A Comprehensive Assessment of Biologicals Contained Within Commercial Airliner Cabin Air

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1.0 EXECUTIVE SUMMARY

Both culture-based and culture-independent, biomarker-targeted microbial enumeration and identification technologies were employed to estimate total microbial and viral burden and diversity within the cabin air of commercial airliners. Samples from each of twenty flights (Phase I: 4; Phase II: 16) spanning three commercial carriers (Phase I [Airline A, B], Phase II [Airline C]) were collected via air-impingement. When the total viable microbial population was estimated by assaying relative concentrations of the universal energy carrier ATP, values ranged from below detection limits (BDL) to 4.1 x 10^6 cells/m^3 of air. The total viable microbial population was extremely low in both of Airline A (~10% samples) and C (~18% samples) compared to the samples collected aboard flights on Airline A and B (~70% samples). When samples were collected as a function of time over the course of flights, a gradual accumulation of microbes was observed from the time of passenger boarding through mid-flight, followed by a sharp decline in microbial abundance and viability from the initiation of descent through landing. It is concluded in this study that only 10% of the viable microbes of the cabin air were cultivable and suggested a need to employ state-of-the art molecular assay that measures both cultivable and viable-but-non-cultivable microbes.

Among the cultivable bacteria, colonies of *Acinetobacter* sp. were by far the most profuse in Phase I, and Gram-positive bacteria of the genera *Staphylococcus* and *Bacillus* were the most abundant during Phase II. The isolation of the human pathogens *Acinetobacter johnsonii, A. calcoaceticus, Janibacter melonis, Microbacterium trichotecenolyticum, Massilia timonae, Staphylococcus saprophyticus, Corynebacterium lipophiloflavum* is concerning, as these bacteria can cause meningitis, septicemia, and a handful of sometimes fatal diseases and infections.

Molecular microbial community analyses exhibited presence of the α-, β-, γ-, and δ-proteobacteria, as well as Gram-positive bacteria, Fusobacteria, Cyanobacteria, Deinococci, Bacterioidetes, Spirochetes, and Planctomyces in varying abundance. *Neisseria meningitidis* rDNA sequences were retrieved in great abundance from Airline A followed by *Streptococcus oralis/mitis* sequences. *Pseudomonas synxantha* sequences dominated Airline B clone libraries, followed by those of *N. meningitidis* and *S. oralis/mitis*. In Phase II, Airline C, sequences representative of more than 113 species, enveloping 12 classes of bacteria, were retrieved. Proteobacterial sequences were retrieved in greatest frequency (58% of all clone sequences), followed in short order by those stemming from Gram-positives bacteria (31% of all clone sequences). As for overall phylogenetic breadth, Gram-positive and α–proteobacteria seem to have a higher affinity for international flights, whereas β–and γ–proteobacteria are far more common about domestic cabin air parcels in Airline C samples. Ultimately, the majority of microbial species circulating throughout the cabin airs of commercial airliners are commensal, infrequently pathogenic normal flora of the human nasopharynx and respiratory system. Many of these microbes likely originate from the oral and nasal cavities, and lungs of passengers and flight crew and are disseminated unknowingly via routine conversation, coughing, sneezing, and stochastic passing of fomites. The data documented in this study will be useful to generate a baseline microbial population database and can be utilized to develop biosensor instrumentation for monitoring microbial quality of cabin or urban air.

2.0 INTRODUCTION AND BACKGROUND

2.1 Overview

Every year more than 600 million passengers board commercial airline flights in the United States (11). Travelers in these closed environments have no choice but to continuously breathe in the very air (and the airborne biologicals within it) that they and their fellow passengers so recently exchanged. Microorganisms are ubiquitous throughout cabin air systems, and passengers and crewmembers are thought to be the predominant source of microbial contamination (10, 42). When coupled with the burdens of stress, fatigue, fluctuating pressure, and arid conditions, the bombardment of the human immune system with such an array of foreign microbiota can result in illness shortly after flying (13, 20, 24, 28). Given that the presence of viable microorganisms circulating about cabin air is of particular consequence to human health, it is critical to thoroughly and accurately assess the diversity and total microbial burden associated with commercial cabin air systems. With well-founded concerns regarding the transmission of emerging diseases on commercial airliners worldwide ever on the rise, the monitoring and control of air quality aboard commercial aircraft has become all the more imperative (30). To date, the majority of investigations of cabin air quality have focused on chemical contaminants, such as cigarette smoke, carbon dioxide, ozone, and total organic carbon (21-23). However, the Federal Aviation Administration has recently begun to emphasize the significance of microbial contamination aboard commercial airliners (The Associated Press; Jan. 19, 2005). Of the few studies to address microbiological burden (10, 15, 42), nearly all have relied on conventional cultivation dependent approaches, which are fraught with bias since fewer than 1% of all bacteria are capable of being cultivated on conventional media (2). As a result, very little remains known about the true diversity of microbial populations circulating about the cabin air of commercial airliners.

Prior to this investigation, the works of Wick ('95), DeChow ('97), and Lee ('99), regarding the microbiological composition of commercial airliner cabin air stood alone and unchallenged. Collectively, these studies reported a typical microbial burden in the range of 10 to 300 CFU/m^3. While these investigations greatly bolstered our general understanding of microbial concentrations in aircraft cabins, they all suffer the same limitation. In failing to account for non-cultivable microbiota when calculating total bioburden, their data is subject to the "great plate count anomaly," a phrase coined by Staley and Konopka (1985) that describes the enormous disparity between what can be recovered from a sample using culturing techniques and the much larger biodiversity that is discernible using molecular methods. Since the time of Pasteur and Koch, it has been acknowledged that cell counts of environmental samples obtained by cultivation are much lower, by orders of magnitude, than direct microscopic cell counts. The factors contributing to this are: a) differing metabolic requirements of varying organisms, b) organisms' ability/tendency to enter a non-cultivable state, and c) potential symbiotic relationships that precludes isolation of specific microbes.

Today it is clear that cultivable microbes represent a miniscule fraction of what is actually present in a sample, and any the microbes that *are* capable of growth are unlikely to be representative of the population as a whole. The novelty of the research described herein is the utilization of molecular, culture-independent methods of assessing total biodiversity and overall bioburden. Whereas previous works were constrained by their

technological approach and could report only the cultivable portion of their samples, our methods circumvented such limitations and facilitated a far more encompassing account of the abundance and diversity of microbiota present within airliner cabin air parcels. In this examination, molecular methods of nucleic acid detection, quantification, and manipulation are employed to both enumerate bacteria and select viruses present, and describe, sans bias associated with culture-based methods, the microbial diversity contained in the cabin air of airliners.

A study using molecular technologies to comprehensively describe the microbial diversity and microbial load inside commercial airliners is timely. In the wake of September 11th and ensuing fears of weaponized pathogens such as anthrax and smallpox, concern over bioterrorism has moved to the forefront. Commercial airliners have been identified as likely means of dispersing biological agents to a worldwide audience. As a measure of prevention, the implementation of biological threat detection systems within aircraft cabins has been suggested. Such a system would sense harmful biological agents in time to facilitate the timely treatment of affected passengers and prevent the spread of infectious agents. In order to develop, fabricate, and calibrate such a detection system, however, the background microbial diversity and total bioburden associated with such environments must first be thoroughly defined.

2.2 Description of Bioaerosol Collection Technologies

A variety of technologies are available for the collection of bioaerosol samples. A summary of the capabilities and limitations of each sampling method follows, as reviewed by Pillai and Ricke (2002). The general strengths and weaknesses of each approach is found in Table 2.2-1.

Impaction. Bioaerosol collection via impaction requires the forced deposition of particles onto a solid or semi solid surface. While this method is ideal for the isolation of known microbiota whose propagation is easily promoted with defined medium, it suffers from the "great plate count anomaly," that is – only a select set of microbes will grow on any particular medium. Such a collection strategy was not appropriate for this study as we are concerned predominantly with elucidation of total microbial diversity and enumeration of selected viruses. Potentially, the media bias associated with impaction sampling can be avoided by collection onto a glass slide rather than predefined media. Collection onto glass remains problematic, however, as a significant amount of cellular injury is associated with this method (Stuart *et al*, 1995), which is only exacerbated by dehydrating storage conditions. Furthermore, the efficient and aseptic transfer of captured particles from the glass slide to a buffer solution presents challenges in the field and laboratory alike.

Filtration. Bioaerosol collection by means of filtration relies on the passage of airflow through, and collection of airborne particles onto a porous material, typically a membrane filter. One drawback of this technique is its dependence on both the physical properties of the airborne particles and the air flow rate of the sampler. Furthermore, the arid environment of commercial airliner cabins is not conducive to efficient filtration sampling, as this collection strategy is greatly affected by relative humidity. Li et al. have reported that the survival of *E. coli* collected via filtration was lower than that observed when collected by impingement, which, in their assessment, was a result of dehydration.

Finally, the results arising from filtration vary greatly with ultrasonic agitation and vortexing, potentially limiting sample work up.

Electrostatic precipitation. The electrostatic precipitation of airborne particles offers a far gentler means of bioaerosol collection, often circumventing the cellular damage associated with impaction or impingement. This technology collects bioaerosols by conferring on airborne particles an electric charge, causing them to drift onto appropriate collection substrates. While the gentle particle collection and the minimization of cellular damage is appealing, preliminary studies have reported differences in culturability of electrostatically precipitated microorganisms depending on the collection substrate. Moreover, this sampling method would be virtually possible to adapt to aircraft sampling as the sampler utilizes electrically charged components for particle collection that undoubtedly generate electromagnetic fields incompatible with aircraft instruments. Ultimately the development of this sampling technology remains immature, and though such a strategy seems highly advantageous in laboratory-based sampling events, its application in field studies of this nature is not yet feasible.

Gravity sampling. Gravity sampling is by far the least effective method of bioaerosol collection. According to Pillai and Ricke (2002), "many studies have shown that gravity-collected samples are so inaccurate that they are neither qualitatively nor quanitatively comparable to those obtained by other samplers." Gravity sampling introduces a large bias in particle deposition due to particle size and shape, and the dynamics of the surrounding air. Since none of these factors can be controlled on a passenger flight, this option for sample collection was quickly eliminated.

Impingement. Impingement is the forced deposition of particles into a liquid substrate, most often a buffer that can sustain its biomolecular load for future culture, microscopic, and molecular methods. Impingement-based bioaerosol samplers are commercially available, and depending on the manufacturer, can be modified to meet the needs of a particular sampling event. The use of self-contained, commercially available sample cartridges ensures consistent buffer composition and eliminates bias associated with vortexing, ultrasonic mixing, and preparation of media or substrates.

Sampling efficiency for impingement samplers has been previously reported (Ref) to vary between 10 and 100%, depending on the biological entities under examination and the specifications of the particular sample used for collection, i.e. very light, portable and high volume air samplers have lower efficiency than ones that are large, bulky, and slow. The trade off between efficiency and practical field deployment, however, is an inescapable consequence of all available air sampling technologies. Based on the shortcomings and limitations of the collection strategies discussed above, and the strengths offered by impingement, we opted to utilize this method of bioaerosol collection for all experimentation discussed herein.

3.0 MATERIALS AND METHODS

3.1 Sample Collection Strategy

The BioCapture BT-550 (Mesosystems Technology Inc., Kennewick, WA) sampler employed in this study was selected for a number of reasons. The unit collects particles in the size range of 0.5 to 10 µm (optimum for cell and particle size) from ambient air, and its compact size and lightweight design (4.5kg) make it ideal for use in the aircraft cabin. Since it was originally DC battery operated, the unit was modified by the manufacturer to operate directly from seat power available on each plane. Batteries were not an acceptable power source for long domestic or international flights, as the unit requires heavy lead-acetate batteries and sustained a charge for only a few samplings before requiring replacement. Finally, the BioCapture sampler offered a high flow rate of 150L per min (0.15 cubic meters per min) and a high sampling efficiency of approximately 50%.

Flow rate and performance may vary when sampling from a blowing source, such as a gasper (the individually controlled ceiling air inlet device located above each seat). To ensure that gasper samples were not contaminated with ambient cabin air, a 2-foot long sterile hose adapter was attached to the sampling unit to and the to gasper outlet. Each 750L air parcel (roughly equivalent to the volume of air human lungs exchange every 2 hours) was impinged into 5 mL of sterile buffered saline by running the sampler for 7 minutes.

Average domestic flights traverse 1,000 miles and last approximately 2.5 hours, whereas intercontinental flights often exceed 5,000 miles and can last more than 15 hours (Wick et al., 1995). Airline cabin exchange rates are typically between 15 and 20 times per hour, whereas, for reference, the air in a private office building or private home is turned over 12, and 5 times per hour, respectively. Stated differently, commercial airline cabin air volumes are exchanged once every 3 to 4 minutes, while exchange of volumes of office or private home air occur every 5 and 12 minutes, respectively. Furthermore, the cabin volume in a standard commercial airliner is, on average, about 566 m^3. For every 7 minute Biocapture BT-550 sampling event, 0.75 m^3 of air is collected, less than 0.13% of the total cabin volume. Therefore, values reported herein, representative of individual samplings, must be multiplied by ~ 1,000 to realize the actual burden associated with the entire aircraft cabin.

In Phase I of this project, 70 air samples were collected via impingement aboard 4 domestic flights on 2 different commercial carriers (Airlines A and B). Samples were collected prior to boarding, upon reaching cruise altitude, mid-flight, prior to descent, and post-landing from each of the following coach section locations: seat, floor, lavatory, and gasper. Phase I sampling events relied on the standard, battery operated device and samples were collected prior to take-off, hourly throughout the course of the flight, and post-deplaning. Samples were returned to the laboratory within 24 hours of being procured. Upon arriving at the lab, samples were either processed immediately or stored at -80ºC, depending on the urgency involved with particular assays (cultivation-based and ATP assays need be carried out immediately). Since original samples were to be partitioned out for numerous assays, no cryoprotectant was used. After suitable aliquots (~ 1 mL each) were removed for culture-dependent plate counts and ATP analyses, the

remaining samples were stored away at -80°C for future DNA extraction and phylogenetic analyses.

In Phase II, 125 air samples were collected from a business class seat location aboard 16 domestic and international flights on a single commercial carrier (Airline C). Business class seats were obtained because the larger scope of the project required space for storing/refrigerating samples and because business class eats offered access to electric power. All Phase II samples were taken using the same model sampler used in Phase I, retrofitted to operate from this power supply. Because the device was tethered by the power cord, sample collection for Phase II was typically restricted to the seat location. Only a few locations in the coach section are powered, used primarily for operating cleaning equipment. A small subset of samples from Phase II was collected from coach when these electrical outlets were available. Three replicate samples were taken at each time point. Immediately following sample collection, two replicates were stored on dry ice, one for viral enumeration, another to be archived for future analysis. A third replicate was placed in a cooler chilled with ice packs to approximately 4°C. Chilled samples were processed as soon as possible for viability assays (ATP and plate counts), typically soon after arrival for international flights which usually landed before noon, and the following morning for domestic flights all of which arrived during evening hours. As with Phase I, aliquots of sample remaining after viable assaying were frozen for DNA extraction and phylogenetic analyses. No samples from flight 4-S (Los Angeles to Sydney, Australia) were processed and were instead archived for later analysis. A total of 116 samples were analyzed at JPL.

There are no previous molecular studies of the airbone viral load contained in commercial airliner cabin air. As no such protocols or procedures exist in current literature, an attempt to optimize the efficiency of viral particle collection, transport, and processing prior to sampling aboard in-flight aircraft was carried out on a set of air samples collected from a metropolitan train depot during morning commute hours (7:00 - 9:30 AM). The samples were transported on icepacks to the University of Southern California (Los Angeles) where they were immediately stored at - 80ºC for viral analysis. From the results of such work, airborne virion collection and analysis protocols were designed to determine the lower limits of viral detection, and maximize the sensitivity and specificity of each viral assay to be performed in Phase II.

3.2 Bioassay Strategies: Biologicals assayed and quantified

Numerous biological assays were utilized in this study in an attempt to thoroughly describe the total bioburden and microbial diversity associated with commercial airliner cabin air. Some of these methods are specific, and report or enumerate the presence of a specific biological entity – 16S biased PCR for bacteria, for example. Other strategies detect a specific biomolecule in a wide range of organisms. The ATP assay for instance, measures ATP molecules regardless of whether they stem from bacterial or eukaryotic sources.

For discussion purposes, the following terms are used as defined below. The values reported by the ATP assay are of two types: i) total ATP values reflect the total amount of ATP present within a given sample, regardless of origin (live and dead microbes, human skin cells, misc. exogenous sources), ii) intracellular ATP values discriminate the

portion of this ATP pool that is derived from viable, in-tact cells. Finally, TaqMan Q-PCR values are indicative of all procaryotic cells present (both dead and alive) and are expressed in terms of total ribosomal gene copy number (each cell bearing between 1 and 15 chromosomal rRNA gene copies).

The main thrust of this study was to use molecular methods to better understand A) total burden of select DNA and RNA human viruses, B) total bacterial burden, and C) the "true" microbial diversity associated with commercial airliner cabin air parcels.

3.3 Total burden of select DNA and RNA viruses

3.3.1 Viral Reference Strains

The abundance of six types of viruses, three DNA and three RNA, were quantified during the course of this study. Reference strains for Human Coronavirus 229E and OC43, Influenza virus A/Port Chalmers/1/73 and B/Lee/40, Human Respiratory Syncytial virus B/Wash/18537/1962, Epstein-Barr virus B95-8, Varicella-Zoster virus AV92-3 were provided by the American Type Culture Collection (Manassas, VA). Adenovirus strains 5 and 40 were kindly provided by Sunny Jiang, Dept. of Environmental Analysis & Design, University of California at Irvine (Irvine, CA). Viral stocks were stored at -80ºC until the time of extracting nucleic acids.

DNA Viruses

Varicella-Zoster virus (VZV), also known as human herpes virus 3 (HHV-3), is one of the eight herpes viruses known to affect humans. At any given time, 5 people in 1,000 present symptoms of VZV. Primary VZV infection results in chickenpox (*Varicella*), which may rarely present complications including VZV encephalitis or pneumonia. Even when clinical symptoms of *Varicella* have resolved, VZV remains dormant in the nervous system. In about 10-20% of cases, VZV reactivates later in life to produce *Herpes Zoster* (shingles). This year, more than 500,000 people will develop shingles. The virus is very susceptible to disinfectants, notably sodium hypochlorite.

Adenoviruses are viruses of the family Adenoviridae. Adenoviruses were first isolated in human adenoids (tonsils), from which the name is derived. They're medium-sized (60-90 nm), nonenveloped icosahedral viruses containing double-stranded DNA. Adenoviruses represent the largest nonenveloped viruses, because they are the maximum size able to be transported through the endosome (i.e. envelope fusion is not necessary). The virion also has a unique "spike" associated with each penton base of the capsid that aids in attachment to the host cell via the coxsackie-adenovirus receptor on the surface of the host cell. There are 51 immunologically distinct human adenovirus serotypes (6 species: Human adenovirus A through F) that can cause human infections ranging from respiratory disease (mainly species HAdV-B and C), and conjunctivitis (HAdV-B and D), to gastroenteritis (HAdV-F serotypes 40 and 41). Adenoviruses are unusually stable to chemical or physical agents and adverse pH conditions, allowing for prolonged survival outside of the body and water. Adenoviruses are primarily spread via respiratory droplets, however they can also be spread by fecal routes as well.

Adenovirus infections most commonly cause illness of the respiratory system; however, depending on the infecting serotype, they may also cause various other illnesses, such as gastroenteritis, conjunctivitis, cystitis, and rash illness. Symptoms of respiratory illness

caused by adenovirus infection range from the common cold syndrome to pneumonia, croup, and bronchitis. Patients with compromised immune systems are especially susceptible to severe complications of adenovirus infection.

Epstein-Barr virus, frequently referred to as EBV, is a member of the herpesvirus family and one of the most common human viruses. The virus occurs worldwide, and most people become infected with EBV sometime during their lives. In the United States, as many as 95% of adults between 35 and 40 years of age have been infected. Infants become susceptible to EBV as soon as maternal antibody protection (present at birth) disappears. Many children become infected with EBV, and these infections usually cause no symptoms or are indistinguishable from the other mild, brief illnesses of childhood. When infection with EBV occurs during adolescence or young adulthood, it causes infectious mononucleosis 35% to 50% of the time.

RNA viruses

Coronaviruses were first isolated from chickens in 1937. After the discovery of Rhinoviruses in the 1950's, ~50% of colds still could not be ascribed to known agents. In 1965, Tyrrell and Bynoe used cultures of human ciliated embryonal trachea to propagate the first human coronavirus (HCoV) in vitro. There are now approximately 15 species in this family, which infect not only man but cattle, pigs, rodents, cats, dogs and birds (some are serious veterinary pathogens, especially chickens).

Coronavirus is a genus of animal virus belonging to the family Coronaviridae. They are enveloped viruses with a positive-sense single-stranded RNA genome and a helical symmetry. The most publicized human coronavirus, SARS-CoV, has a unique pathogenesis because it causes both upper and lower respiratory tract infections and can also cause gastroenteritis. Because the primary route of transmission for this virus is respiratory or via fomites, coronaviruses are believed to cause a significant percentage of all common colds in human adults. Common symptoms include throat discomfort, followed by sneezing, runny nose, nasal congestion, coughing and decreased energy level. Fever is uncommon, except in young children. The significance and economic impact of coronaviruses as causative agents of the common cold are hard to assess because, unlike rhinoviruses (another common cold virus), human coronaviruses are difficult to grow in the laboratory.

In 2003, following the outbreak of Severe acute respiratory syndrome (SARS) which had begun the prior year in Asia, and secondary cases elsewhere in the world, the World Health Organization issued a press release stating that a novel coronavirus identified by a number of laboratories was the causative agent for SARS. The virus was officially named the SARS coronavirus (SARS-CoV).The SARS epidemic resulted in over 8000 infections, about 10% of which resulted in death.

Respiratory Syncytial Virus (RSV) is a negative sense, single-stranded RNA virus of the family Paramyxoviridae, which includes common respiratory viruses such as those causing measles and mumps. RSV is a member of the paramyxovirus subfamily Pneumovirinae, and causes respiratory tract infections in patients of all ages. It is the most common cause of respiratory disease in infancy and childhood. In the United States, 60% of infants are infected during their first RSV season, and nearly all children will have been infected with the virus by 2-3 years of age. RSV causes 50% of all bronchiolits,

25% of all pneumonia, and is responsible for more than 4,500 deaths per year in the US. Transmission occurs via direct contact and aerosols. RSV is highly transmissable! In temperate climates there is an annual epidemic during the winter months. Natural infection with RSV does not induce protective immunity, and thus people can be infected multiple times.

For most people, RSV produces only mild symptoms, often indistinguishable from common colds and minor illnesses. RSV is ubiquitous in all parts of the world; avoidance of infection is not possible. Vaccine development has been fraught with difficult obstacles and so far remains elusive.

Influenza

Until the advent of AIDS, influenza was the last uncontrolled pandemic killer of humans. One historic measure of influenza's potential lethality is that more people died in the 1918-19 pandemic than in World War I. In the United States, influenza currently causes more morbidity and mortality than AIDS. In nonpandemic years, 10,000 to 20,000 people die of influenza-related illness in the U.S. In pandemic years, such deaths can exceed 100,000; the morbidity, of course, far exceeds the mortality. In addition to this human toll, influenza is annually responsible for a total cost of over $10 billion in the U.S. Measured resistance to standard antiviral drugs amantadine and rimantadine in the dominant strain (H3N2) has increased from 1% in 1994 to 12% in 2003 to 91% in 2005.

The influenza viruses are a group of RNA viruses designated as types A, B, and C. Influenzavirus B is a genus in the virus family Orthomyxoviridae. The only species in this genus is called "Influenza B virus". Influenza B virus usually causes a minor illness, but it does have the potential to cause more severe disease in older persons. Influenza A virus, however, causes pandemics. Influenzavirus A is a genus of a family of viruses called Orthomyxoviridae in virus classification. Influenzavirus A has only one species in it; that species is called "Influenza A virus". Influenza A virus causes "avian influenza" (also known as bird flu, avian flu, Influenzavirus A flu, type A flu). It is hosted by birds, but may infect several species of mammals. All known subtypes are endemic in birds.

The reason for the recurrent outbreaks is that the virus undergoes periodic antigenic shifts in its two outer membrane glycoproteins-hemagglutinin (H) and neuraminidase (N)-for example, from H1N1 to H2N2 in 1957 and from H2N2 to H3N2 in 1968, thus introducing a new virus into a population that has no protective serum antibody. No different subtypes of H and N have been identified for influenza B.

Influenza virus infection causes three syndromes, an uncomplicated rhinotracheitis, a respiratory viral infection followed by bacterial pneumonia, and viral pneumonia. All three syndromes have incubation periods of one to two days. In the early stages, they are often clinically indistinguishable from many other viral infections. One distinguishing feature of influenza virus infection, present in a small percentage of patients, is very rapid onset of profound malaise. In as little as one or two minutes, patients who feel well may suddenly feel so tired that they can hardly move.

3.3.2 Isolation of Nucleic Acid from Virus Stocks

RNA and DNA extraction from viral stocks was performed using the QiaAmp Minelute Virus Spin Kit (Qiagen) according to the kit directions. All stocks of Qiagen protease

were resuspended in RNase-free water instead of Qiagen Resuspension Buffer because the flight samples were stored in cartridges containing a phosphate-containing buffer (phosphate buffered saline). The nucleic acids were eluted into 40µL of elution buffer, split into aliquots of 20µl and stored at -80ºC until further use.

3.3.3 Viral Nucleic Acid Isolation from Union Station Samples

Initially, samples volumes of 0.5mL to 13mL were filtered through 0.02 µm pore size Anodisc (Whatman) filters and nucleic acids were extracted immediately from the filter. Nucleic acid extraction was performed using the QiaAmp Minelute Virus Spin Kit (Qiagen) with the following modification at the beginning of the procedure: Qiagen protease, lysis buffer AL, and carrier RNA and buffer AVE were added directly to the filter and incubated in flat-bottom non-tissue culture treated plates (Falcon) at 56ºC for 15 minutes prior to transferring the liquid to the column provided. Results for viral controls were positive, but the final extraction and recovery efficiency with samples collected on filters was greatly reduced compared to direct extraction from small (0.2mL to 2mL) sample volumes. We therefore adjusted the extraction procedure and samples were extracted directly from 0.2mL to 2mL of sample. The extraction efficiencies of 0.2mL, 1mL, and 2mL were compared for Epstein-Barr and Coronavirus OC43 assays, and the 0.2mL volume was determined to be sufficient. We observed no improved overall recovery from 1mL vs 0.2mL samples, and a decrease in copy number detected in the 2mL samples. While the reasons for this were not known (we thought it involved saturation and even washing off of the viral nucleic acid from the Qiagen cartridge used to capture it), we used this as an empirical guide. Therefore, 0.2mL samples were used subsequently.

3.3.4 Viral Nucleic Acid Isolation from Flight Samples and Blank Cartridges

RNA and DNA extraction from unused cartridges (blanks) and flight samples were performed by use of the QiaAmp Minelute Virus Spin Kit (Qiagen). Three 200µl subsamples were extracted from each cartridge. The remaining volume in each cartridge (~12.4mL) which was not extracted was stored in falcon tubes at -80ºC. At the end of the extraction procedure, each column was eluted with 35µL elution buffer, yielding approximately 30 ul each. The three subsamples were then mixed by gentle pipetting and then divided into three aliquots and frozen at -80ºC. Throughout the extraction and detection procedure, work was done in a freshly cleaned (bleach) Type II safety hood, with barrier-type pipette tips and frequent changes of gloves. Particular care was taken in opening of tubes to minimize cross-contamination.

3.3.5 RT-QPCR and Q-PCR of viral nucleic acids

Each assay was verified initially using a dilution series of viral nucleic acids (from $5x10^{-4}$ to 5uL undiluted nucleic acids) using previously published Taqman-based QPCR assays (Kimura, Morita et al. 1999; Cohrs, Randall et al. 2000; van Elden, Nijhuis et al. 2001; Heim, Ebnet et al. 2003; Hu, Colella et al. 2003; van Elden, van Loon et al. 2004) (Table 3.3.5-1). These assays were initially designed for clinical samples (e.g. plasma),. All amplification and detection were performed by the Stratagene MX3000P QPCR System. All samples, PCR standards, and negative controls were run in duplicate. To minimize the chance of contamination, all samples wells for the assays were filled and closed before the positive control was opened. We were unable to amplify human coronavirus

229E and influenza B from viral RNA stocks, and did not include these stocks in further analysis.

3.3.6 Generation of Real-Time virus DNA PCR standards/standard curves

The target genes for each assay were amplified using the published RT-QPCR and QPCR protocols, with an added 7 minute extension at 72ºC. The PCR products were then cloned with the Qiagen Cloning Kit for Sequencing and sequenced at the Biotechnology Program at the University of Florida (Gainesville, FL). Plasmids containing the target gene were purified with the QiaPrep Spin Miniprep Kit (Qiagen), eluted into 50 ul EB buffer, and quantified with PicoGreen dsDNA Quantitation Kit (Molecular Probes). The copy number of the target gene in the miniprep was then determined based upon the amount of DNA, the length of the cloned PCR product, and the length of the vector. A dilution series from $5x10^5$ to 5 copies of each plasmid were added to each appropriate QPCR assay to generate a standard curve. These standard curves were used to determine the gene copy number in each subsequent set of assays.

3.3.7 RT-QPCR and QPCR of flight samples and blank cartridges

Each assay was conducted on nucleic acid subsamples that had not been thawed previously (we had found previously that a second freeze-thaw step can lead to significant loss of nucleic acid, Fuhrman et al 2005). Standard curves were not run with each 96-well plate, in order to reduce the risk of contamination by multiple positive controls, but a single low-level positive control (in duplicate) was run on each plate. The positive control worked as expected for each set of data presented in this report.

3.4 Total bacterial burden

Three methodologies were employed in concert to more effectively ascertain total bioburden about the cabin air of airliners: culture-based plate count assays, total- and intracellular-ATP-based biomarker assays, and 16S rDNA Taqman Q-PCR.

3.4.1 Cultivation-based plate count assay

Duplicate 100 µL aliquots of each sample were spread-plated onto minimal media (R2A agar; Difco Laboratories, Detroit, MI) and colony-forming units (CFU) of total heterotrophs were enumerated following 7 days incubation at 25°C. Isolates were picked, purified and cultured, and stored at –80°C for further processing and analysis. When fewer than 5 colonies arose on a plate, all colonies were picked. When >10 colonies were present, 10 colonies were picked in a non-duplicative manner, with the aim of capturing the most diverse collection of isolates as possible. Colony morphology was considered when selecting isolates for further characterization. Identification of purified strains was determined via 16S rDNA sequence analysis (Agencourt Bioscience, Beverly, MD).

3.4.2 ATP-based bioluminescence assay

ATP-based microbial detection was made popular in the early 1960s to evaluate industrial hygiene quality (8), and recent innovations have promoted the applicability of this technique to the forefront of many facets of microbial monitoring (40). Today it is possible to measure strictly intracellular ATP (indicative of viable microbes) within a given sample as well as total ATP (indicative of total microbial burden), inclusive of extracellular and intracellular ATP (36, 40). To determine total ATP, 0.1 mL of sample

was combined with 0.1 mL of a detergent for lysing cells (benzalkonium chloride) and then incubated at room temperature for 1 min prior to the addition of 0.1 mL of luciferin-luciferase reagent, an enzyme resistant to inactivation by detergent. The sample was mixed and the amount of bioluminescence measured with a luminometer. To determine intracellular ATP, 0.1 mL of an ATP-eliminating reagent (apyrase, adenosine deaminase) was added to a 1-mL portion of the sample, mixed, and allowed to incubate for 30 min to remove any extracellular ATP, after which the assay for ATP was carried out as described above. The dynamic range of this assay is from 5x 10^{-12} M to 10^{-7} M ATP, with one relative luminescence unit (RLU) corresponding to 2 x 10^{-14} M ATP as determined by linear regression analysis of standard curves with known ATP concentrations (40). This rapid and simple assay (60-min) is commercially available (Kikkoman Corp., San Francisco, CA).

A thorough and comprehensive examination of the correlation between traditional colony forming units (CFU) and modern ATP-assay derived relative luminescence units (RLU) in enumerating microbes and determining bioburden of surfaces/samples has yet to be thoroughly explored. Previous studies, however, have reported that for most Gram-negative bacteria, 1 RLU corresponds to 1 CFU, as compared to Gram-positive bacteria where 1 CFU corresponds to roughly 5 RLU. It should also be assumed that in regularly cleaned, low humidity, HEPA filtered environments such as aircraft cabins; cells exist in a less active metabolic state and contain less ATP than actively growing cells (36). While yeast and fungi contain 100 times more ATP than typical Gram-negative bacteria, 18S-biased eukaryotic PCR failed for all samples, and Rose-Bengal agar plates specific for mycelial growth were devoid of fungal colonies, suggesting that all RLU are attributable to procaryotic microflora. Considering these circumstances, we conservatively estimate that 1 RLU is approximately equal to 1 CFU (17, 40).

3.4.3 DNA extraction and PCR amplification

In Phase I, 5 mL from each of a few samples was used to extract DNA via standard lysozyme/phenol-chloroform procedures (Ausubel et al.). In an attempt to reduce the shearing of DNA during this process, and minimize loss of DNA product while transferring between several reaction tubes, samples were not concentrated. We have previously reported the percent DNA recovery using this solvent-based DNA extraction method for low biomass samples to be ~ 10% (7). DNA was extracted from individual or pooled samples following standard lysozyme/organic solvent extraction protocols (16). Briefly, samples were treated with lysozyme (final conc. 10mg/ml) to degrade cell walls, followed by Proteinase K and RNAse treatment to remove unwanted bioploymers, and finally phenol-chloroform to clean-up cellular debris. DNA was precipitated with 2 volumes ice-cold ethanol, washed briefly with 70% ethanol, and resuspended in TE buffer (30 µL) before storing at - 80ºC. Bacterial small subunit rRNA genes (~1.5 kb) were PCR-amplified (1 to 5 µL template) with a PTC-100 thermal cycler (MJ Research, Waltham, MA) using eubacterially biased primers 8F and 1492R, as previously described (32).

In Phase II, steps were taken to improve DNA recovery. Portions of all samples from each flight were pooled and concentrated in Amicon-Ultra 15 centrifugal filters (Millipore) using a Sorvall RC-5B refrigerated superspeed centrifuge (DuPont Instruments) at 3000 rpm for 20 minutes. These filter units have a molecular weight cut-

off of 50,000 daltons, facilitating the isolation of all intact bacterial cells and naked DNA fragments of greater than 100bp. This is accomplished by placing two filters into each 50mL Amicon tube in a "V" configuration, nearly parallel to the length of the tube. Centrifugal force pushes cells and particles downward into a ~250 µL reservoir while water exits from the sides resulting in less shearing force on intact cells. Amicon units were reusable for each sample set, allowing for all samples (~20-50mL) from a single flight to be pooled and concentrated to a volume of 200uL in a single filter tube. A comparable amount of sterile, unused cartridge buffer was concentrated in a separate filter tube to act as a control for each extraction. After concentrating pooled samples from each flight, total DNA was collected for each flight sample via a standard organic solvent DNA extraction protocol similar to the one used in Phase I except for the addition of a CTAB precipitation step aimed at further purifying removing polysaccharides that could inhibit DNA amplification.

3.4.4 Taqman® Quantitative PCR (as adapted from Brunk et al., 2002).

Recently, a real-time analytical technique for QPCR analysis, Taqman (Applied Biosystems, Foster City, California), has become available which provides remarkably rapid and accurate determination of target sequences in a sample (10-12). A PCR machine is used to record the fluorescence activity of individual PCR tubes during the PCR amplification. Taqman analysis measures the increase in PCR product for each cycle of the PCR amplification. During the early cycles the amount of PCR product is below the detection limit of the machine, but by cycle number 12 to 15, the PCR product can usually be detected. When the PCR product is first detectable, the PCR is still in the linear range and the amount of PCR product is proportional to the amount of target sequence in the original sample.

In QPCR, a short fragment of the small subunit ribosomal RNA (SSU rRNA) gene (~100 base pairs) is PCR amplified; the amount of amplification product produced is monitored by the release of fluorescence from a target specific probe during the PCR process. The probe hybridizes specifically with the target sequence at a position between the PCR primers. There are two kinds of probe for the Taqman assay. The standard probe contains a fluorescent reporter dye and a non-fluorescent a quencher dye, in close proximity. The minor grove binder (MGB) probe contains a fluoresenct reporter, a non-fluorescent quencher and a MGB, which significantly increases the binding of the probe to the target sequence. The MGB probe is usually shorter (13-18 bp) than the standard probe (15-40 bp) and produces increased sequence specificity. The interaction between the reporter dye and quencher prevents the fluorescence of the reporter dye. The interaction between the two dyes prevents the fluorescence of the reporter dye. During PCR amplification the exonuclease activity of the polymerase degrades the probe and separates the reporter dye and the quencher dye, which allows fluorescence of the reporter dye. Although this QPCR analysis requires sophisticated and expensive instrumentation, it is a very rapid and accurate means of determining the amount of target DNA in a sample. This approach requires no post PCR analysis. The MJ Research Chromo4 we employed performs 96 parallel wells of Taqman analyses. Generally, 12 wells are used for controls and standards leaving 84 wells for sample analysis. The machine determines a standard curve and calculates the amount of target DNA in each of the sample wells. Thus the output is a direct measure of the abundance of target sequence in the sample. Extensive

documentation on the principles, techniques, and applications of Taqman assays is provided on the Applied Biosystems website (www.appliedbiosystems.com/molecularbiology/about/ pcr/ sds/).

For the purposes of this study, 16S Ribosomal RNA gene copy numbers were quantified in triplicate using two chromophore systems (MJ Research Chromo4, Biorad, Hercules, CA; Applied Biosystems 7700; Applied Biosystems Inc, Foster City, CA). A TaqMan Q-PCR standard curve ranging from 1 x 10^1 to 1 x 10^8 16S rDNA fragments was generated by purifying and decimal diluting *E. coli* 16S rDNA-ligated cloning vector plasmids. Universal eubacterial primers targeting the 16S rRNA gene, 1369F (5'-CGGTGAATACGTTCYCGG-3') and 1492R (5'-GWTACCTTGTTACGACTT-3'), and the fluorescent-labeled probe TM1389F (5'-FAM-CTTGTACACACCGCCCGTC-TAMRA-3'), were used in this quantitative analysis (37). Each 50 µL reaction consisted of 1X Taqman Universal PCR Master Mix (Cat. #: 4352042; Applied Biosystems Inc.), 0.8 µM of each oligonucleotide primer, 0.5 µM of oligonucleotide probe, and 1 µL of template DNA. Reaction conditions were as follows: 95ºC denaturation for 15 min., followed by 40 cycles of denaturation at 95ºC for 15 s and a combined annealing and extension at 60ºC for 1.5 min.

3.5 Molecular microbial community analyses

Bacterial diversity was assessed by two distinct and independent molecular strategies. Both microarray technologies and rDNA cloning techniques rely heavily on efficient extraction of total genomic DNA from samples, and subsequent PCR amplification of desired genes. Once DNA amplification products are purified, they can then be both analyzed via hybridizations with RNA microarrays and cloned into vectors to generate rDNA clone libraries.

3.5.1 Conventional cloning and sequencing

As reviewed by Brunk et al. (2002), in the past decade molecular techniques have provided a means for detecting the presence of virtually any type of bacteria in environmental samples, independent of the ability to culture them in the laboratory. Bacterial DNA can be isolated from the sample and the entire 16S rRNA gene (~1400 base pairs) can be amplified using the polymerase chain reaction (PCR). The sequences of the SSU rRNA genes uniquely identify the bacteria in the sample. PCR typically results in a 10^5 to 10^6-fold amplification of target DNA for 20 to 30 cycles, thus the sensitivity of this approach is very high. The selection of PCR primer sequences allows a wide spectrum of specificity. If general primers are used, almost all SSU rRNA sequences can be amplified. If unique PCR primers are chosen, a single species or small group of bacterial SSU rRNA sequences in the sample will be preferentially amplified.

3.5.1.1 16S rDNA Clone Library Analysis

Sample selection for cloning during Phase I

Since none of the initial DNA extractions yielded positive PCR amplification products as described in section 3.4.3, and to circumvent problems and biases encountered in processing low-biomass volumes, subsequent samples of conserved location were pooled from each flight (rationale given below). PCR conditions were as follows: 1 min. 95°C

denaturation, 2 min. 55°C annealing, and 3 min. 72°C extension for 35 cycles followed by a 10 minute incubation at 72°C.

Cloning: Phase I

Ribosomal DNA amplification products were purified with a gel excision kit (Qiagen, Chatsworth, CA), ligated in the pCR4-TOPO cloning vector (Invitrogen, Carlsbad, CA) and transformed into chemically competent *E. coli* Top10 cells (Invitrogen, Calsbad, CA) according to manufacturer's protocols. For each of the 10 pooled samples, 100 transformant colonies were picked and plasmid DNA was purified with a Qiaprep kit (Qiagen, Chatsworth, CA). Inserts from each clone were amplified as described above with T7 and M13R primers targeted to vector regions flanking the insert. Amplicons were digested with *Hha*I restriction endonuclease (Promega, Madison, WI) for 4 hours and analyzed on a 2% low melting point agarose gel (Shelton Scientific, Prosta, IA). Clones were grouped according to similarity of banding patterns and representatives of each group were fully, bi-directionally sequenced (Davis Sequencing, Davis, CA).

Sample selection for cloning during Phase II

Samples from all flights were pooled in Phase II and DNA was extracted as described in section 3.4.3. Bacterial small subunit rRNA genes were amplified with the following three universal bacterial primer sets (in order of increasing sensitivity): 27F (AGA GTT TGA TCM TGG CTC AG) and 1492R (GGT TAC CTT GTT ACG ACT T), 27f.1 (AGR GTT TGA TCM TGG CTC AG) and 1492R, and 9bF (GRG TTT GAT CCT GGC TCA G) and 1406uR (ACG GGC GGT GTG TRC AA). The 27F/1492 primer set was used exclusively in the Phase I study and was used preferentially and as often as possible for the Phase II DNA samples. Of the three sets, it had the least propensity for signal amplification of the control buffer. More sensitive primer sets were used as needed for samples that showed no PCR amplification product when tested using the 27F and 1492R primer set. Clone libraries generated by using either the 27f.1/1492 or 9bF/1492R primer sets are indicated in Table 3.5.1.1-1.

Cloning: Phase II

PCR amplification products were ligated into pCR4-TOPO cloning vector and transformed into chemically competent E. coli cells using commercially available kits (Invitrogen). For each pooled sample, a total of 96 colonies were randomly picked either manually or mechanically. In contrast to Phase I, which used RFLP analysis to down-select clone libraries, all clones from Phase II were fully and bidirectionaly sequenced by Agencourt Bioscience Corporation (Beverly, MA). In total about 2,000 clones were selected and sequenced for 1.5 kb length. An assembly and search algorithm created in-house was used to automatically assemble each clone sequence, and locate their nearest phylogenetic neighbor in the public database (http://www.ncbi.nlm.nih.gov/BLAST). Phylogenetic analysis of assembled clone sequences and nearest neighbors was carried out using PAUP and ARB software.

3.5.1.2 Sequence analysis and phylogenetics

An alignment of ca. 20,000 homologous full and partial sequences available in the public ARB database (25) was used. Novel 16S sequences (ca. 1,400 nucleotides) were aligned to their nearest neighbor using automated tools of the ARB software suite (Technische

Universitat Munchen, Munich, Germany [http://www.mpi- 180bremen.de/molecol/arb/]). The resulting alignment was checked manually and corrected if necessary. Phylogenetic trees were reconstructed via maximum parsimony and neighbor joining methods.

3.5.2 Microarray Technology

3.5.2.1 Microarray Design

Microarray technology was utilized for the second phase of this project to complement data from clone libraries. In summary, a collection of 500K 16S rDNA probes comprising 9K species were spotted onto glass slides. Environmental DNA extracts were PCR amplified, fragmented, biotinylated and hybridized to the microarray chip.

The microarray probe design approach previously described for differentiating Staphylococcacae (DeSantis, 2003) was applied to the all known prokaryotic sequences of substantial length. Briefly, 16S rDNA sequences (*Escherichia coli* base pair positions 47 to 1473) were obtained from approximately 30,000 16S rDNA sequences that were at least 600 nucleotides in length in the 15 March 2002 release of the prokMSA database 16S rDNA database, greengenes (greengenes.lbl.gov). This region was selected because it is bounded on both ends by universally conserved segments that can be used as PCR priming sites to amplify bacterial or archaeal (Dojka *et al.*, 1998) genomic material using only 2 to 4 primers. Putative chimeric sequences were filtered from the data set using the software package Bellerophon (Huber et al., 2004) preventing them from being misconstrued as novel organisms (Hugenholtz & Huber, 2003). The filtered sequences are considered to be the set of putative 16S rDNA amplicons. Sequences were clustered to enable each sequence of a cluster to be complementary to a set of perfectly matching (PM) probes. Putative amplicons were placed in the same cluster as a result of common 17-mers found in the sequence. The resulting 8,988 clusters, each containing less than 5% sequence diversity, were considered operational taxonomic units (OTUs) representing all 262 (91) known prokaryotic orders. The taxonomic family of each OTU was assigned according to the placement of its member organisms in Bergey's Taxonomic Outline (Bergey's Manual of Systematic Bacteriology, 2nd Edition 2001, George M. Garrity, Editor-in-Chief Springer-Verlag, New York). The Hugenholtz Taxonomic Outline (Hugenholtz, P. 2002. Exploring prokaryotic diversity in the genomic era. Genome Biology. 3(2):reviews0003.1-0003.8.) was consulted for phylogenetic classes containing uncultured environmental organisms or unclassified families belonging to named higher taxa. The OTUs comprising each family were clustered into subfamilies, each containing 85% transitive sequence identity according to a previously described method (DeSantis, 2003). Altogether, 842 subfamilies were found. The taxonomic position of each OTU as well as the accompanying NCBI accession numbers of the sequences composing each OTU can be viewed at http://greengenes.lbl.gov/Download/Taxonomic_Outlines/ G2_SeqDescByOTU_outline.txt.

The objective of the probe selection strategy was to obtain an effective set of probes capable of correctly categorizing mixed amplicons into their proper OTU. For each OTU, a set of 11 or more specific 25-mers (probes) that were prevalent in members of a given OTU but were dissimilar from sequences outside the given OTU were sought. In the first step of probe selection for a particular OTU, each of the sequences in the OTU were separated into overlapping 25-mers, the potential targets. Then each potential target

was matched to as many sequences of the OTU as possible. Since each sequence in an OTU could range from 600 to 1,500 nucleotides, it was not sufficient to use a simple text search. Therefore, the multiple sequence alignment provided by greengenes was necessary to provided discrete measurement of group size at each potential probe site. For example, if an OTU containing seven sequences possessed a probe site where one member was missing data, then the site-specific OTU size was only six. In ranking the possible targets, those having data for all members of that OTU were preferred over those found only in a fraction of the OTU members. In the second step, a subset of the prevalent targets were selected and complemented into probe orientation, avoiding those capable of mis-hybridization to an unintended amplicon. Probes presumed to have the capacity to mis-hybridize were those 25-mers that contained a central 17-mer matching sequences in more than one OTU (Urakawa, Stahl et al. 2002). Thus, probes that were unique to an OTU solely due to a distinctive base in one of the outer four bases were avoided. Also, probes with mis-hybridization potential to sequences having a common tree node near the root were favored over those with a common node near the terminal branch. As each PM probe was chosen, it was paired with a control 25-mer (mismatching probe, MM), identical in all positions except the thirteenth base. The MM probe did not contain a central 17-mer complimentary to sequences in any OTU. The target probe and MM probes constitute a probe pair analyzed together. The chosen oligonucleotides were synthesized by a photolithographic method at Affymetrix Inc. (Santa Clara, CA, USA) directly onto a 1.28 cm by 1.28 cm glass surface at an approximate density of 10,000 probes per μm^2 (Chee, Lockhart et al. 1996). Each unique probe sequence on the array had a copy number of roughly 3.2×10^6 (personal communication, Affymetrix). The entire array of 506,944 features was arranged as a square grid of 712 rows and columns. Of these features, 297,851 were oligonucleotide 16S rDNA PM or MM probes, and the remaining were used for image orientation, normalization controls, or other unrelated analyses. Each DNA chip has two kinds of controls on it: 1) probes that target amplicons of prokaryotic metabolic genes spiked into the 16S rDNA amplicon mix in defined quantities just prior to fragmentation and 2) probes complimentary to a pre-labeled oligonucleotide added into the hybridization mix. The first control collectively tests the fragmentation, biotinylation, hybridization, staining and scanning efficiency. It also allows the overall florescent intensity to be normalized across all the arrays in an experiment. The second control directly assays the hybridization, staining and scanning.

3.5.2.2 Environmental Sampling and DNA extraction

DNA extracted as mentioned in chapter 3.4.3 is used for this study.

3.5.2.3 16S rDNA Amplification

The 16S rDNA was amplified from the gDNA using universal Bacterial primers 27f.1 (5' AGR GTT TGA TCM TGG CTC AG) and 1492R (5' GGT TAC CTT GTT ACG ACT T). Polymerase chain reaction (PCR) was carried out using the *TaKaRa Ex Taq* system (Takara Bio Inc, Japan). Each PCR reaction mix contained 1X *Ex Taq* buffer, 200 uM total final concentration of *TaKaRa* dNTP mixture, 0.02U/µL *TaKaRa Ex Taq* polymerase, 0.4mg/mL bovine serum albumin (BSA), and 300 pmol of each primer. PCR conditions were 1 cycle of 3 min at 95°C, followed by 35 cycles of 30 sec at 95°C,

30 sec at 48-58°C (gradient block), and 1 min at 72°C, and finishing with 7 min incubation at 72°C.

3.5.2.4 Microarray Processing

For each array, amplicons were concentrated to a volume less than 40µL with a by isopropanol precipitation. The PCR products (500 ng) were spiked with known concentrations of amplicons derived from prokaryotic metabolic genes. This mix was fragmented to 50-200 bp using DNAse I (0.02 U/µg DNA, Invitrogen) and One-Phor All buffer per Affymetrix's protocol. The complete mixture was incubated at 25 C for 10 min., 98 C for 10 min., and then labeled. Biotin labeling was accomplished using an Enzo® Bioarray™ Terminal Labeling Kit (Affymetrix) per the manufacturer's directions. The labeled DNA was then denatured (99 C for 5 min) and hybridized to the DNA microarray at 48 C overnight (> 16 hr). The arrays were subsequently washed and stained. Reagents, conditions, and equipment are detailed elsewhere (Masuda and Church, 2002).

3.5.2.5 Scanning and Probe Set Scoring

Arrays were scanned using a GeneArray Scanner (Affymetrix, Santa Clara, CA, USA). The scan was recorded as a pixel image and analyzed using standard Affymetrix software (Microarray Analysis Suite, version 5.1) that reduced the data to an individual signal value for each probe. Background probes were identified as those producing intensities in the lowest 2% of all intensities. The average intensity of the background probes was subtracted from the fluorescence intensity of all probes. The noise value (N) was the variation in pixel intensity signals observed by the scanner as it read the array surface. The standard deviation of the pixel intensities within each of the identified background cells was divided by the square root of the number of pixels comprising that cell. The average of the resulting quotients was used for N in the calculations described below.

Probe pairs scored as positive were those that met two criteria: (i) the intensity of fluorescence from the perfectly matched probe (PM) was greater than 1.3 times the intensity from the mismatched control (MM), and (ii) the difference in intensity, PM minus MM, was at least 130 times greater than the squared noise value (>130 N^2). The positive fraction (PosFrac) was calculated for each probe set as the number of positive probe pairs divided by the total number of probe pairs in a probe set. A subfamily was considered present when at least one of its subordinate OTUs had a PosFrac >0.92 in all three replicates.

4.0 RESULTS

Previous attempts at defining the microbiological burden of airliner cabin air parcels have surely underestimated the number of organisms present since such investigations have been limited to plate count enumerations of bacteria and molds on predefined media. These methods are inherently biased as "studies of several types of environments estimate that more than 99% of organisms seen microscopically are not cultivated by routine techniques" (Pace, 1997). This is the first report to use moleculer-based microbial enumeration techniques to more accurately determine the quantities and/or identities of bacteria and viruses typically present within commercial aircraft cabins.

For the sake of relevance and increased significance, it is important to address the findings of this study with respect to the Hong Kong Indoor Air Quality (IAQ) standard of 1,000 CFU/m^3 (Lee et al., 1999). This is not a trivial task as direct comparisons between population estimates that include non-cultivable microbes and those that do not are futile. As numerous tables in this report depict, when assessed by culture-independent, molecular methods, the bioburden present almost always exceeds 1,000 CFU/m3. A standard so heavily biased toward culturability is of questionable use when one imagines an air parcel littered with pathogenic viruses or unculturable microbes. This study underscores the fact that in order for this IAQ standard significantly serve its purpose, it must be reassessed to take into account both cultivable and non-cultivable portions of the total microbial load.

4.1 Total viral burden

Samples collected from the Union Station rail depot (Los Angeles, CA) were used primarily for methods development and optimization to spare precious airline samples. It should be stressed that the work-up for these samples in no way constitutes a comprehensive study, as analyses ceased once we had demonstrated the efficacy of the sampling method and its appropriate application to airline cabins. The Union Station samples confirmed the presence of all viruses assayed with the exception of influenza. Based on the positive results from this trial we proceeded with extraction and enumeration of air cabin samples using the same protocols.

A total of fourteen cabin air sample sets were screened to assess viral loads of varicella-zoster virus (VZV), Epstein-Barr virus (EBV), adenovirus (AV), coronavirus (CV), respiratory syncytial virus-B (RSV-B), influenza A (INF-A), and influenza B (INF-B). Assays were selected based on their abilities to detect multiple types of each target virus. Adenovirus, RSV-B, and Influenza A and B were not detected in any of the flight samples or the blank cartridges and positive controls for these viruses worked consistently. Figures 4.1-1 to 4.1-14 and Table 4.1-1 provides complete results for the three viruses detected: EBV, VZV, and CV. Only positive results above blank levels are considered (see below for rationale). While certain flights presented a much greater viral burden than others, the presence of one virus was by no means indicative of the presence of other viruses, ruling out the ability to judge overall air quality based on detection of a single virus.

The theoretical minimum detectable DNA copy number per sample cartridge is 187.5 copies for a 50µl QPCR reaction or 375 copies for a 25µl QPCR reaction volume. This calculation is based on the detection of one copy of the target gene (i.e. one viral genome) in one of the two replicates per sample. However, we often detected what appeared to be below that level (i.e. easily detected but calculated at much less than 1 copy per tube), and we speculate that this may be due to partial cross-reaction with related viruses that amplify with lower efficiency than the authentic target viruses. In our interpretation of results, we exclude those from consideration. We also note that for two viruses (VZ, EBV) this level was below that found in certain partitions of buffer originating from the blank (unused) sample cartridges, grounds for the exclusion of these results anyway. This consideration effected the interpretation of only few of the coronavirus results.

As shown in Figure 4.1-1, samples collected aboard Flight 2N yielded no detectable viruses above the lower threshold (375 virion copies). However, samples from flight 2-R-N demonstrated low and persistent, but barely detectable levels of VZV and EBV (Figure 4.1-2). Coronavirus was also detected, but the levels were below the reliable range of detection. For flights 3-L and 3R-L, there were scarce levels of VZV, most of which was below the reliable limit. Sample 3R-L-9 was the only sample from these two flights having a detectable level of EBV (Figure 4.1-4). Coronavirus was not found in either of these two London flights. All three viruses were detected in the 4-S and 4R-S Sydney flight series. The levels of VZV fell into a questionable range, above the lower limit of detection but below the value of the blank cartridges. Sample 4-S-7, however, presented $5.5 \times 10^3 \pm 881.7$ copies of VZV per cartridge (Figure 4.1-5). The 5-NY and 5R-NY flights contained all three viruses above the lower limit of detection. 5-NY had low levels (from 1-2 copies) of VZV, EBV, and CV detected per QPCR reaction, corresponding to 200-500 copies per entire cartridge. However, 5R-NY showed an increased amount of viral contamination with an average of 5.77 to 21.47 VZV copies/assay and 5.08 to 23.90 CV copies/assay. This corresponds to 4×10^3 and 1.8×10^4 viral copies per cartridge of VZV and CV, respectively (Figure 4.1-8). EBV remained at a level similar to 5-NY. Like 5-N New York trip, flights to Narita, Japan 6-J and 6R-J had significant levels of all three viruses. The Los Angeles to Narita segment (6-J) exhibited 3, 2, and 0 positives out of 8 samples for VZV, EBV, and CV, respectively. In general, the Narita, Japan to Los Angeles flight (6R-J) was more virally laden than was 6-J exhibiting 7. 5. 4 positives out of 7 samples for VZV, EBV, and CV, respectively. Significant coronaviral burden measured in 6R-J Gasper sample should be noted (Figure 4.1-10). Of the 7N and 7R-N flights to New York segment, only 7N-1 preboard sample showed any significant viral presence (2×10^3 +/- 1×10^3 VZV copies/cartridge). For flights 8S and 8R-S, two samples collected during Sydney trip, 8R-S-2 and 8R-S-3, had 6×10^3 +/- 2.1×10^3 and 1×10^4 +/- 8.5×10^3 VZV copies/cartridge. In addition, 8R-S-2 yielded 843 EBV copies/cartridge but no CV was detected. In all other samples, no virus copies were detected.

4.2 Total microbial burden

The microbial populations housed within various air parcels collected from several locations aboard Airlines A (Figure 4.2.1-1 to 4.2.1-20), Airline B (Figure 4.2.1-21 to 4.2.1-40), and Airline C (Figure 4.2.1-41 to 4.2.1-55), as estimated by culture-dependent and –independent analyses, are depicted as well as tabulated (Tables 4.2.1-1 to 4.2.1-26)..

4.2.1 Cultivable bacterial population as estimated by plate count assay

Collectively, the number of CFU arising from a typical cubic meter of air ranged from below detectable limits (BDL) to 4.6×10^5 among the 30 samples collected aboard Airline B flights (Tables 4.2.1-3 and 4.2.1-4). Between the two flights (flights #3 and #4), samples collected on flight #4 (Table 4.2.1-4; Figure 4.2.1-31) harbored more cultivable microbes than those from flight #3 (Table 4.2.1-3; Figure 4.2.1-21). Roughly 73% of the samples collected (11 of 15 samples) aboard flight #4 exhibited $>10^4$ CFU/m^3 of air, whereas only ca. 26% of samples (4 of 15 samples) collected on flight #3 contained $>10^4$ CFU/m^3 of air. Gasper and seat height air samples yielded high CFU numbers from both flights. The order of magnitude increase in CFU between gasper samples collected pre-board (ca. 10^4 CFU/m^3 of air) and mid-flight (ca. 10^5 CFU/m^3 of air) could reflect an

accumulation of passenger-borne respiratory-associated microbes over the course of the flight. Given their small, enclosed space, lavatory air parcels were expected to be the most highly laden with microbial matter. This was not the case, as lavatory samples yielded between BDL and 6.4 x 10^4 CFU/m^3 of air. As was seen with the gasper air samples, lavatory air parcels generally gave rise to more CFU as the flight progressed. Ten air samples were systematically collected at 20-25 minute intervals from an economy-class seat location from the time prior to boarding (pre-board; time zero), throughout the 3 hour 40 minute flight, and through the landing of flight #3 on Airline B. A gradual accrual of cultivable microbes was witnessed (Figure 4.2.1-21a) from pre-board through mid-flight (ca. 75 minutes; ca. 3 log increase). After 75 minutes the cultivable portion of the population declined (ca. a log per sampling), and the population became completely non-cultivable (with the culture media employed; R2A) just prior to landing.

In contrast, Phase II plate count results for Airline C were far lower. No samples yielded plate counts comparable to the 10^4 CFU/m^3 found in some of the Phase I samples, and only 1.8% of the samples (2 of 111 samples) showed cultivable counts of 10^3 CFU/m^3 or higher, none of which exceeded 2.7 x 10^3 CFU/m^3. Approximately 50% of the Airline C samples were below detection level, showing no bacterial growth on R2A media (56 of 111 samples). About 53% of samples (25 of 47 samples) taken on the domestic flights were below detection level compared to only 48% of samples (31 of 64 samples) taken on the international flights. Average results for each Phase II flight ranged from BDL (2 of 16 flights) to 363 CFU/m^3. Domestic flights showed a slightly lower total cultivable count than international flights with only 2 of 8 flights yielding plate counts higher than 50 CFU/m^3. Of the international flights that were analyzed 6 of 7 flights had an average CFU count above 50 CFU/m^3. Only two international flights (8-S and 8R-S) and one domestic flight (1R-N) had plate counts higher than 100 CFU/m^3. Plate counts tended to fluctuate within each flight, and no particular trend was noticeable over the course of any individual flight.

Total aerobic, heterotphic microbiota, as measured by minimal media plate count assays, ranged from 0 to 10^2 CFU/m^3 of air for samples collected from Airline C. This is far cleaner (3 orders of magnitude) than that observed for air samples collected aboard Airlines A and B in Phase I, likely a consequence of the different cabin locations sampled: Phase I samples arose from the coach cabin, and Phase II samples were predominantly business class. Furthermore, of the few samples collected from coach class during Phase II, all presented plate counts above 10^2 CFU/m^3. (Tables 4.2.1-18 to 4.2.1-23). The 52 samples collected while aboard domestic flights on Airline C (Phase II) ranged in cultivable burden from BDL to 2.5 x 10^2 CFU/m^3 of air. Samples collected from international flights (64 samples) shared this range of cultivable burden, but values were consistently higher and tended to aggregate in the upper zone of this spectrum. Of particular note, flight #8 (to and fro Sydney) contained the greatest incidence of cultivable microbes, where 40 to 60% of the samples collected during these Sydney trips contained more than 10^2 CFU/m^3 of air (Tables 4.2.1-24 and 4.2.1-25).

4.2.2 Total and viable microbial population as measured by ATP

Based on the results of the intracellular ATP assay, the total viable microbial population across samples in Phase I varied from BDL to 4.1 x 10^6 cells/m^3 of air. A threshold of

10^3 RLU/m^3 of air was established to strengthen the conclusions drawn from this data [15, 17]. Only 5 to 15 % of the samples collected aboard Airline A yielded elevated RLU values (3 of 20 samples analyzed from flight #1 and 1 of 20 samples analyzed from flight #2 had RLU values above threshold). Airline B was considerably more laden, as nearly 70% of samples yielded RLU values above threshold (11 of the 15 samples examined on flight 3 and 10 of the 15 examined from flight 4). In examining the resulting RLU values across several samplings, microbial numbers appear to amass from the time of boarding, through reaching cruise altitude, and into early mid-flight (Figure 4.2.1.21a), a phenomenon thought to be attributable to the build-up of respiration-associated microflora in this enclosed space. Furthermore, a scarcity of microbes (intracellular ATP) was observed in the late mid-flight time period.

The percentage of cultivable microbes with respect to the total microbial population (plate count values divided by intracellular ATP values) for flights #3 and #4 is given in Table 4.2.1-5. There were no discernable patterns observed among the various locations sampled. Samples retrieved from the lavatories aboard both flights of Airline B exhibited only 1.5% to 22% cultivable microbes, whereas this ratio of cultivable to total viable microflora was >100% from the air samples collected from the seat height locations of flight #3 and the gaspers of flight #4. Fifty to seventy percent of the microbes present in Airline A samples were viable, as derived from dividing intracellular ATP values by total ATP values. In contrast, among the 20 Airline B samples that exhibited viable microbes, 5, 6, and 9 of these samples yielded viable percentages of >50 to 80%, 25 to 50%, and <25% of the total microbial population, respectively (data not shown).

The results of total microbial burden (derived via total-ATP assay) and the viable portion of this burden (derived via intracellular-ATP assay) for samples collected aboard Phase II flights are shown in Table 4.2.1-5a to 4.2.1-5d. Samples collected aboard Airline C in Phase II were a ranged in total-ATP content from 7.31 x 10^3 to 1.78 x 10^5 RLU/m^3 of air. In general, total microbial populations numbered 10^4 cells/m^3 of air. The flight from Sydney, Australia (4R-S) to Los Angeles was an exception, presenting a total burden of 1.1 x 10^5 cells/m^3 of air. A mere 1 – 10% of this microbial load was found to be viable, as values obtained from the intracellular-ATP assay were consistently 1 to 2 logs lower than those resulting from the total-ATP assessment (Table 4.2.1-26). To simplify the discussion of these results, and to remain consistent with the procedures followed in Phase I, we set a significance threshold of 10^3 RLU/m^3 of air (equivalent to the Hong Kong IAQ standard). When the data are examined in this manner there are no apparent trends to speak of, as values tend to be much more leg/flight dependent. Unlike the trends observed in Phase I, contamination was not observed to change with varying phases of the flight. In Phase II, burden seemed to be uniformly distributed throughout the duration of these flights (Figure 4.2.1-21 to 4.2.1-55). There are definitely differences in accumulated microbial burden by the end of domestic and international flights, as a function of difference in time. As one would expect, international flights, being of longer flight duration, consistently presented greater accumulations of microbiota by flights end. Only 0 – 14% of samples collected aboard domestic segments of Airline C yielded RLU values above threshold, whereas 14 – 60% of samples collected from international flights aboard this airline were above this 10^3 cells /m^3 of air level (Tables 4.2.1-8 and 4.2.1-9). As observed in the results of the plate count assay (above), both legs of Flight #8 (to and

fro Sydney) were considerably laden with viable microbiota, as 40-60% of the samples collected while on these flights were above threshold.

4.2.3 Total microbial population as measured by DNA

Q-PCR assay was performed on ten location-conserved samples generated by pooling 2 to 6 individually collected air-impinged samples (Table 4.3.2-1). Samples yielded from BDL to 3.9 x 10^7 gene copies/m^3 of air. The gasper sample contained a moderately high number of 16S gene copies (1.56 x 10^5 copies/m^3 of air), when compared to other location-dependent pooled samples aboard Flight #1. Since bacterial chromosomes house 1 to 15 copies of the 16S rRNA gene, the results of this assay suggest a presence of between BDL and 10^6 procaryotic cells/m^3 of air. It should be noted here that 7 of these pooled samples arose from Airline A and 3 of these samples were collected aboard Airline B in which 3 of the 7 samples of Airline A did not show any detectable 16S rRNA gene copies where as all 3 Airline B samples tested exhibited >10^6 16S rRNA gene copies per m^3 of air.

Even though considerable variation was observed across samples and between carriers in both the plate count and molecular-based assays, when averaged, the samples collected aboard Airline B were an order of magnitude more laden with ribosomal RNA gene copies than were samples collected on Airline A. A similar observation was documented when samples were assessed for viable microbes via ATP analysis and cultivable counts. This supports our conclusion that Airline A air samples are less laden with microbial matter than those of Airline B.

The results of 16S rDNA-based quantitative PCR bacterial enumeration analyses for all samples examined in Phase II are given in Table 4.2.1-6. It must be noted here that all samples collected from each individual flight were pooled, as to increase the likelihood of obtaining a sufficient quantity of DNA for this and other analyses. Much like the results observed in Phase I, in general, total bacterial populations, as derived via Q-PCR, ranged from BDL to 3 x 10^7 gene copies per m^3 of air. A majority of all domestic flights (6 of 8) presented bacterial burdens from 10^6 to 10^7 gene copies/m^3 (Table 4.2.1-8). With the exception of the flights to and fro London (3L and 3R-L), all international flights were burdened with 10^5 – 10^6 gene copies per m^3. The very low gene copy number (10^3) observed in the London flights is likely attributable to problems encountered with sample processing, as these samples failed to yield a sufficient amount of DNA for PCR amplification and subsequent clone library construction. Unlike those obtained from other measurements, Q-PCR results for international flights exhibited a minimum of one to two logs less bacterial burden than domestic flights. Because more than double the volume of sample is pooled for international flight samples, it is our hypothesis that some form of inhibition is accumulating and interfering with these amplifications.

4.3 Molecular microbial community analysis

4.3.1 Identification and diversity of cultivated population

A phylogenetic presentation of the identities and overall diversity of the cultivable populations present in Phase I samples is given in Figure 4.3.1-1. Representatives of the α-, β-, and γ-proteobacteria, as well as high G+C Gram-positive bacteria, were identified in varying abundance. Cells of *Acinetobacter johnsonii* (36%) dominated samples

retrieved from Flight #3, while Flight #4 contained equal allotments of *Pseudomonas reactans, Methylobacterium* spp., and *Microbacterium phyllosphaerae*, based on morphometric comparison between colonies and sequence identification data (Table 4.3.1-2). No discernable phylotypic correlations were evident between the time and/or location of sampling and the types of microbes detected.

A 16S rDNA-based identification (Table 4.3.1-2) and phylogenetic affiliation (Figure 4.3.1-2) of isolates collected from samples aboard flights of Phase II is given. Representative of α, β, and γ-proteobacteria, as well as sporulating and non-sporulating Gram-positive bacteria were isolated in varying abundance. Of the 112 bacterial isolates sequenced, ca. 86% were Gram-positive, and 60% of these were of high G/C content, including *Micrococcus*, *Kytococcus*, and *Staphylococcus*. As one would expect a significant abundance of Staphylococci and Micrococci were isolated (38 of 57 High G/C non-spore-forming isolates). Cells in these genera are found in tight association with human skin cells, which are shed countinuously. Spore-forming *Bacillus* species were also quite prevalent, as 19 of the 57 high G/C Gram-positive cultured cell lines arose from this lineage. Members of the α-proteobacteria, such as *Methylobacterium* spp., comprised 10% of all bacterial strains isolated, complementary to results obtained from 16S rDNA-based clone library analyses. Over the course of the Phase II study, five novel bacterial lineages were uncovered about the cabin air samples analyzed: two members of the α-proteobacteria (*Paracoccus* sp. and *Paracraurococcus* sp.), one CFTB (Taxeobacter sp.), and 2 Gram-positive bacteria (*Corynebacterium* sp. and *Bacillus* sp.). The novelty of these strains was determined based on 16S-rDNA sequence similarities of less than 97% with nearest neighbors in the Genbank public database (Goebel and Stackenbrandt, 19XX). Several bacteria implicated in the cause of human illness were also isolated, including *Janibacter melonis* – the causative agent of bactaeremia, *Microbacterium trichotecenolyticum* – the causative agent of neutropenia, *Massilia timonae* – a cause of cerebellar lesions, *Staphylococcus saprophyticus* – a cause of urinary tract infections, and *Corynebacterium lipophiloflavum* – the cause of bacterial vaginosis. The 16S rDNA sequences of most of these isolates were also retrieved and observed in clone libraries, lending further support to the conclusion that these species are indeed prevalent about these cabin air parcels. These isolations are sporadic and cannot be linked to any given time points or locations about any specific aircraft.

4.3.2 Prokaryotic diversity documented by cloning and sequencing

Approximately 660 clones representative of Airline A (7 samples) and 275 clones representative of Airline B (3 samples) were analyzed as a means of elucidating the molecular microbial diversity of these cabin air parcels (Table 4.3.2-1; Figures 4.3.2-1 to 4.3.2-10). The identity and relative abundance of each clone obtained from rDNA cloning analyses are shown in Table 4.3.2-2, displayed according to their phylogenetic relationships. Ribosomal DNA sequences closely related to species of *Abiotrophia*, *Fusobacterium, Gemella, Haemophilus, Neisseria, Prevotella, Rothia, Streptococcus,* and *Terrahaemophilus* were recovered in varying abundance. Sixteen phylogenetically distinct bacterial sequences were retrieved from each carrier analyzed. A slightly higher microbial diversity was observed for samples representative of Airline A (15 sequences) compared to 9 different sequences retrieved from Airline B samples, however this may be an artifact of the smaller sample size analyzed from Airline B. An overwhelming

abundance of *Neisseria meningitidis* sequences (62.3% of 660 clones) was observed in Airline A followed by *Streptococcus oralis/mitis* sequences. In contrast, *Pseudomonas synxantha* (69.2%) sequences dominated Airline B clone libraries, followed by *N. meningitidis* (13.2%) and *S. oralis/mitis* (9.2%) sequences.

The results of molecular microbial community analyses as a function of sample location (category A), sample time (category B), airline (category C), and flight (category D) are depicted in Table 4.3.2-3. Variations in microbial community structure diversity at different locations (category A), such as gasper, lavatory, and seat area were elucidated by analyzing samples collected aboard flight #1 of Airline A. In comparing category A samples, one striking trend that can be observed is the high percentage of DNA sequences closely related to the *Neisseriaceae* present in the gasper samples. Ribosomal DNA sequences arising from *N. meningitidis* and close relatives accounted for 100% of the clone library from gasper samples while this group comprised only ca. 50% of the sequences from other locations. Sequences representative of *Streptococcus, Gamella, Terrahaemophilus, Prevotella,* and *Abiotrophia* species comprised the remaining clones analyzed from other locations (lavatory, seat, floor). An elevated incidence of *S. oralis* sequences was observed in samples collected from the lavatory, as well as seat and floor air parcels. Differences in diversity corresponding to temporal variations (category B; cruise altitude, mid-flight, pre-descent, pre-board, and post-landing) were assessed by comparing samples from flight #2 of Airline A. Samples of this category set revealed a change in microbial diversity as the flight progressed. *N. meningitidis* rDNA sequences were retrieved in abundance from the seat and floor air samples prior to boarding (74%), though such recovery dropped significantly (41%) upon reaching cruise altitude and through mid-flight and pre-descent.

To compare inter-airline microbial diversity variations, lavatory-borne air samples (flight # 2 of Airline A and flight #4 of Airline B) collected at varying time periods were analyzed (category C). This set of air samples exhibited a clear distinction in the microbial incidence between the two airlines, with samples collected aboard Airline A being more diverse, despite the fact that these samples were less laden with microbial matter. Sequences arising from 6 distinct microbial phylotypes were retrieved, for the most part mirroring the diversity profiles comprising categories A and B. Samples collected from Airline B also presented novel, airline-specific microbes, as lavatory samples housed a predominance of *Massilia timonae* (97%). *Pseudomonas synxantha* was also isolated exclusively from samples collected aboard Airline B. In addition, a set of samples collected during mid-flight (time-course study of flight #3 seat and floor samples) were pooled and compared to analogous samples from flight #4 to understand the variation in microbial diversity across distinct flight segments of the same carrier (flight #3 and #4 of Airline B; category D). *P. synxantha* sequences were retrieved exclusively from flight #4 whereas *N. meningitidis* sequences were highly prevalent in samples collected aboard flight #3.

The microbial diversity detected via rDNA cloning strategies in samples collected aboard flights of Phase II on Airline C was astounding. Sequences representative of more than 113 species, enveloping 12 classes of Bacteria, were retrieved from these air parcels (Figure 4.3.2-11). Table 4.3.2-4 shows a class-based consolidation of the molecular microbial diversity observed over various flight-legs. Pooled samples collected aboard

flights 3L, 3R-L, and 2R-N failed to yield PCR amplifiable DNA, and are thus excluded from this discussion of results. Among all bacterial classes (Figure 4.3.2-12 to 4.3.2-17), sequences arising from Proteobacteria were collected in greatest frequency (58% of all clone sequences), followed in short order by those stemming from Gram-positives bacteria (31% of all clone sequences). The percent incidence of clones, as a function of Bacterial class, retrieved from all flights aboard Airline C was as follows: 30% α-proteobacteria, 8% β-proteobacteria, 20% γ-proteobacteria, 1% δ-proteobacteria, 10% Actinobacteria, 21% Firmicutes, 0.1% Spirochaetes, 0.25% Fusobacteria, 8% Deinococci, 4% Cyanobacteria, 0.5% Planctomycetes, 0.2% Bacterioidetes. It is easy to see that proteobacteria, alpha and gamma proteobacteria in particular, predominate these samples. In each and every flight leg, α-proteobacteria constituted at least 10% of the detected microbial diversity, and in some instances, a-proteobacteria comprised 100% of the diversity collected on a particular flight-leg (New York to Los Angeles; 1R-N; Tables 4.3.2-5 to 4.3.2-14).

Overall, there were 62 distinct species of Proteobacteria detected via cloning methods, 14 of which had previously been reported to take part in human illness. Of the 23 species of α-proteobaceria detected (Figure 4.3.2-14), 7 species were consistently retrieved at frequencies greater than 10% of the library, none of which had ever been implicated with human disease (although sequences of *Agrobacterium tumefaciens*, a renown plant pathogen were in this group). Of the 19 clone sequences arising from β-proteobacteria (5 human pathogens), 4 were consistently retrieved at frequencies greater than 10% per library (Figure 4.3.2-15), and 2 of those 4 were human pathogens (*Burkholderia cepacia* and *Massilia timonae*). An astounding 50% of all γ-proteobacterial sequences collected (20 species), arose from human pathogens (Figure 4.3.2-16). Of the greatest interest, *Salmonella typhi* has historically caused a great deal of human mortality and morbidity. Though clones of this microbe were not retrieved in great abundance (only 2 occurrences), its presence is nonetheless worthy of note. There were, however, pathogenic γ-proteobacterial sequences retrieved in elevated abundance (>10%), *Acinetobacter junii* and *Psychrobacter meningitidis.* A lone member of the δ-proteobacteria, *Bacteriovorax stolpii*, a non-pathogenic bacterium, was observed in low incidence (2 clones).

A roughly equivalent species richness was observed between the Proteobacteria (62 species) and the Gram-positive bacteria (57 species). Of these 57 Gram-positive species, 1/3 were of Actinobacterial origin and the remaining 2/3 were Firmicutes. Of the 20 Actinobacterial clones recovered, 6 were pathogenic to humans, and 3 of these 6 pathogenic clones were retrieved at elevated frequency (>10% per library; Figure 4.3.2-12). Of particular note, clones of *Actinomyces viscosus*, the causative agent of cervicofacial actinomycosis, were retrieved while aboard the flight from Sydney to Los Angeles (4-S). In addition, clones arising from the causative agent of Cat-Scratch Disease, Propionibacterium acnes, were recovered while aboard a flight from Los Angels to New York (2-N). This bacterium has been frequently isolated from arthritis patients and is often found in association with prosthetic limbs and joints. The Firmicutes was the most highly represented of any Bacterial class, as 37 distinct species of clone belonging to this class were recovered aboard Airline C (Figure 4.3.2-13). Six species of Firmicute were recovered at frequencies in excess of 10% per library. Clones indicative of the presence

of numerous Firmicute pathogens (6 clones) were retrieved in varying abundance, including *Staphylococcus aureus* and several Streptococci. Clones of *Streptococcus pneumoniae*, a cause of human pneumonia, were collected in high numbers aboard a flight from New York to Los Angeles (7R-N). Of all of the Firmicute clones retrieved, only 1 species spore-forming species (of 9 in total), *Bacillus pallidus*, was recovered at a frequency greater than 10% per library, suggestive of a somewhat scarce population of spore-forming bacteria about these air samples.

Other classes of Bacteria were also represented in varying fashion, as discussed below (Figure 4.3.2-17). The Deinococci class was represented by clones of 4 distinct species, 2 of which were recovered at a frequency greater than 10%. *Deinococcus murrayi* sequences were collected in large numbers from both legs of the round trip flight to Sydney (8-S and 8R-S). Cyanobacterial sequences representing 4 distinct species were retrieved in varying abundance. *Oscillatoria sancta* clones were observed in samples collected from flights to and fro Sydney and Tokyo. There was but one spirochete clone recovered in this study, *Leptospira parva*, a human pathogen causing leptospirosis, arose from a sample collected on a flight from Los Angeles to Tokyo (6-J). Numerous clones of Bacterioidetes and Fusobacteria having been previously linked to environmental stewardship efforts were also recovered in varying abundance over the course of this Phase II study.

Because this rDNA cloning approach speaks nothing of enumeration, we are limited to reporting on the presence or absence of given bacterial lineages. Therefore, rather than attempt to assess pathogenic burden via raw cell counts with integrated percent regression, we report relative pathogenic burden as a function of phylogenetic breadth and diversity. Generally speaking, international flights tended to house a greater phylogenetic diversity of pathogenic bacteria than domestic flights. Of all pathogenic clones (species richness: 29) recovered in this Phase II study, 15 pathogenic species were collected aboard international samplings and 12 pathogenic species were collected aboard domestic samplings. Only two species of human pathogen, *B. cepacia* and *P. acnes*, were collected from both international and domestic flight samples. While the diversity of pathogenic microbiota aboard international flights tended to encompass all classes of Bacteria, domestic flights were inundated with pathogenic bacteria belonging to the g-proteobacteria (*S. typhi* and *Haemophilus* spp.).

As for overall phylogenetic breadth, Gram-positive and α–proteobacteria seem to have a higher affinity for international flights (Table 4.3.2-14), whereas β–and γ–proteobacteria are far more common about domestic cabin air parcels (Table 4.3.2-13). Ultimately, the majority of microbial species circulating throughout the cabin airs of commercial airliners are commensal, infrequently pathogenic normal flora of the human nasopharynx and respiratory system. Many of these microbes likely originate from the oral and nasal cavities, and lungs of passengers and flight crew and are disseminated unknowingly via routine conversation, coughing, sneezing, and stochastic passing of fomites.

4.3.3 Prokaryotic diversity elucidated by microarray analysis

The high-density DNA microarrays showed more bacterial subfamilies detected than cloning and sequencing alone in every sample. There was a much greater diversity of

subfamilies detected by microarray as well. The largest discrepancies were regarding the Actinobacteria and the Firmicutes, both Gram-positive bacterial phyla. While sequences in these bacterial groups were only occasionally detected in the clone libraries, they were the most prominent bacterial subfamilies detected by microarrays. Occasionally, subfamilies were detected though cloning and sequencing that were not detected on the microarrays. Because the microarray detection method is more inclusive than the cloning and sequencing method for detecting bacterial groups, especially when the clone libraries are small, they are useful for doing surveys of systems to compare diversity. It is also possible to compare all samples in one analysis to determine how similar their profiles are to one another.

4.3.4 Disclaimer

Any discussion of results stemming from DNA extraction-based microbial enumeration assays must include a caveat on the difficulties and limitations involved with collecting samples from environments of very low biomass. Potential sources and mechanisms for bias are detailed below.

Sampling Technology

In this project we were forced to contend with the limitations of available sampling technology. While the sample cartridges (MesoSystems) we used for sample collection, are guaranteed by the manufacturer to be sterile with respect to cultivability, they are not promised to be devoid of biological molecules such as DNA. Consequently, unused sampling cassettes, yielded significant PCR products and gave rise to clone libraries of substantial microbial diversity. As a result, virtually all unused sample cartridges that we processed as experimental controls demonstrated DNA at detectable levels. To counter this effect, we included an unused, unopened sampling cartridge as a negative control blank alongside each DNA extraction we performed on experimental samples, allowing us to ascertain the contribution of DNA inherent within the buffer. For the purposes of phylogenetic analysis, we processed all blank samples identically to experimental samples; each blank was cloned and sequenced and the results of all blank cloning experiments were combined into a single library. Microbial populations appearing in both the experimental sample libraries and this blank library were subtracted from our results. In total over (XXX) clones were sequenced and identified from unused Mesosystems sample buffer compared to the 100 clones analyzed per flight. Microarray analysis of blanks also proceeded alongside processing of experimental cartridges

PCR

A number of universal 16S gene bacteria primer sets were used in the course of this study to elucidate the microbial community structures present in these airline cabin environments. During the course of Phase I, DNA amplification was limited to a single set of universal bacterial primers (27F/1492R). This primer set often lacked the sensitivity to amplify samples to levels required for cloning and sequencing protocols. In Phase II we hoped to circumvent this problem by using two additional sets of primers (27f.1/1492R and 9bF/1406R) with increasing sensitivity (Table 3.5.1.1-1). Every attempt was made to use the original 27F/1492R primer set during the course of Phase II as the more sensitive primer sets had a tendency to amplify DNA contaminants inherent

to the sample buffer. By putting to use multiple primer sets, we were able to uncover a far greater diversity that would have been revealed with any one primer set alone.

Q-PCR

Depending on availability (instrument maintenance), multiple instruments of different manufacturers (MJ Research and Applied Biosystems Inc) were used to quantify bacteria via 16S rRNA gene enumeration. To compensate for differences in sensitivity between DNA detectors, a known concentration of rRNA gene copies were decimally diluted, and this series served as standards from which all measurements could be calibrated. The same standard dilution series was used for standardization in all Q-PCR reactions performed. Standard curves were generated alongside each and every sample Q-PCR reaction, individually, using the same set of physical rDNA standards across all reactions, for the sake of consistency and normalization. The MJ Research Chromo4 unit, used primarily in this study, has a theoretical lower detection limit of 10^2 copies per microliter of sample assayed. Realistically, the results of our reactions assayed with known rDNA copy concentrations have shown this detection limit to be closer to 10^3 copies per microliter of sample. This corresponds to roughly 5×10^4 copies per cubic meter of air. Because each sample DNA extraction was performed juxtaposed a corresponding "blank," accurate enumerations of rRNA genes were easily deduced by subtracting the values of each blanks from its corresponding experimental sample. While blank cartridges housed 16S gene copy numbers ranging from ~ this detection limit to 2×10^5 copies, each and every pooled experimental sample housed more gene copies than their corresponding blank, often by at least an order of magnitude.

5.0 DISCUSSION

There are numerous factors that may indeed have influenced the findings reported herein. Of these, many have not been fully disclosed to the authors, such as aircraft make and model, age of aircraft, passenger load per flight, periodicity of aircraft flight-time, timing of ventilation/bleeding events and the treatment and processing of this incoming airflow, filtration systems in place and the maintenance of such systems, and schedules of routine and/or rigorous cleaning of the cabin. Despite this sizeable list of variables, microbial and viral burden of aircrafts should not be assumed based on a single sample or even a single flight. Instead the data from this study should be considered as a whole to provide an overview of the general biodiversity present and trends in the levels of bioburden present in aircraft cabin air. Our experimental design had to contend with a number of challenges, including the limitations of our sampling technology, and the lower detection limits of our biological assays. Nevertheless, the data compiled herein represent a technological step forward in our ability to evaluate cabin air quality using modern methods, and will hopefully facilitate the development of future safeguards for air travel.

The underlying objective of this study was to utilize molecular strategies to rapidly assess total biological burden and diversity associated with commercial airline cabin air. Accuracy and specificity come at the cost of simplicity, robustness and efficiency – there is no single, perfect assay for the investigation discussed herein. This study, however exploits a multitude of molecular-based microbial enumeration strategies that together provide a more thorough portrayal of microbial burden and diversity than any of them alone.

5.1 Culture-independent analyses

5.1.1 Total viable microbial population

Total ATP

For the purpose of discussion, 1 RLU is presumably equivalent to 1 CFU, as reported earlier (Venkateswaran et al., 2003). Based on the results of the total ATP assay (accounting for both dead and live microbes) the total microbial burden across samples collected in Phase I (Airlines A and B) averaged 2.9 x 10^5 and 2.8 x 10^5 RLU per cubic meter of air for Airlines A and B, respectively. Samples collected aboard Airline C in Phase II were an order of magnitude less biologically laden, averaging 5.3 x 10^4 RLU/m^3 of air. It should be noted however, that the vast majority of Airline C samples were collected from the business class section of the aircraft. The small fraction of samples taken from the coach sections of Airline C (7 samples) had a total-ATP average of 1.0 x 10^5 RLU per cubic meter of air. Based on these findings, we conclude that cabin class does influence the concentrations of biologicals contained within cabin air, most likely as a consequence of differences in the number and density of passengers in the two sections.

When, in Phase I, we compared the microbial burden of different sample locations of each flight, lavatory air samples were laden with at least an order of magnitude more bioburden than samples collected from any other locations. The only exception was flight #2 aboard Airline A, where seat and floor samples were an order of magnitude more burdened than lavatory samples. As a whole, lavatory samples ranged from 2.1 x 10^4 to 2.1 x 10^6 RLU/m^3 of air, while all other samples ranged from 8.0 x 10^3 to 5.9 x 10^5

RLU/m^3 of air. In contrast, gasper samples were consistently less laden with microbes (at least one log) than samples collected from any other location. When plotted against time (pre-board through landing), most samples yielded patterns of increasing total ATP upon reaching cruise altitude followed by decreasing levels of total ATP through the duration of the flight . This might be due to the activation of air circulation and/or filtration systems aboard the aircraft, but no such details were made available for this study. Additional sampling events are required to validate this observed trend. Flight #1 was the only exception to this trend, exhibiting a pattern of elevated microbial burden (total ATP) upon pre-board sampling followed by decreasing burden over the 4 hour flight period. In Phase II we observed international flights to be ~ twice as burdened as domestic flights (international flights' averaged 6.47 x 10^4 RLU/m^3, while domestic flights' averaged = 3.8 x 10^4 RLU/m^3 of air), as would be expected due to the increased duration of international flights.

Intracellular ATP

While the total ATP-assay is rapid and provides a rough estimate of cellular matter present, the derivation of microbial cell abundance via intracellular ATP quantification is not straight-forward. However, unlike the microbial burden assessments derived from total ATP measurements, which take into account cellular remnants, as well as both dead and live microbes, the intracellular-ATP assay reports only the presence of intact living cells. By first enzymatically degrading extracellular ATP associated with dead microbial cells and other extraneous sources, the intracellular ATP arising from both cultivable and non-cultivable microbes can be elucidated (Venkateswaran et al., 2003).

The total viable microbial population was extremely low in both flights of Airline A compared to the samples collected aboard flights on Airline B. A threshold of 1,000 RLU/m^3 was established to strengthen the conclusions drawn from this data. This value represents the instrument detection level, where only 5 to 10% variation was observed when using known ATP standards. A mere 3 of 20 samples analyzed from flight 1, and 1 of 20 samples analyzed from flight 2 (5 to 15 % of the samples collected aboard Airline A) yielded significant RLU values for intracellular ATP. Airline B was considerably more laden, as 10 of 14 samples examined on flight 3 and 10 of 15 examined from flight 4 (ca. 70% of the Airline B samples) yielded RLU values above the threshold. Furthermore, viable microbes (based on intracellular ATP) were less abundant in the late mid-flight time period, suggestive of an in-flight recirculation event.

Only 20 of 109 business class samples (ca. 18%) taken on the 15 flights analyzed for intracellular-ATP in Phase II (Airline C) yielded average intracellular ATP values above the threshold limit of 1,000 RLU/m^3. Furthermore, all but 4 readings of average intracellular ATP content fell within 2.5 times the threshold. This suggests that viable microbes are quite scarce about the circulating cabin air on commercial airliners, and that the bioburden that is present (that which we detect in all other assays described herein) is comprised primarily of non-cultivable, nonviable, and or dead microbiota. Despite the differences in average flight time between domestic and international flights, and unlike the results of total-ATP quantification, no discernable differences in intracellular-ATP content were observed between these types of flights. Presumably any difference between bioburden levels in air sampled on international flights versus domestic flights is too minute to be resolved using the technology presently available.

The ratio of the viable microbial population (intracellular ATP) to the total microbial population (total ATP) in samples yielding above-threshold levels of intracellular ATP (4 of 40 for airline A, 20 of 39 for airline B, 20 of 109 for Airline C) was determined for discussion purposes. In 3 of the 4 Airline A samples this ratio was between 50 and 70%. Ratios of > 50% viable microbes to total microbial burden were observed in 7 of the 20 Airline B samples. Only 3 of 20 samples with above-threshold intracellular ATP readings collected aboard Airline C yielded a ratio greater than 10%, one of which had a intracellular/total ATP ratio greater than 1. This discrepency was most likely due to a defective intracellular ATP vial for that sample (7R-N-6) as the plate counts for that sample were negative and the total ATP value did not vary significantly with the rest of the flight (Figure 4.2.1-53). It appears that the elevated incidence of viable microbes in the cabin air parcels of Airline B is directly related to the maintenance of the system. However, only by incorporating additional data, such as circulation patterns and filtration schedules during each flight, into the aforementioned results will we be able to draw any meaningful conclusions in this regard. In the interest of security, such information was not made available to the authors.

5.1.2 *Total microbial population*

In Phase I of this study, this assay was performed on ten samples generated by pooling multiple spatially-conserved air-impinged samples. Initial attempts at extracting DNA from individual samples failed to yield PCR amplification products. As the ATP analysis shows, many samples housed fewer than 10^5 cells per cubic meter of air. Hence, samples representing a given location collected at several time points were combined. A description of the pooled air samples and their constituent individual samples is given in Table 4.2.1-5e. Pooled samples ranged in total volume, from the combination of 2 to 6 individually collected air-impinged samples. Ribosomal RNA gene copy numbers ranged from 10^2 to 10^5, 10^2 to 10^7, 10^8, and 10^6 gene copies per m^3 of air for flight numbers 1 through 4 of Phase I, respectively. Samples collected aboard Airline C in Phase 2 ranged in rRNA copy number from 8.4×10^3 to 3.95×10^7 copies/m^3 of air. These values could feasibly overestimate or underestimate the microbial population by an order of magnitude, since there are anywhere from 1 to 15 16S rRNA gene loci on any given bacterial chromosome.

We remain a bit skeptical of two Phase II samples, 3L and 3R-L, that yielded rDNA copy numbers of approximately 1×10^4 copies per cubic meter. These values fell far below those resulting from the majority of all other samples, not to mention the detection level of the Q-PCR instrument. It is our presumption that this drastically low rDNA copy is a consequence of inefficient DNA extraction, since both of these samples (and their blank as well) underwent DNA extraction processing simultaneously, in a common lot. This assertion is supported by the inability to derive any phylogenetic diversity by means of cloning or microarray technologies.

Since the total ATP assay predicts the total microbial burden present in a given sample, it seems rational to directly compare the results of this assay with those of the Q-PCR assay. A detailed analysis of the tested samples has been categorized as follows: The first category consists of samples that yielded similar levels of microbial burden, as estimated by both assays (Phase I: samples XXIV and XXX; Phase II: 3-L, 3R-L, 4R-S, 5N, 7R-N). The second category houses samples that showed ca. 3-logs higher ATP-based microbial

burden than that derived via Q-PCR. The ATP of samples belonging to the second category might have originated from non-microbial sources, such as food and human somatic cells (Phase I: samples XXV and XXII; Phase II: no samples in this category). A third category consisted of samples that exhibited 1 to 2-logs higher Q-PCR based microbial burden; the low ATP concentrations measured here might be attributed to the reduced metabolic activity of these microbes (Phase I: samples LXIX and ML; Phase II: 1-N, 1R-N, 2-N, 2R-N, 5R-N, 6R-J, 7-N, 8-S, 8R-S). This would stand to reason, as microbes intermixing between air and oligotrophic surfaces would not be expected to be very metabolically active. In the fourth and final category, samples presenting a < 1-log higher Q-PCR-derived burden are most likely a consequence of the presence of microbes that have multiple16S rRNA gene loci (Phase I: samples XXIII and XXVI; Phase II: 6-J). Molecular microbial diversity analysis should be conducted to confirm this hypothesis. As with any environmental microbiological analysis (Venkateswaran et al., 2003), outliers were common in this study. In Phase I, sample XXI contained a mere 5.0×10^1 16S rRNA gene copies, this low microbial burden might reflect DNA extraction failure, since an appreciable amount of intracellular ATP was detected in this sample (3.6×10^4 RLU per 100 L of air). In contrast, the very high gene copy numbers (ca. 3 logs) observed in sample XX could not be explained.

Our hypotheses concerning the disparity between the results of the QPCR and total ATP assays warrant further study. It should be noted that the total ATP assay has exceptional sensitivity compared to the Taqman assay. We were consistently able to obtain significant (above our 10^3 RLU threshold) and repeatable (results in triplicate) measurements using only 100uL of individual, unconcentrated sample. The QPCR approach to enumeration provides specificity for bacterial organisms but it requires a 500 fold concentration of a pooled set of 5-10 air samples.

5.2 Culture-dependent analysis

5.2.1 Total cultivable population

The number of colony forming units (CFU) arising from one cubic meter of air ranged from 0 to 4.2×10^5 for the 29 samples collected aboard Airline B, and 0 to 3.63×10^2 for the 109 samples taken from Airline C. This discrepancy in cultivable microbiota could be attributed to the fact that the samples collected aboard Airline C were sampled at fixed positions in the business class cabin, whereas those collected aboard Airline B were collected from a series of locations in the coach cabin. Gasper air samples, thought to be the "cleanest" air about the aircraft, yielded high CFU numbers from both Phase I flights (ca. 6.4×10^4 CFU/m^3 of air). The order of magnitude increase in CFU between gasper samples collected pre-flight and during flight (13 fold increase for flight #3 and 3 fold increase for flight #4) most likely reflects the build up of passenger-borne, respiratory tract-associated microbes over the course of the flight. The possible accumulation of microbial biofilms about the surfaces of the gasper conduits could also be responsible for such an observation, as the flux of air passing through these channels increases as the aircraft reaches cruise altitude. Given their small, enclosed space, lavatory samples were expected to be very highly laden with microbes, this was not the case as such parcels yielded a mere 3×10^2 to 2×10^4 CFU/m^3 of air. As was seen with the gasper air samples, lavatory air parcels generally gave rise to more CFU as the flight progressed. The cabin air samples collected from the seat and floor exhibited a great variance in numbers of

cultivable microbes (between 0 and 2.7 x 10^5 CFU/m^3 of air). The somewhat esoteric trend of increasing microbial burden as the flight progressed, as was observed via molecular approaches, was not observed in these samples. One plausible explanation for the absence of such a trend is intermixing and circulation throughout the cabin, however, if this were the case it should also hold true for the gasper samples. A more thorough understanding of the airflow dynamics about the cabin at varying times during the flight is required to better understand these observations.

5.2.2 Ratio of cultivable microbes among total viable microbes

In Phase I, when numbers generated from the plate count assay (total cultivable microbes) were compared with those from the intracellular ATP assay (total viable microbes), about 35% (5 out of 14; Table 4.2.1-5c) and 28% (4 out of 15; Table 4.2.1-5d) of samples from Flight # 3 and #4, respectively, exhibited a higher cultivable population than was suggested by ATP-based analysis. This supported the notion that many microbes were metabolically inactive, thus maintaining lower concentrations of ATP. Of the total population, between 2.4% and 24% was cultivable for Flight #3 samples, and this ratio ranged from 1.6% to 80% (7 out of 11 samples) for Flight #4 samples. In Phase II, 21% (23 of 109 samples) yielded greater average cultivable microbial counts than were suggested by the ATP assay (Table 4.2.1-26). The average CFU counts for 3 of 15 flights showed cultivable to total viable ratios of greater than 1. There are two possible explanations for this: fluctuating accumulations of certain types of microbes, and/or induction of a non-cultivable phenotype. Previous studies have suggested that desiccating environments may induce a viable-but-non-cultivable state (Colwell, 2000), however, an in-depth investigation is required before any cogent conclusions on this matter can be made.

5.2.3 Cultivable bacterial diversity

While representatives of the α-, β-, γ-, and δ-proteobacteria, as well as Gram-positive bacteria, Spirochaetes, Fusobacteria, Deinococci, Bacterioidetes, Cyanobacteria, and Planctomyces were identified in varying abundance from Phase II samples, Phase I samples were limited in cultivable diversity to members of the α-, β-, and γ-proteobacteria, and high G/C content Gram-positive bacteria. This discrepancy in cultivable diversity may be attributable to the different number of isolates characterized in each phase (120 in Phase II, 14 in Phase I). In Phase I, colonies of *Acinetobacter* sp. were by far the most profuse, and Gram-positive bacteria of the genera *Staphylococcus* and *Bacillus* were the most abundant of Phase II isolates, as made evident by morphometric alignment of colony morphologies with sequence identification data (Figure 4.3.1-2). The isolation of the human pathogens *Acinetobacter johnsonii, A. calcoaceticus, Janibacter melonis, Microbacterium trichotecenolyticum, Massilia timonae, Staphylococcus saprophyticus, Corynebacterium lipophiloflavum* is concerning, as these bacteria can cause meningitis, septicemia, and a handful of sometimes fatal diseases and infections (Hunt et al. 2000; Chang et al, 2000; Bergogne-Berezin and Joly-Guillou, 1985). Furthermore, 25% (29 of 113) of the species whose presence was implicated by clone-library analyses were to some degree pathogenic. Though beyond the scope of this study, attempts at culturing microbial populations from these samples with a more encompassing array of media is highly recommended. Indeed, as we increased the number of isolates characterized (from 14 in Phase I to 120 in Phase II), so did the

diversity and novelty of microbiota discovered increase (5 novel microbes isolated in Phase II).

5.3 Concluding Remarks

This multi-year biological study called for air sampling of 20 domestic and international flights on multiple airlines. The traditional culture based approaches and state of the art molecular methods used to describe the bioburden and diversity of these unique environments required hundreds of lab hours and generated a tremendous amount of data. In closing this report the authors hope to put this information into perspective and from it, draw conclusions on how commercial aircraft air affects the health of passengers and the general usefulness of this data in the development of a bio-threat detection system. The detection of viral nucleic acids and bacterial phylogenetic data indicating the presence of opportunistic pathogens should not lead to the assumption that aircraft cabin air presents a hazard for travelers. The biological diversity and bioburden present in aircraft cabin air should not be evaluated solely on the presence of these agents but rather on the comparative risk of contracting illness while traveling as opposed to other activities.

A substantial amount of baseline data has been collected that presents little or no clear spatial or temporal patterns. The majority of microbial species circulating in commercial airline cabins are commensal, infrequently pathogenic normal flora of the human nasopharynx and respiratory system. Many of these microbes likely originate from the oral and nasal cavities, and lungs of passengers and flight crew and are disseminated unknowingly via routine conversation, coughing, sneezing, and stochastic passing of fomites. Additionally, we came across several clones implicating the presence of bacterial pathogens of mites. This is perhaps suggestive of microbial burden originating from the aircraft upholstery and flooring. Regardless of any other trends, it was overly apparent that the cabin air aboard Airline A was much less laden with airborne microorganisms than that of Airline B, and Airline C was cleaner still, albeit that Airline C samples were collected from the business class cabin. Microbial examination of commercial airline cabin air sampled at various times and locations about the cabin, while in flight, revealed that current methods for filtering and purifying cabin air need improvement. The air purification systems on-board the aircraft are allowing the circulation of viable microbial cells of opportunistic pathogens. Additional purification is recommended for the recycling of air throughout the cabins of commercial aircraft.

Anecdotal stories and even published investigations of illness following air travel at best only make correlations between air travel and passenger or crew member health. Because they are drawn from observational interviews and not empirical data, it cannot be said with confidence that pathogenic agents within aircraft caused the malady. Considering the extent to which air is processed by HEPA filters aboard planes and the culture dependent and culture independent results presented in this report, illness is much more likely contracted outside of the plane. The potential subset of illness that actually stems from exposure to the internal environments on planes is more likely due to suppression of the immune system as a consequence of travel related stress than to increased levels of pathogens. Indeed, the frequency of microbes and viruses in the vast majority of samples that we examined in this study suggest walking about in the airport is more dangerous to traveler health than sitting in the plane itself.

The findings of this study have the potential to enhance the preservation and safety of air travel. Such research is paramount to the development and implementation of biological sensors aboard aircraft. In order to calibrate and train sensing devices to detect a particular group of "select" microorganisms the background microbial population must first be thoroughly defined.

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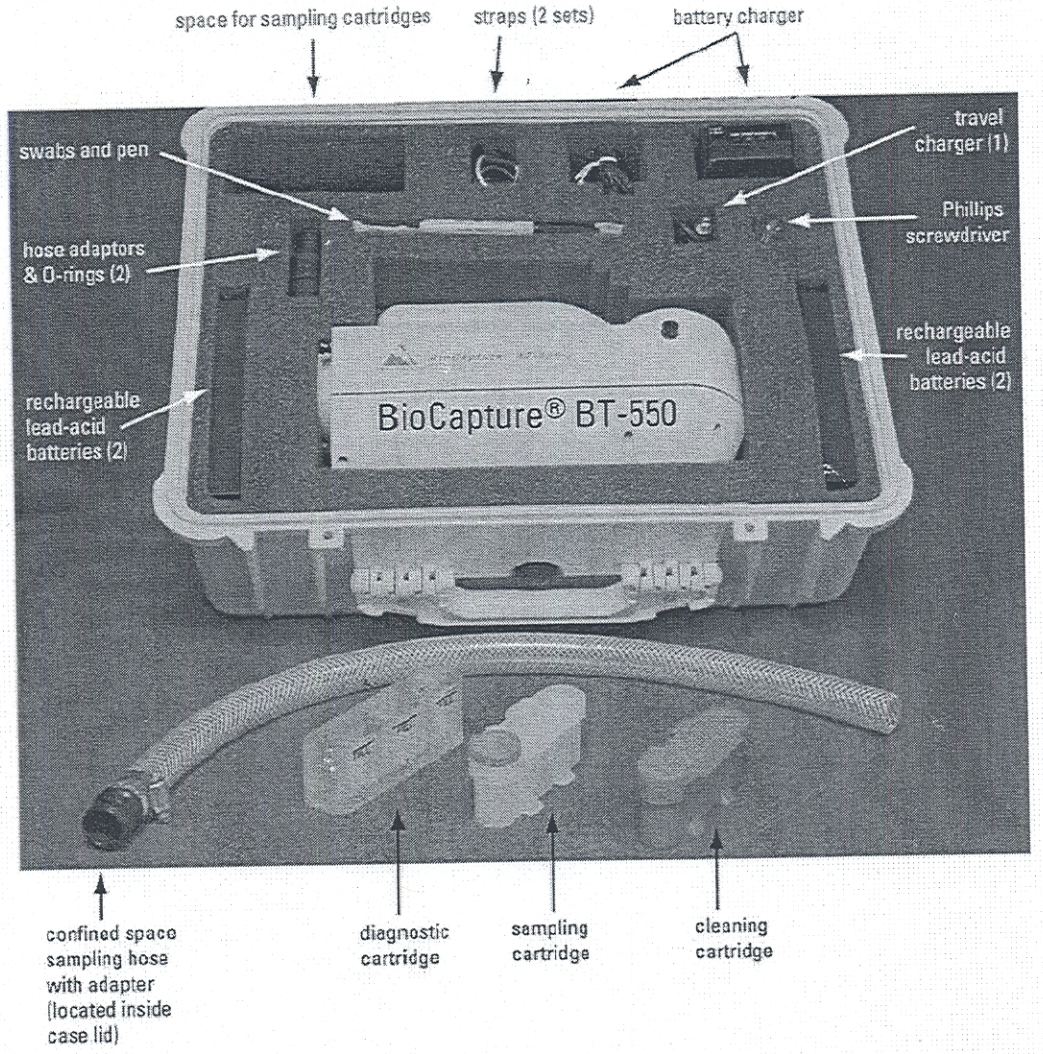
2006 1 5

PRODUCT SPECIFICATIONS

Characteristics	Description
Sampling flow rate	5.3 CFM 150 liters per minute
Dimensions	12" x 5" x 6" 30 cm x 13 cm x 15 cm
Weight	10 lbs 4.5 kg
Particle size collection	range 0.5 to 10.0 microns
Power requirements	12VDC lead-acid battery pack
Collection fluid media	buffered saline and detergent
User interface	individually labeled LEDs
Detection media port	Tetracore BioThreat Alert™ test strips (handheld assays)

PELICAN CASE CONTENTS



Not pictured: User's Manual, laminated Quick Reference Guide

Note: sampling and cleaning cartridges are packaged separately.

FIGURE 2.2-1

MESOSYSTEMS 550

Figure 3.3.6-1 Viral Standard Curve: Varicella-Zoster Virus

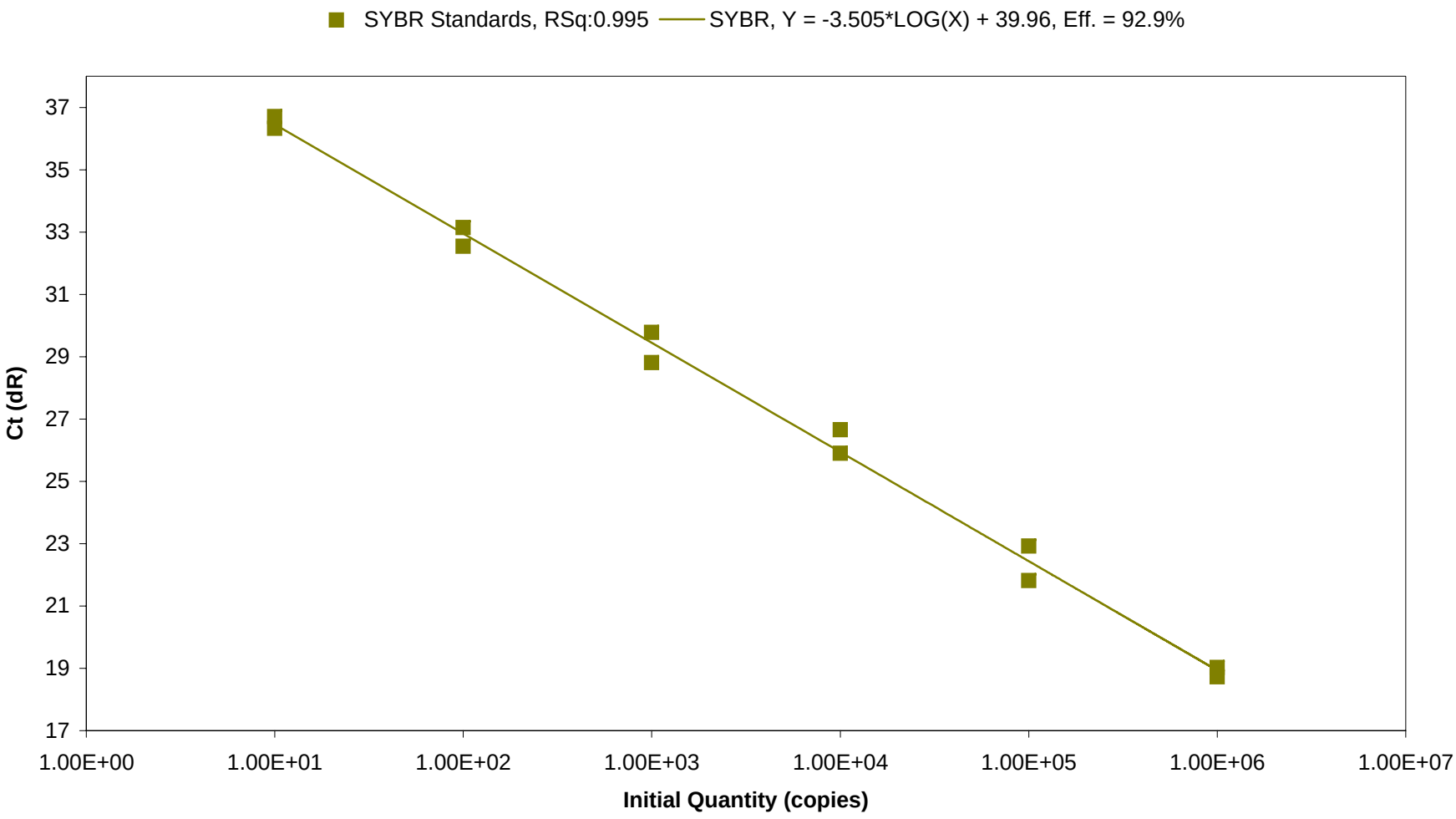


Figure 3.3.6-2 Viral Standard Curve: Epstein Barr Virus

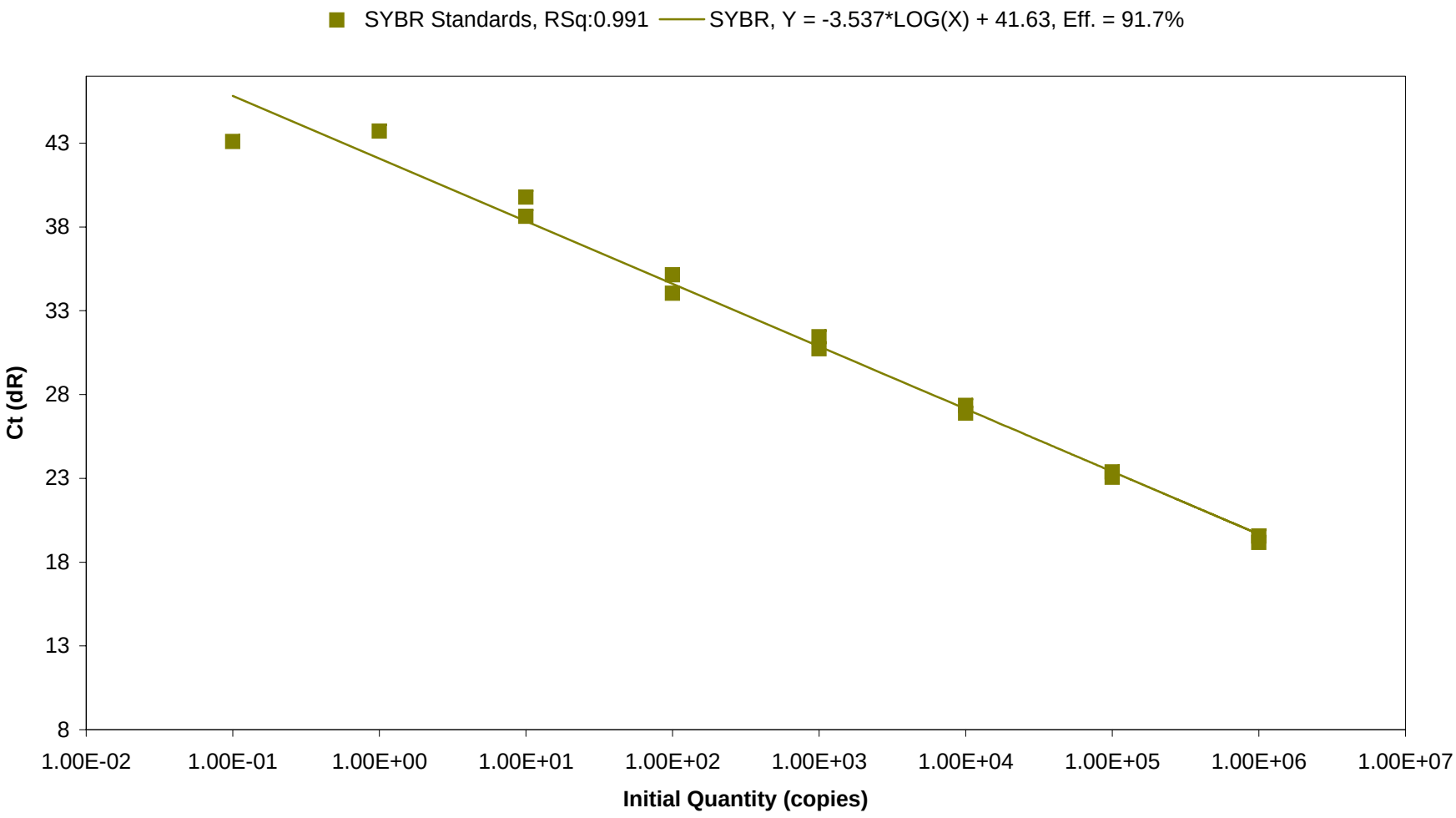


Figure 3.3.6-3 Viral Standard Curve: Corona Virus

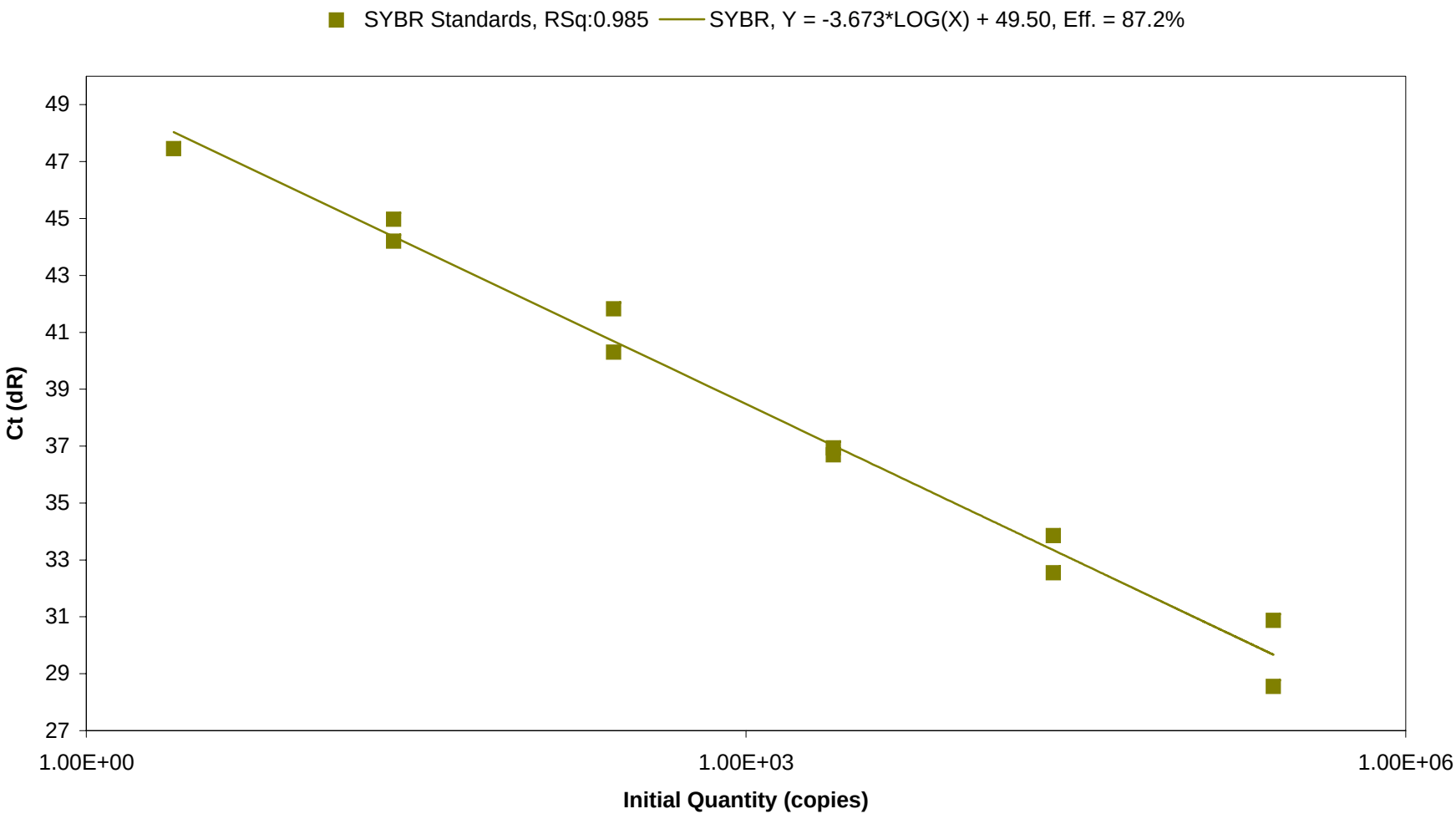


Figure 3.4.3-1 QPCR analysis of varying extraction methods (Class 10K cleanroom samples)

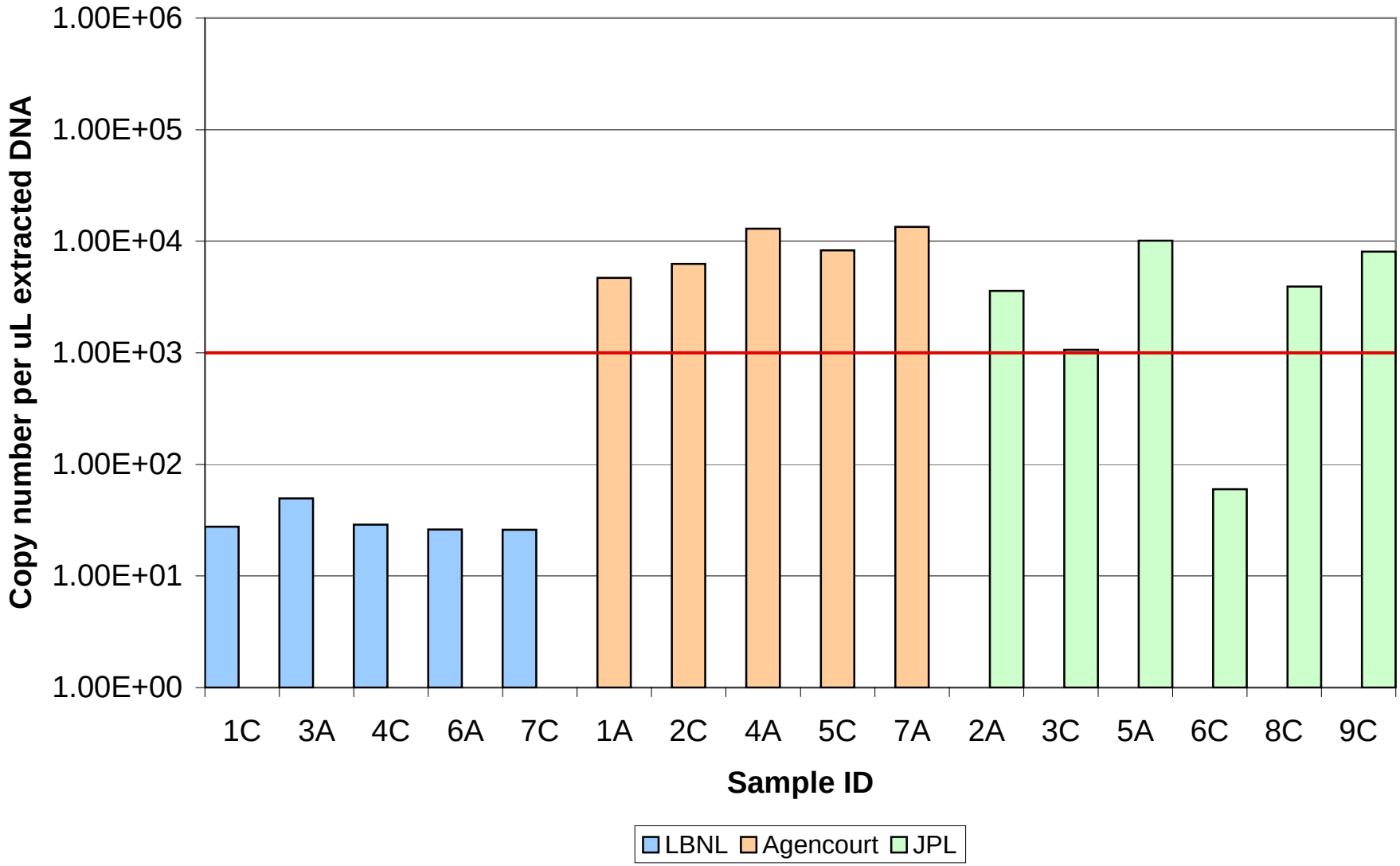


Figure 3.4.3-2 QPCR analysis of varying extraction methods (Class 100K cleanroom samples)

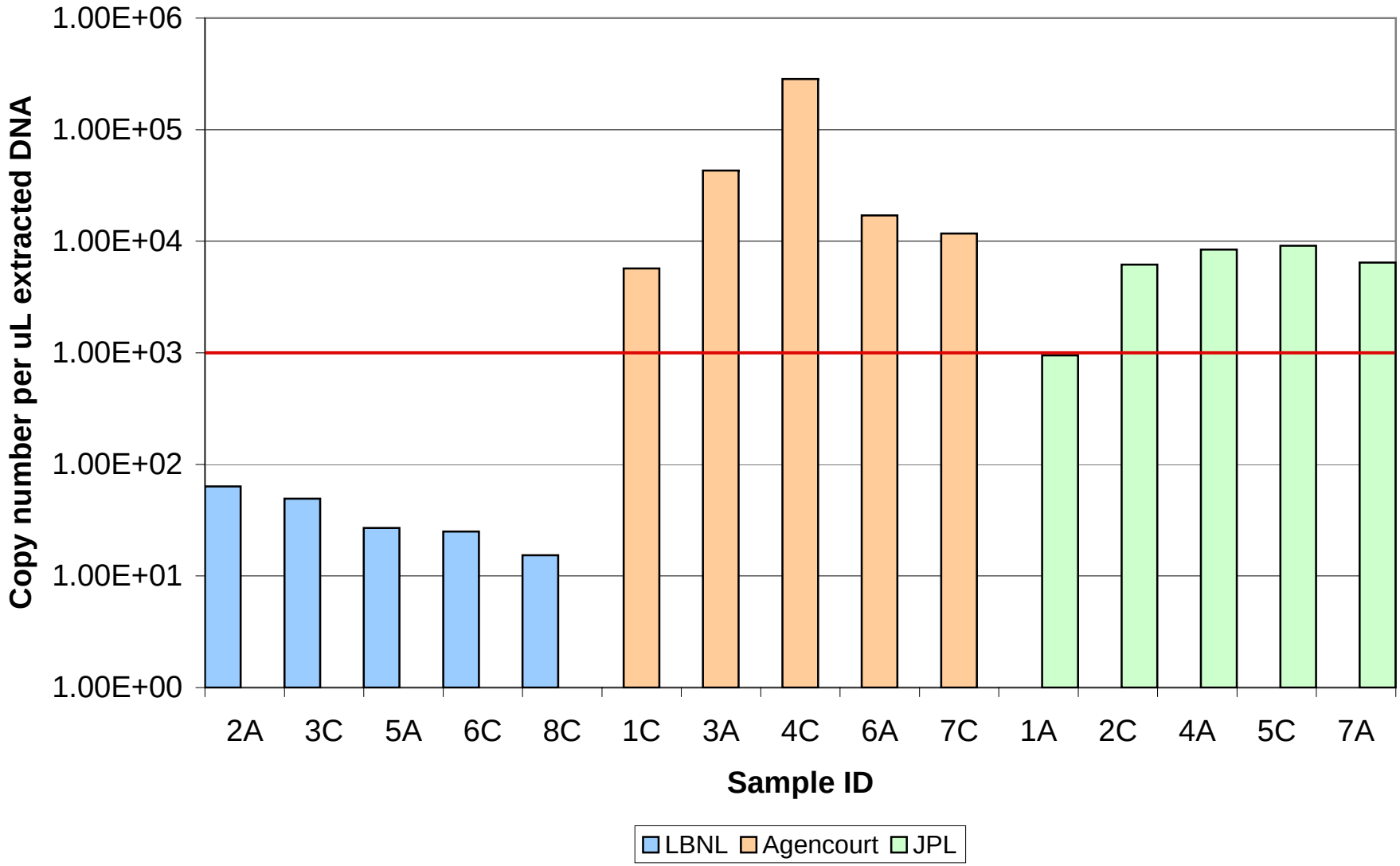
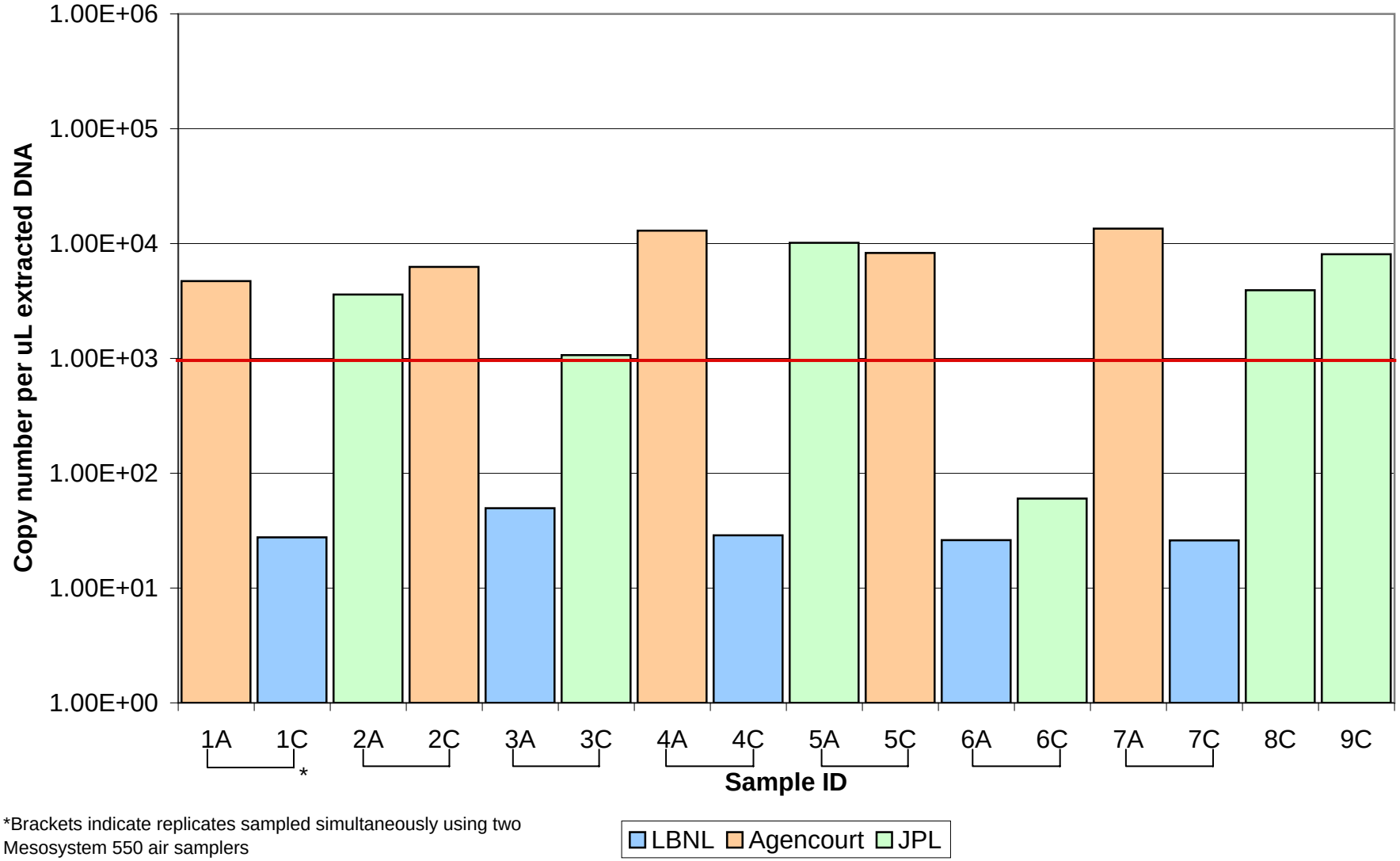
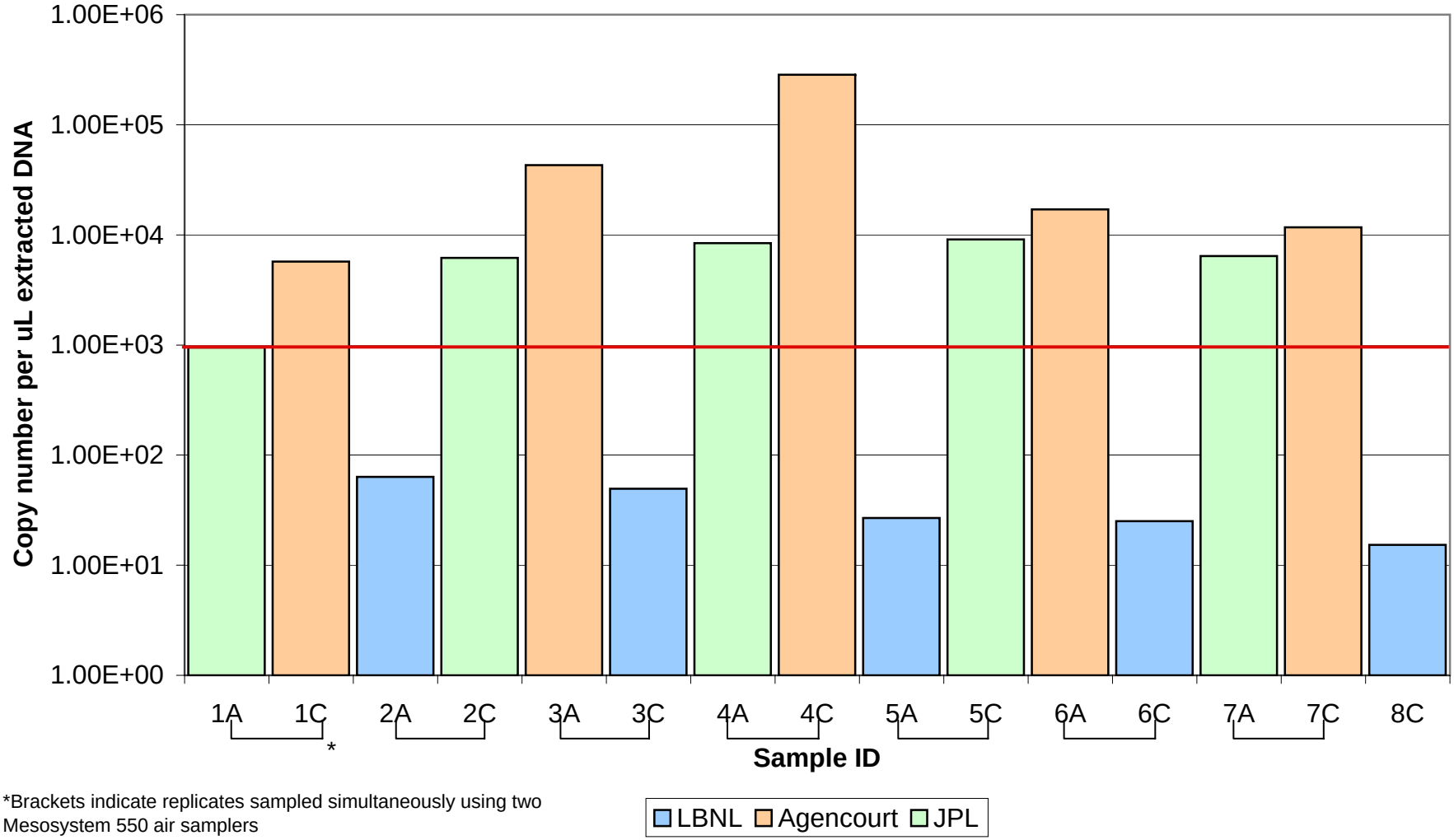


Figure 3.4.3-3 QPCR analysis of varying extraction methods (Class 10K cleanroom samples)



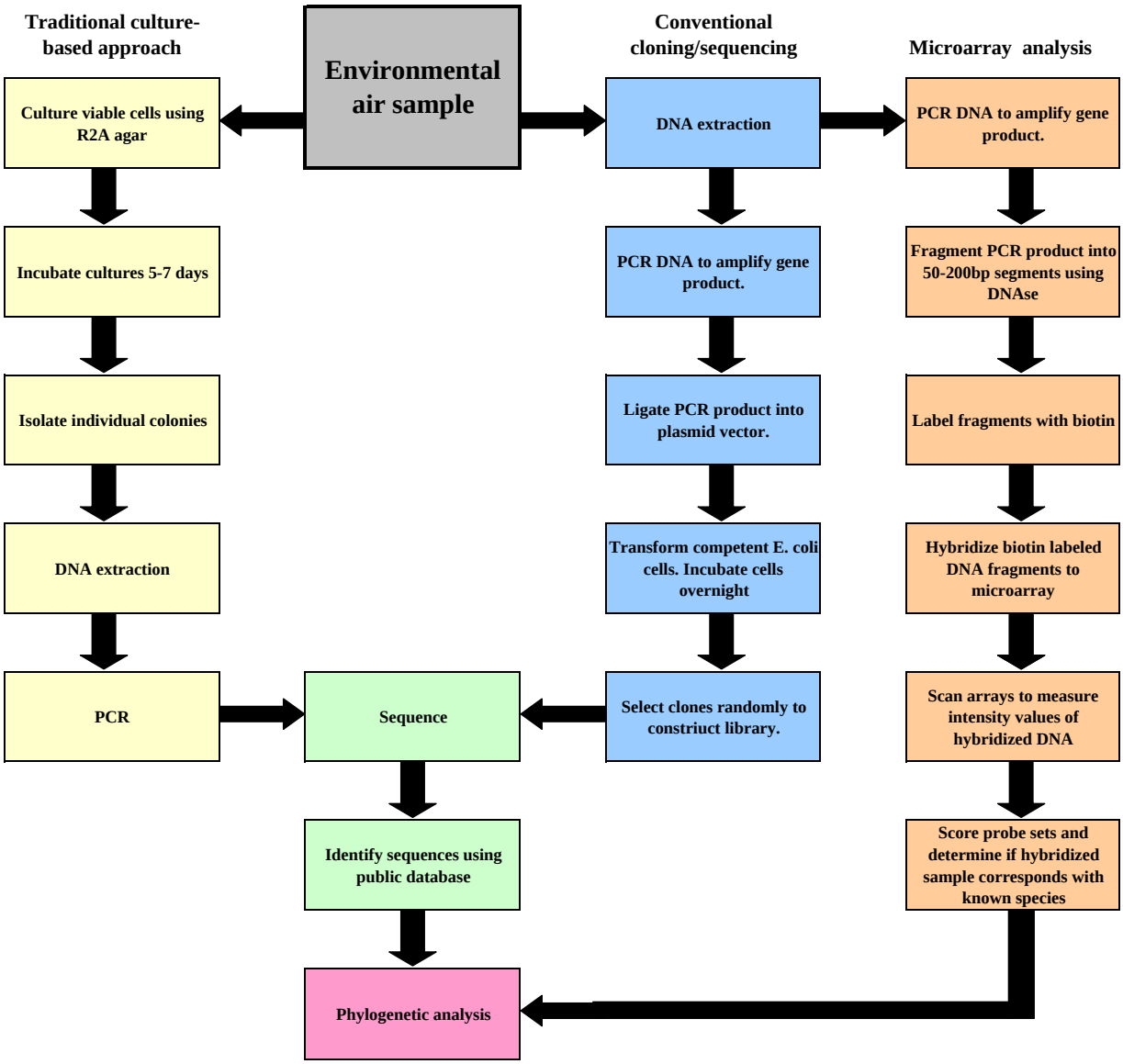
*Brackets indicate replicates sampled simultaneously using two Mesosystem 550 air samplers

Figure 3.4.3-4 QPCR analysis of varying extraction methods (Class 100K cleanroom samples)



*Brackets indicate replicates sampled simultaneously using two Mesosystem 550 air samplers

Figure 3.4.3-5 Bacterial diversity assays utilizing DNA extracted from Phase II samples



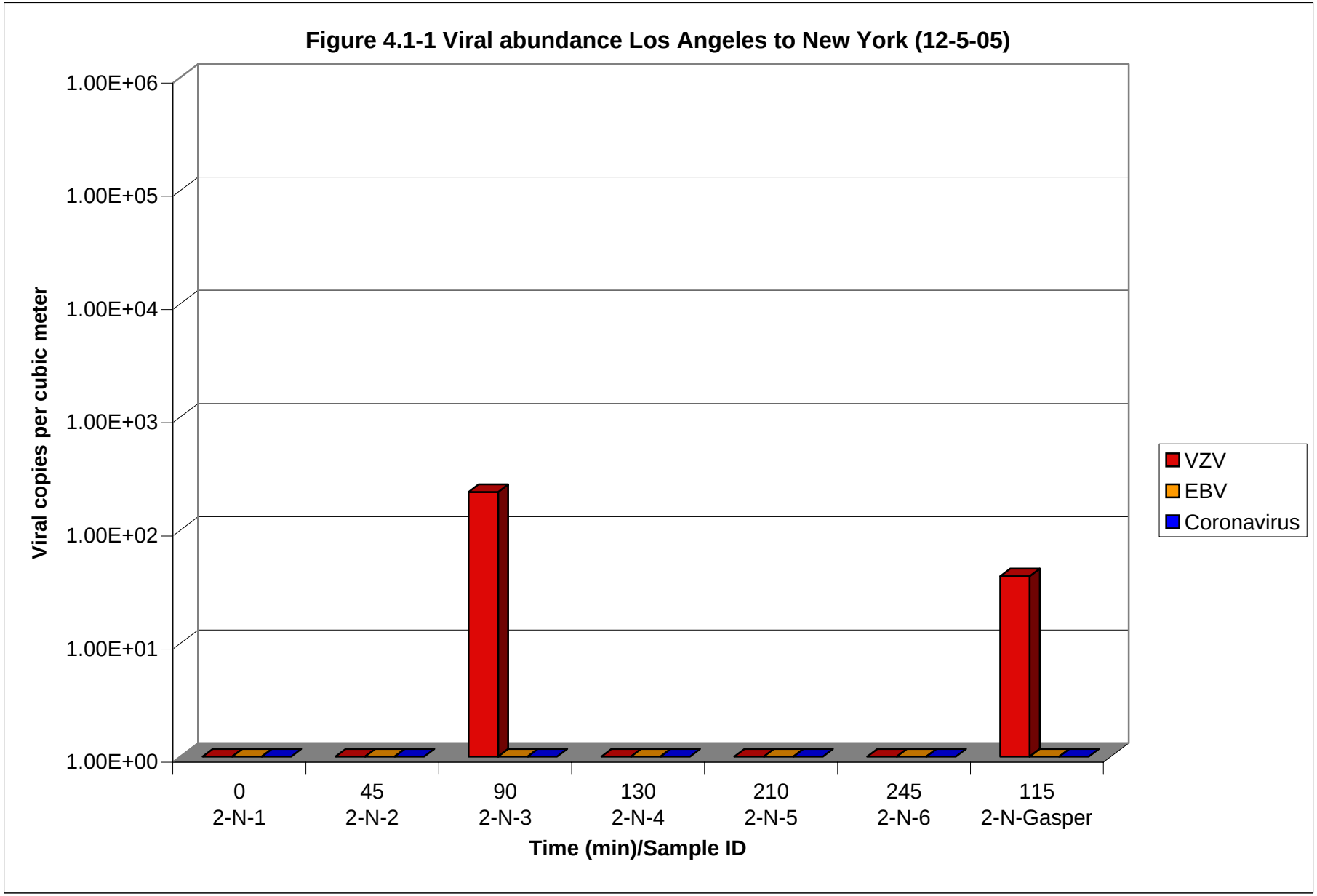


Figure 4.1-1 Viral abundance Los Angeles to New York (12-5-05)
Viral copies per cubic meter
1.00E+06
1.00E+05
1.00E+04
1.00E+03
1.00E+02
1.00E+01
1.00E+00
0
2-N-1
45
2-N-2
90
2-N-3
130
2-N-4
210
2-N-5
245
2-N-6
115
2-N-Gasper
Time (min)/Sample ID
VZV
EBV
Coronavirus

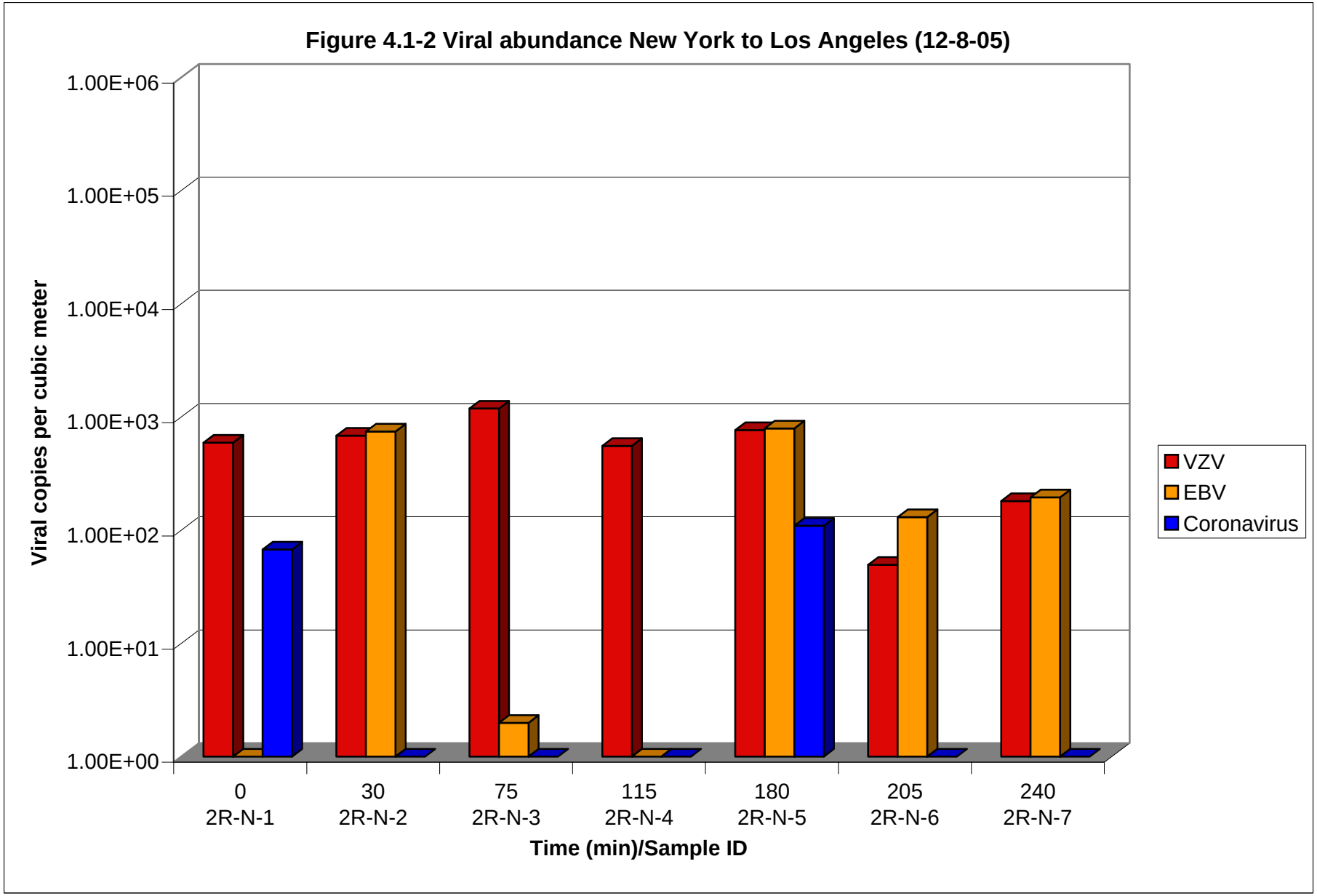


Figure 4.1-2 Viral abundance New York to Los Angeles (12-8-05)
Viral copies per cubic meter
1.00E+06
1.00E+05
1.00E+04
1.00E+03
1.00E+02
1.00E+01
1.00E+00
0
2R-N-1
30
2R-N-2
75
2R-N-3
115
2R-N-4
180
2R-N-5
205
2R-N-6
240
2R-N-7
Time (min)/Sample ID
VZV
EBV
Coronavirus

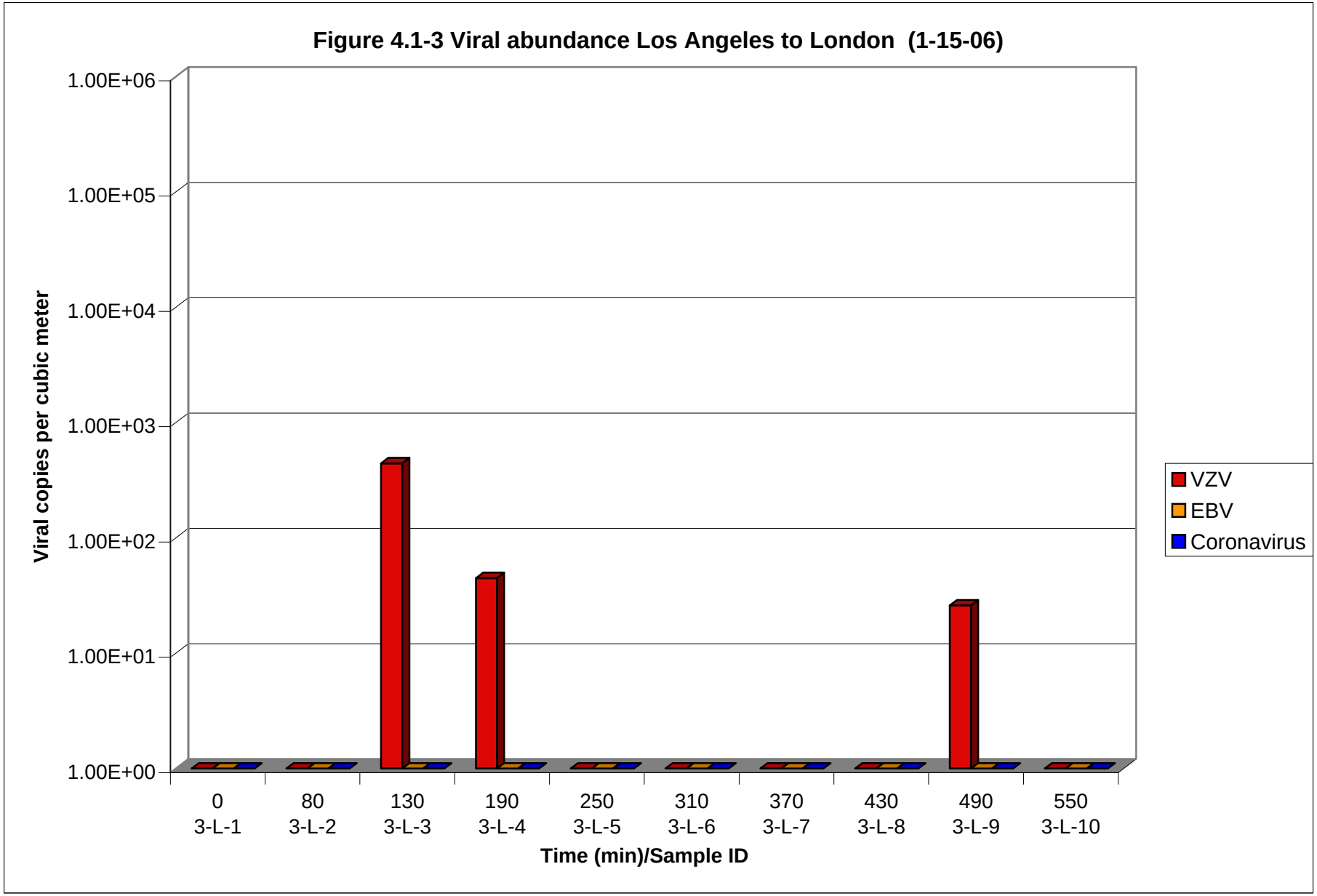


Figure 4.1-3 Viral abundance Los Angeles to London (1-15-06)
Viral copies per cubic meter
1.00E+06
1.00E+05
1.00E+04
1.00E+03
1.00E+02
1.00E+01
1.00E+00
0
3-L-1
80
3-L-2
130
3-L-3
190
3-L-4
250
3-L-5
310
3-L-6
370
3-L-7
430
3-L-8
490
3-L-9
550
3-L-10
Time (min)/Sample ID
VZV
EBV
Coronavirus

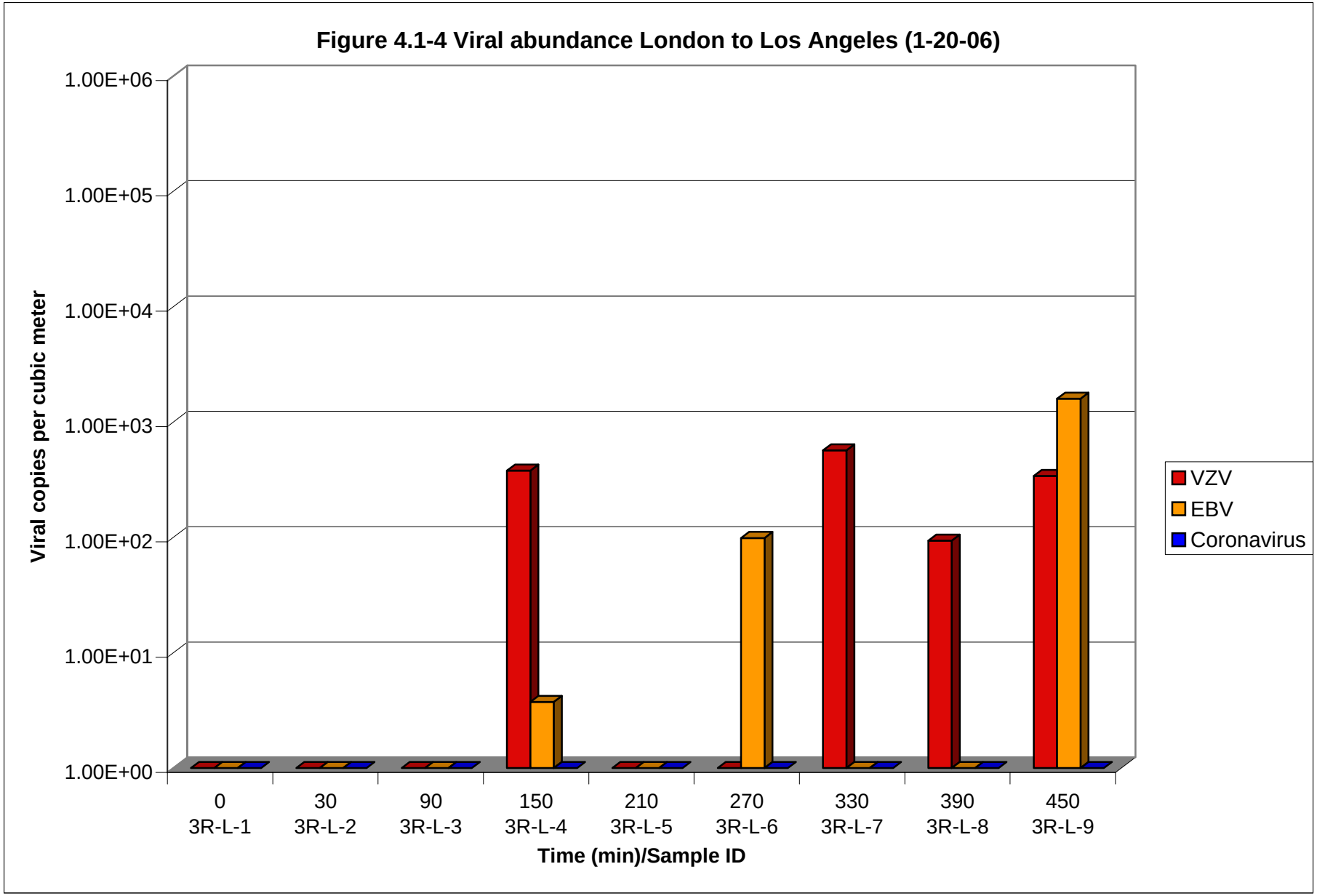


Figure 4.1-4 Viral abundance London to Los Angeles (1-20-06)
Viral copies per cubic meter
1.00E+06
1.00E+05
1.00E+04
1.00E+03
1.00E+02
1.00E+01
1.00E+00
0
3R-L-1
30
3R-L-2
90
3R-L-3
150
3R-L-4
210
3R-L-5
270
3R-L-6
330
3R-L-7
390
3R-L-8
450
3R-L-9
Time (min)/Sample ID
VZV
EBV
Coronavirus

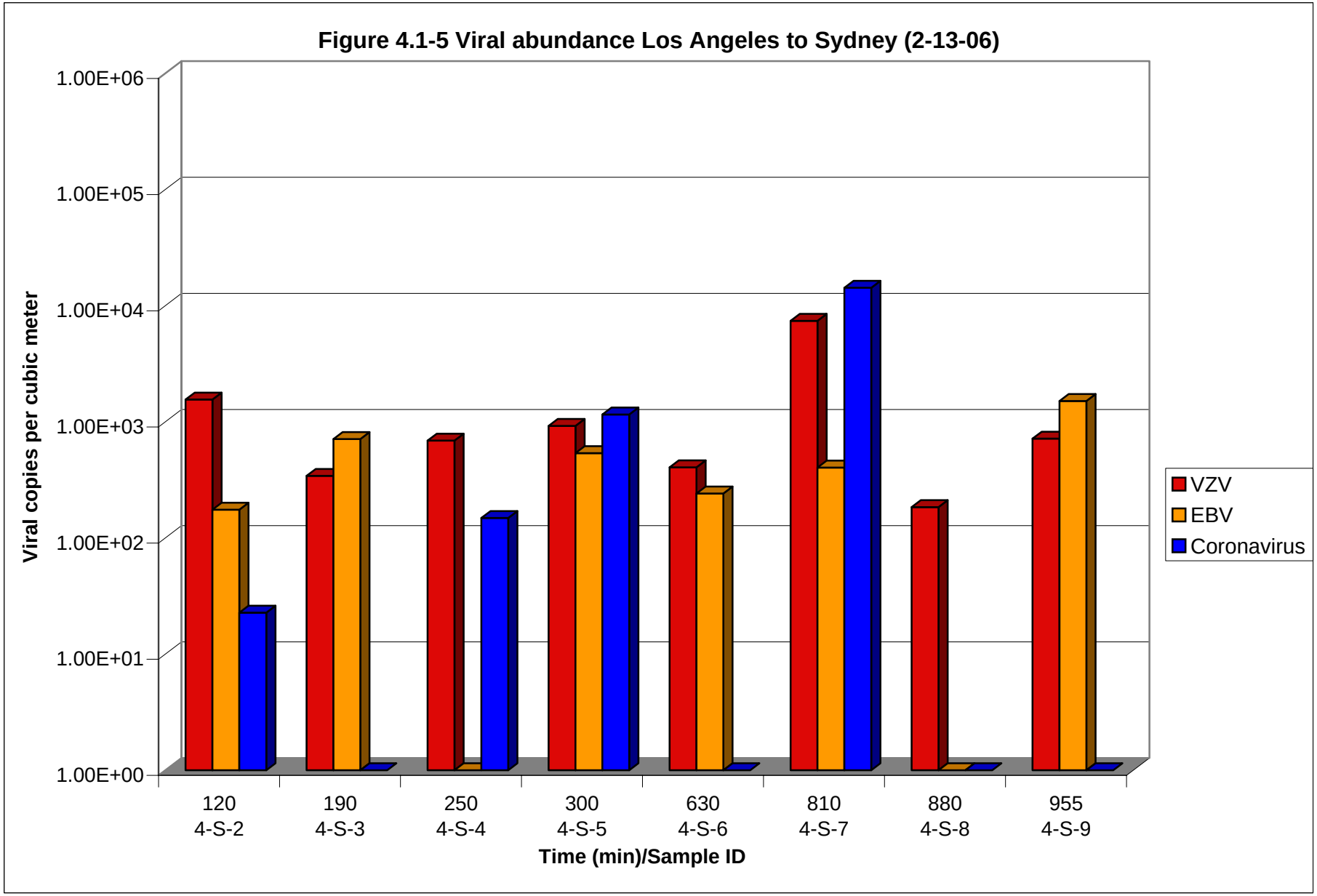


Figure 4.1-5 Viral abundance Los Angeles to Sydney (2-13-06)
Viral copies per cubic meter
1.00E+06
1.00E+05
1.00E+04
1.00E+03
1.00E+02
1.00E+01
1.00E+00
120
4-S-2
190
4-S-3
250
4-S-4
300
4-S-5
630
4-S-6
810
4-S-7
880
4-S-8
955
4-S-9
Time (min)/Sample ID
VZV
EBV
Coronavirus

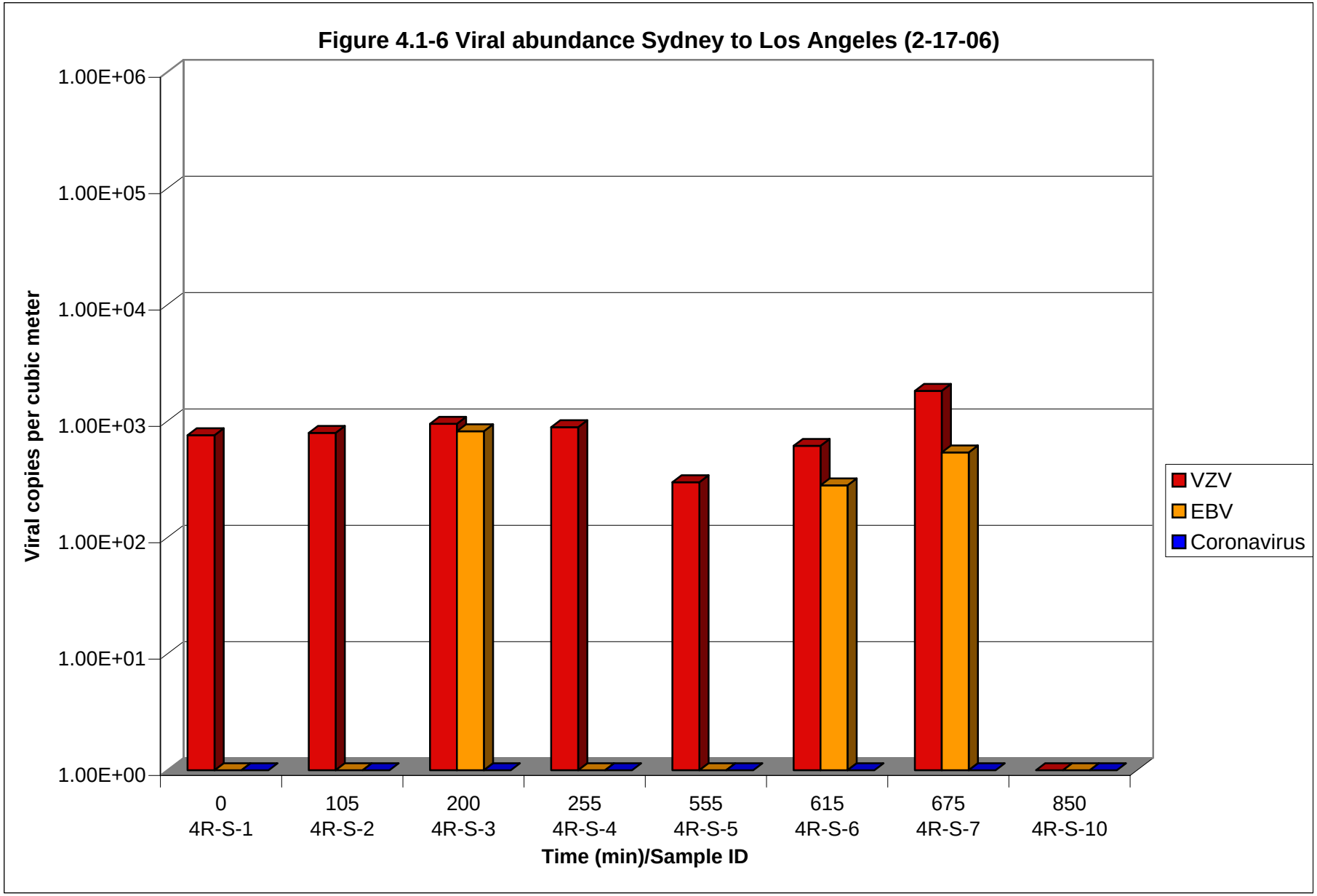


Figure 4.1-6 Viral abundance Sydney to Los Angeles (2-17-06)
Viral copies per cubic meter
1.00E+06
1.00E+05
1.00E+04
1.00E+03
1.00E+02
1.00E+01
1.00E+00
0
4R-S-1
105
4R-S-2
200
4R-S-3
255
4R-S-4
555
4R-S-5
615
4R-S-6
675
4R-S-7
850
4R-S-10
Time (min)/Sample ID
VZV
EBV
Coronavirus

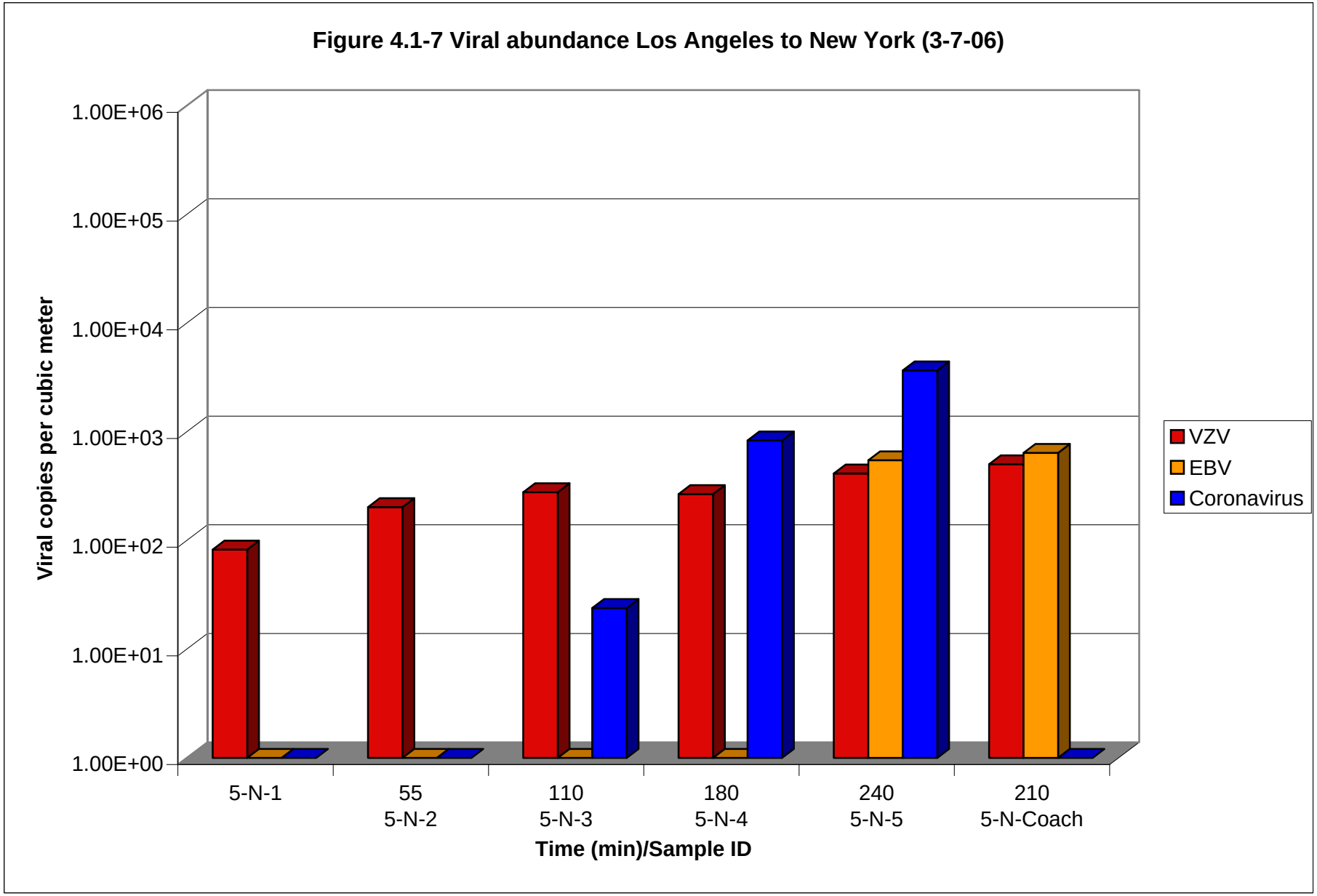


Figure 4.1-7 Viral abundance Los Angeles to New York (3-7-06)
Viral copies per cubic meter
1.00E+06
1.00E+05
1.00E+04
1.00E+03
1.00E+02
1.00E+01
1.00E+00
5-N-1
55
5-N-2
110
5-N-3
180
5-N-4
240
5-N-5
210
5-N-Coach
Time (min)/Sample ID
VZV
EBV
Coronavirus

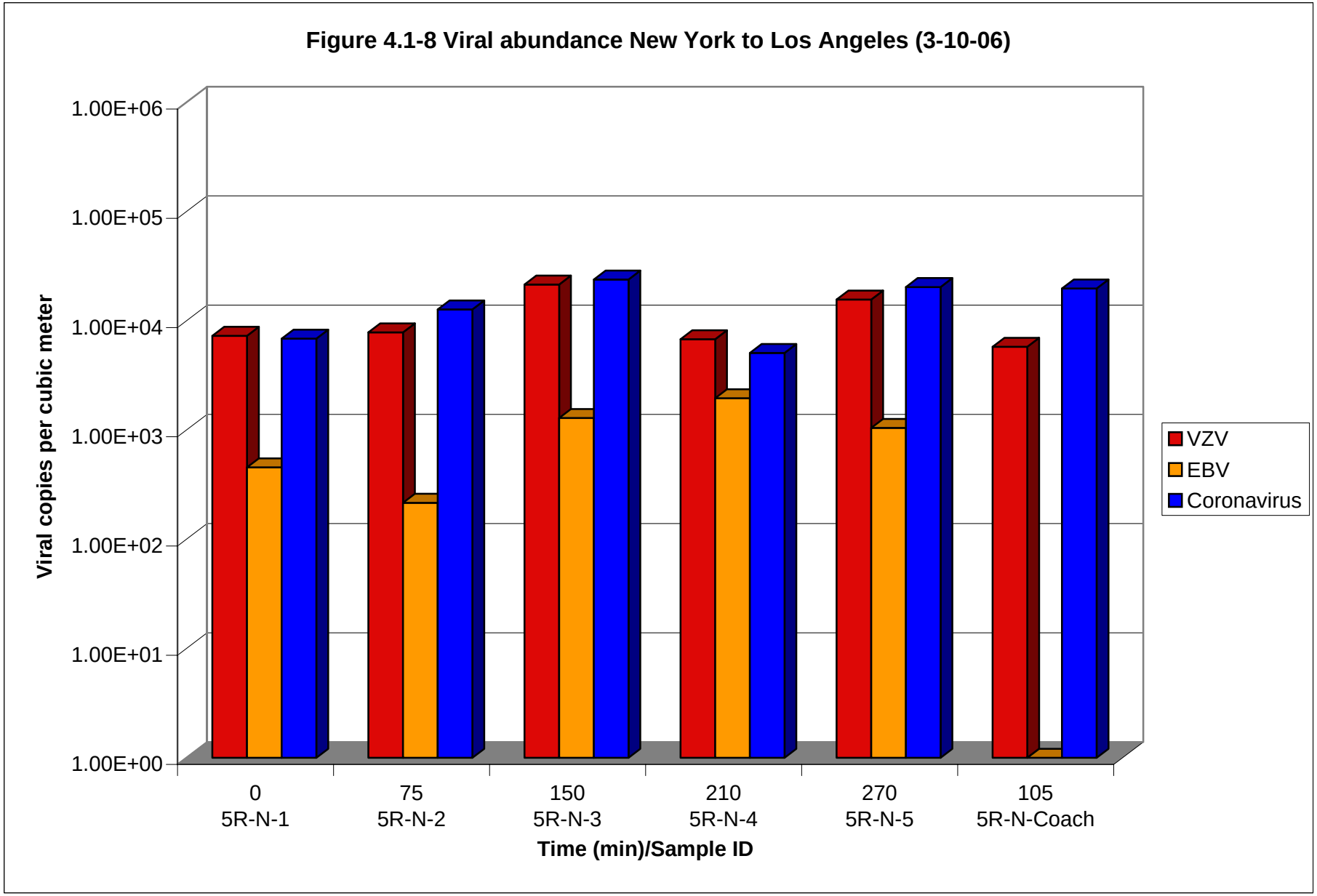
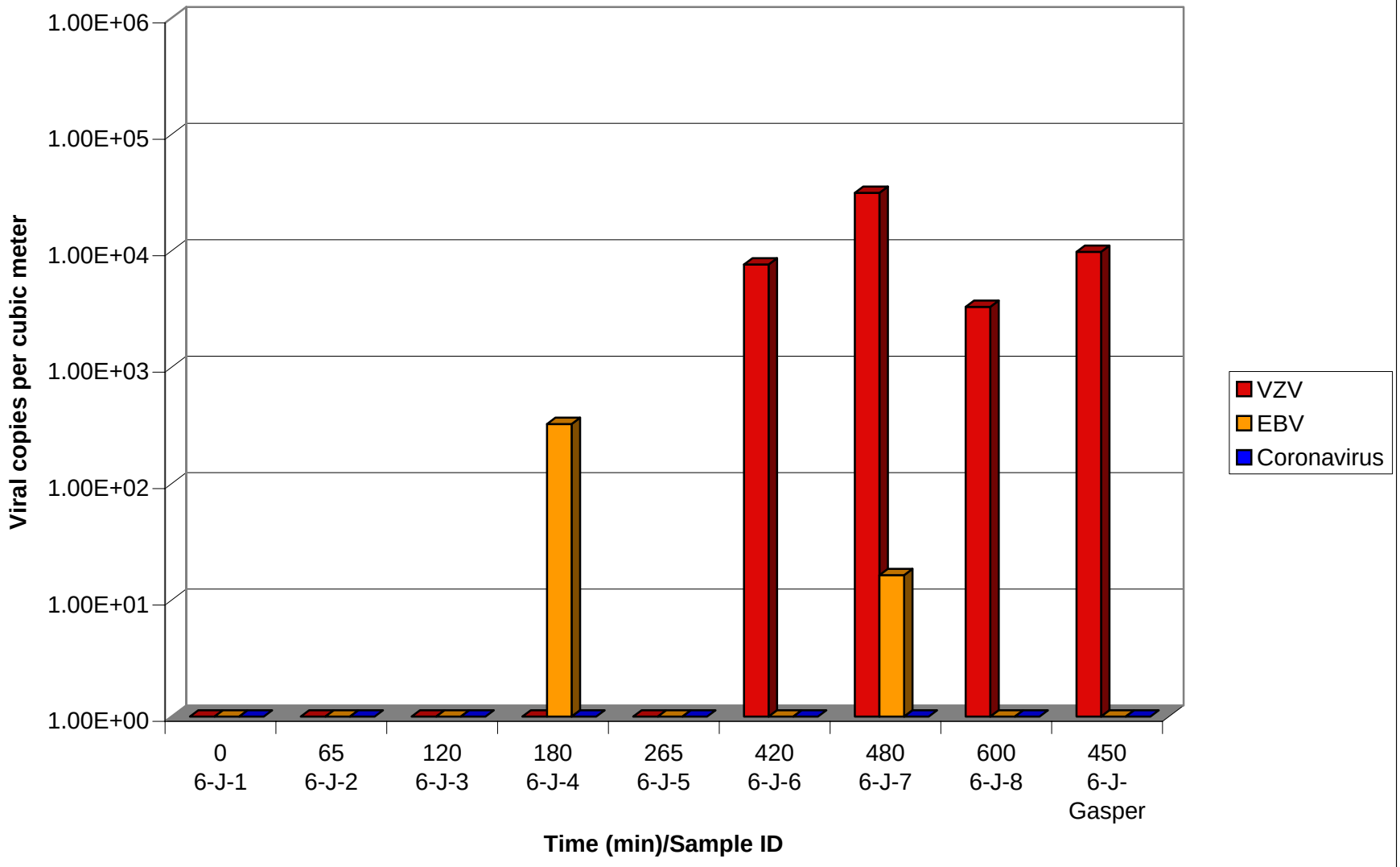


Figure 4.1-8 Viral abundance New York to Los Angeles (3-10-06)
Viral copies per cubic meter
1.00E+06
1.00E+05
1.00E+04
1.00E+03
1.00E+02
1.00E+01
1.00E+00
0
5R-N-1
75
5R-N-2
150
5R-N-3
210
5R-N-4
270
5R-N-5
105
5R-N-Coach
Time (min)/Sample ID
VZV
EBV
Coronavirus

Figure 4.1-9 Viral abundance Los Angeles to Tokyo (4-3-06)



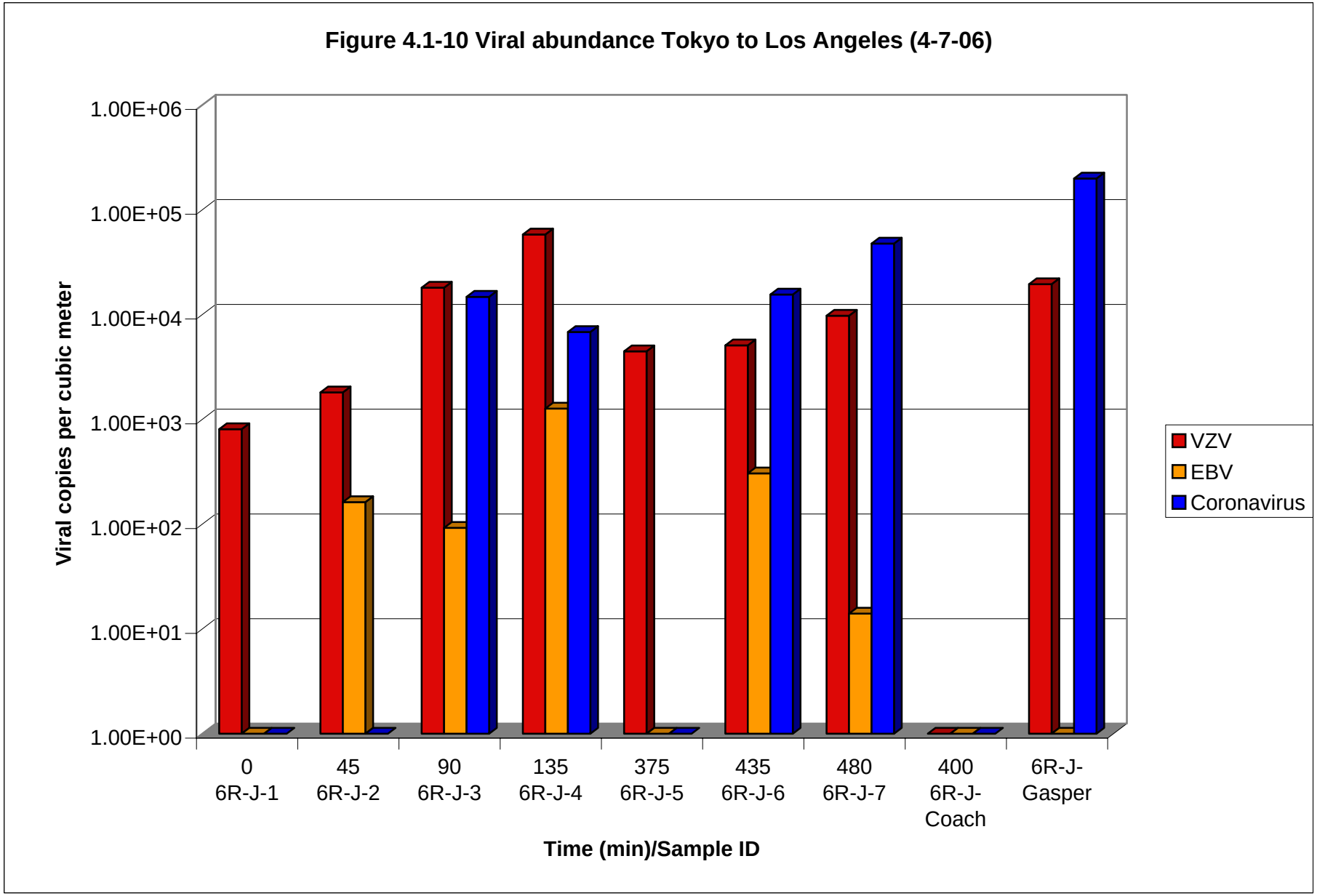


Figure 4.1-10 Viral abundance Tokyo to Los Angeles (4-7-06)
Viral copies per cubic meter
1.00E+06
1.00E+05
1.00E+04
1.00E+03
1.00E+02
1.00E+01
1.00E+00
0
6R-J-1
45
6R-J-2
90
6R-J-3
135
6R-J-4
375
6R-J-5
435
6R-J-6
480
6R-J-7
400
6R-J-Coach
6R-J-Gasper
Time (min)/Sample ID
VZV
EBV
Coronavirus

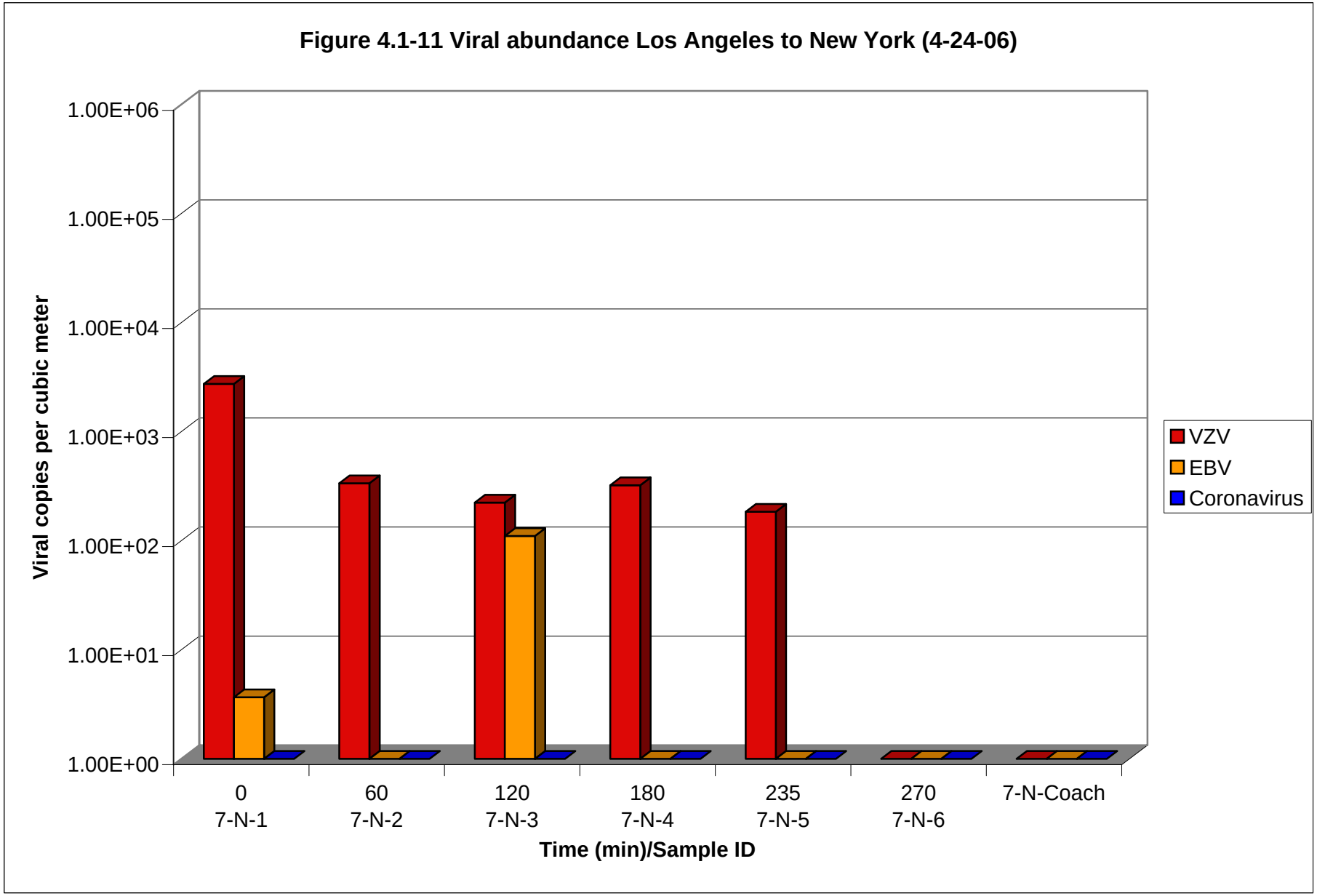


Figure 4.1-11 Viral abundance Los Angeles to New York (4-24-06)
Viral copies per cubic meter
1.00E+06
1.00E+05
1.00E+04
1.00E+03
1.00E+02
1.00E+01
1.00E+00
0
7-N-1
60
7-N-2
120
7-N-3
180
7-N-4
235
7-N-5
270
7-N-6
7-N-Coach
Time (min)/Sample ID
VZV
EBV
Coronavirus

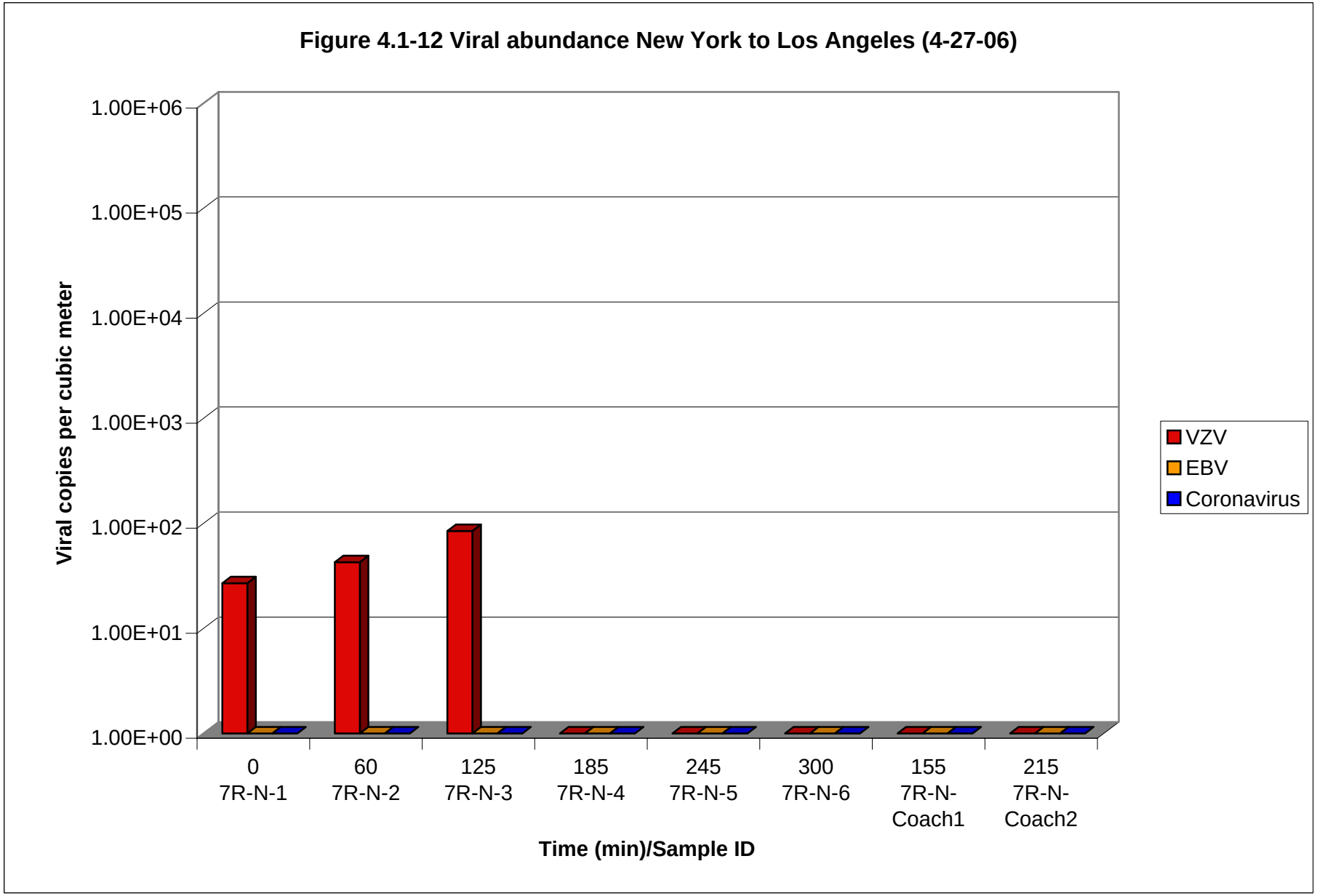


Figure 4.1-12 Viral abundance New York to Los Angeles (4-27-06)
Viral copies per cubic meter
1.00E+06
1.00E+05
1.00E+04
1.00E+03
1.00E+02
1.00E+01
1.00E+00
0
7R-N-1
60
7R-N-2
125
7R-N-3
185
7R-N-4
245
7R-N-5
300
7R-N-6
155
7R-N-Coach1
215
7R-N-Coach2
Time (min)/Sample ID
VZV
EBV
Coronavirus

Figure 4.1-13 Viral abundance Los Angeles to Sydney (5-7-06)

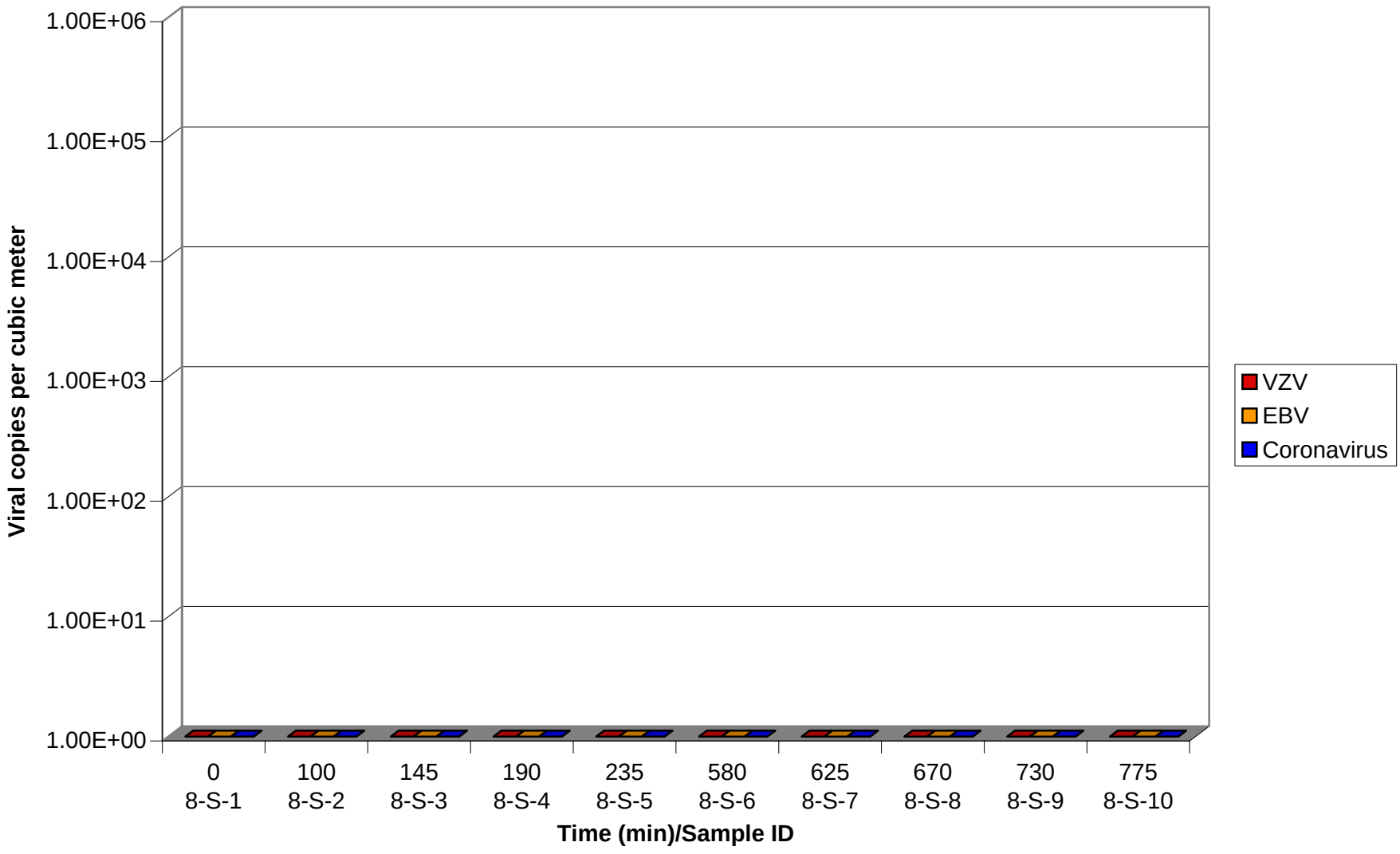
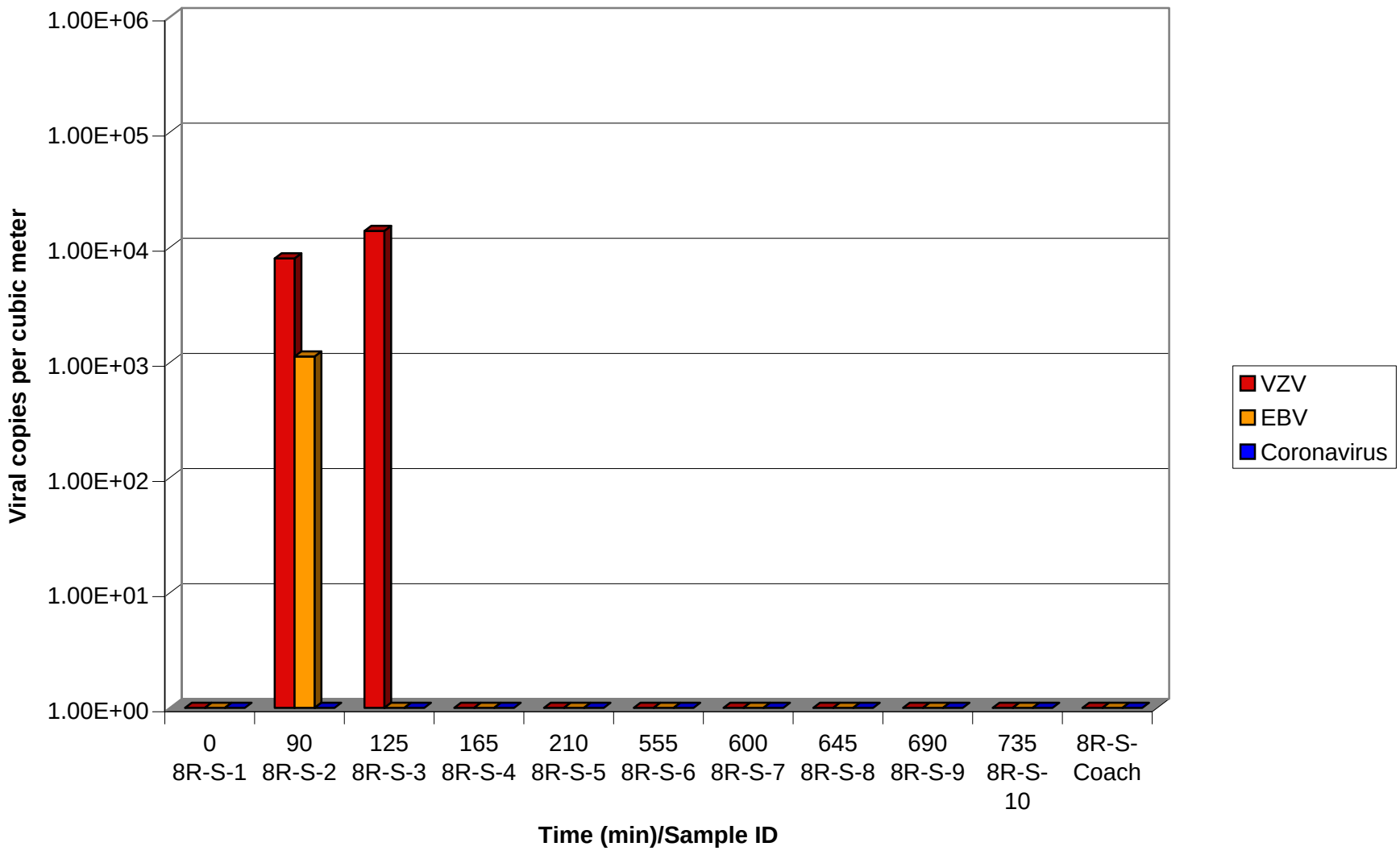


Figure 4.1-14 Viral abundance Sydney to Los Angeles (5-12-06)



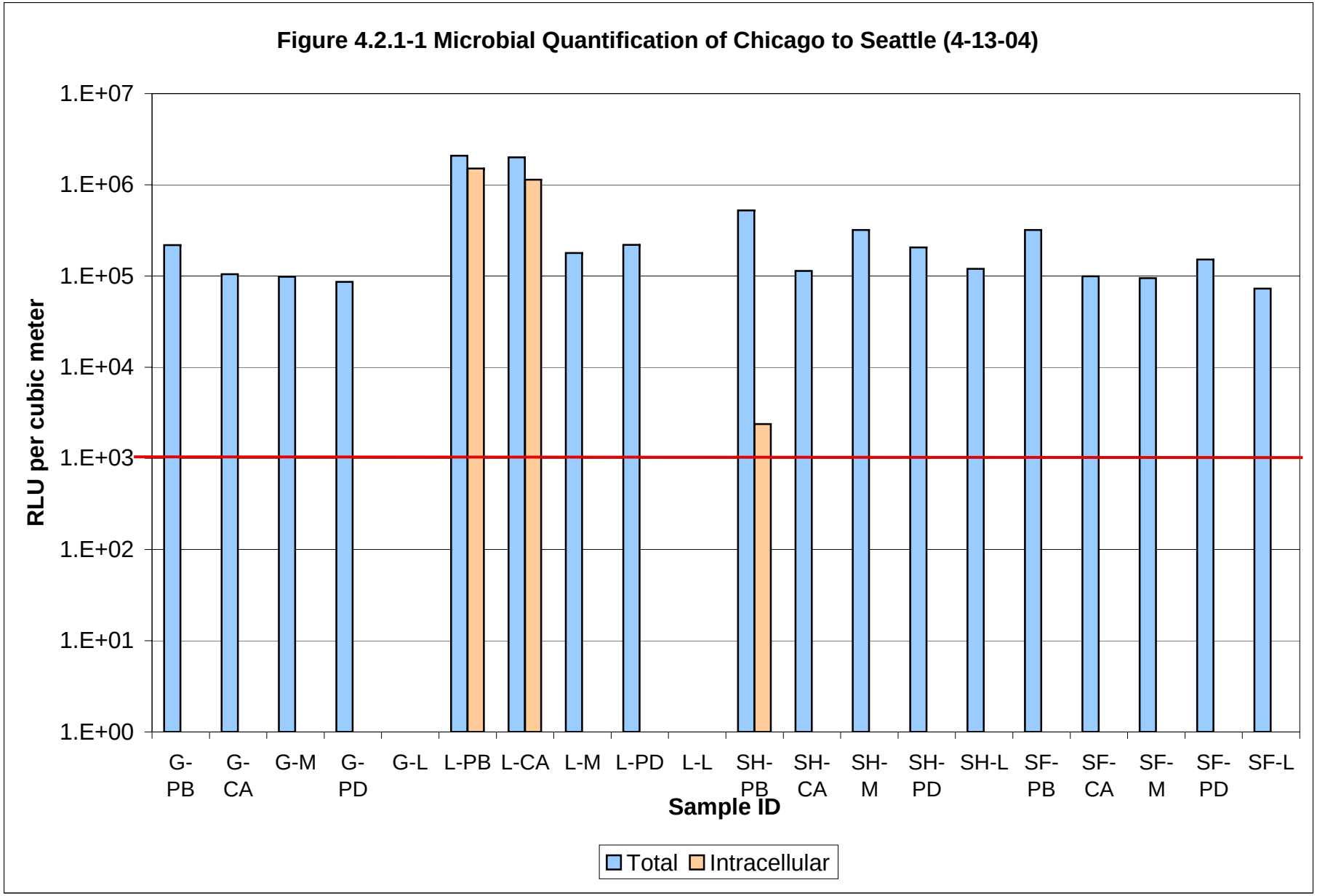


Figure 4.2.1-1 Microbial Quantification of Chicago to Seattle (4-13-04)
RLU per cubic meter
1.E+07
1.E+06
1.E+05
1.E+04
1.E+03
1.E+02
1.E+01
1.E+00
G-PB
G-CA
G-M
G-PD
G-L
L-PB
L-CA
L-M
L-PD
L-L
SH-PB
SH-CA
SH-M
SH-PD
SH-L
SF-PB
SF-CA
SF-M
SF-PD
SF-L
Sample ID
Total
Intracellular

Figure 4.2.1-2 Microbial Quantification of Chicago to Seattle (4-13-04), Gasper Samples

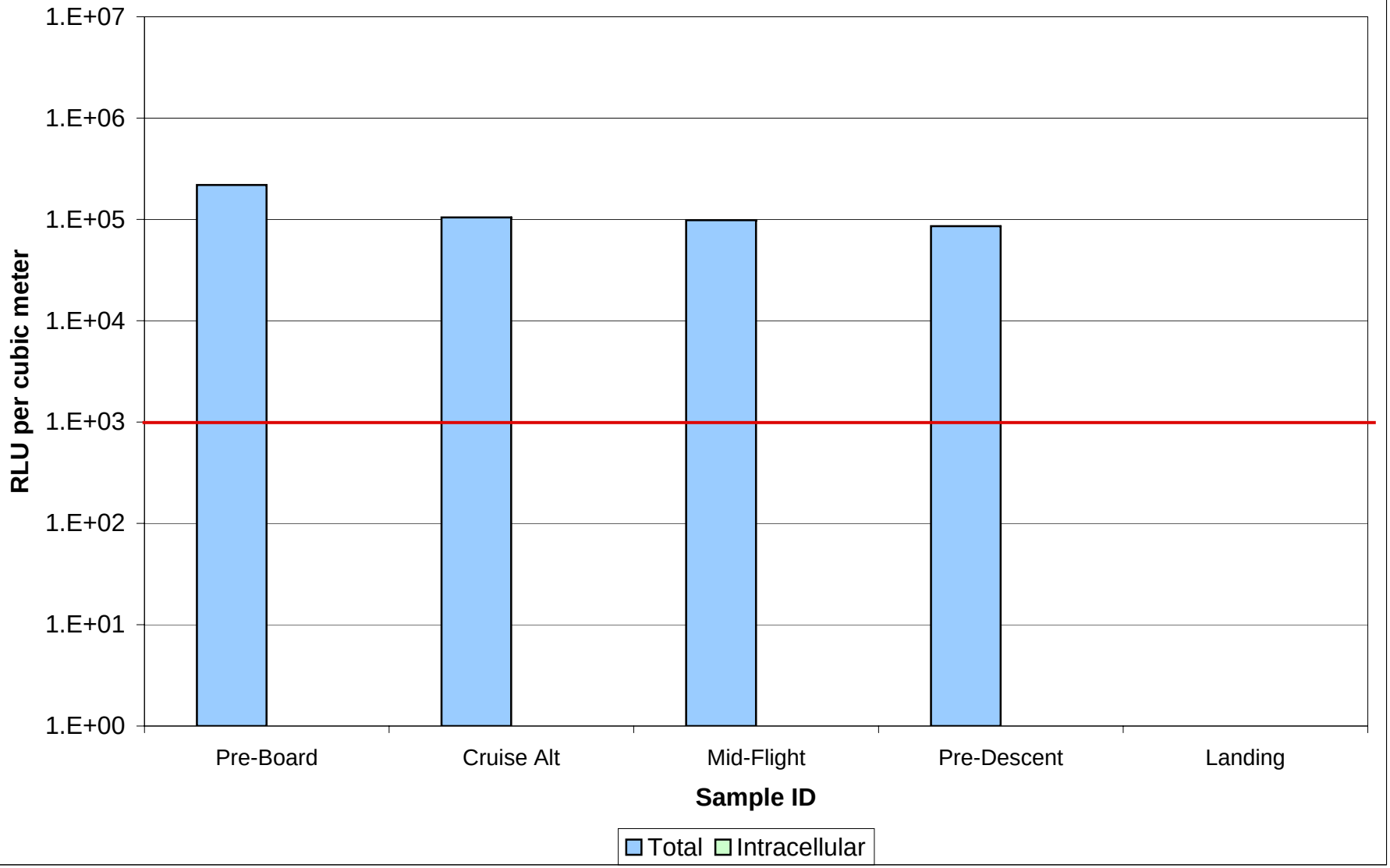
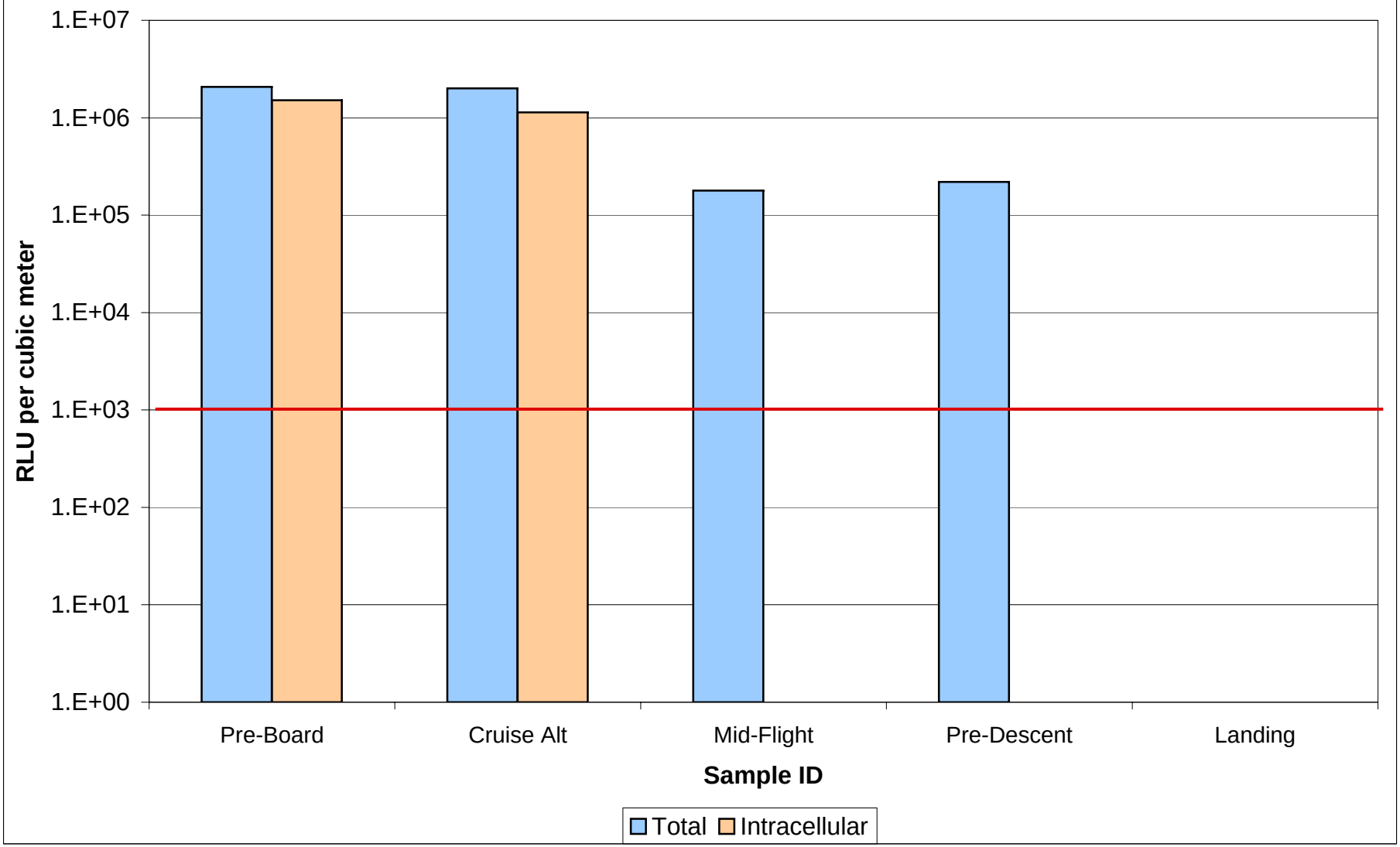


Figure 4.2.1-3 Microbial Quantification of Chicago to Seattle (4-13-04), Lavatory Samples



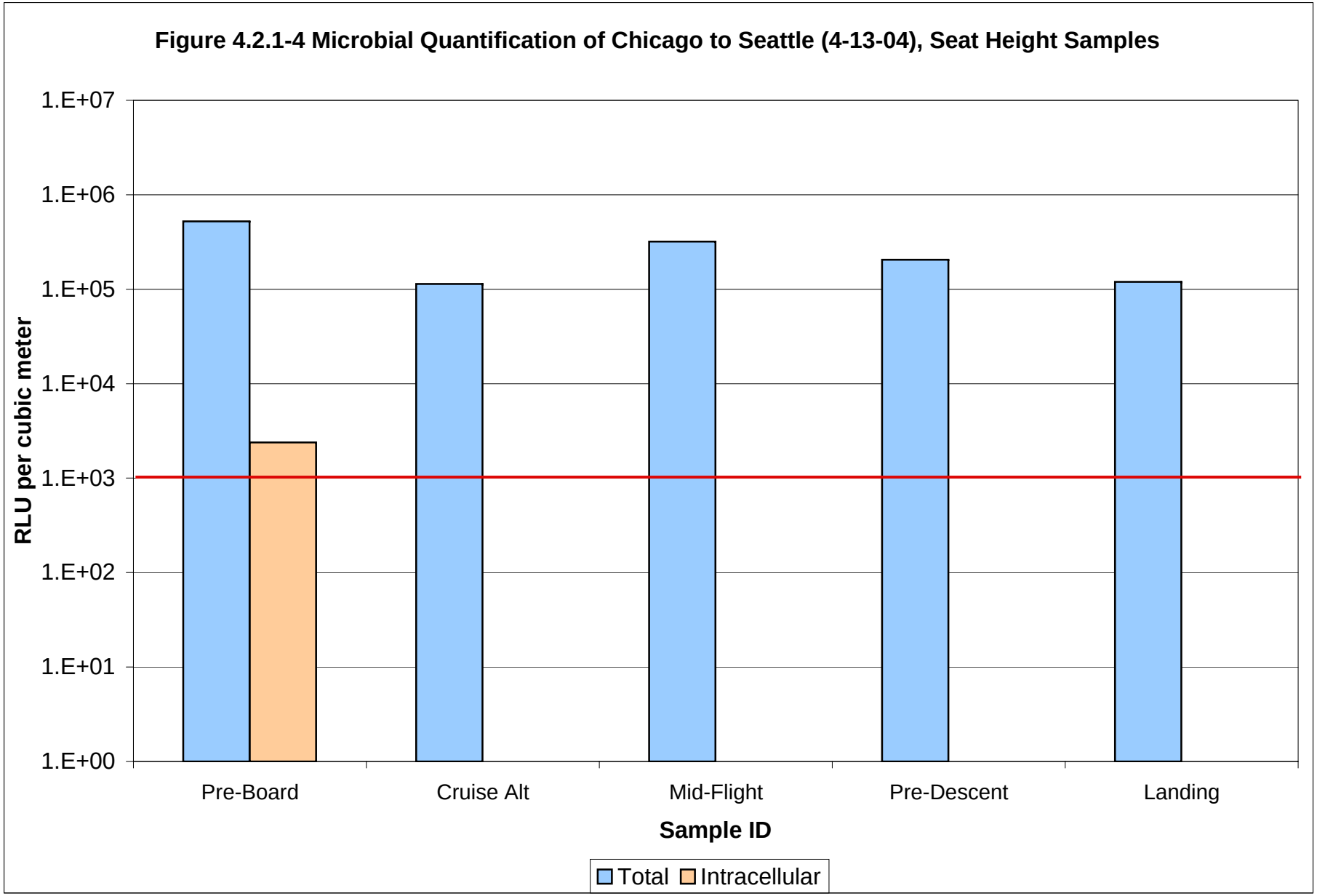


Figure 4.2.1-4 Microbial Quantification of Chicago to Seattle (4-13-04), Seat Height Samples
1.E+07
1.E+06
1.E+05
1.E+04
1.E+03
1.E+02
1.E+01
1.E+00
RLU per cubic meter
Pre-Board
Cruise Alt
Mid-Flight
Pre-Descent
Landing
Sample ID
Total
Intracellular

Figure 4.2.1-5 Microbial Quantification of Chicago to Seattle (4-13-04), Floor Samples

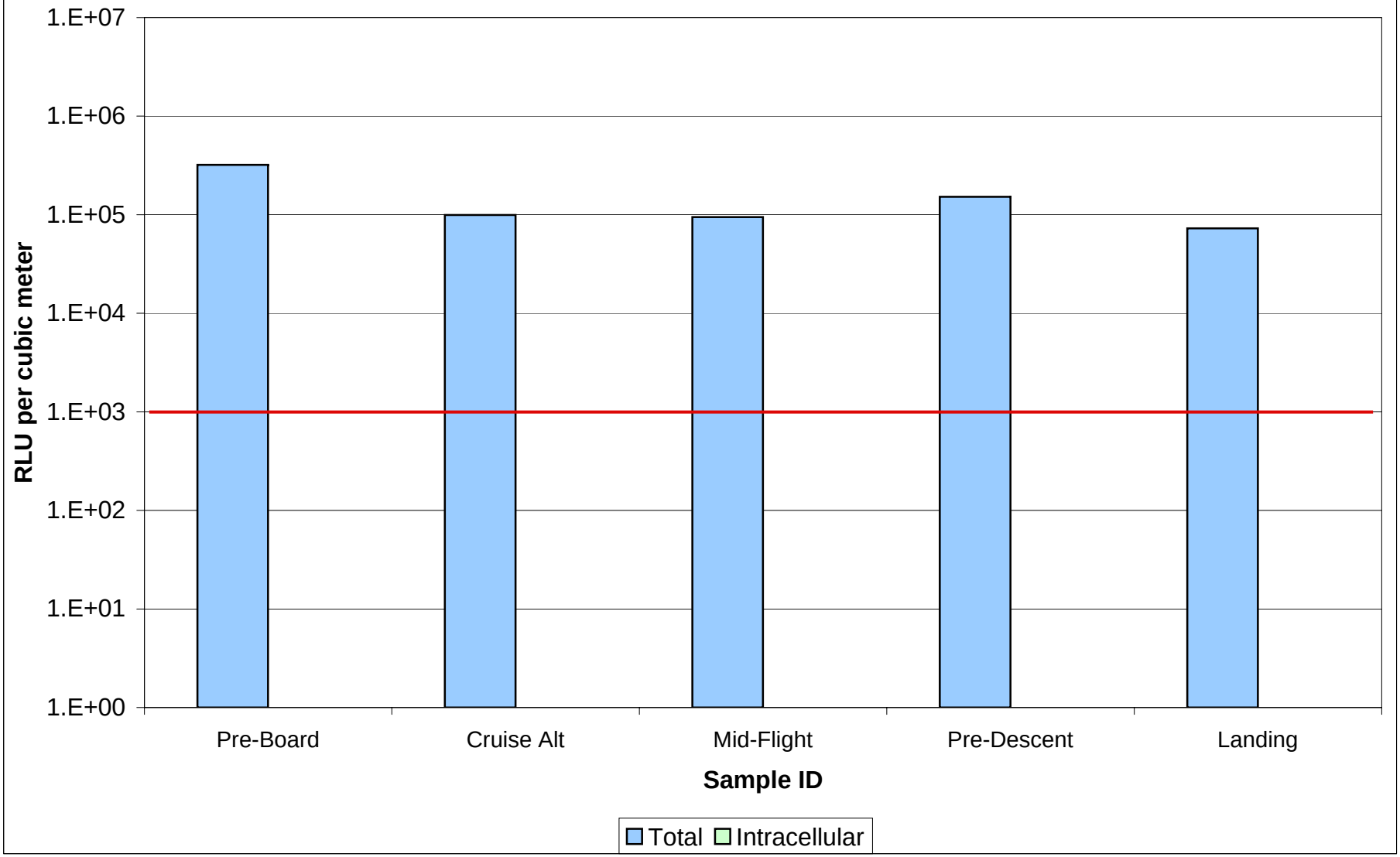


Figure 4.2.1-6 Microbial Quantification of Chicago to Seattle (4-13-04), Pre-Board Samples

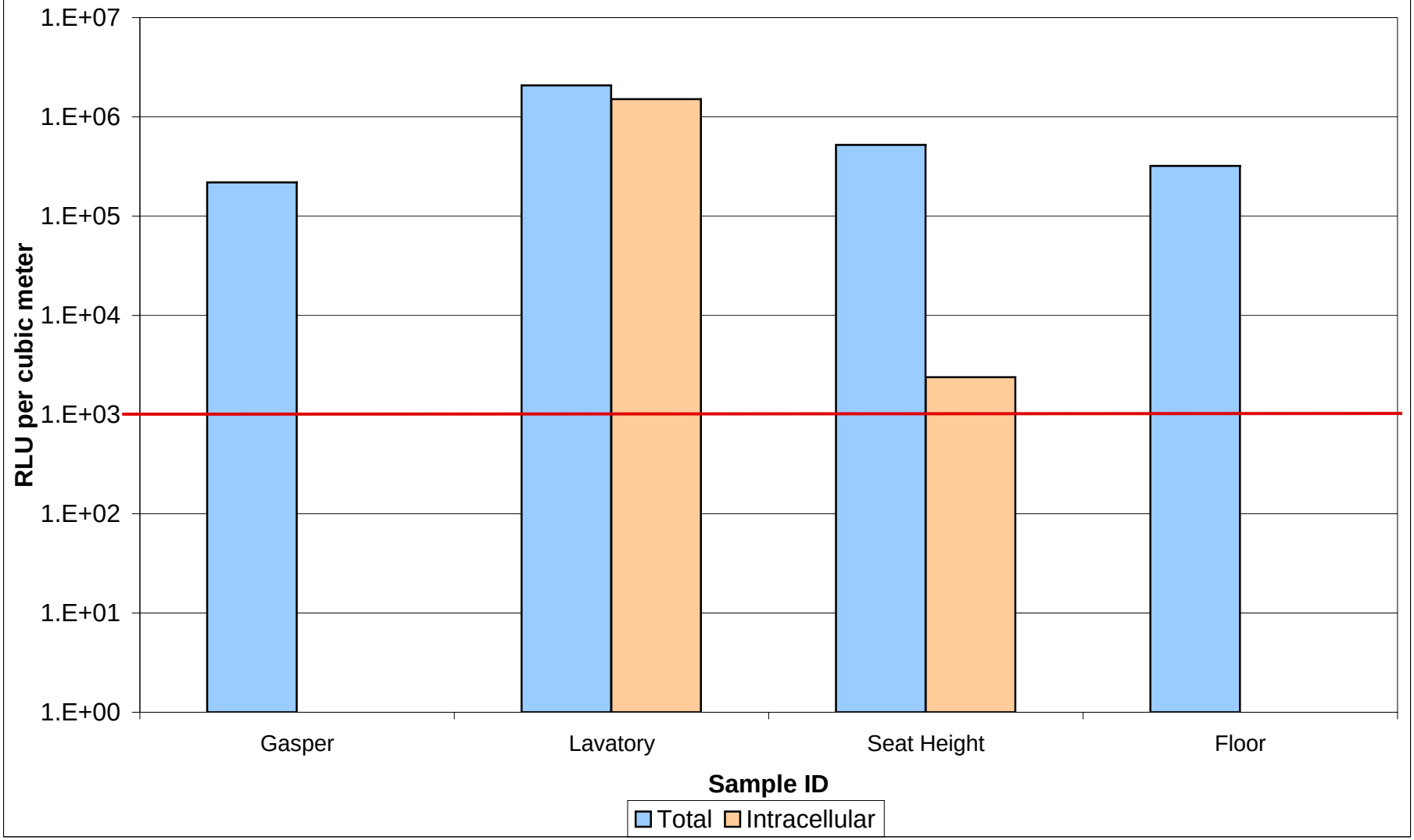


Figure 4.2.1-7 Microbial Quantification of Chicago to Seattle (4-13-04), Cruise Altitude Samples

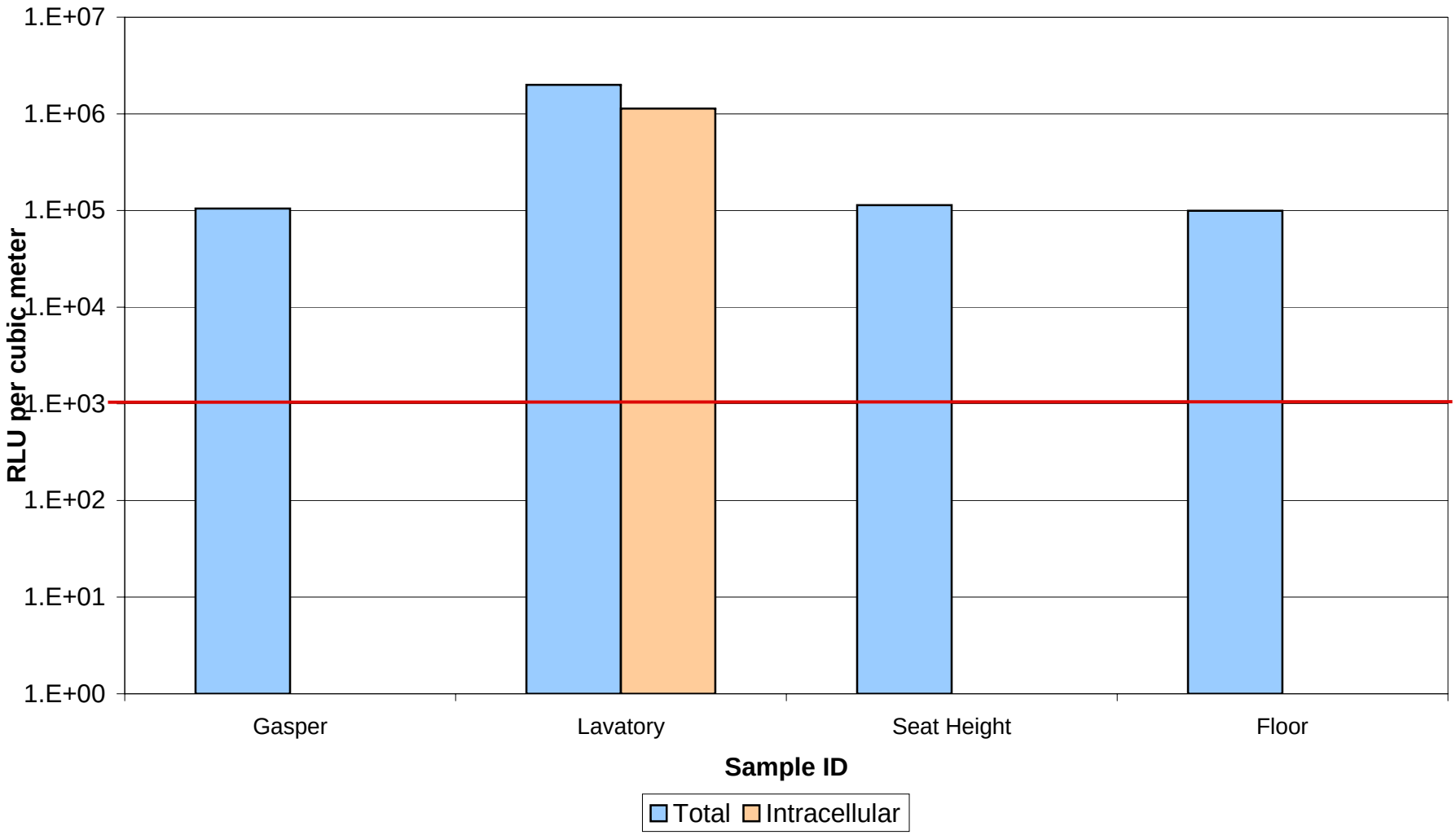


Figure 4.2.1-8 Microbial Quantification of Chicago to Seattle (4-13-04), Mid-Flight Samples

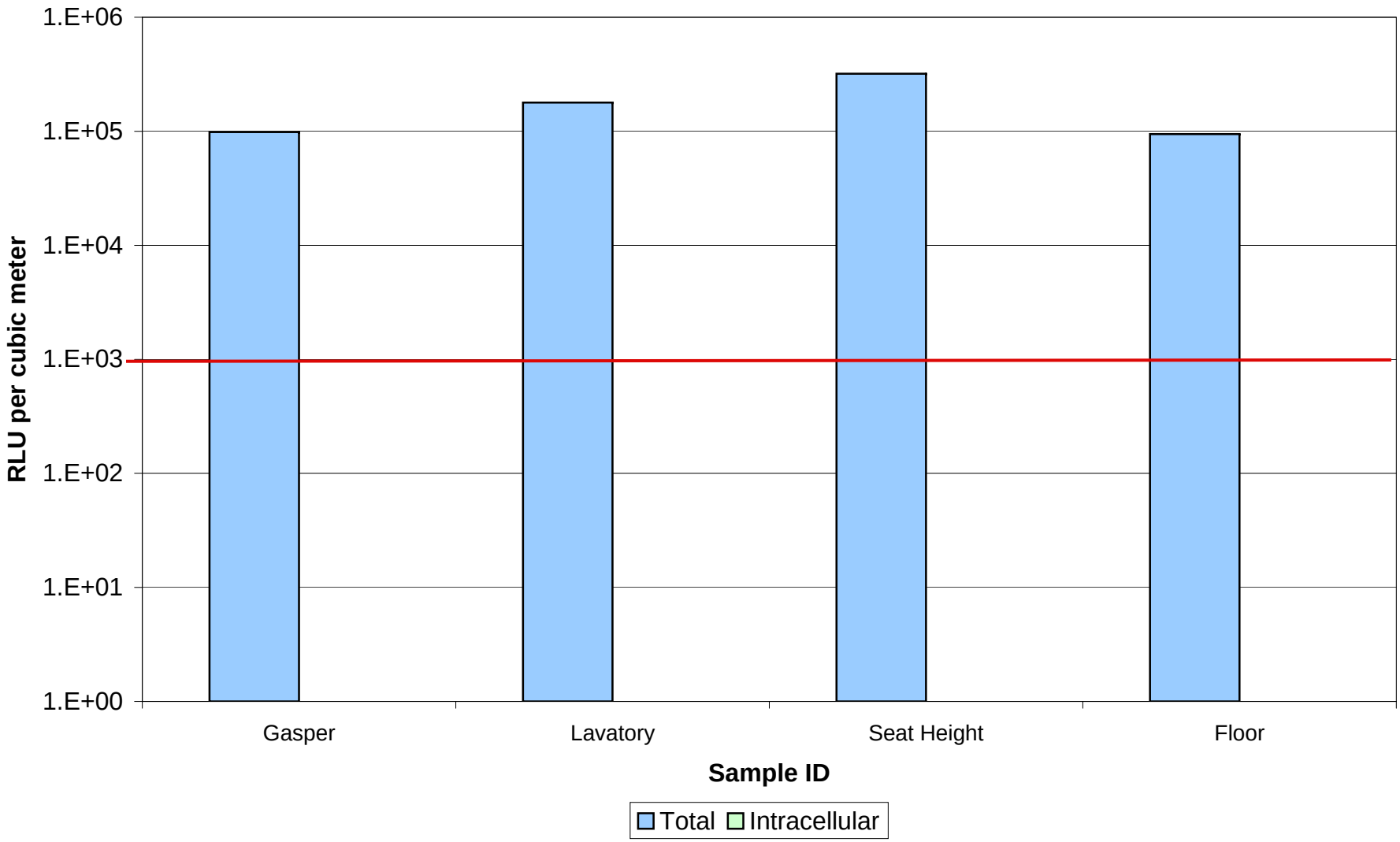


Figure 4.2.1-9 Microbial Quantification of Chicago to Seattle (4-13-04), Pre-Descent Samples

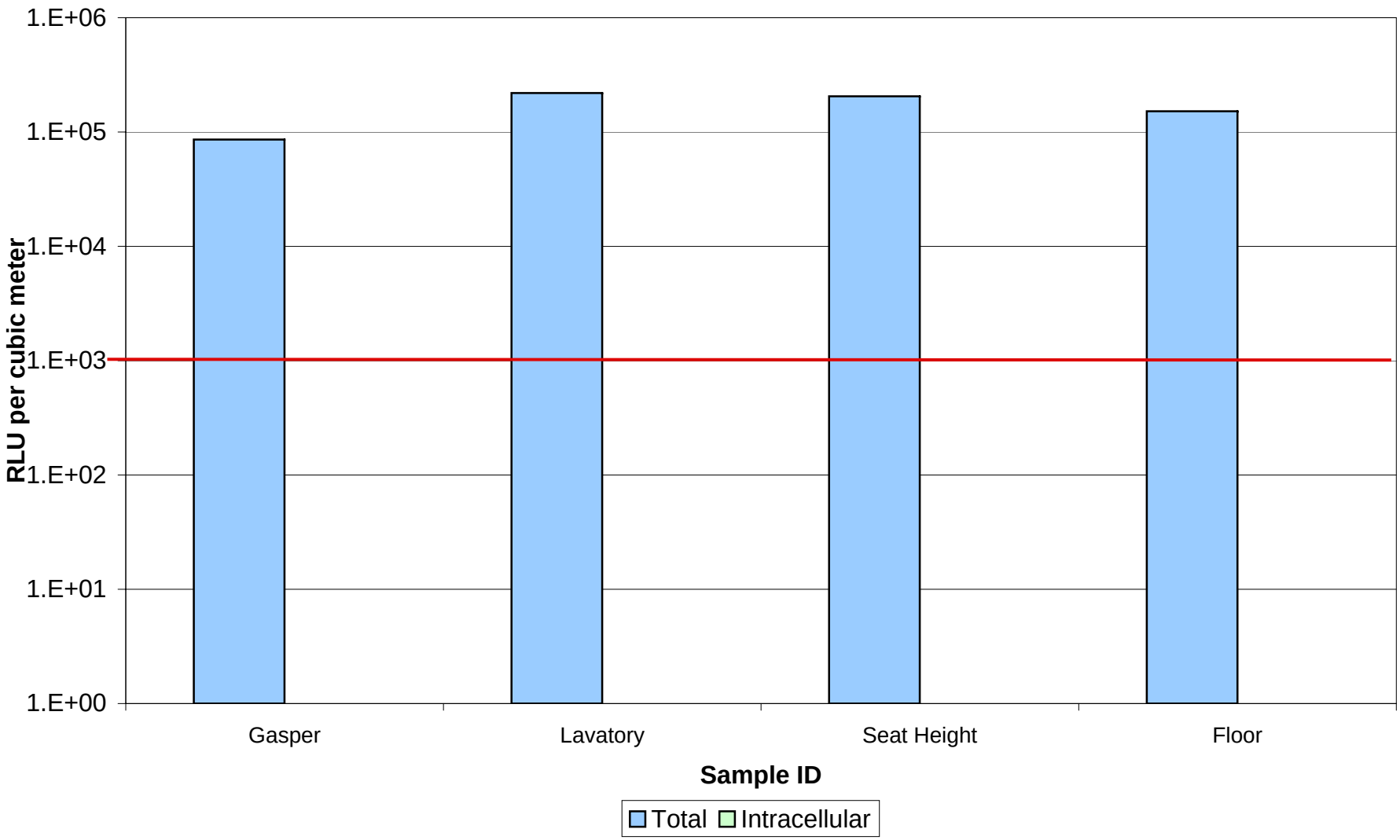


Figure 4.2.1-10 Microbial Quantification of Chicago to Seattle (4-13-04), Landing Samples

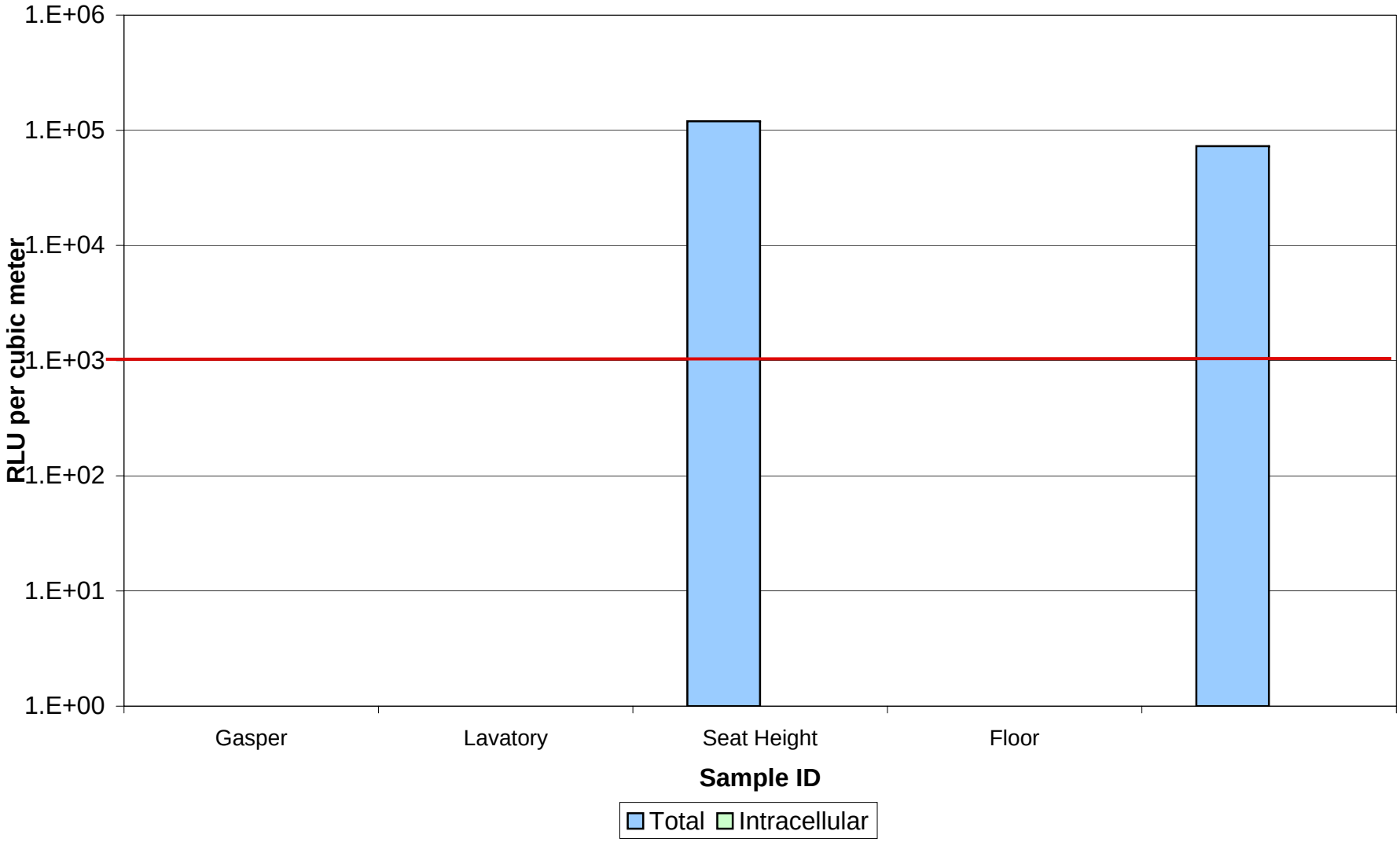


Figure 4.2.1-11 Microbial Quantification of Seattle to Dallas; 4-14-04

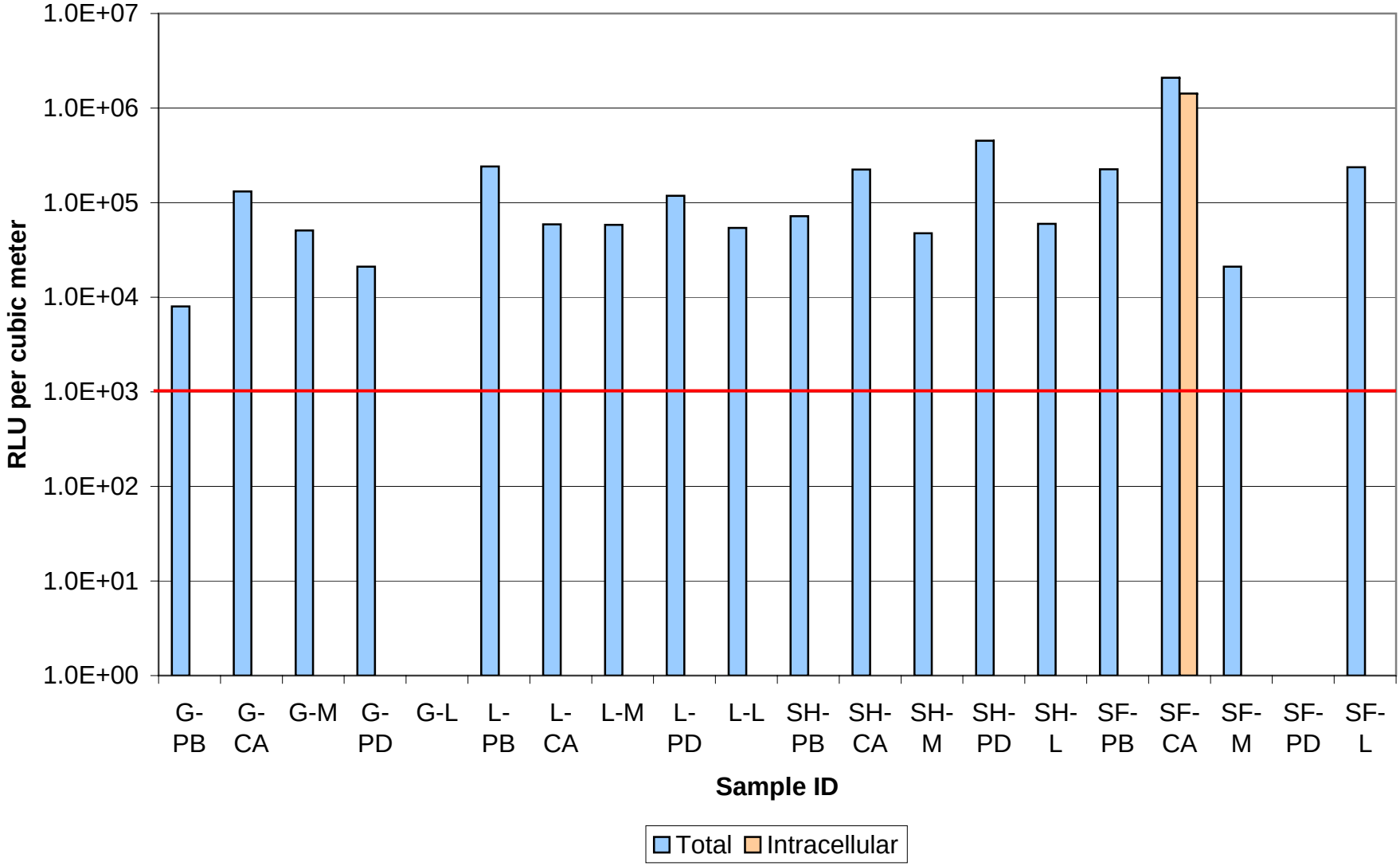


Figure 4.2.1-12 Microbial Quantification of Seattle to Dallas; 4-14-04 Gasper Samples

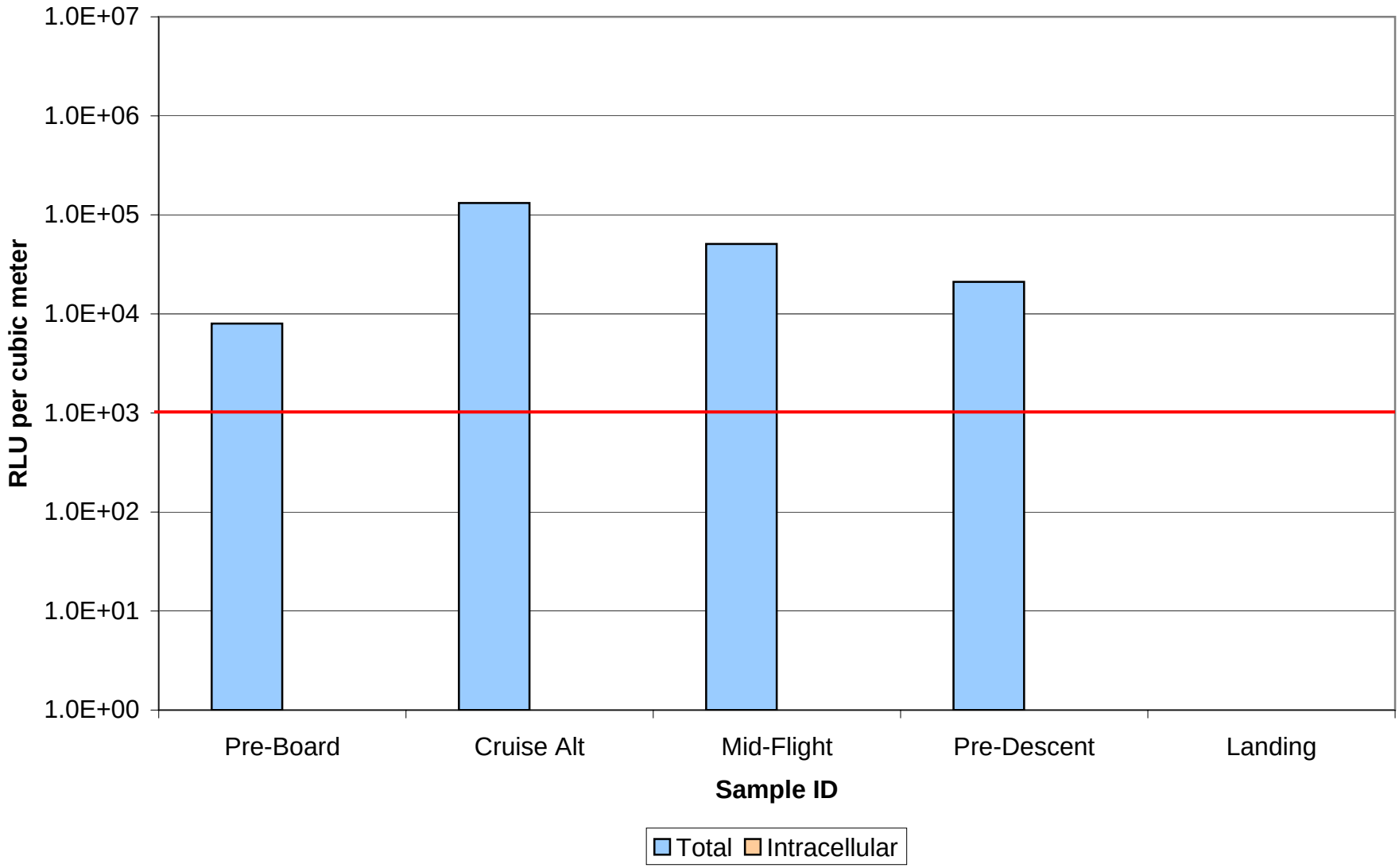


Figure 4.2.1-13 Microbial Quantification of Seattle to Dallas; 4-14-04 Lavatory Samples

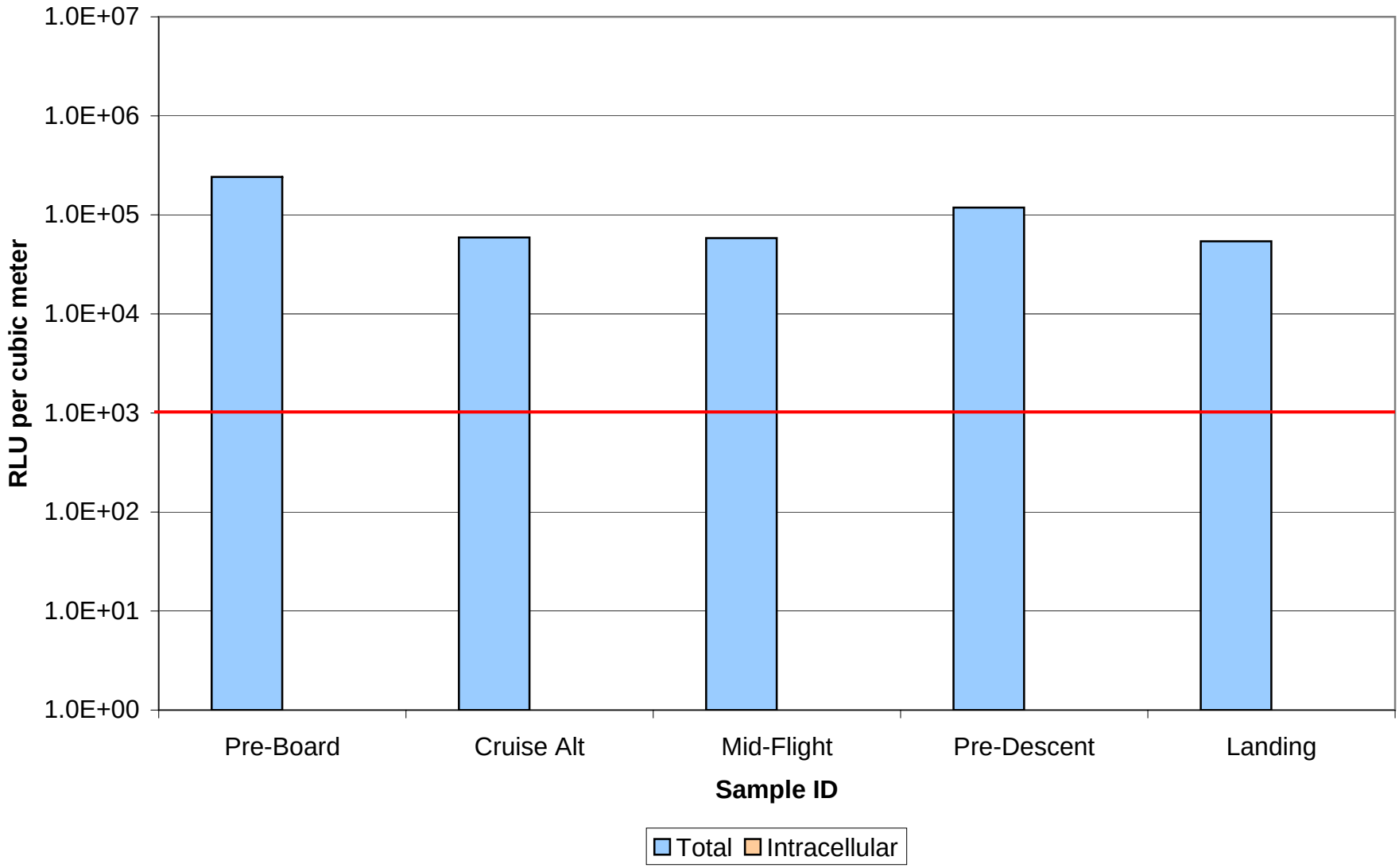


Figure 4.2.1-14 Microbial Quantification of Seattle to Dallas; 4-14-04 Seat Height Samples

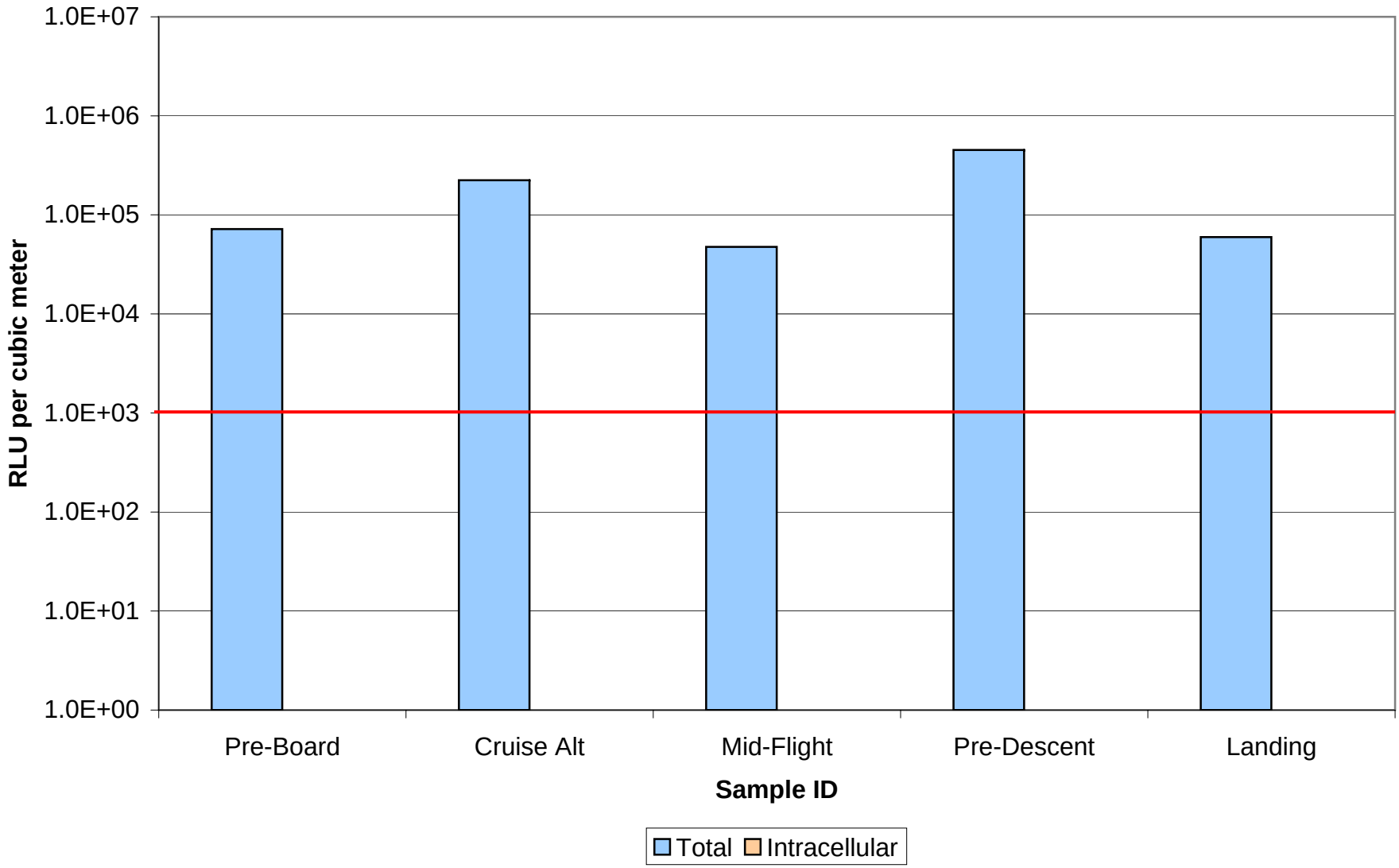


Figure 4.2.1-15 Microbial Quantification of Seattle to Dallas; 4-14-04 Floor Samples

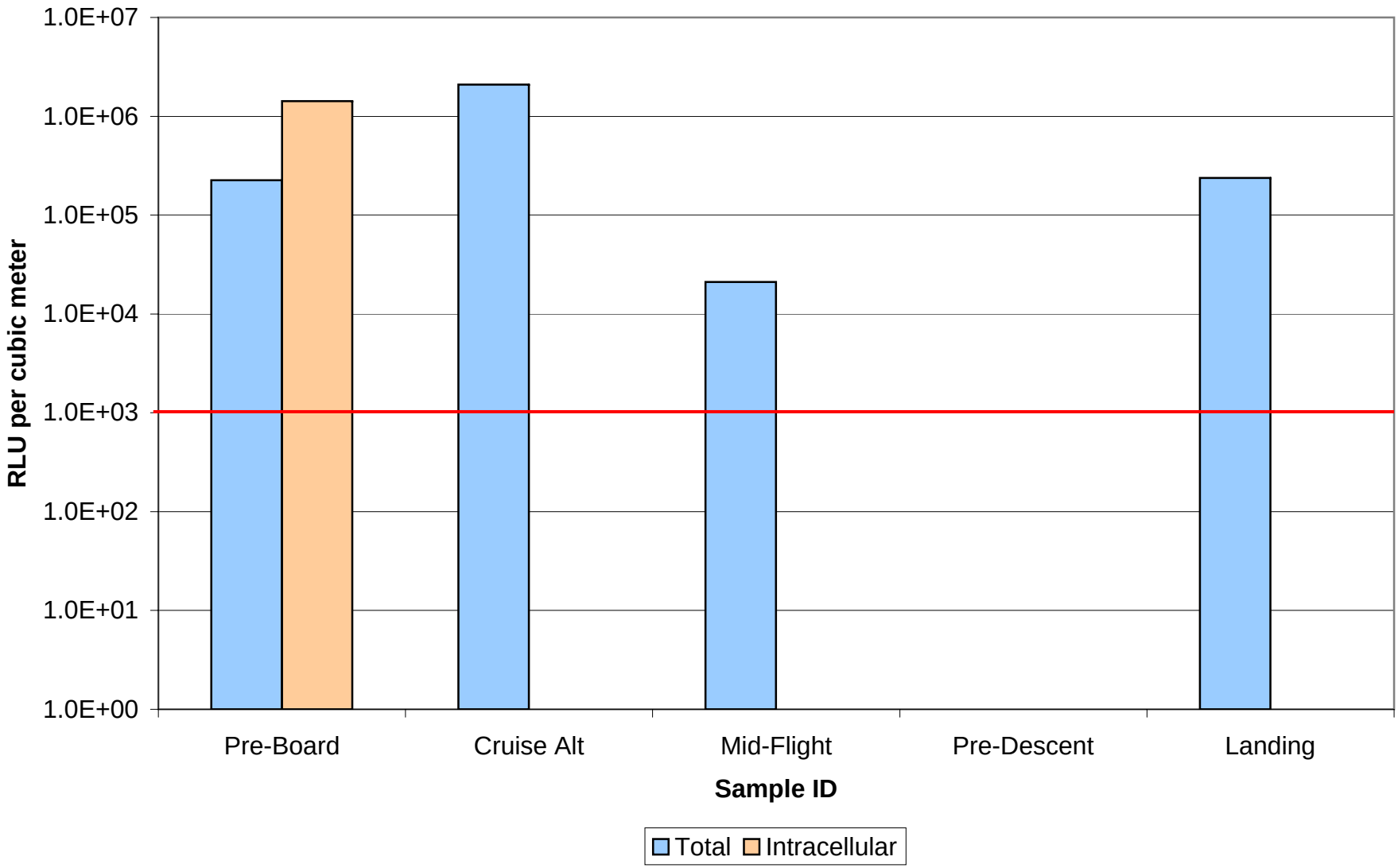


Figure 4.2.1-16 Microbial Quantification of Seattle to Dallas; 4-14-04 Pre-Board Samples

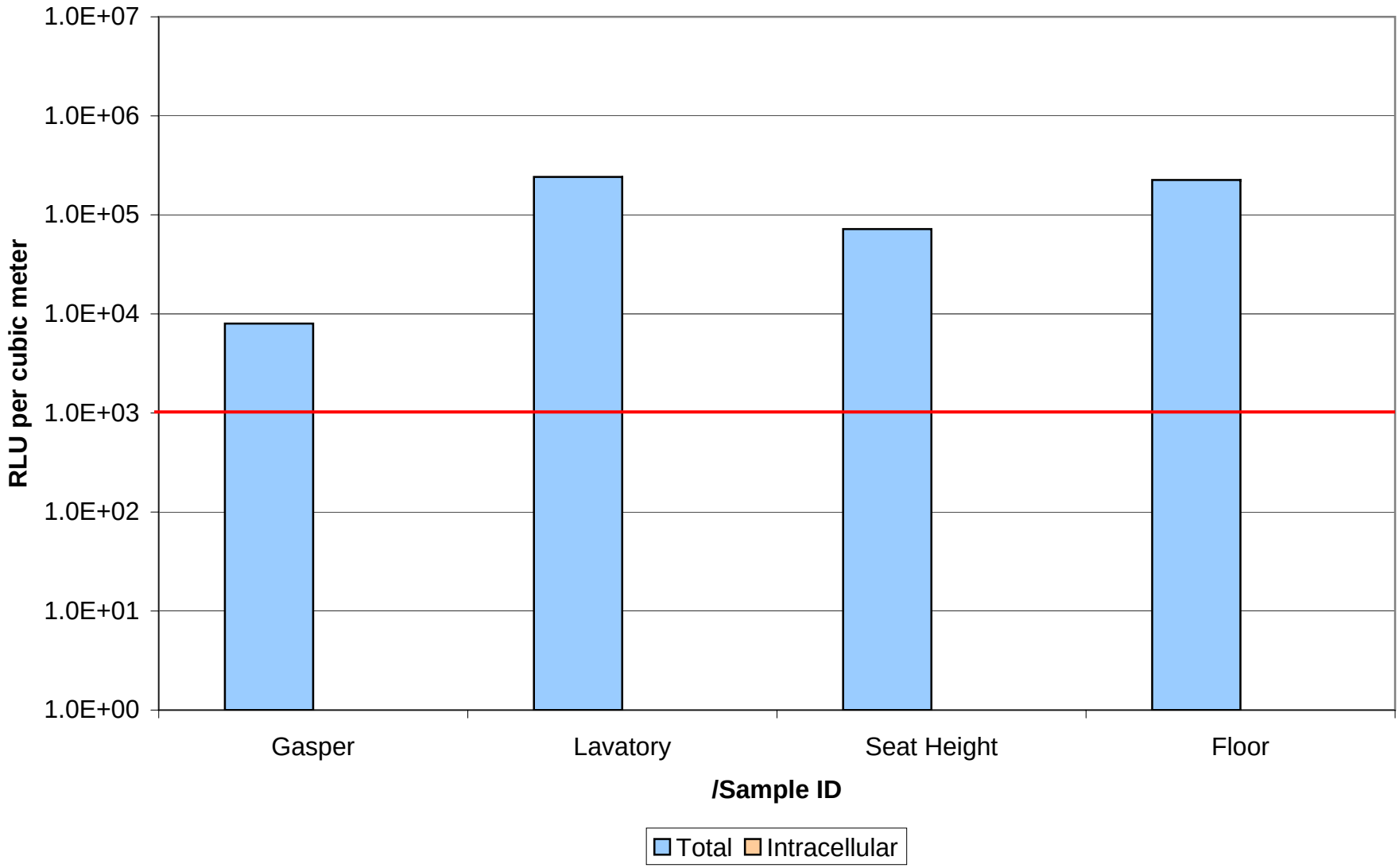


Figure 4.2.1-17 Microbial Quantification of Seattle to Dallas; 4-14-04 Cruise Altitude Samples

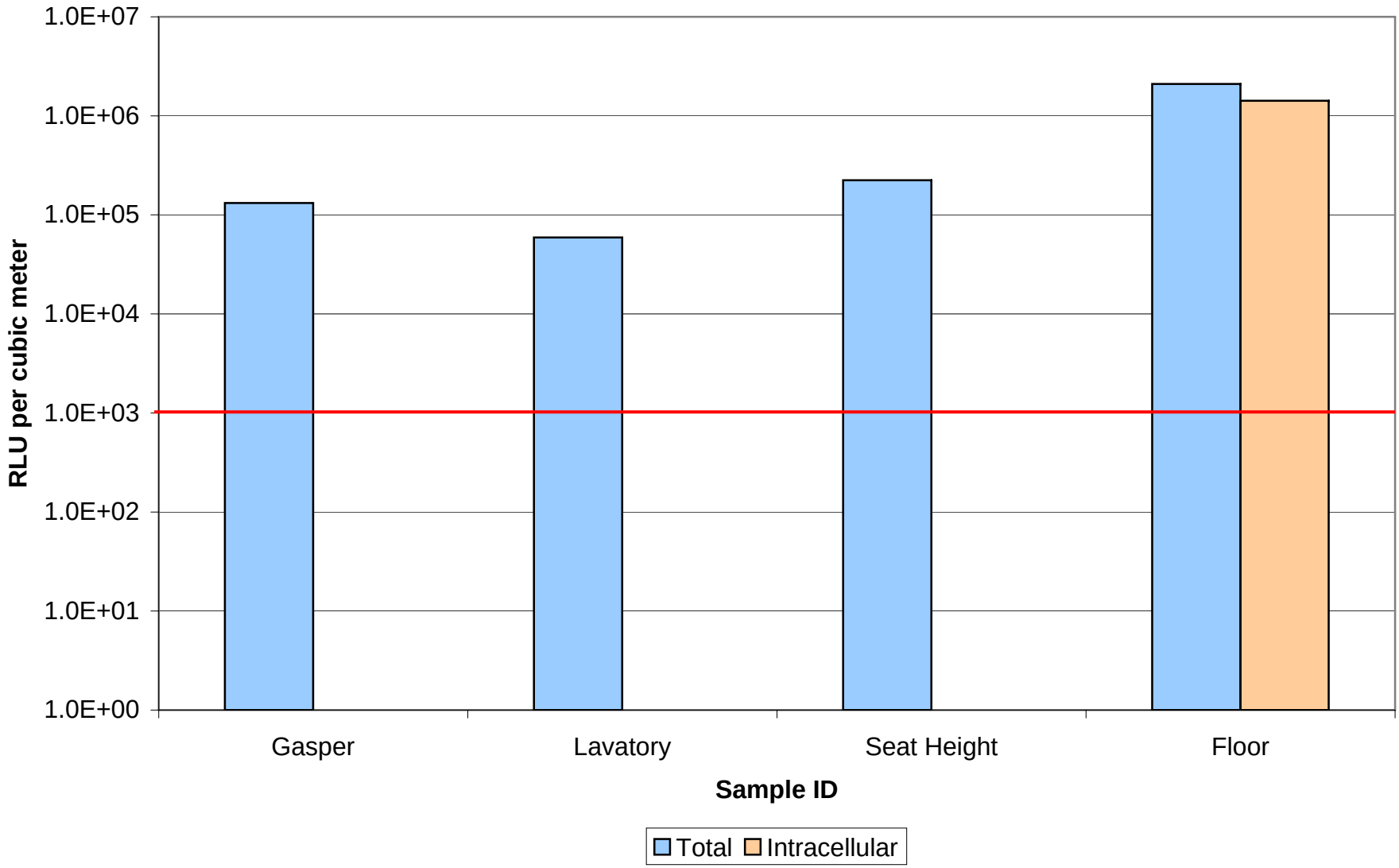


Figure 4.2.1-18 Microbial Quantification of Seattle to Dallas; 4-14-04 Mid-Flight Samples

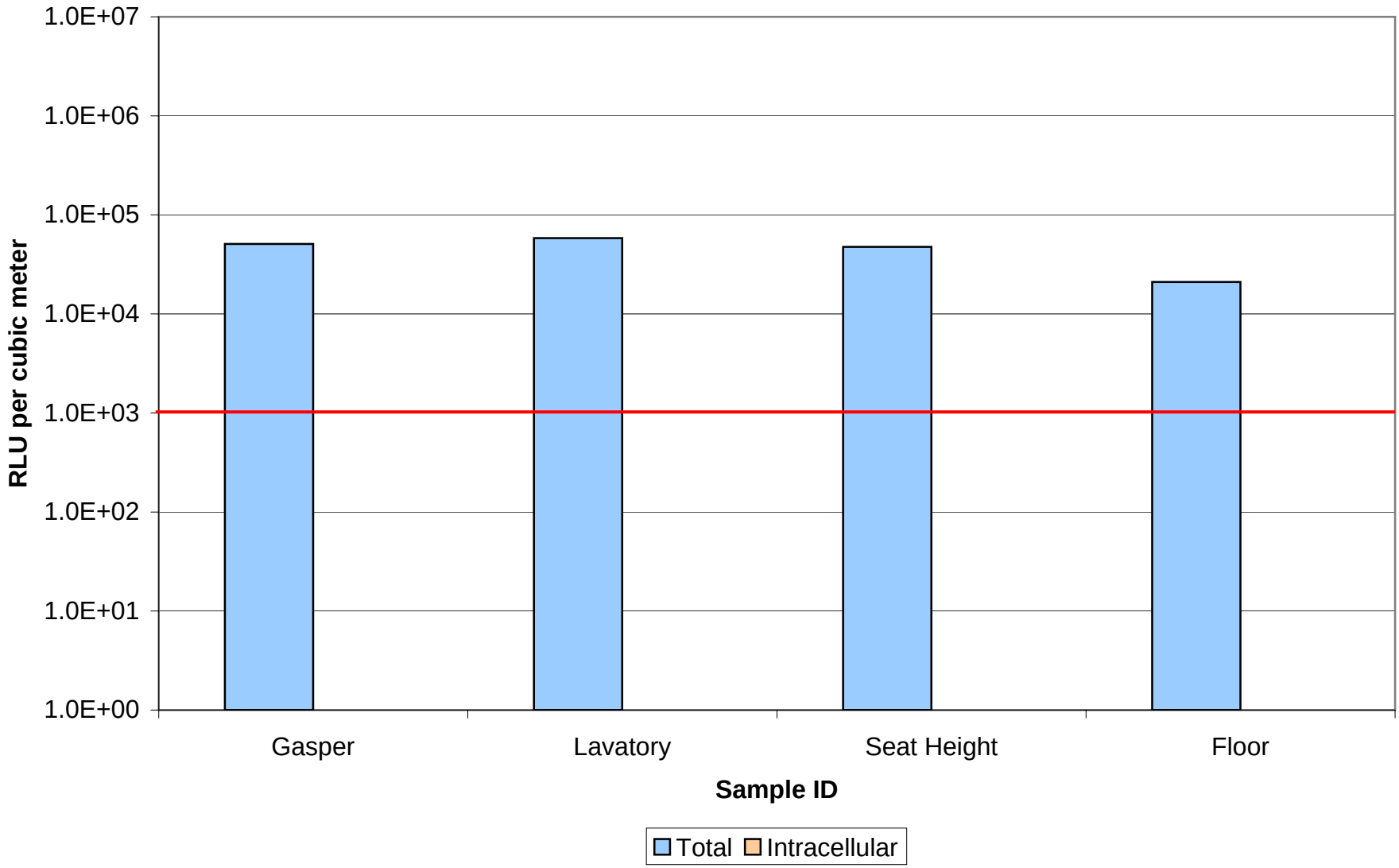


Figure 4.2.1-19 Microbial Quantification of Seattle to Dallas; 4-14-04 Pre-Descent Samples

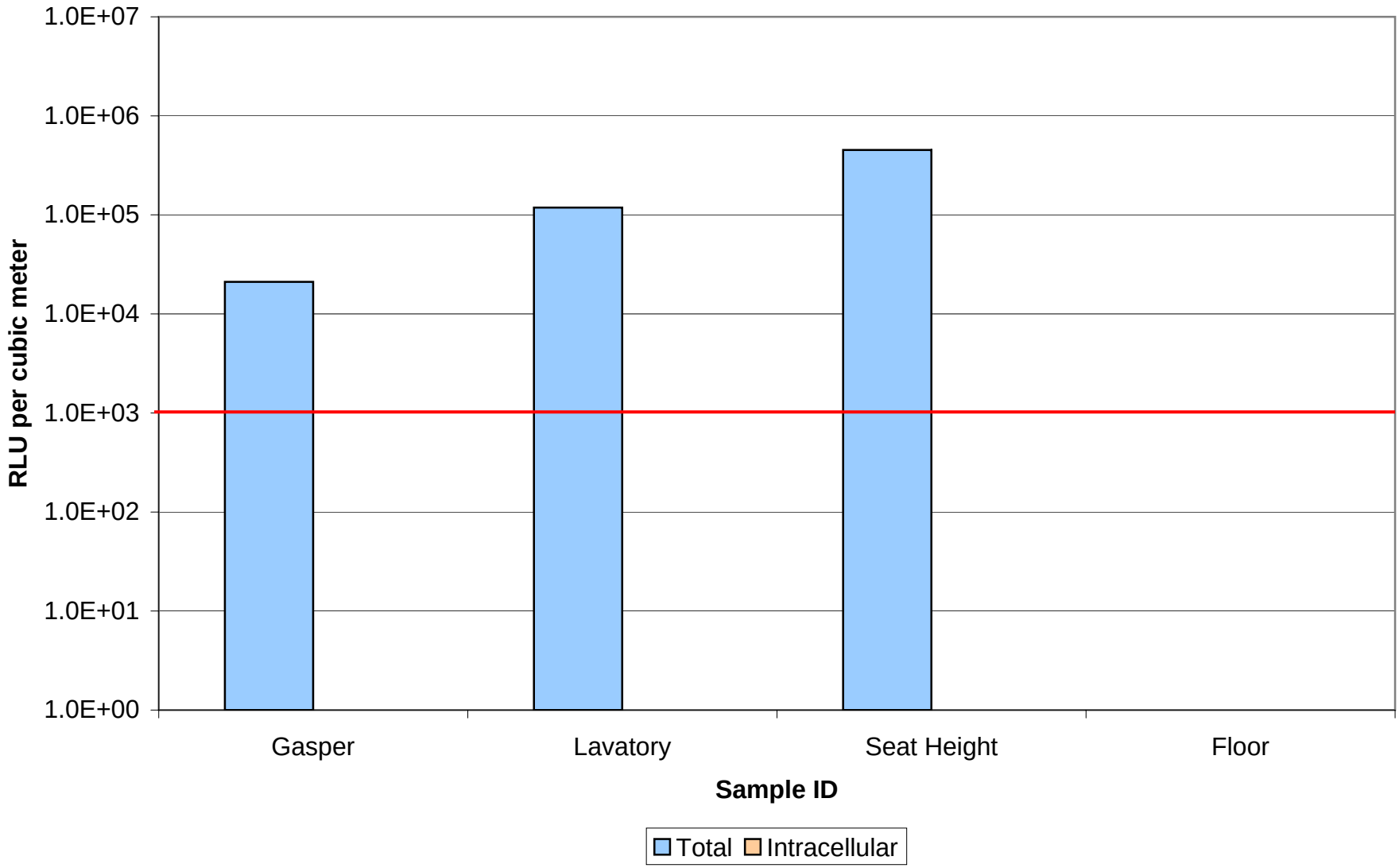


Figure 4.2.1-20 Microbial Quantification of Seattle to Dallas; 4-14-04 Landing Samples

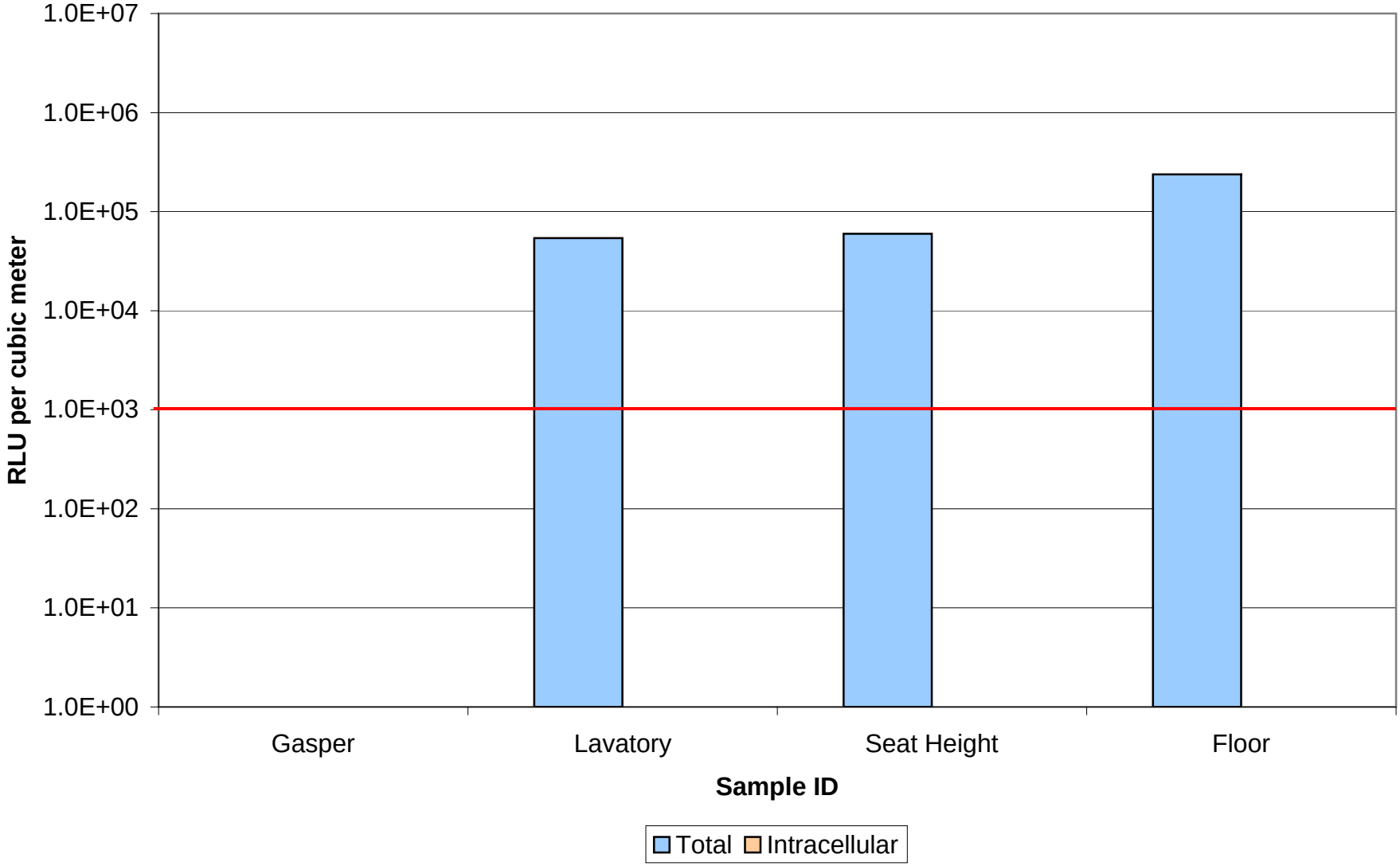


Figure 4.2.1-21 Microbial Quantification of Salt Lake City to Cincinnati; 4-21-04

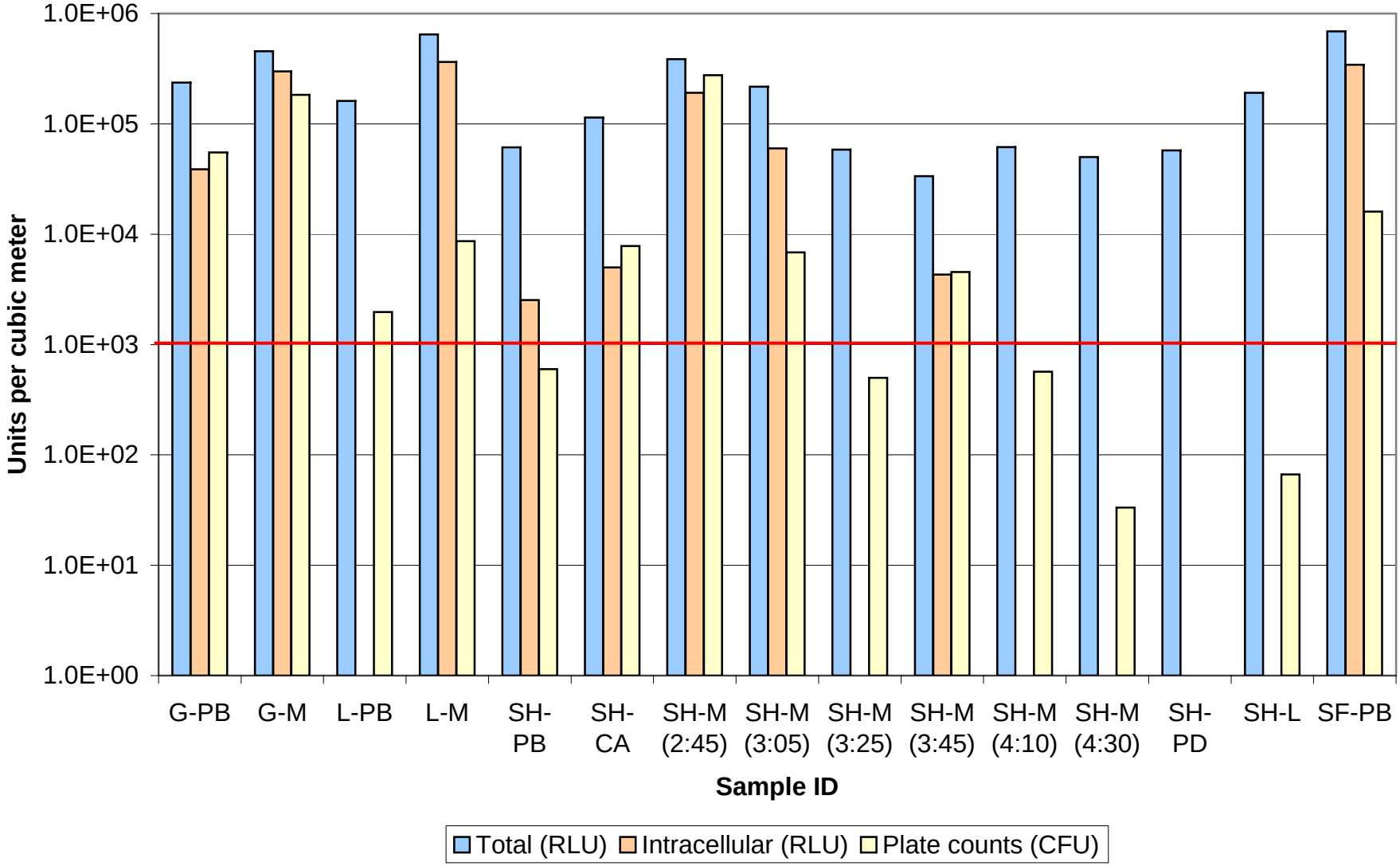


Figure 4.2.1-21a. Changes in microbial burden of cabin air collected at seat height of commercial airline B; flight #3 for the Salt Lake City to Cincinnati segment. Air samples were collected at about 20 to 25 minutes of interval for a period of 3h 40 minutes flight duration at the seat height. Systematic air sampling was performed during Pre-Board (0:00), upon reaching cruise altitude (0:45), half-way through the flight (1:10 to 2:55), just before beginning to descent (3:15), and after landing (3:40). RLU (solid bars): Relative luminescence unit; CFU (crosshatch bars): colony forming unit.

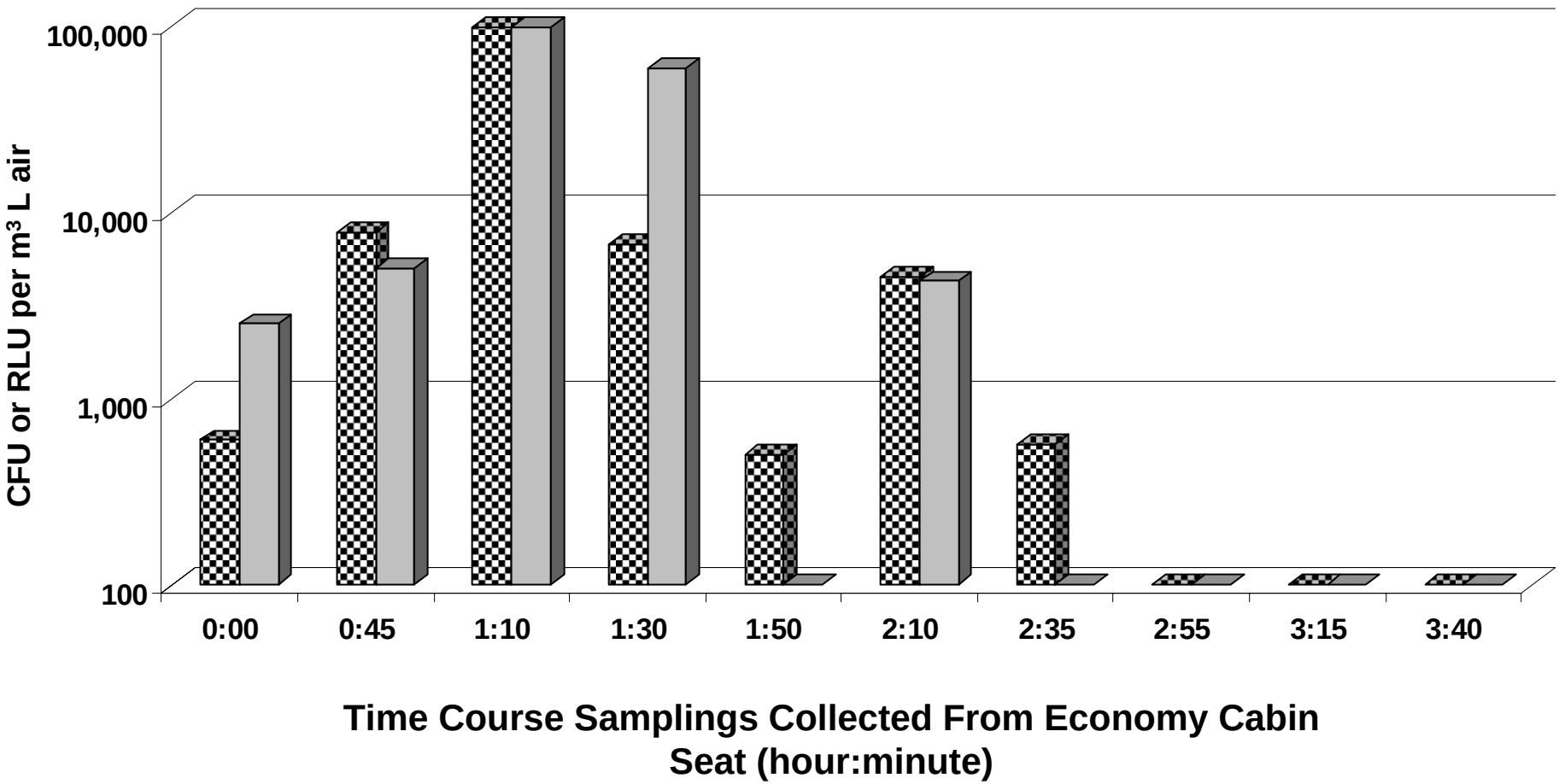


Figure 4.2.1-22 Microbial Quantification of Salt Lake City to Cincinnati; 4-21-04 Gasper Samples

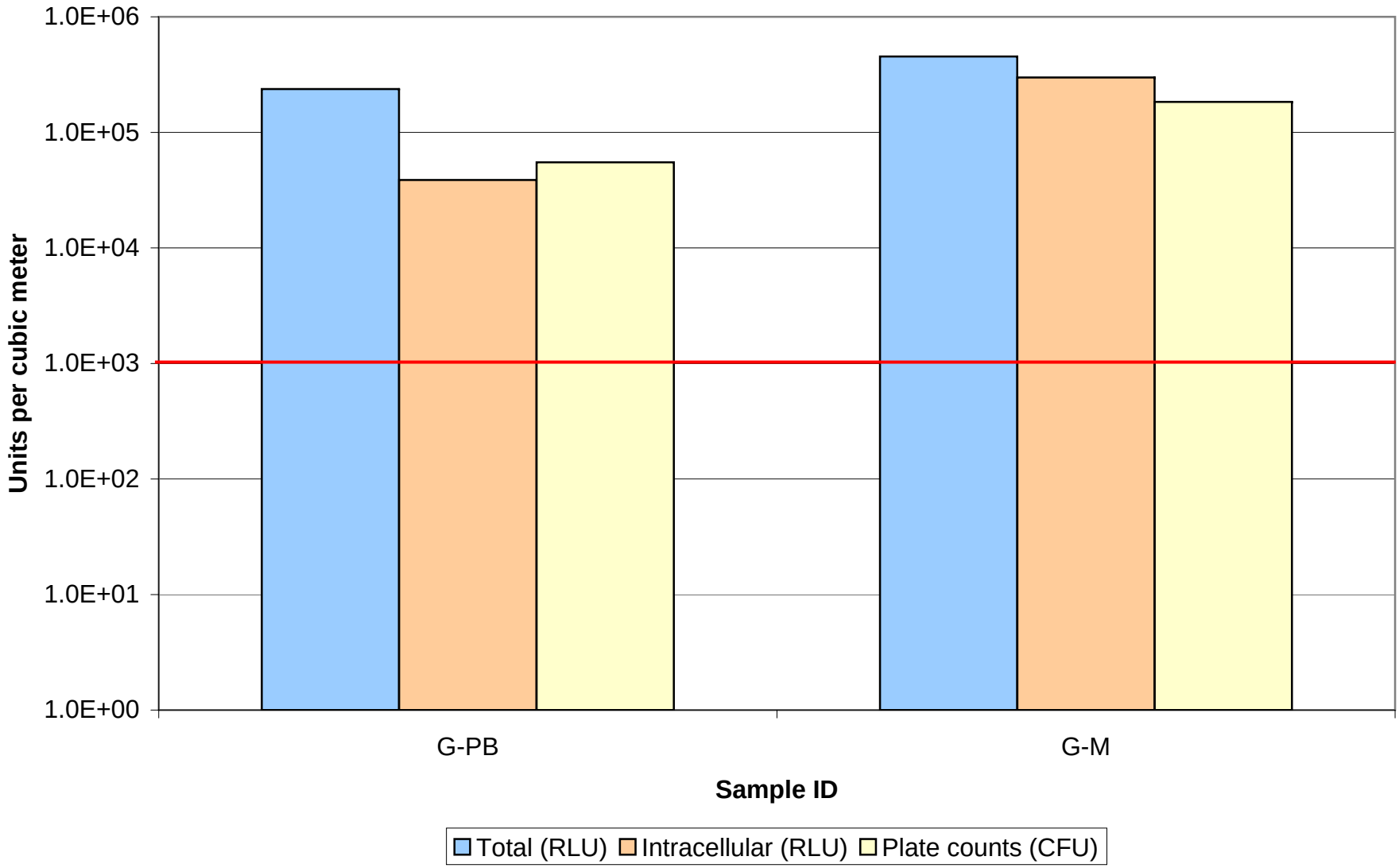


Figure 4.2.1-23 Microbial Quantification of Salt Lake City to Cincinnati; 4-21-04 Lavatory Samples

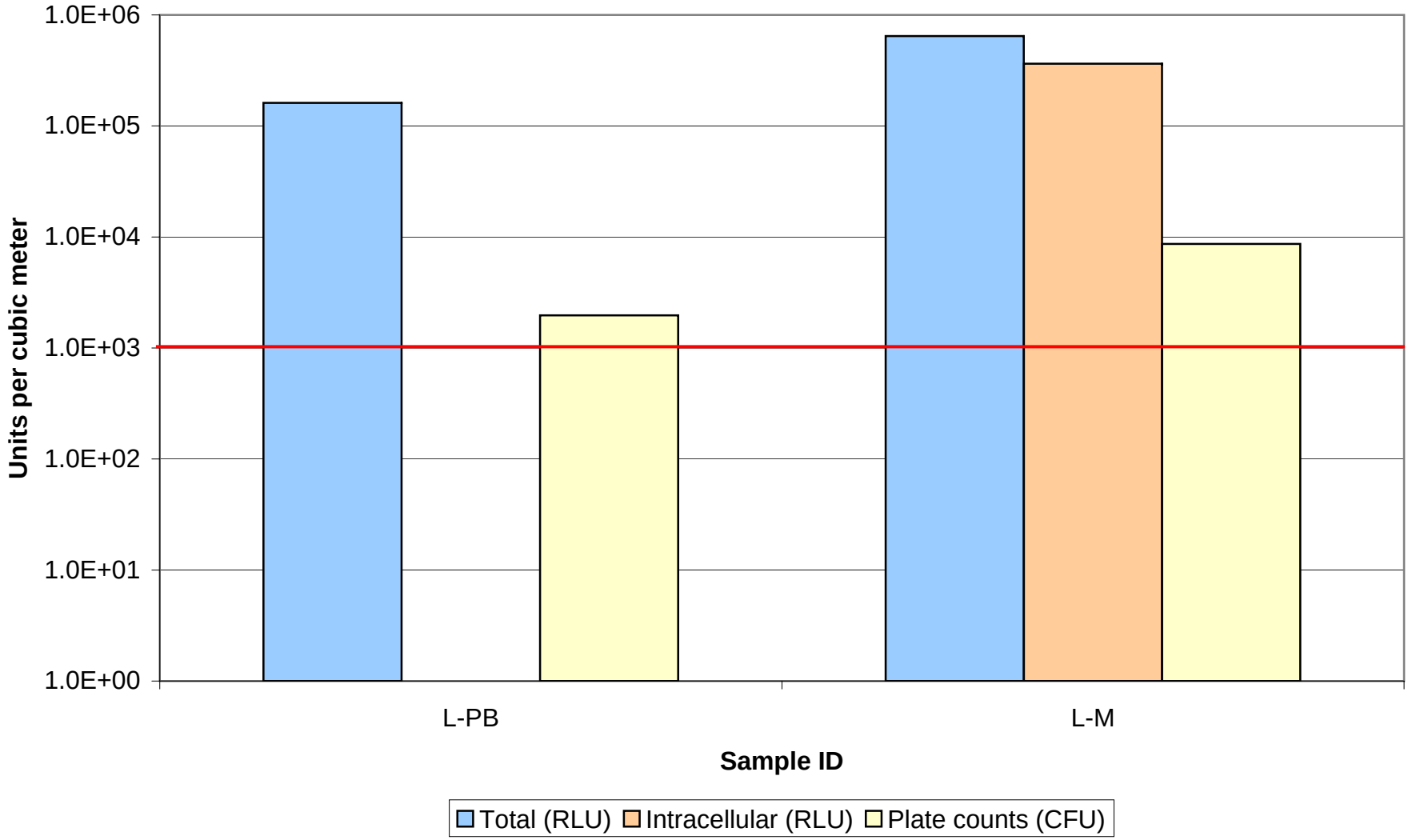


Figure 4.2.1-24 Microbial Quantification of Salt Lake City to Cincinnati; 4-21-04 Seat Height Samples

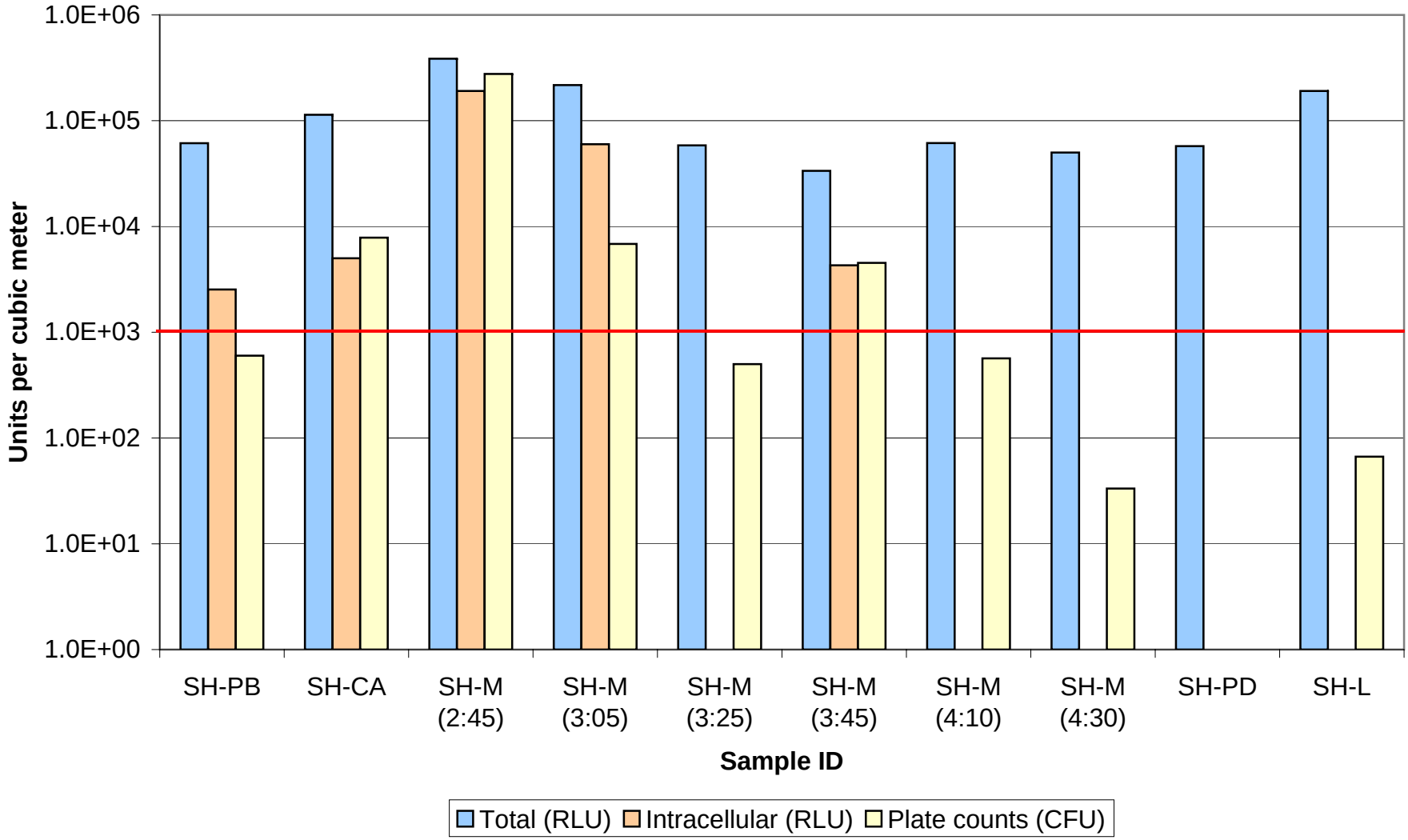


Figure 4.2.1-25 Microbial Quantification of Salt Lake City to Cincinnati; 4-21-04 Floor Samples

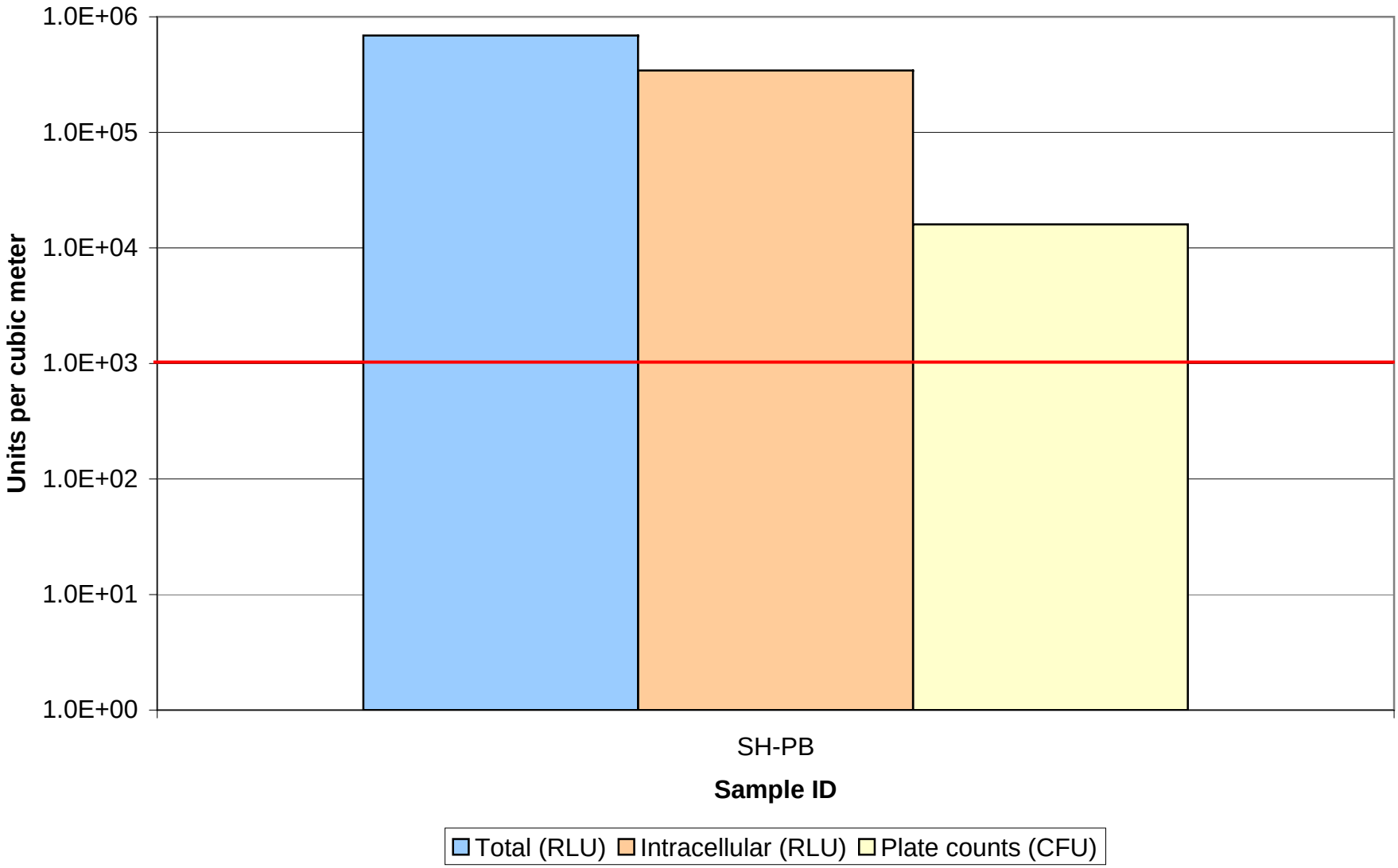


Figure 4.2.1-26 Microbial Quantification of Salt Lake City to Cincinnati; 4-21-04 Pre-board Samples

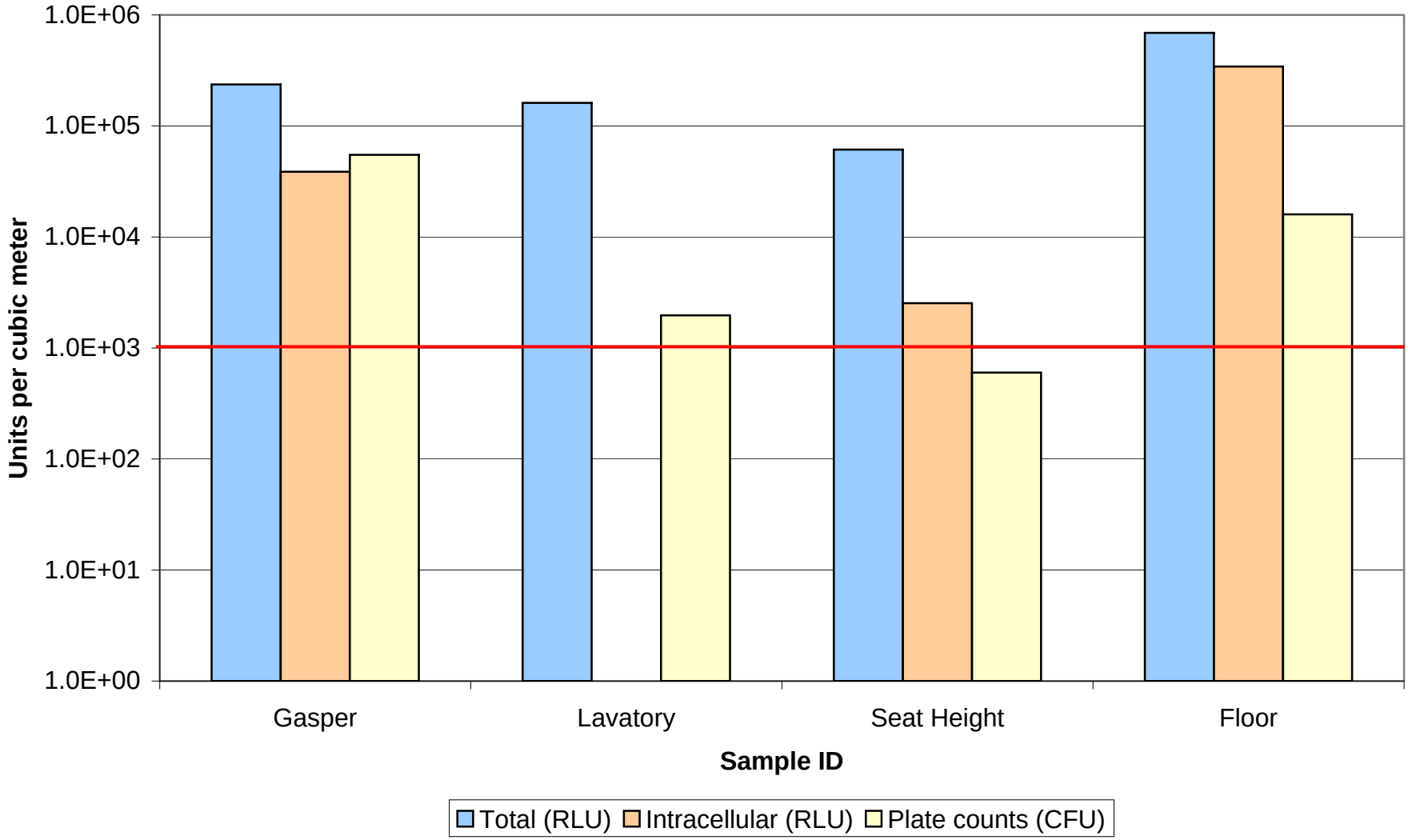


Figure 4.2.1-27 Microbial Quantification of Salt Lake City to Cincinnati; 4-21-04 Cruise Altitude Samples

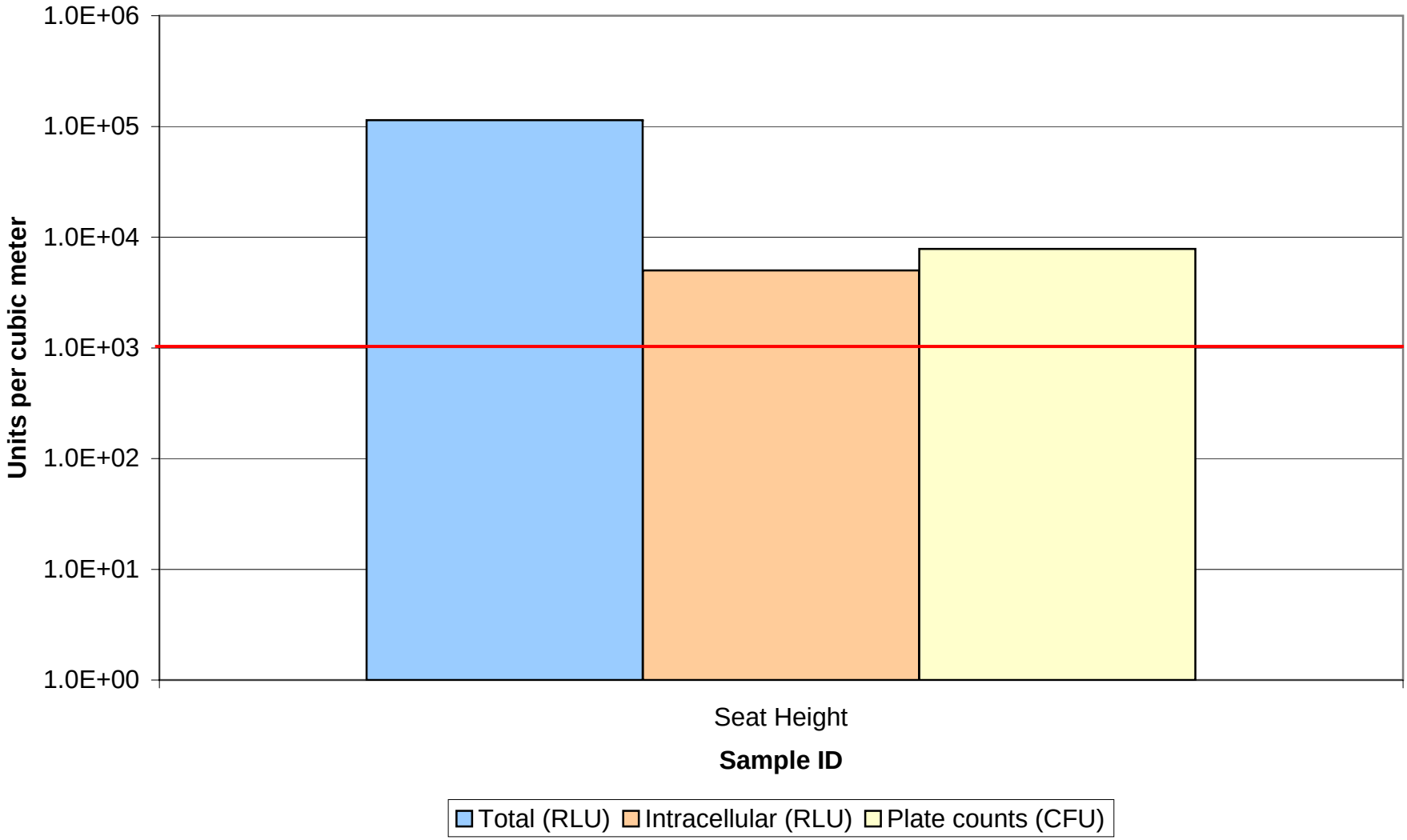


Figure 4.2.1-28 Microbial Quantification of Salt Lake City to Cincinnati; 4-21-04 Mid-Flight Samples

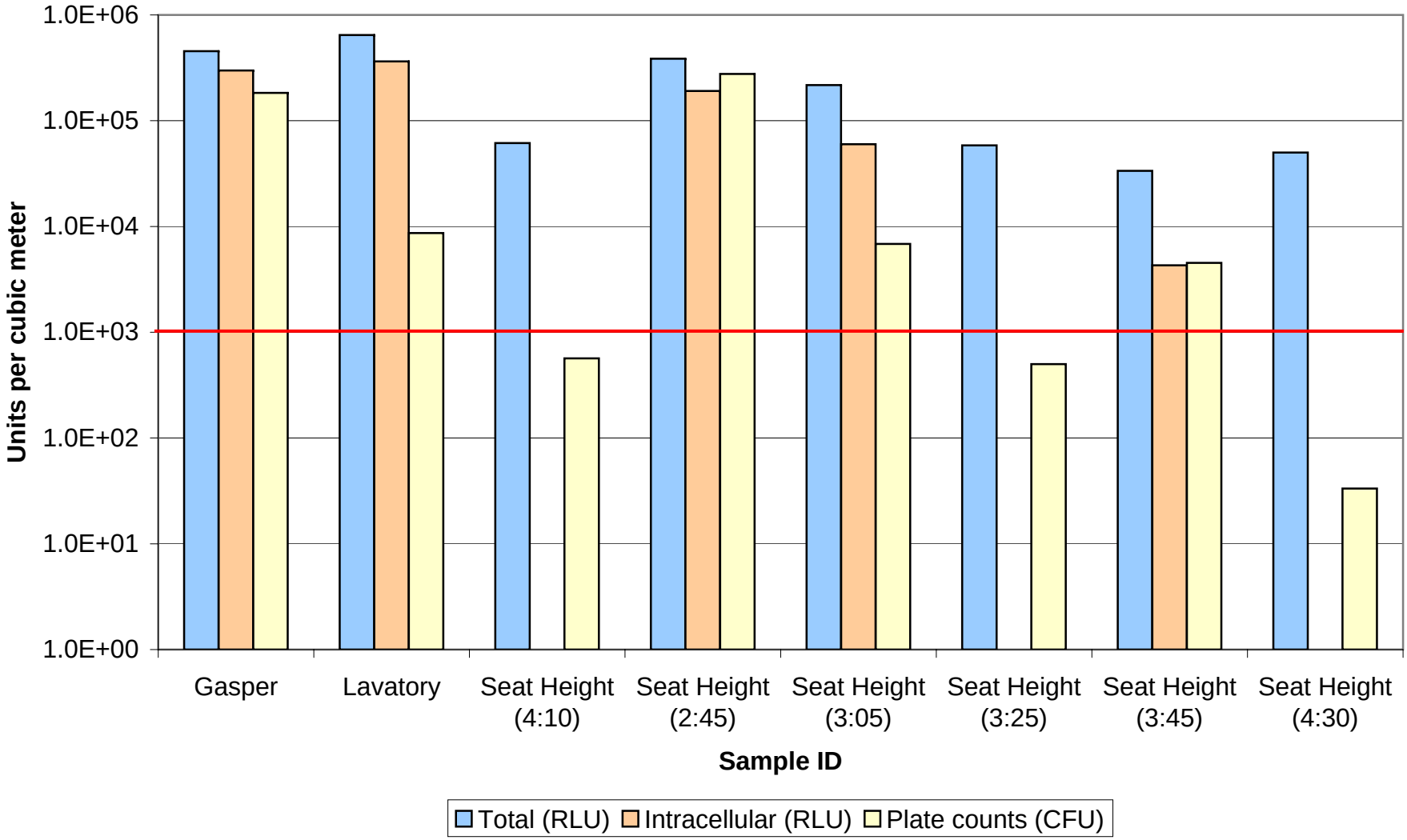


Figure 4.2.1-29 Microbial Quantification of Salt Lake City to Cincinnati; 4-21-04 Pre-Descent Samples

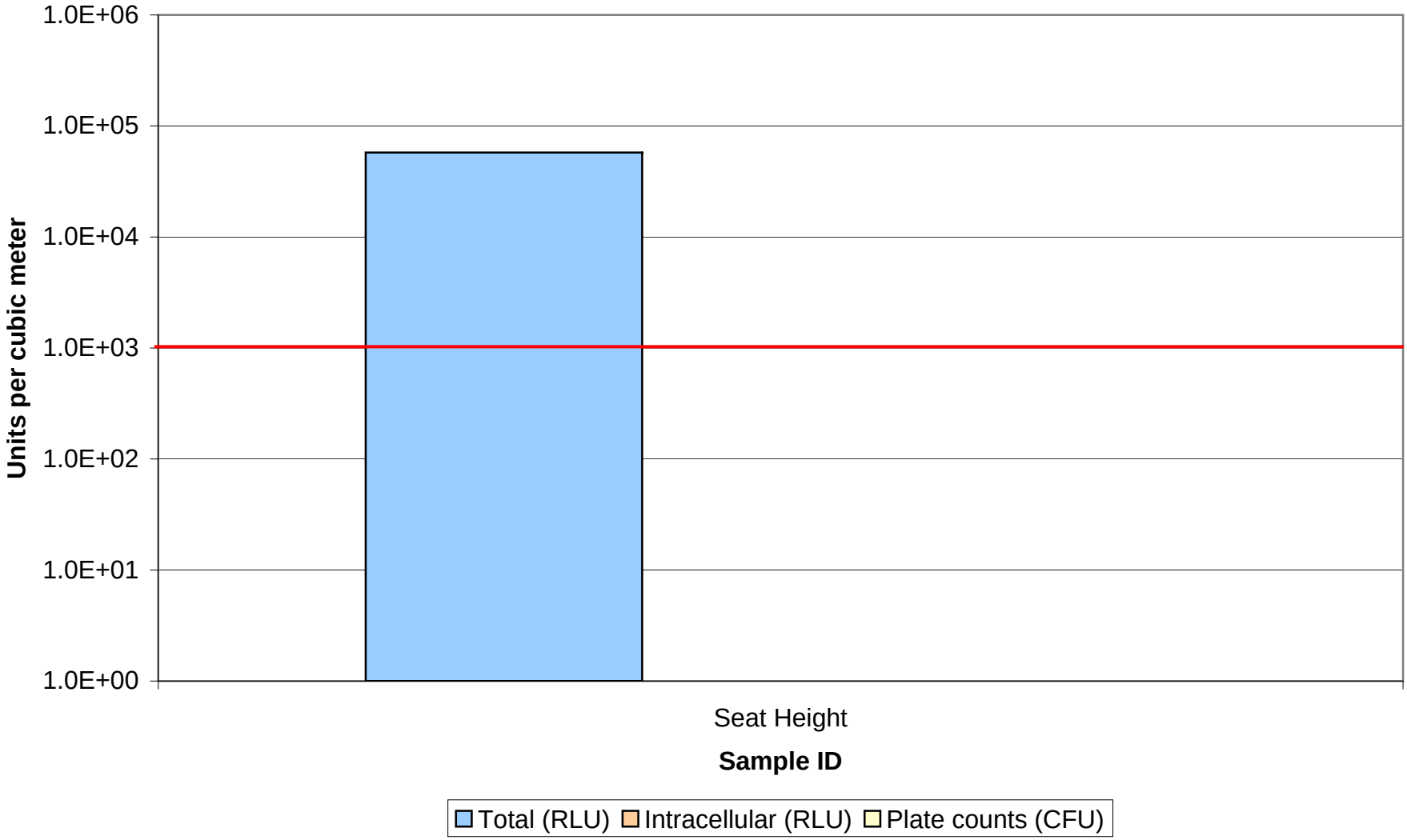


Figure 4.2.1-30 Microbial Quantification of Salt Lake City to Cincinnati; 4-21-04 Landing Samples

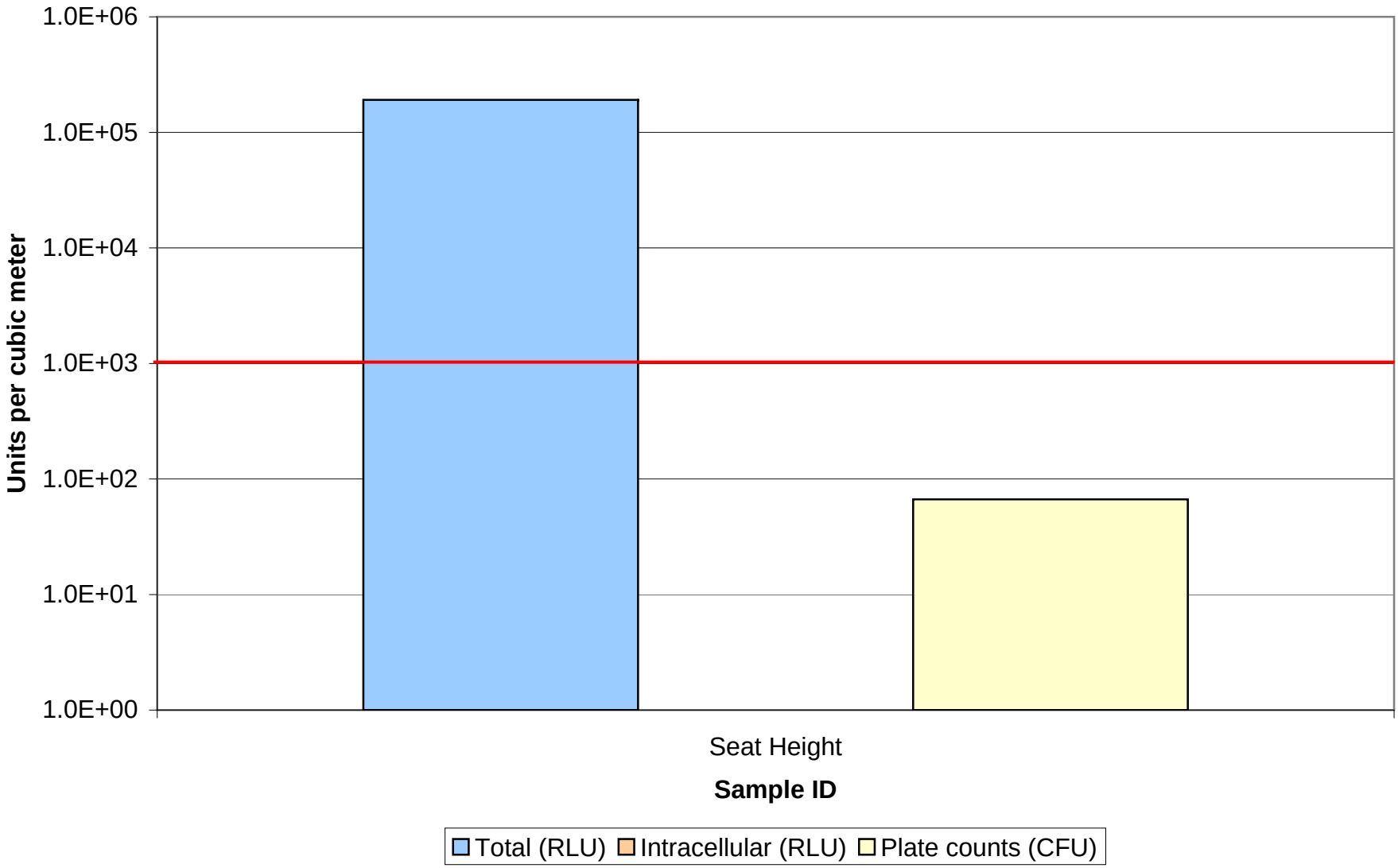


Figure 4.2.1-31 Microbial Quantification of Cincinnati to Salt Lake (4-21-04)

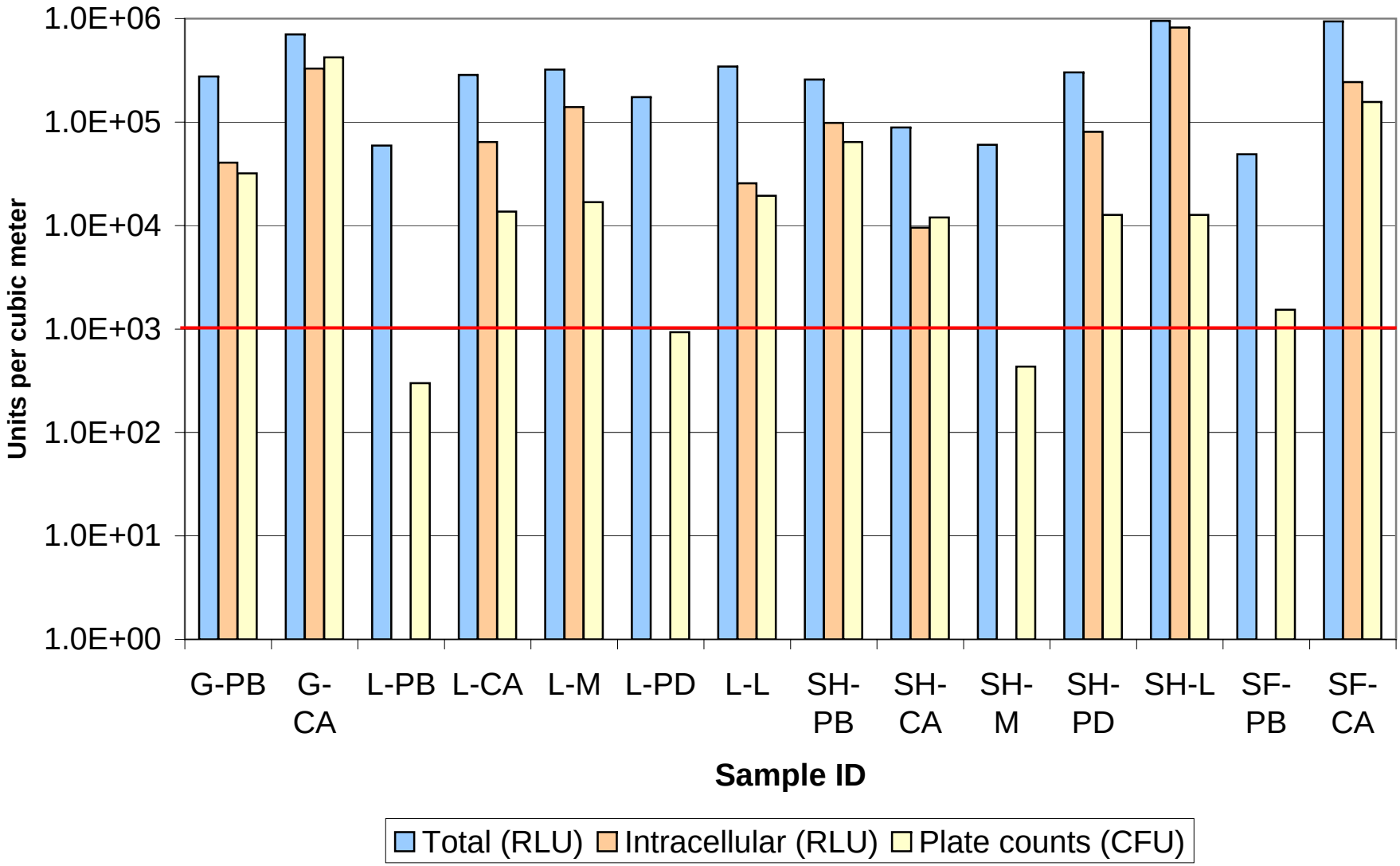


Figure 4.2.1-32 Microbial Quantification of Cincinnati to Salt Lake (4-21-04) Gasper Samples

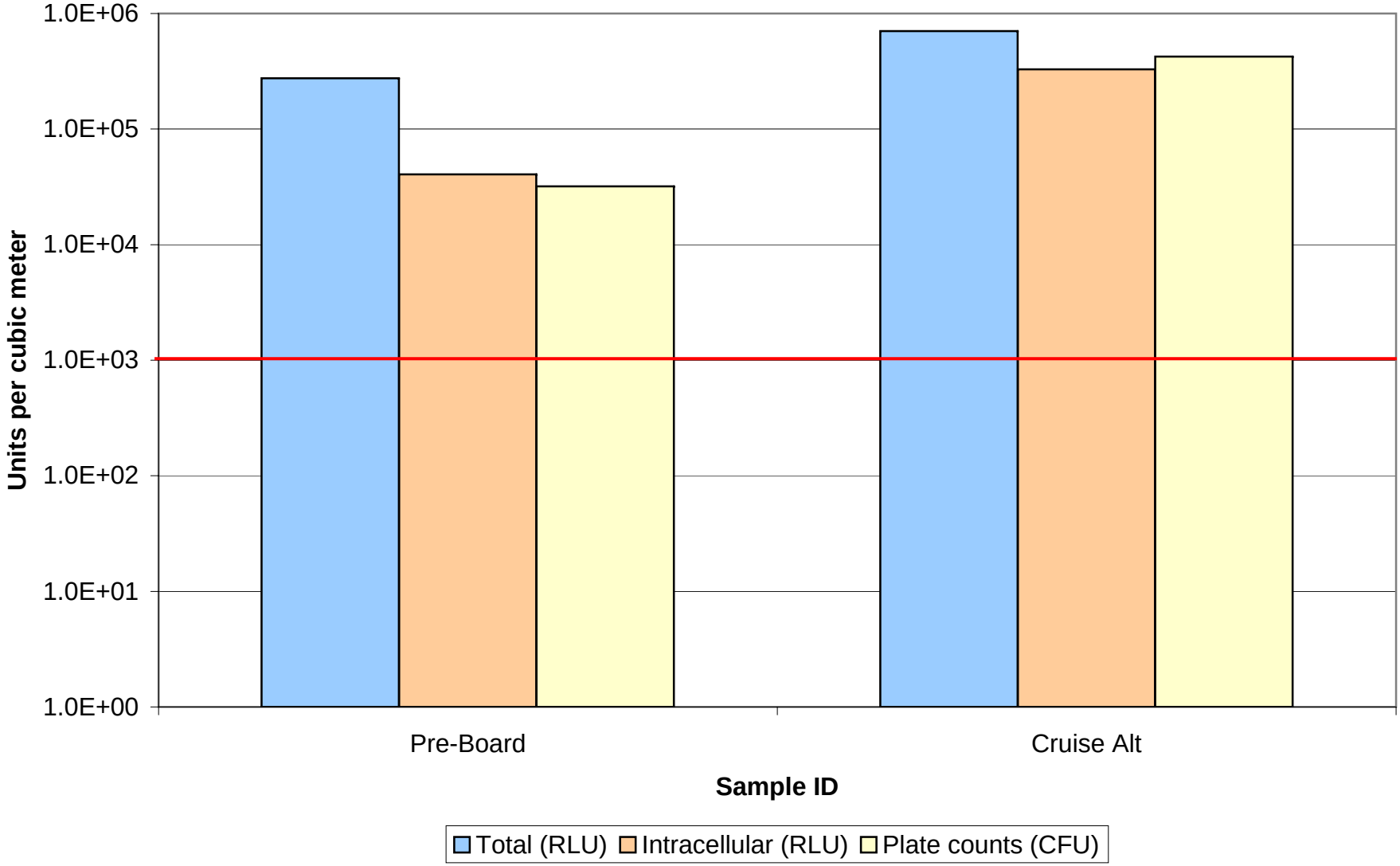


Figure 4.2.1-33 Microbial Quantification of Cincinnati to Salt Lake (4-21-04) Lavatory Samples

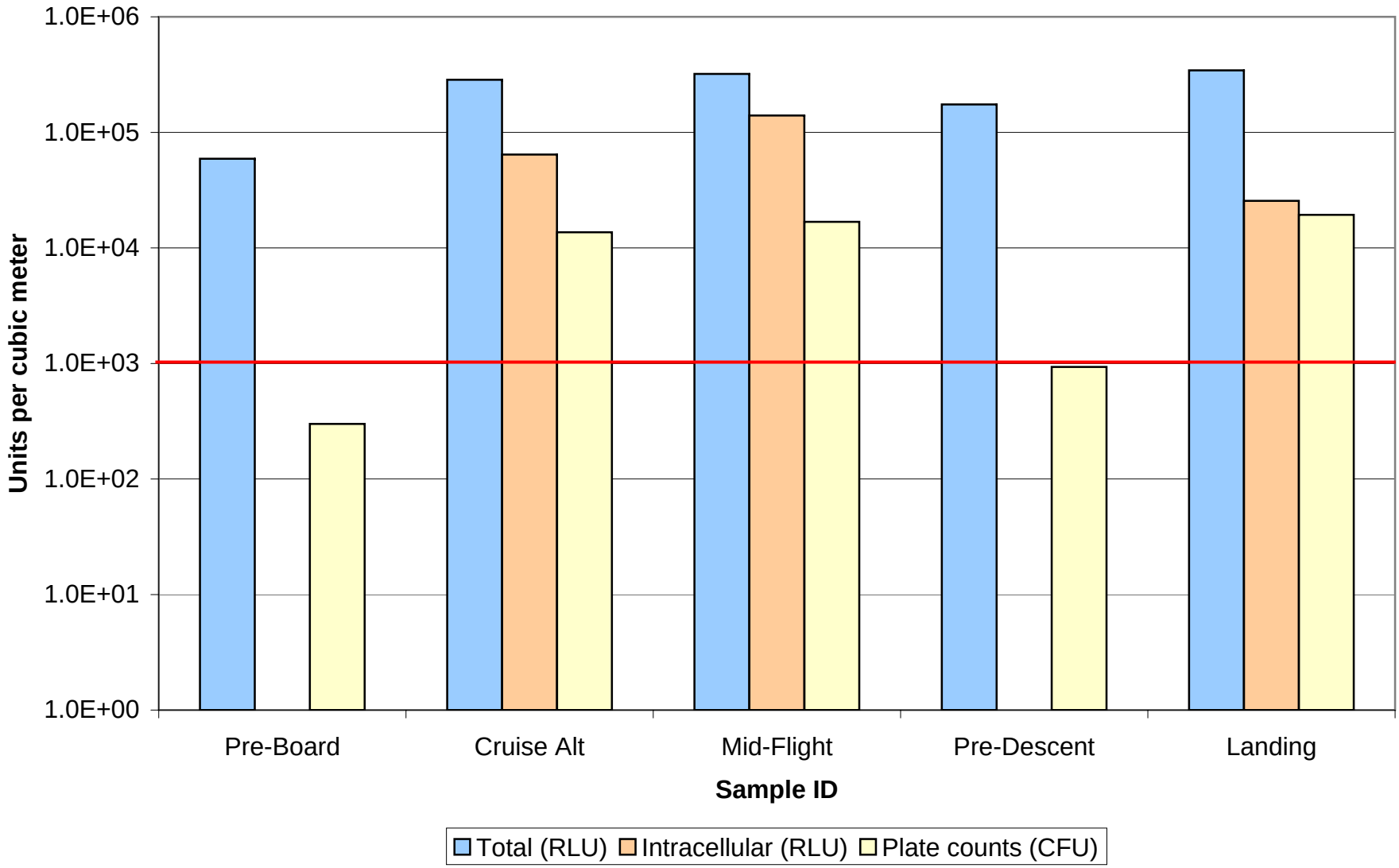


Figure 4.2.1-34 Microbial Quantification of Cincinnati to Salt Lake (4-21-04) Seat Height Samples

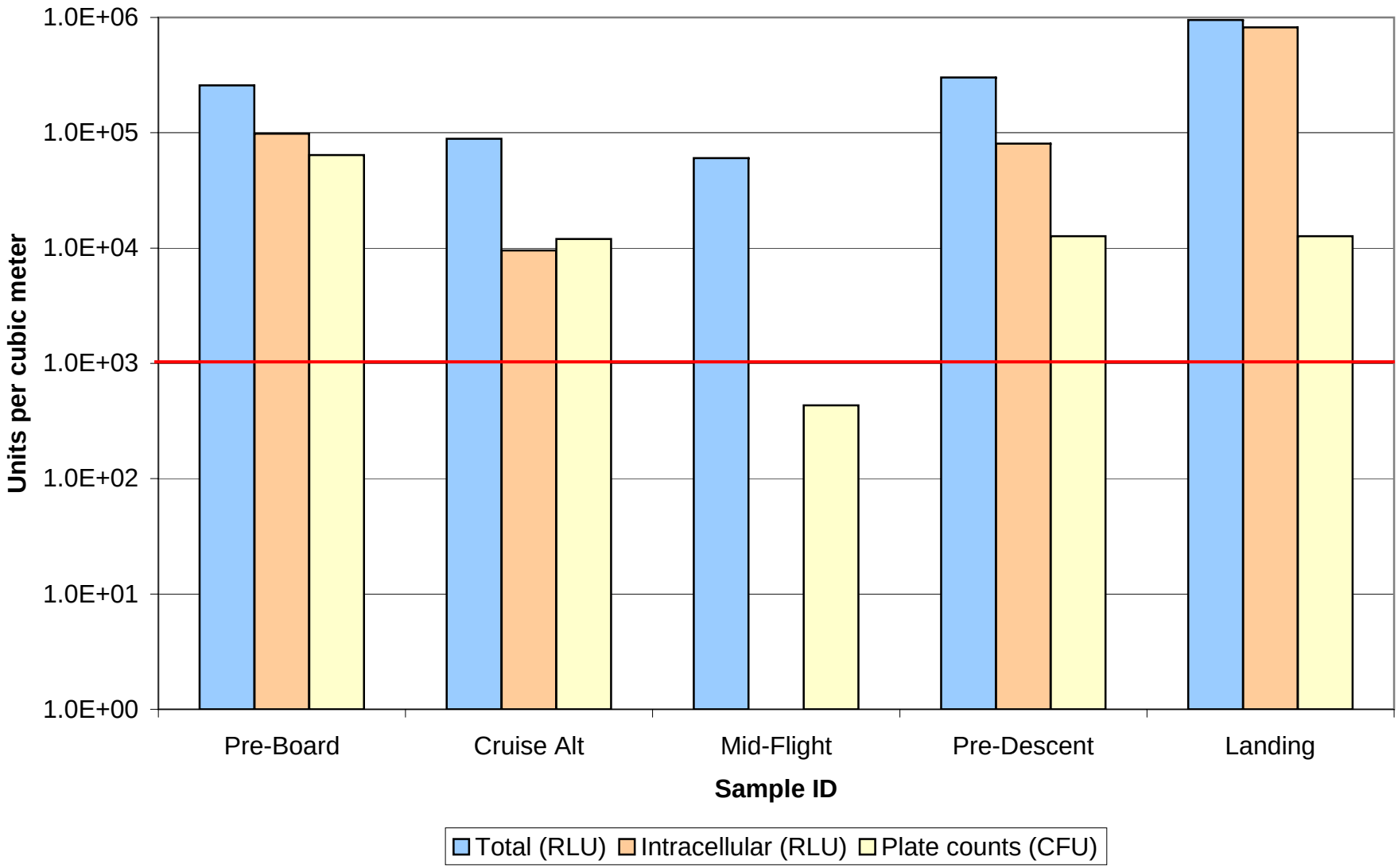


Figure 4.2.1-35 Microbial Quantification of Cincinnati to Salt Lake (4-21-04) Floor Samples

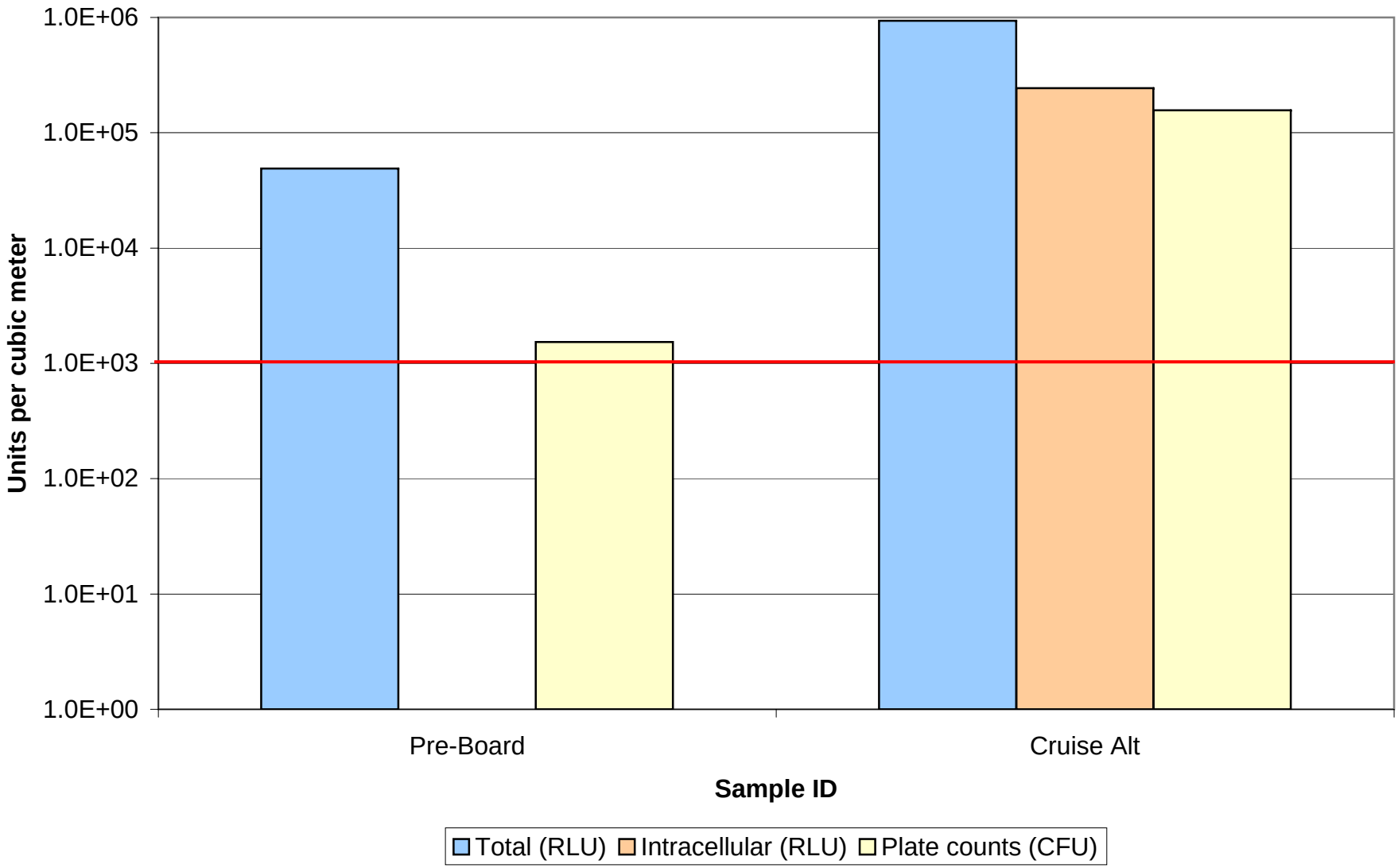


Figure 4.2.1-36 Microbial Quantification of Salt Lake (4-21-04) Pre-Board Samples

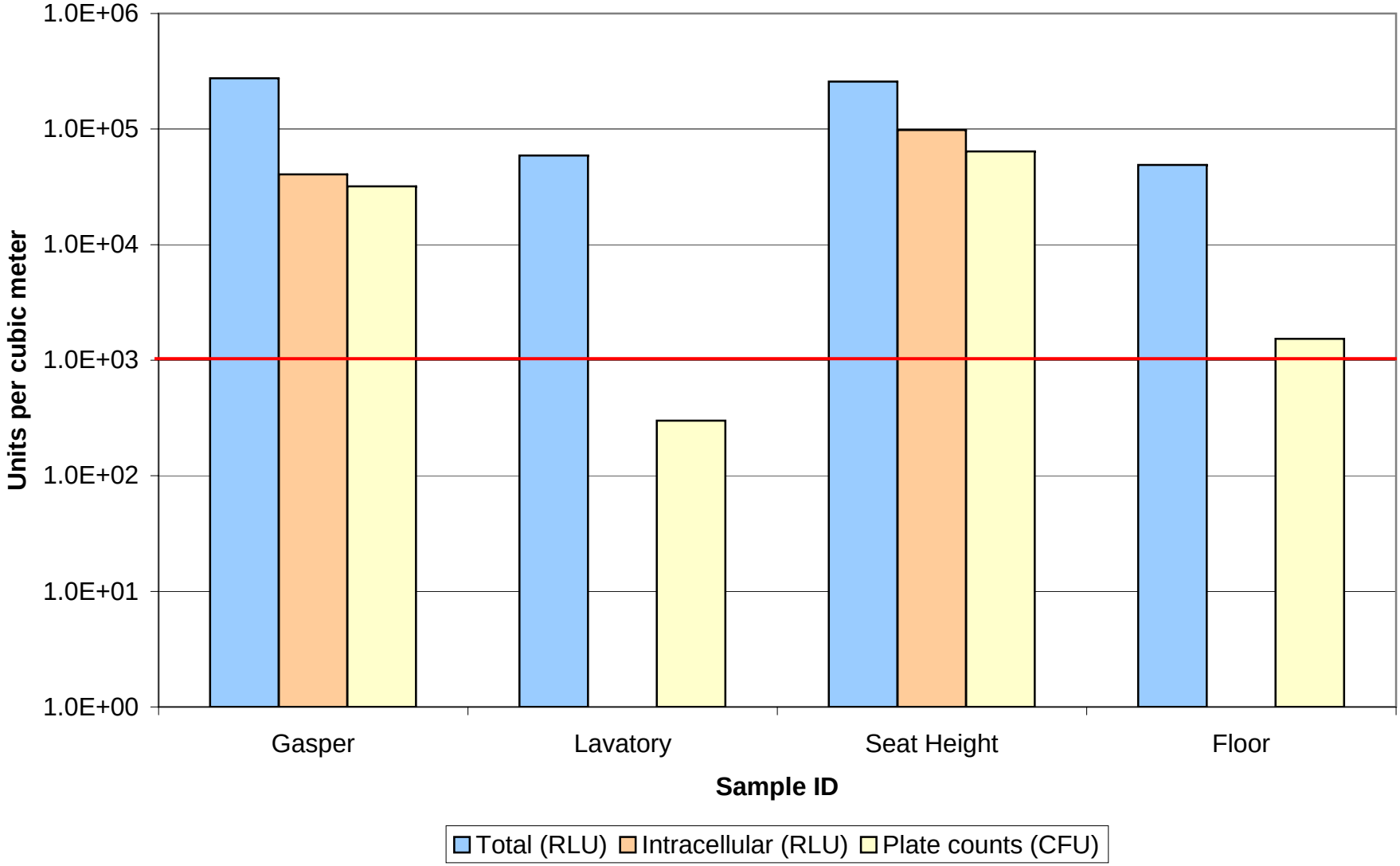


Figure 4.2.1-37 Microbial Quantification of Cincinnati to Salt Lake (4-21-04) Cruise Altitude Samples

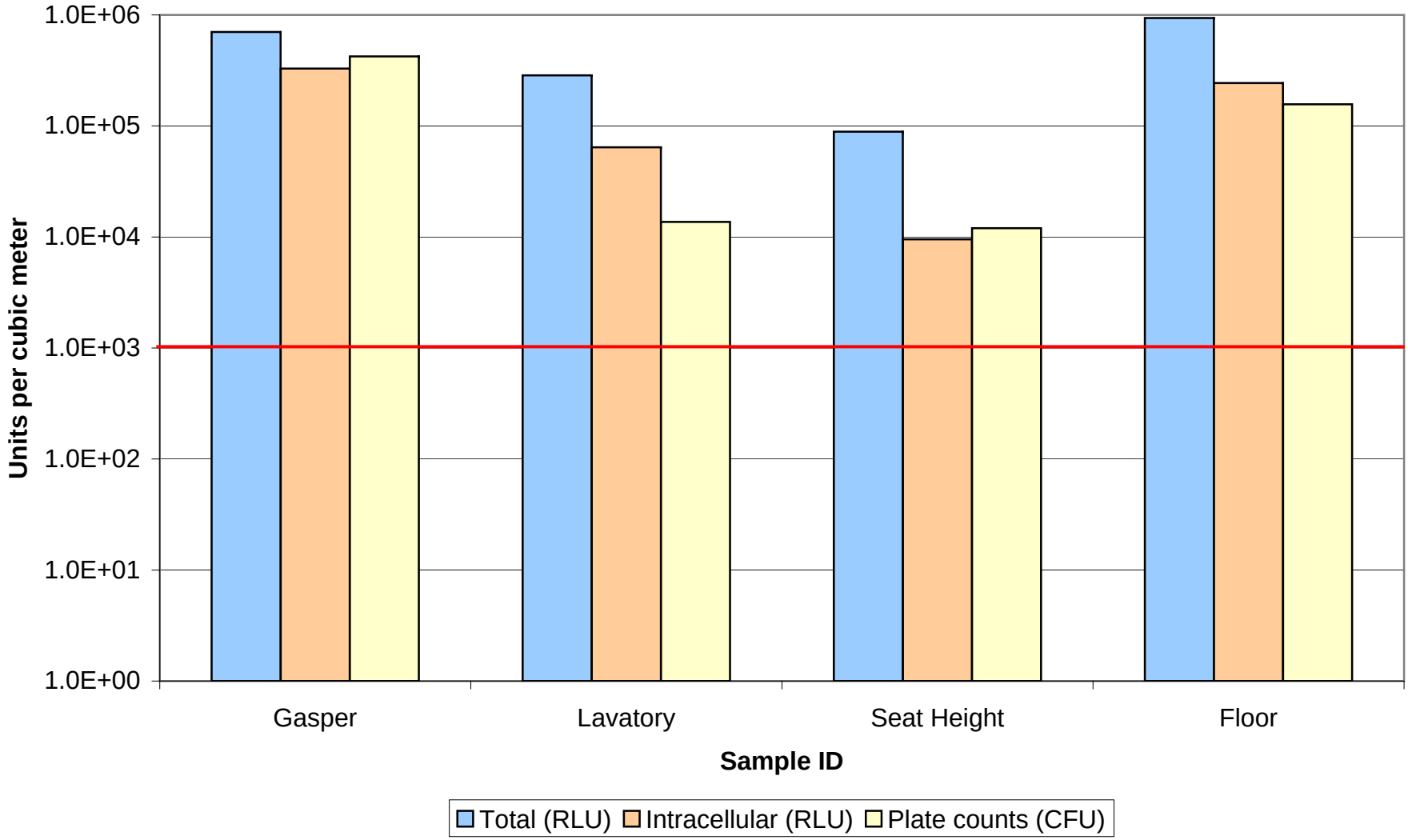


Figure 4.2.1-38 Microbial Quantification of Cincinnati to Salt Lake (4-21-04) Mid-Flight Samples

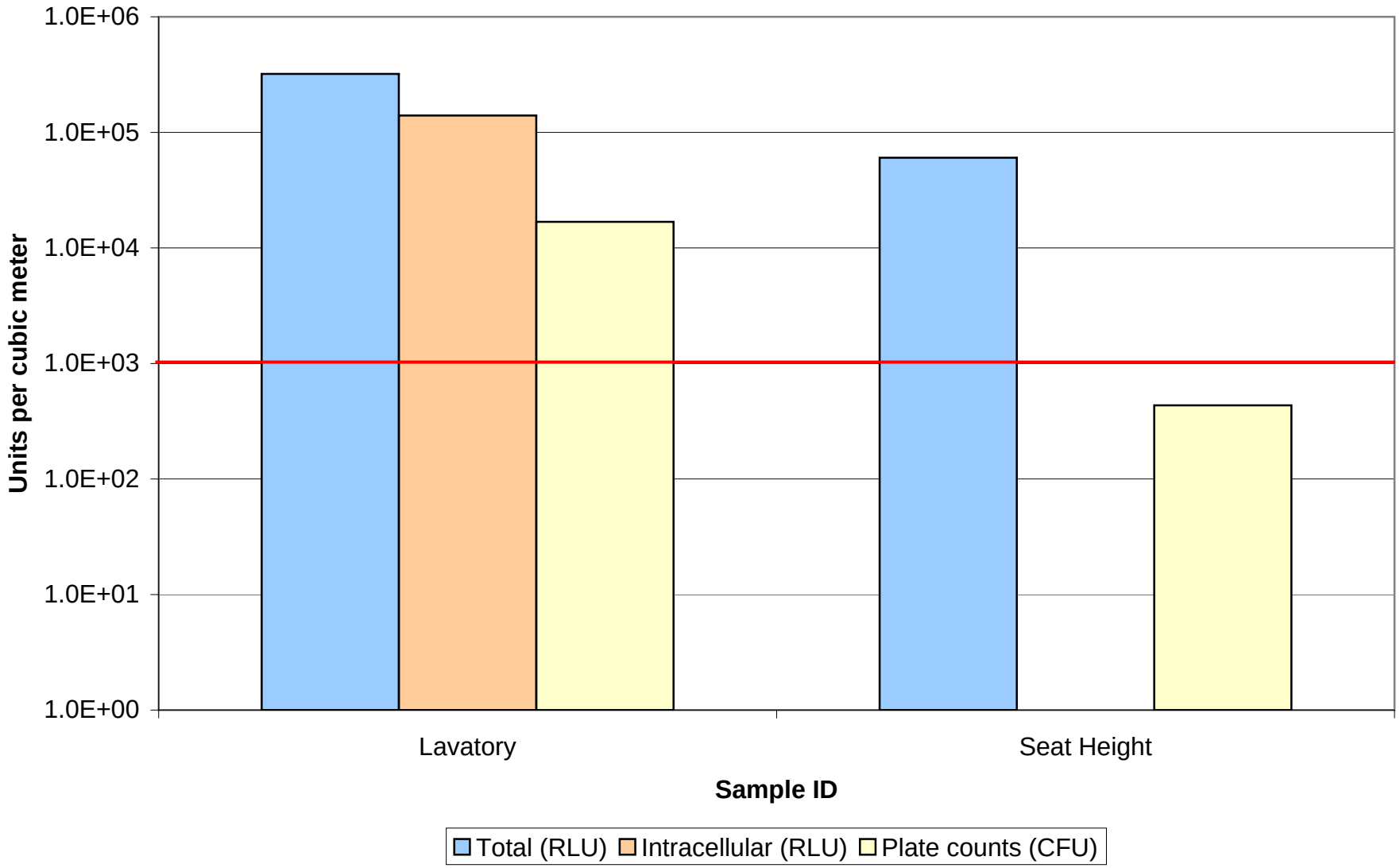


Figure 4.2.1-39 Microbial Quantification of Cincinnati to Salt Lake (4-21-04) Pre-Descent Samples

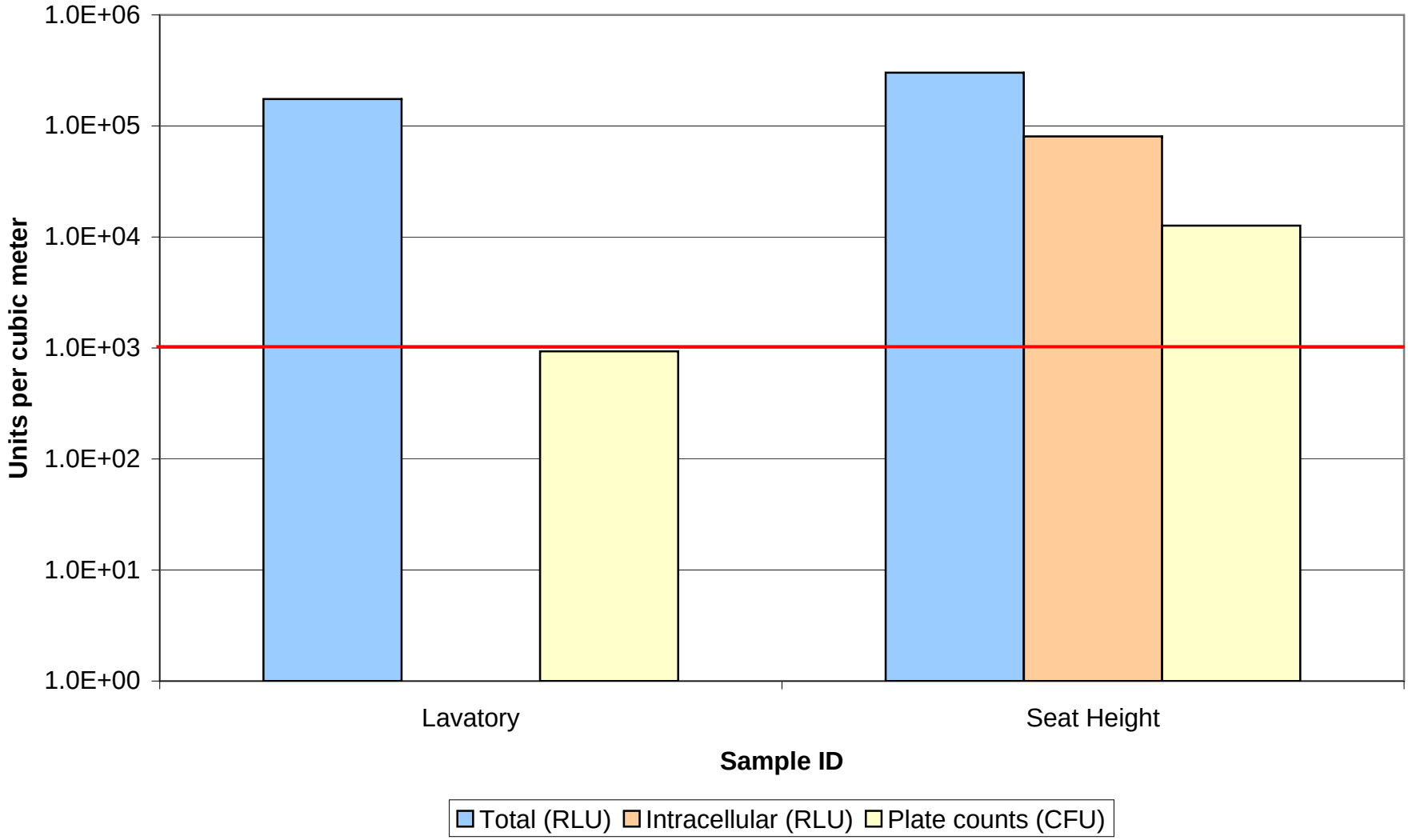


Figure 4.2.1-40 Microbial Quantification of Cincinnati to Salt Lake (4-21-04) Landing Samples

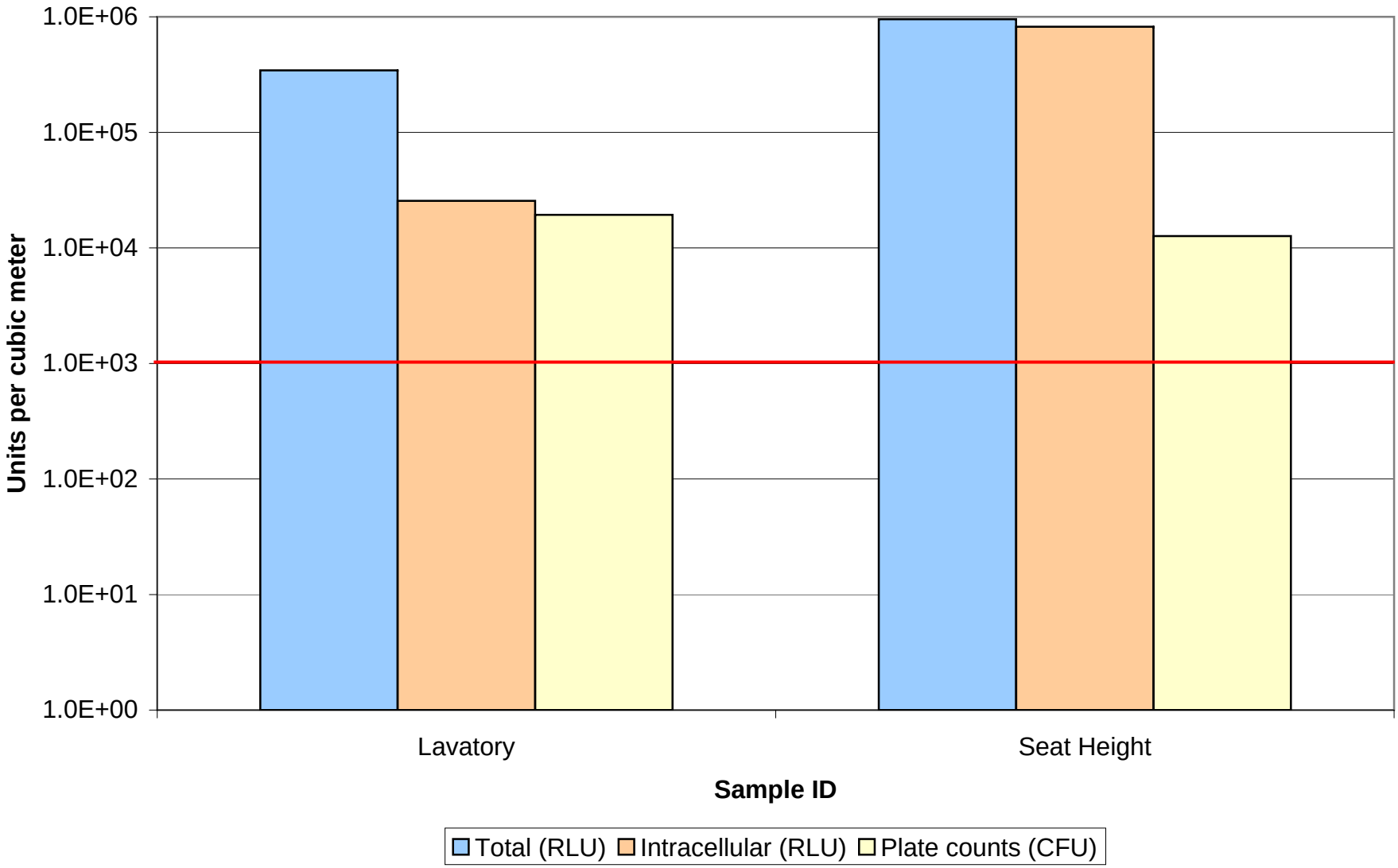


Figure 4.2.1-41 Microbial Quantification of Los Angeles to New York (11-14-05)

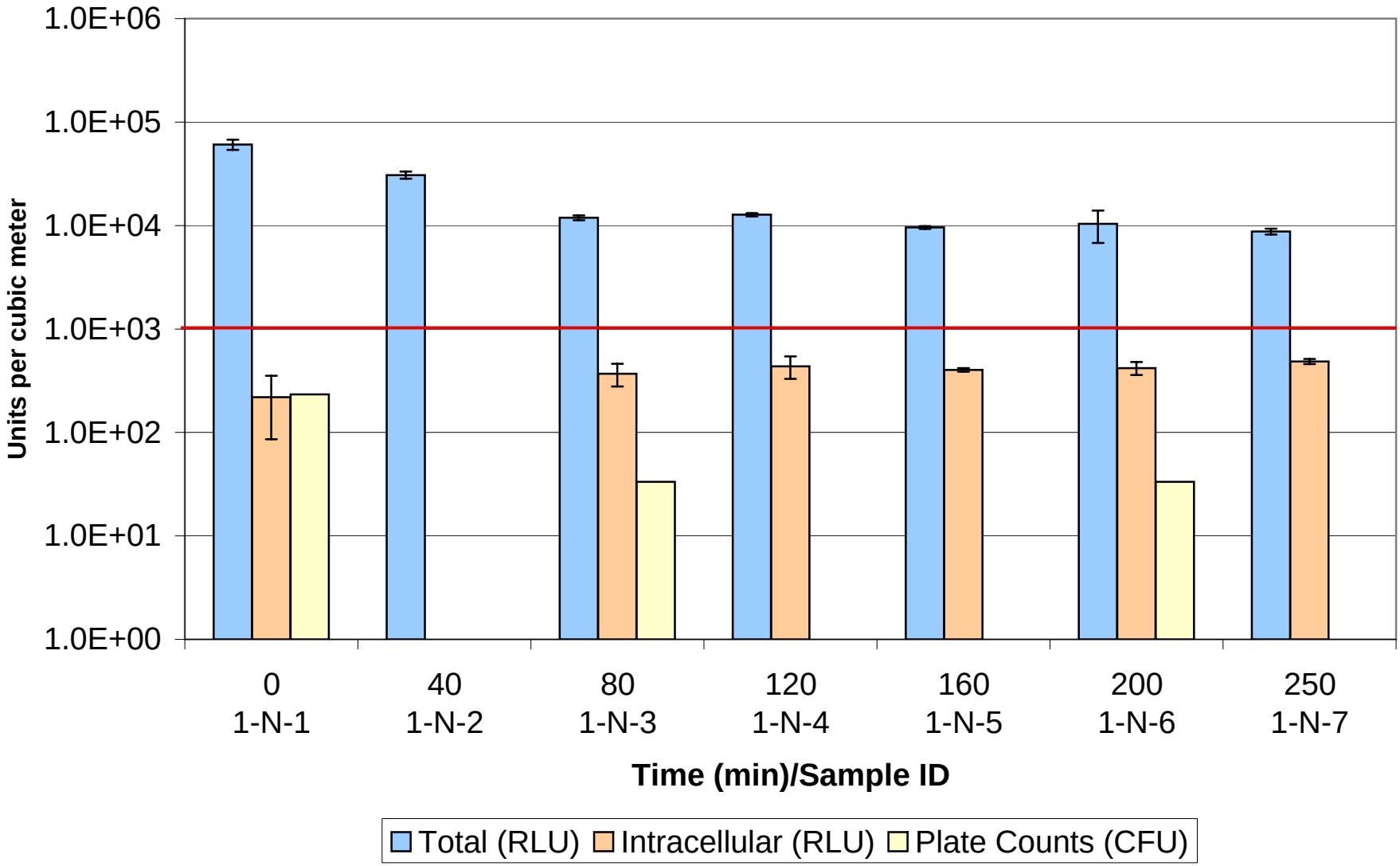


Figure 4.2.1-42 Microbial Quantification of New York to Los Angeles (11-17-05)

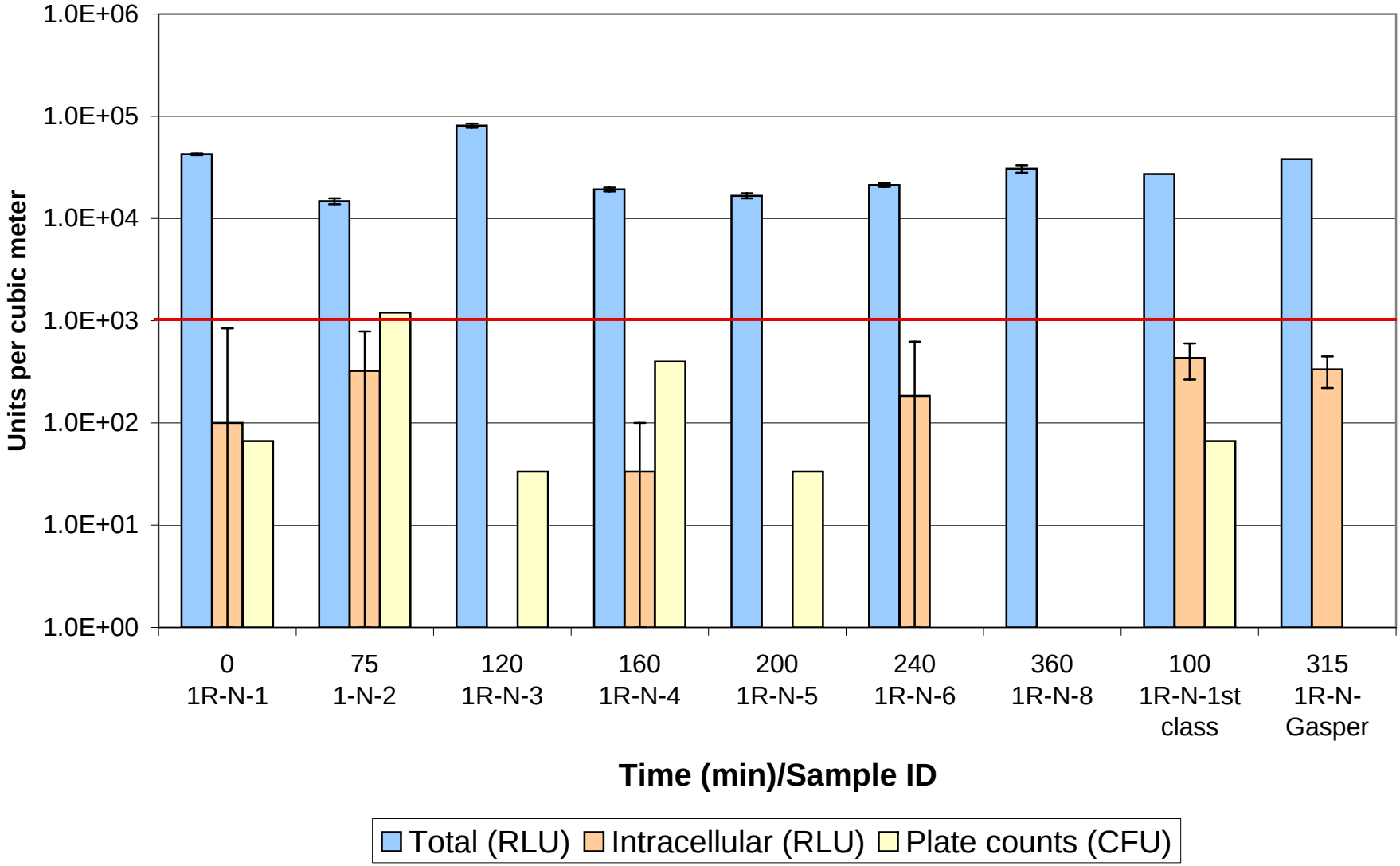


Figure 4.2.1-43 Microbial Quantification of Los Angeles to New York (12-5-05)

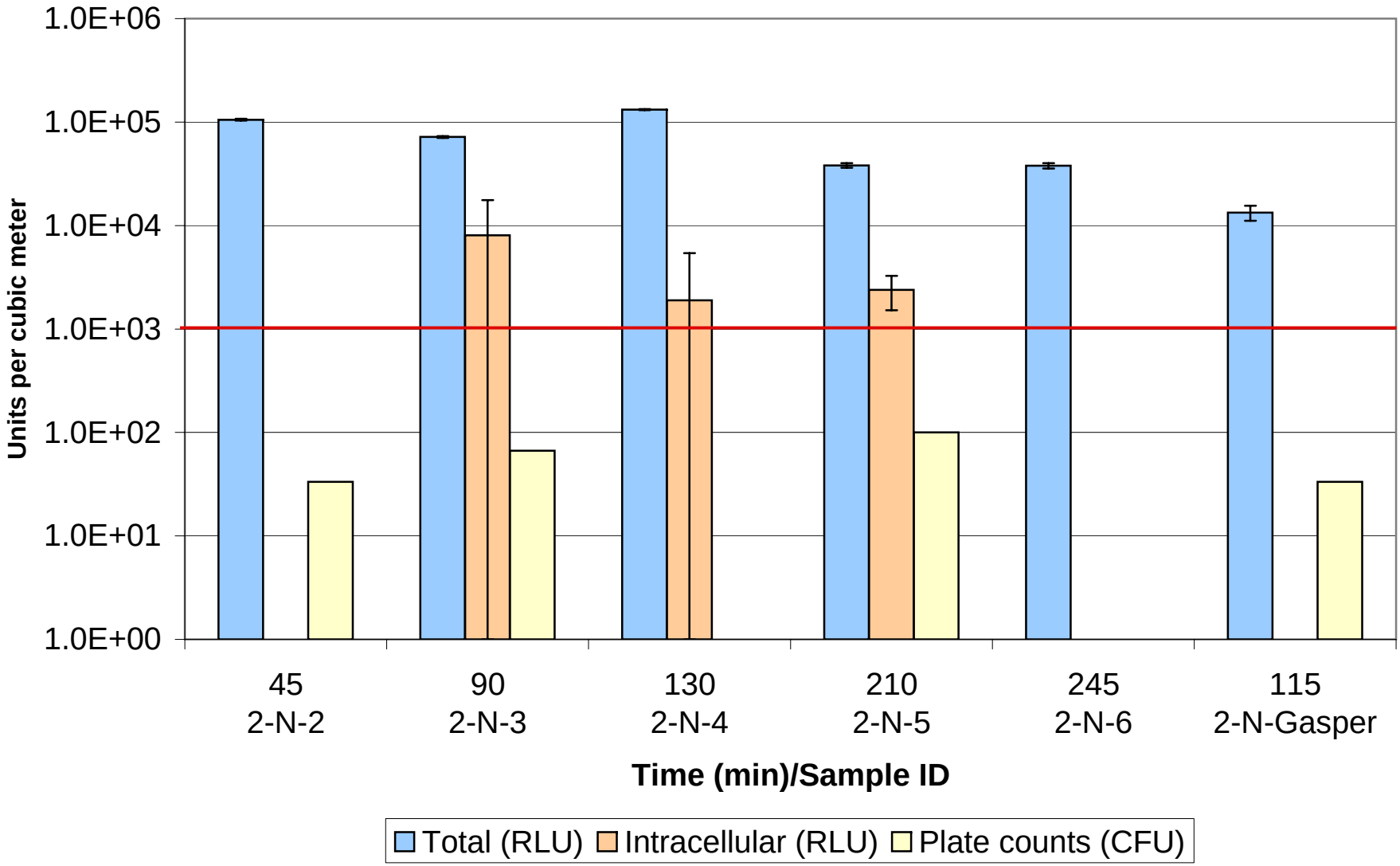


Figure 4.2.1-44 Microbial Quantification of New York to Los Angeles (12-8-05)

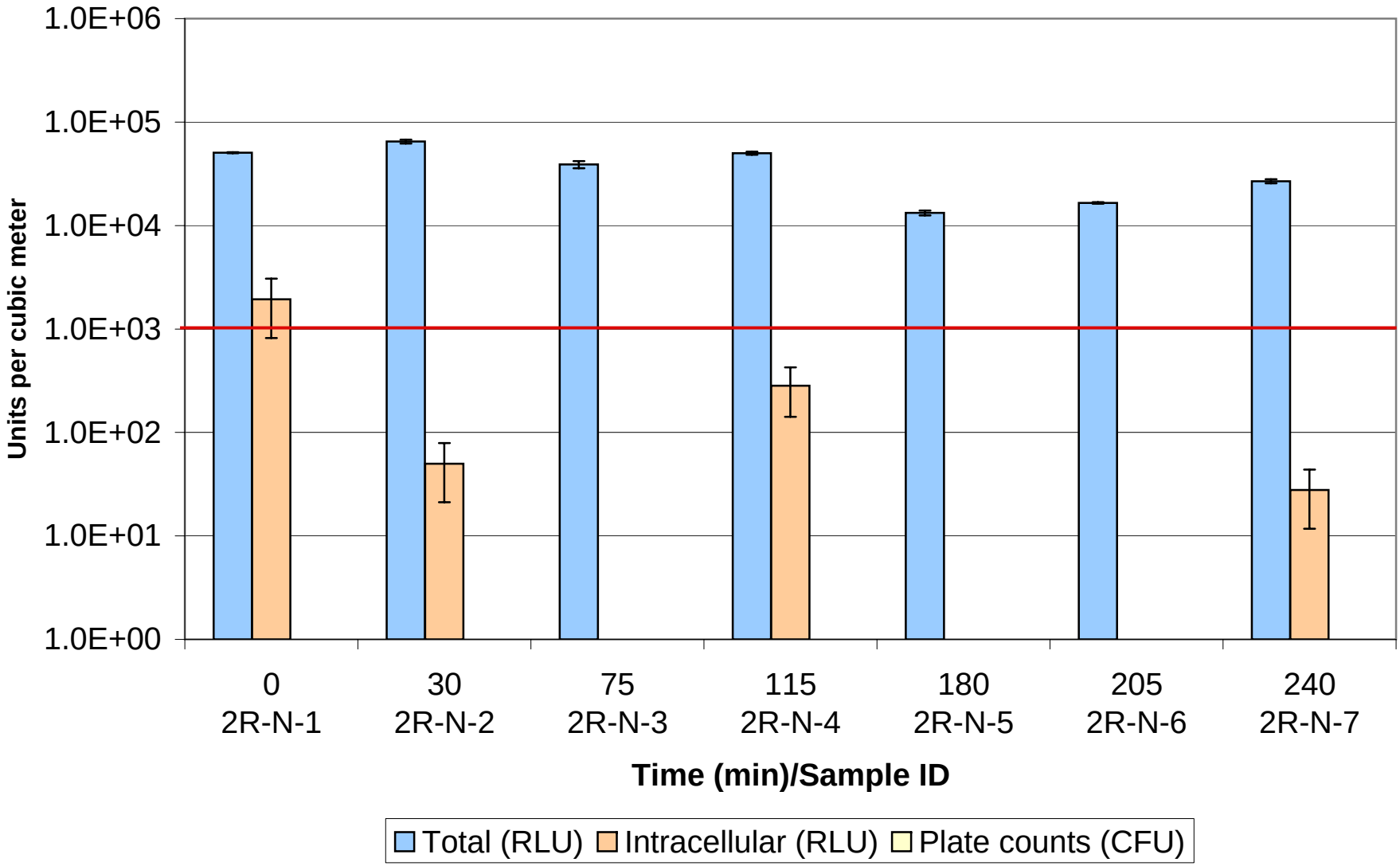


Figure 4.2.1-45 Microbial Quantification of Los Angeles to London flight (1-15-06)

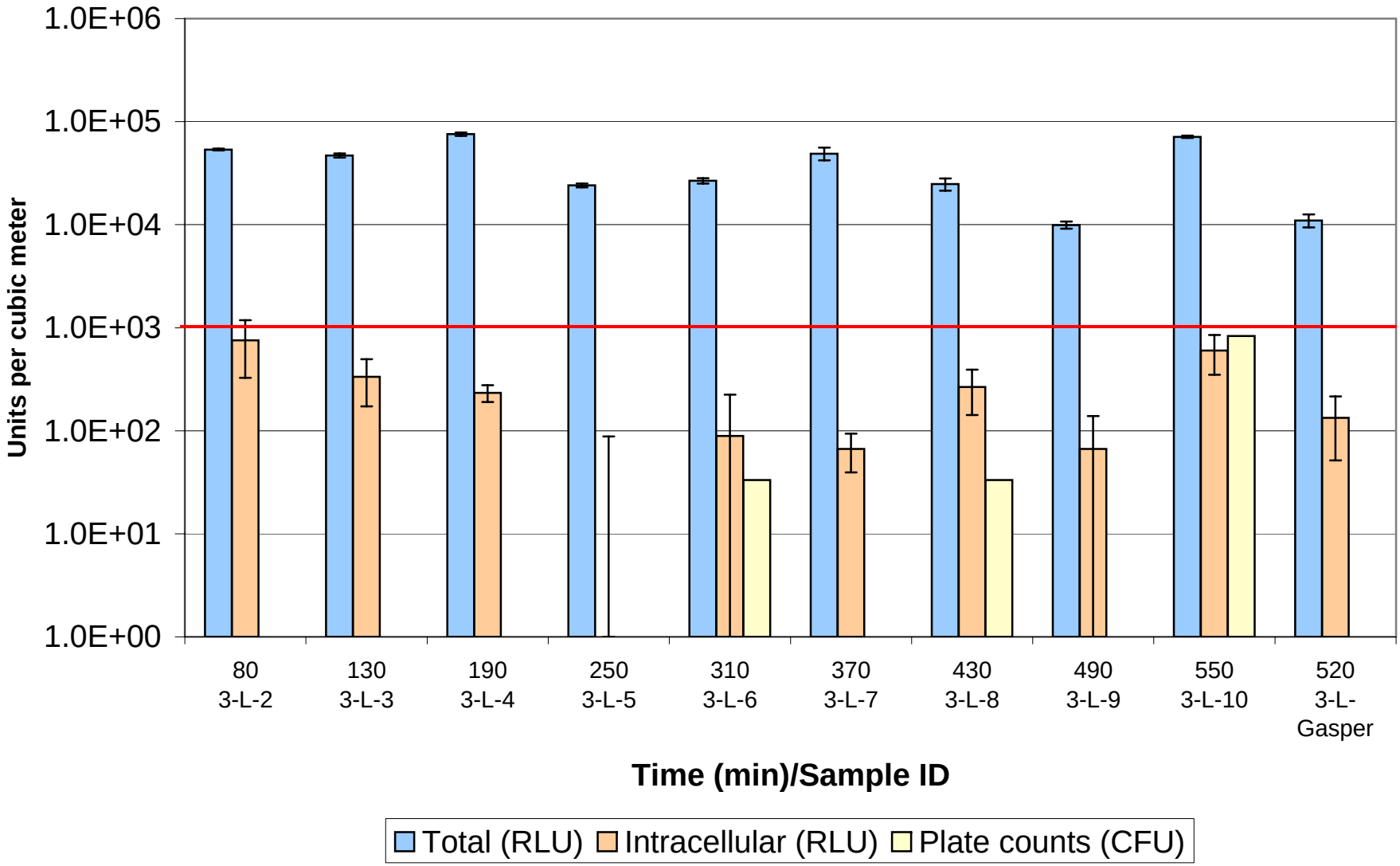


Figure 4.2.1-46 Microbial Quantification of London to Los Angeles (1-20-06)

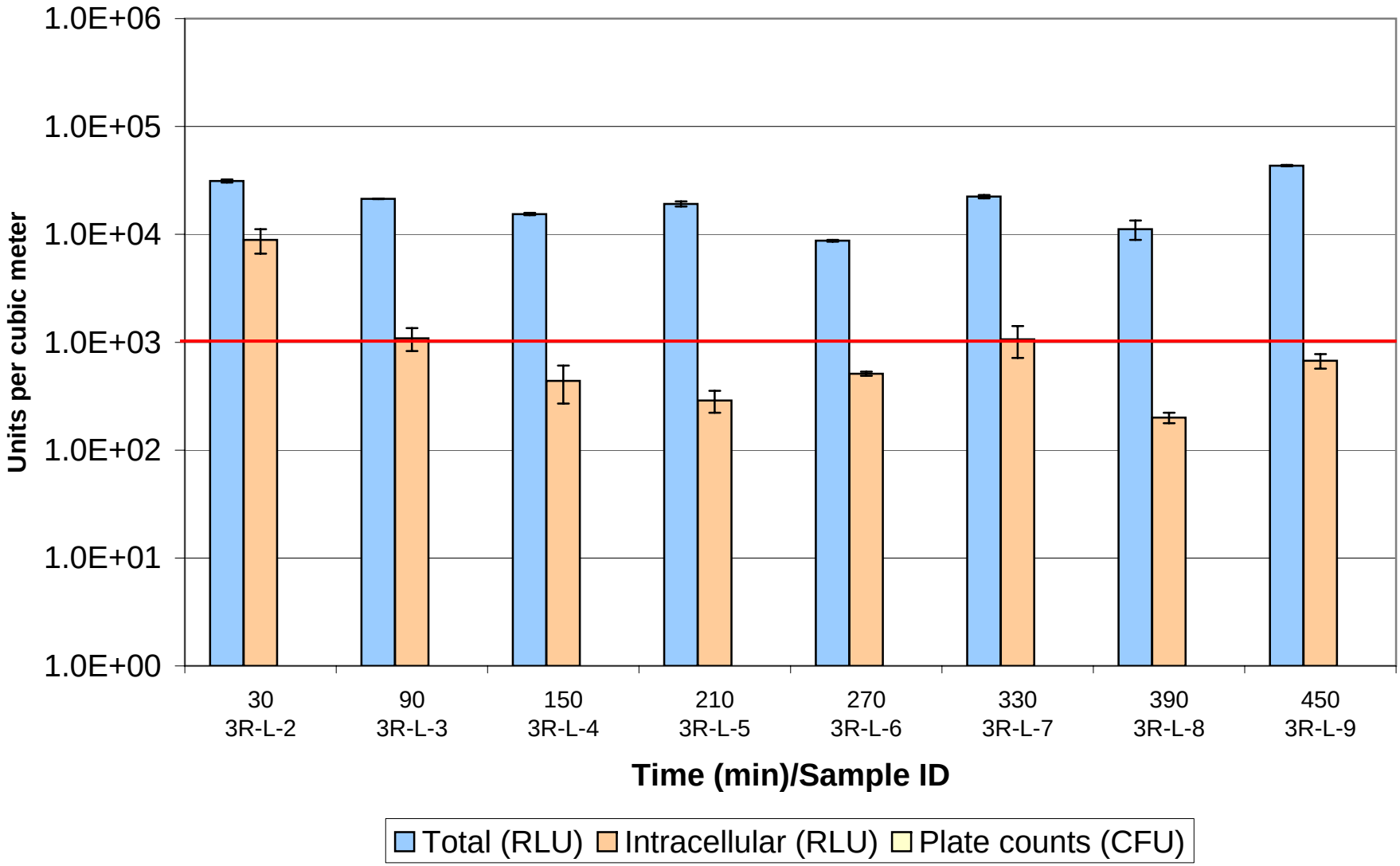


Figure 4.2.1-47 Microbial Quantification of Sydney to Los Angeles (2-17-06)

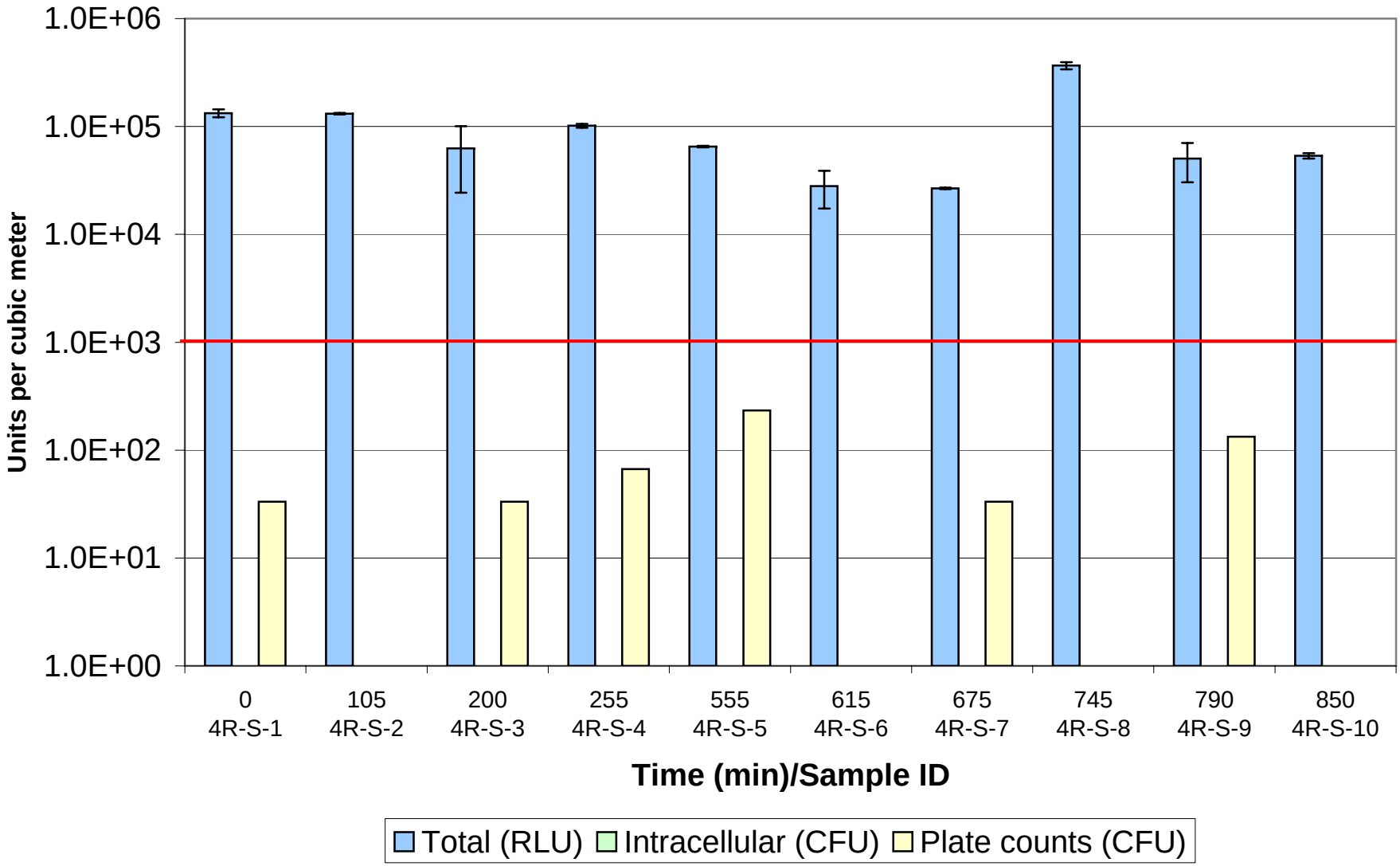


Figure 4.2.1-48 Microbial Quantification of Los Angeles to New York (3-7-06)

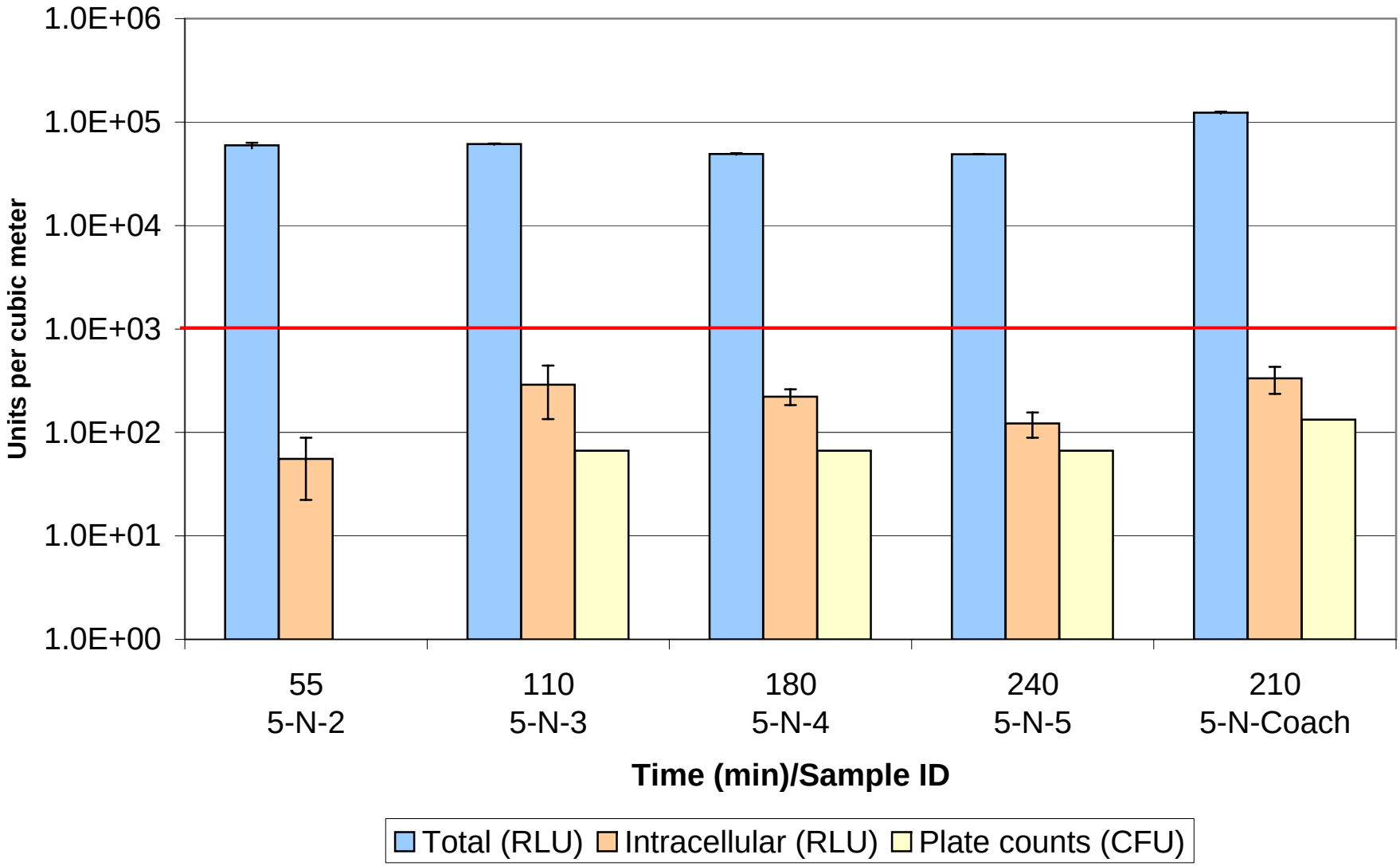


Figure 4.2.1-49 Microbial Quantification of New York to Los Angeles (3-10-06)

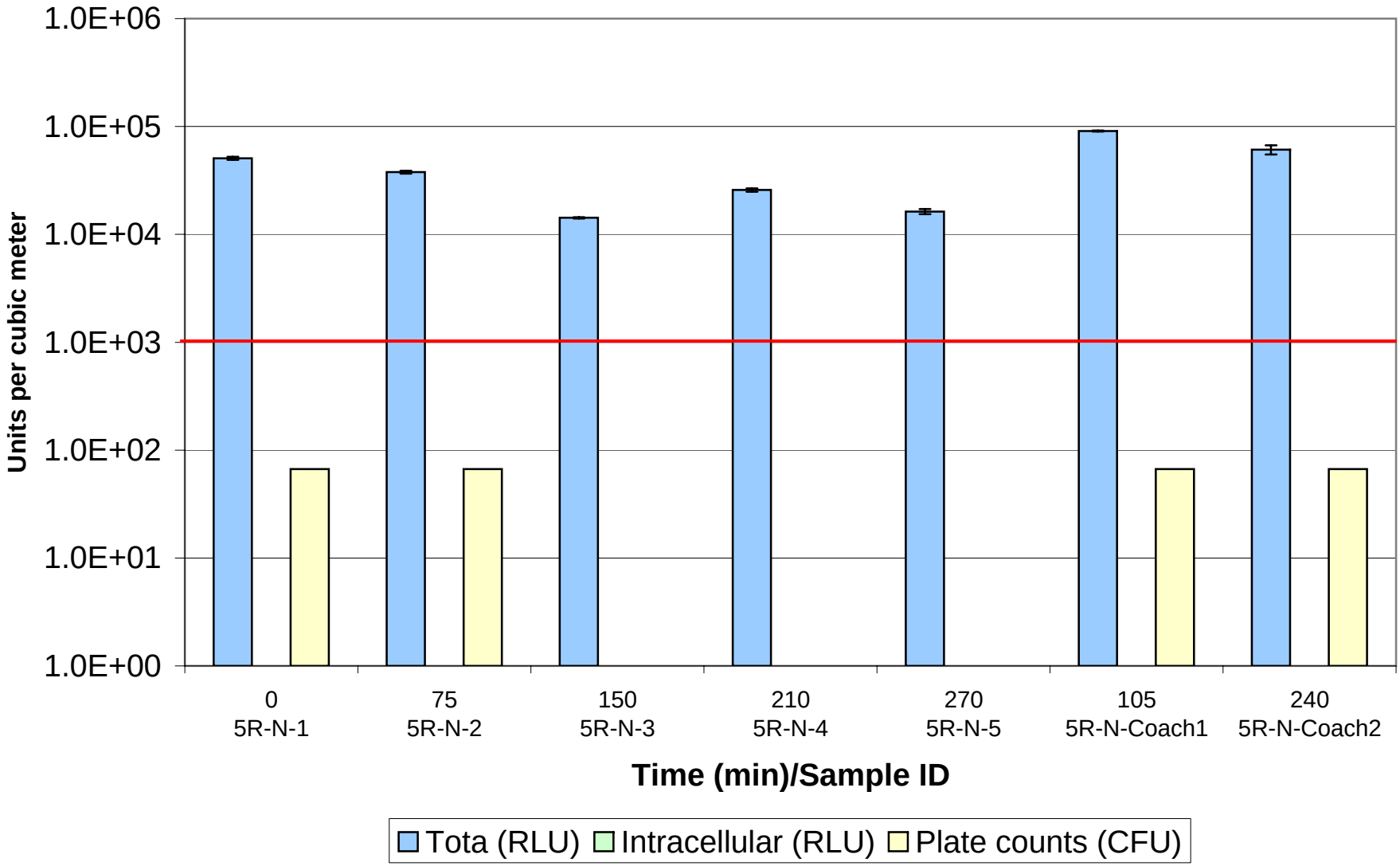


Figure 4.2.1-50 Microbial Quantification of Los Angeles to Tokyo (4-3-06)

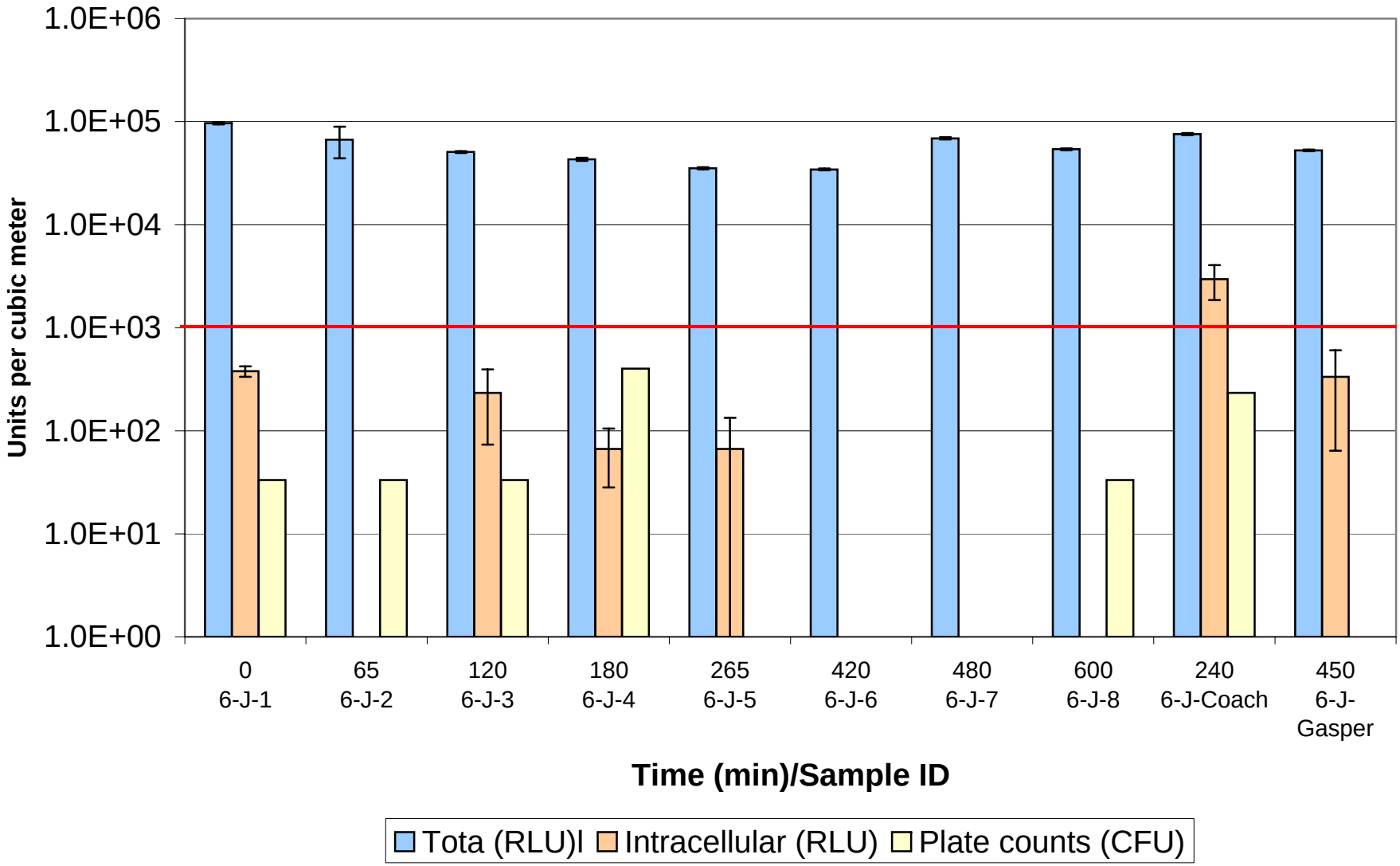


Figure 4.2.1-51 Microbial Quantification of Tokyo to Los Angeles (4-7-06)

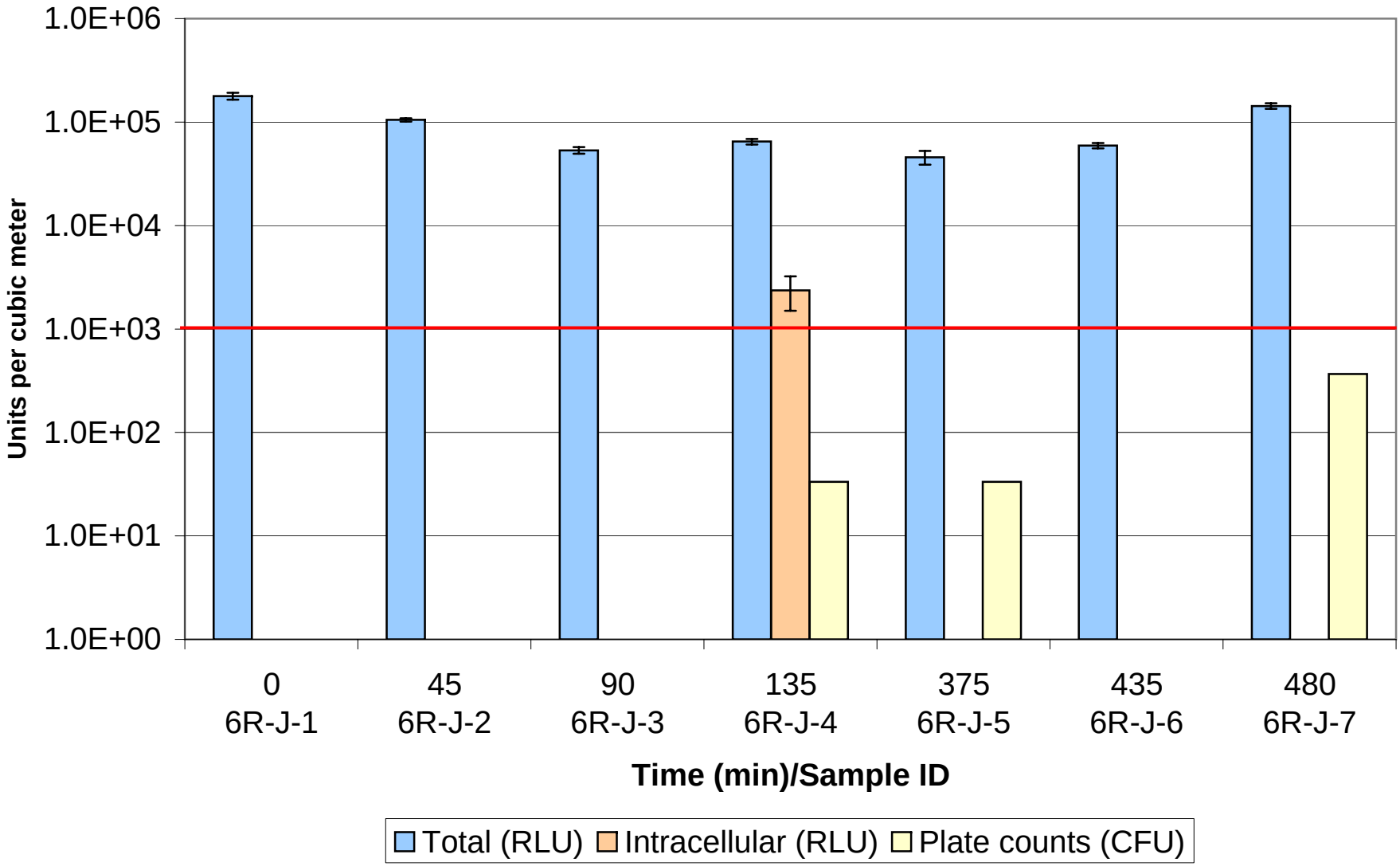


Figure 4.2.1-52 Microbial Quantification of Los Angeles to New York (4-24-06)

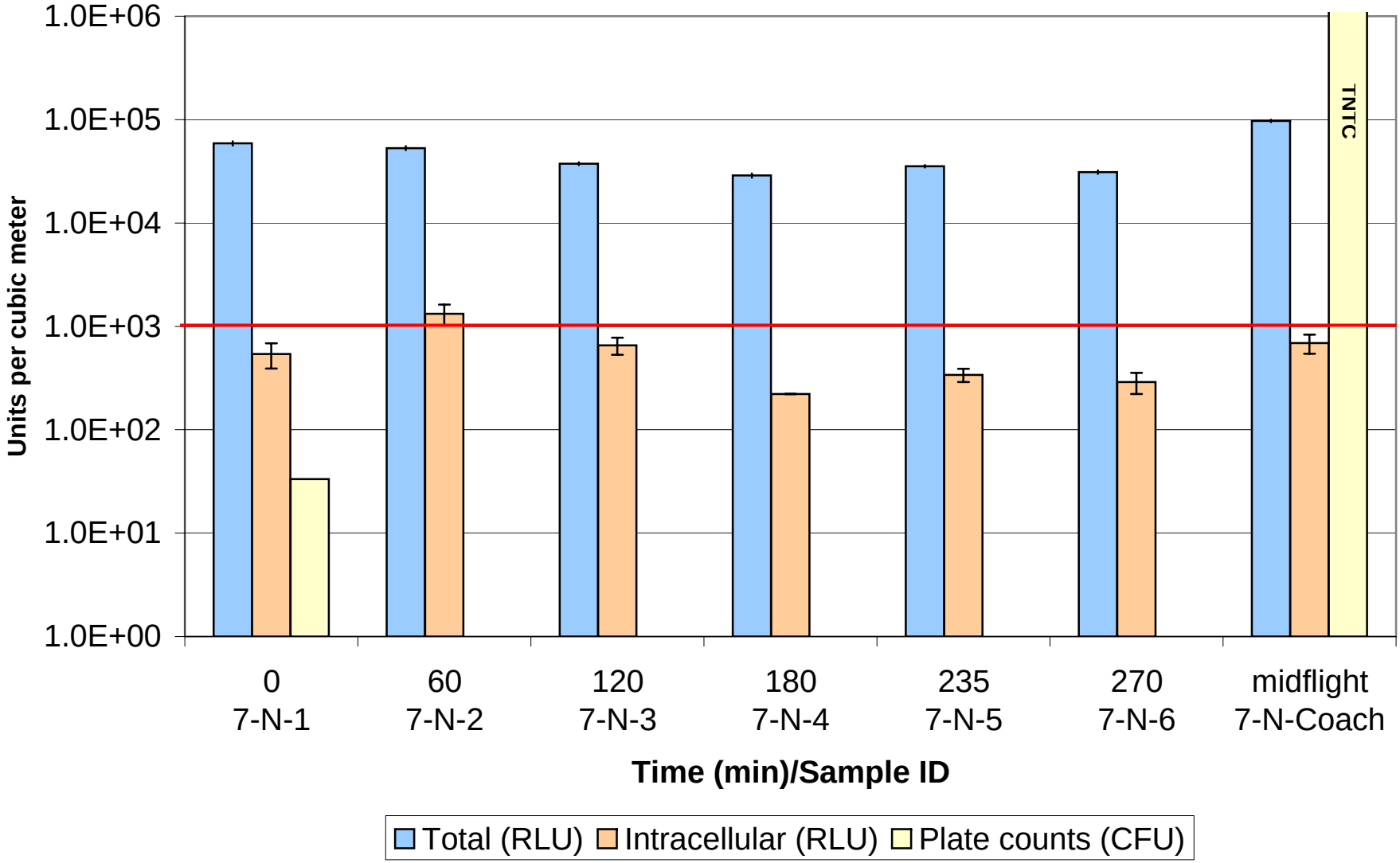


Figure 4.2.1-53 Microbial Quantification of New York to Los Angeles (4-27-06)

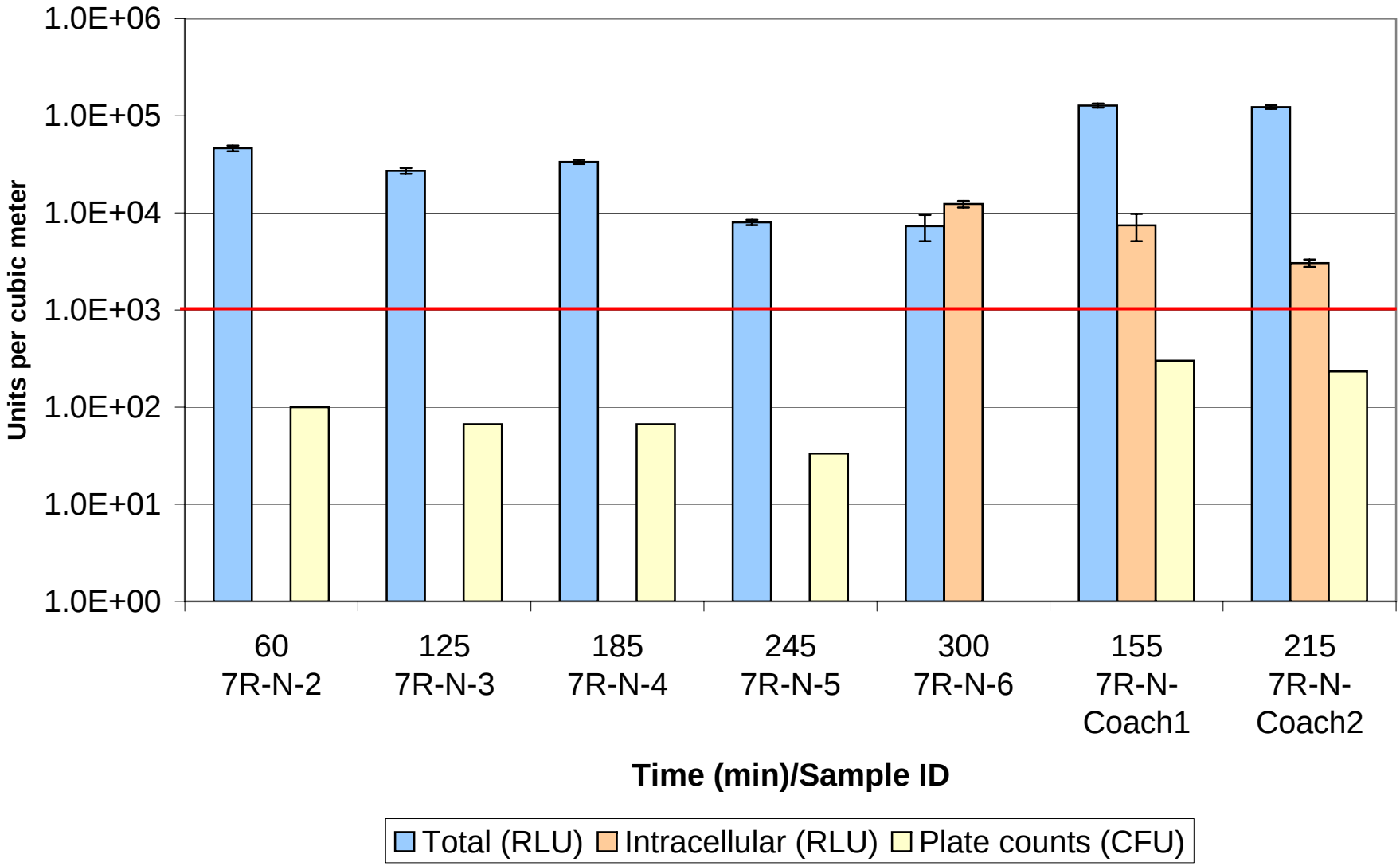


Figure 4.2.1-54 Microbial Quantification of Los Angeles to Sydney (5-7-06)

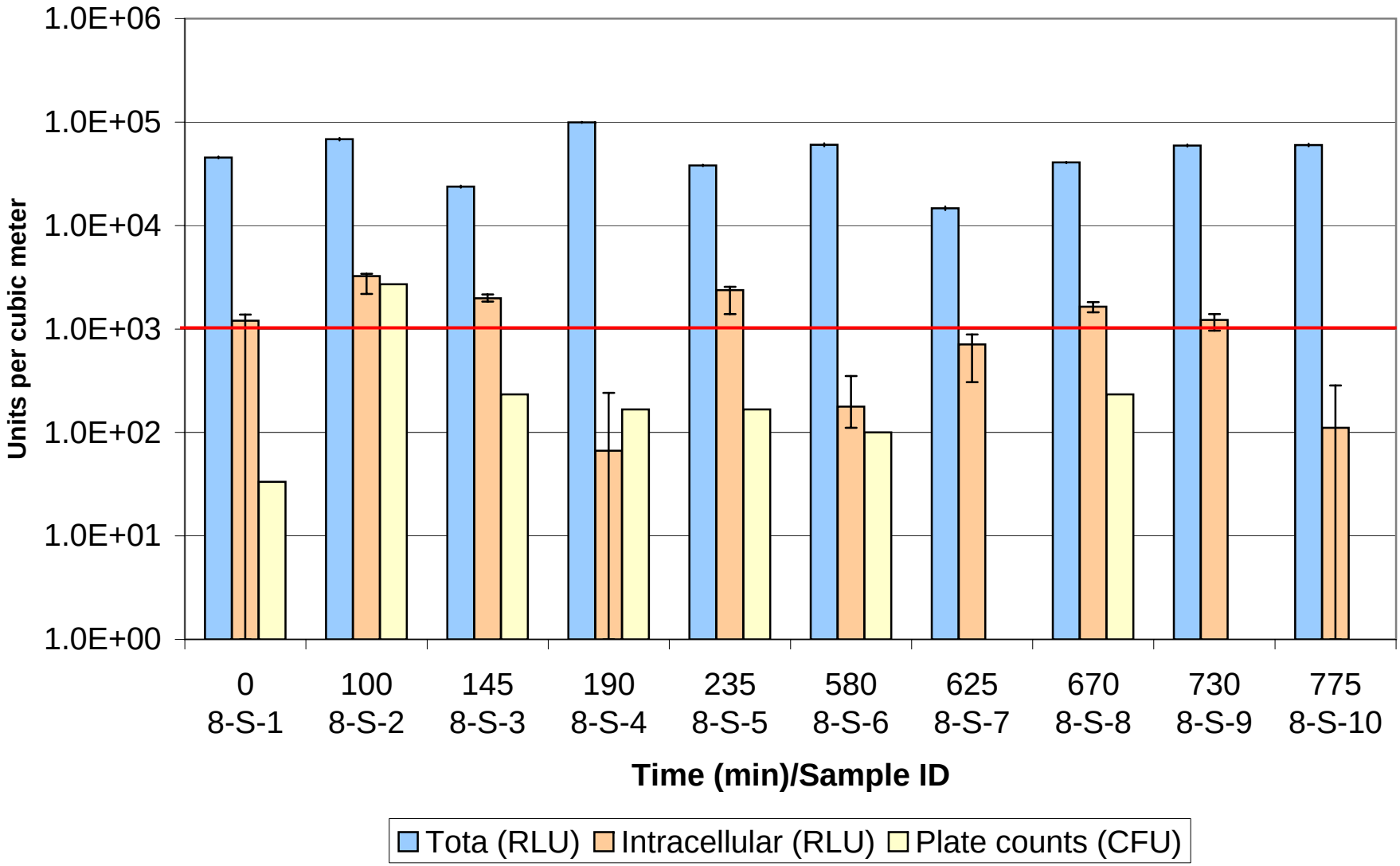


Figure 4.2.1-55 Microbial Quantification of Sydney to Los Angeles (5-12-06)

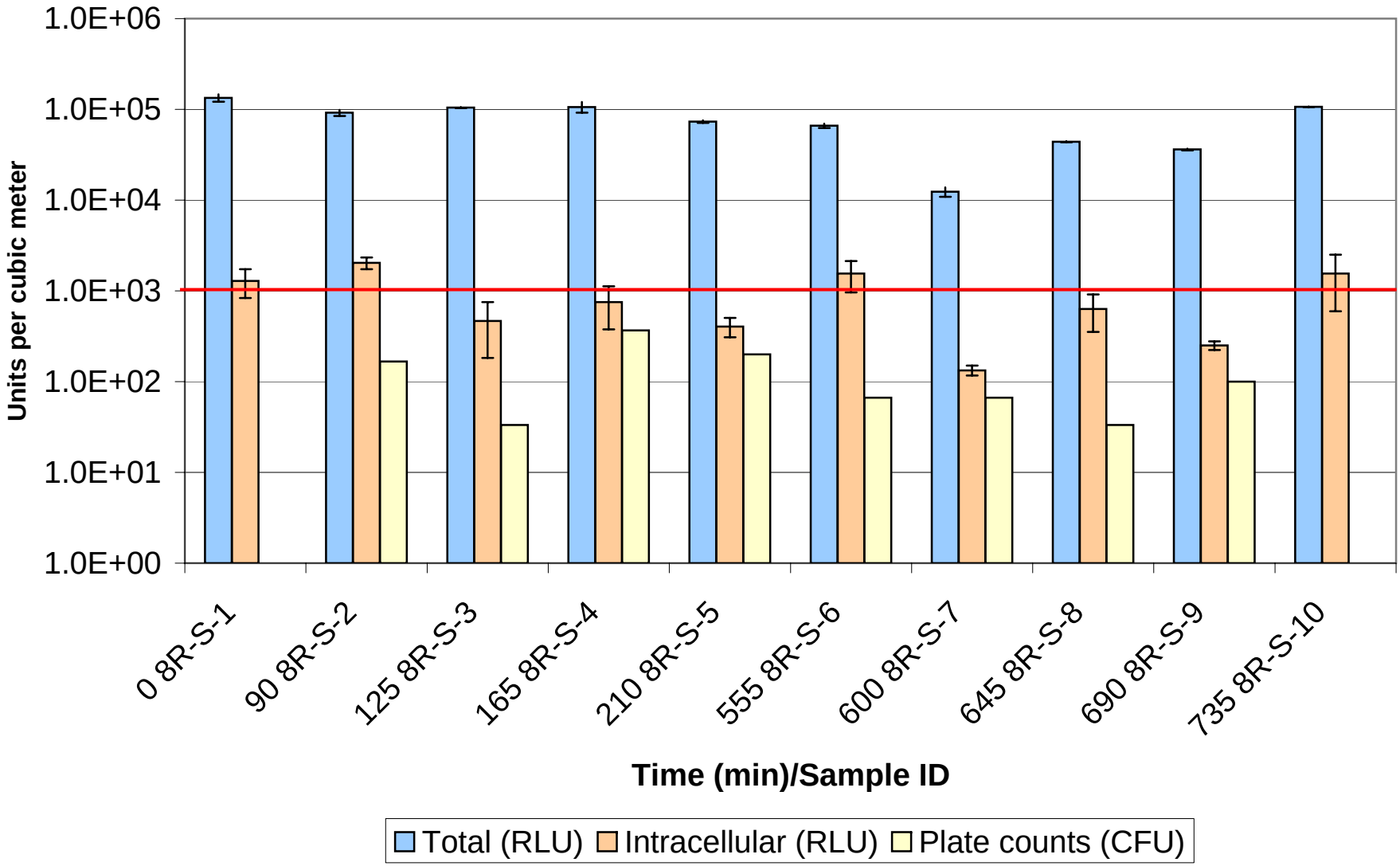


Figure 4.2.1-56 DNA Based Bacterial Quantification of Domestic Flights

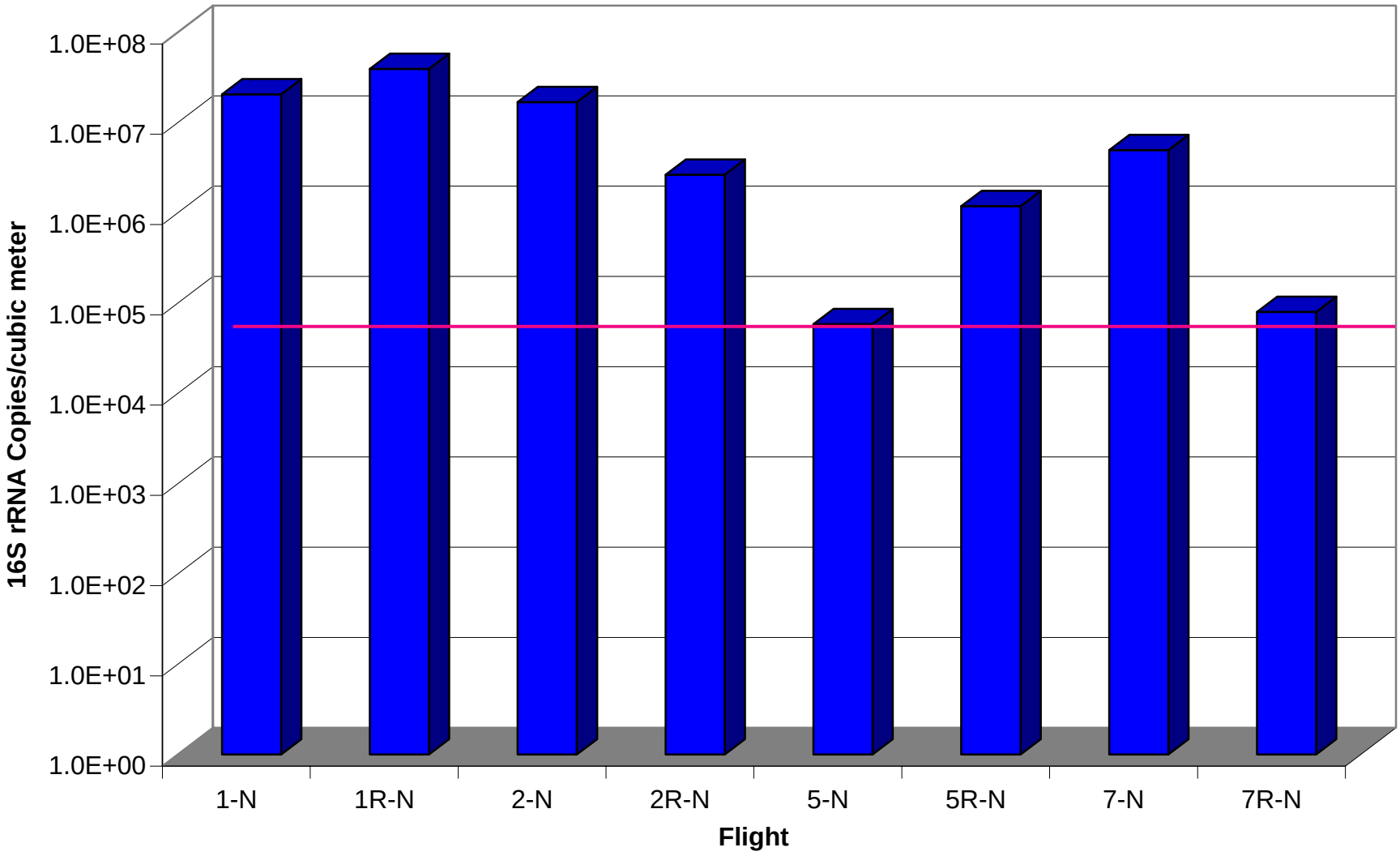


Figure 4.2.1-57 DNA Based Bacterial Quantification of International Flights

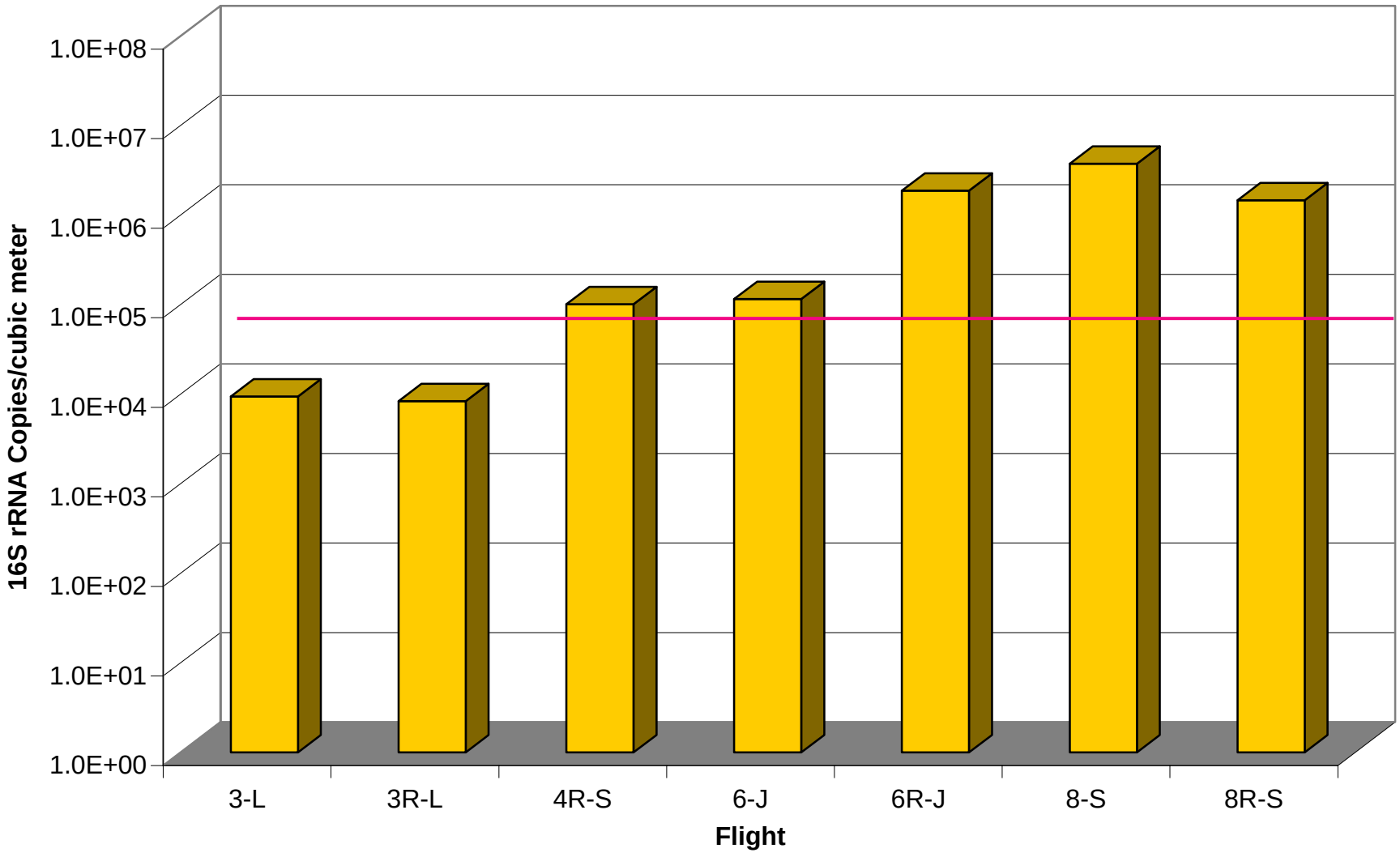


Table 2.2-1: Comparison of commonly utilized air sampling technologies

Sampler type	Method of collection	Strengths	Weaknesses
Impaction	Forced deposition of particles onto a solid or semi solid surface, typically agar media.	Excellent for isolating microbes known to grow on specific media.	Capable of isolating <1% of total biodiversity due to media bias.
Filtration	Collection of airborne particles on a porous material, typically a membrane filter.	Varying pore size of filters allows for concentration of particles of specific sizes.	Cell loss due to dehydration. Affected by airflow rate and properties of airborne particles (e.g. charge).
Electrostatic precipitation	Confers an electric charge onto particles causing them to drift onto appropriate collection substrates	Gentler means of bioaerosol collection, reduced cellular damage due to sampling process.	Possible differences in culturability of electrostatically precipitated microorganisms
Gravity sampling	Passive collection of particles due to fall out.	Inexpensive due to the absence of mechanized equipment.	Large bias in deposition due to particle size and shape, and the dynamics of surrounding air. Least accurate means of air collection.
Impingement	Deposition of particles into a liquid substrate, most often a buffer that can sustain biomolecular load for future use.	Commercially available, portable, samples are immediately available for biological analysis.	Varying sampling efficiency depending on sampler manufacturer, and biological targets.

Table 3.1-1. Summary of analyses performed on Phase II flight samples

Flight	Destination	Sample cartridges				Additional Samples	Cultivation			ATP	DNA Analysis							
		# of Regular Samplings	USC replicates (A)	JPL replicates (B)	Archived replicates (C)		Cultivation	Archiving	Identification		# of samples pooled	DNA extraction	PCR- signal	QPCR	Cloning	Sequencing	Phylogenetic Analysis	Samples for microarray
1-N	LAX to JFK	8	8	7		none	yes	yes	yes	yes	7	Done	+	X	X	X	X	X
1R-N	JFK to LAX	9	9	7		1,G	yes	yes	yes	yes	7	Done	+	X	X	X	X	X
2-N	LAX to JFK	6	6	5	4	G	yes	yes	yes	yes	5	Done	+	X	X	X	X	X
2R-N	JFK to LAX	7	7	7	6	G	yes	yes	yes	yes	7	Done	-	X				X
3-L	LAX to Lon	10	10	10	9	G	yes	yes	yes	yes	10	Done	-	X				X
3R-L	Lon to LAX	9	9	8	8	none	yes			yes	8	Done	-	X				X
4-S	LAX to Syd	9	9	18		none	Not applicable				18	Done	+	X	X	X	X	X
4R-S	Syd to LAX	10	10	10	6 (4)*	none	yes	yes	yes	yes	10	Done	+	X	X	X	X	X
5-N	LAX to JFK	5	5	4	4	C(2)	yes	yes	yes	yes	4	Done	+	X	X	X	X	X
5R-N	JFK to LAX	5	5	5	5	C(2)	yes	yes	yes	yes	4	Done	+	X	X	X	X	X
6-J	LAX to Tok	8	8	8	8	C,G, S*, swabs*	yes	yes	yes	yes	8	Done	+	X	X	X	X	X
6R-J	Tok to LAX	7	7	7	7	C,G, swabs*	yes	yes	yes	yes	7	Done	+	X	X	X	X	X
7-N	LAX to Tok	6	6	6	6	C	yes	yes	yes	yes	6	Done	+	X	X	X	X	X
7R-N	Tok to LAX	6	6	6	6	C(2)	yes	yes	yes	yes	6	Done	+	X	X	X	X	X
8-S	LAX to Tok	10	10	10	10	none	yes	yes	no	yes	10	Done	+	X	X	X	X	X
8R-S	Tok to LAX	10	10	10	10	none	yes	yes	no	yes	10	Done	+	X	X	X	X	X

*Used for viral nucleic acid optimization

1=1st class
G=Gasper
C=Coach
B=Between
S=Seat
* DNA extracted

no colonies

no clones

Tabe 3.3.5-1 Primer sets used to quantify DNA and RNA viruses from Phase II samples

Virus and Target Gene	Viral type	Primer name	Sequence (5'-3')	bp	PCR Concentration (µM)	Reference
Adenovirus *Hexon Gene*	**DNA**	Adenovirus AQ1F	GCCACGGTGGGGTTTCTAAACTT	23	0.5	Heim et al 2003
Generic for all five fully		Adenovirus AQ2R	GCCCCAGTGGTCTTACATGCACATC	25	0.5	Heim et al 2003
sequenced types.		Adenovirus probe	[fam]TGCACCAGACCCGGGCTCAGGTACTCCGA[bhq]		0.4	Heim et al 2003
Coronavirus	**RNA**	Coronavirus N229E-1F	CAGTCAAATGGGCTGATGCA	20	0.15	van Elden et al 2004
N gene		Coronavirus N229E-2R	AAAGGGCTATAAAGAGAATAAGGTATTCT	29	0.15	van Elden et al 2004
Specific for human strains		Coronavirus N229E-p probe	[fam]CCCTGACGACCACGTTGTGGTTCA[bhq]		0.05	van Elden et al 2004
229E and OC43		Coronavirus NOC43-1F	CGATGAGGCTATTCCGACTAGGT	23	0.45	van Elden et al 2004
		Coronavirus NOC43-2R	CCTTCCTGAGCCTTCAATATAGTAACC	27	0.45	van Elden et al 2004
		Coronavirus NOC43-p probe	[fam]TCCGCCTGGCACGGTACTCCCT[bhq]		0.1	van Elden et al 2004
Epstein-Barr Virus	**DNA**	Epstein-Barr Virus F	CCCTGTTTATCCGATGGAATG	21	0.2	Kimura et al 1999
BALF-5 Viral DNA		Epstein-Barr Virus R	CGGAAGCCCTCTGGACTTC	19	0.2	Kimura et al 1999
Polymerase Gene		Epstein-Barr virus probe	[fam]TGTACACGCACGAGAAATGCGCC[bhq]		0.1	Kimura et al 1999
Influenza A and B	**RNA**	Influenza A INFA-1F	GGACTGCAGCGTAGACGCTT	20	0.9	van Elden et al 2001
INF-A: *Matrix Protein Gene*		Influenza A INFA-2R	CATCCTGTTGTATATGAGGCCCAT	24	0.9	van Elden et al 2001
INF-B: *Hemagglutinin Gene*		Influenza A INFA-3R	CATTCTGTTGTATATGAGGCCCAT	24	0.9	van Elden et al 2001
		Influenza A INFA-probe	[fam]CTCAGTTATTCTGCTGGTGCACTTGCCA[bhq]		0.1	van Elden et al 2001
		Influenza B INFB-1F	AAATACGGTGGATTAAATAAAAGCAA	26	0.3	van Elden et al 2001
		Influenza B INFB-2R	CCAGCAATAGCTCCGAAGAAA	21	0.3	van Elden et al 2001
		Influenza B INFB-probe	[fam]CACCCATATTGGGCAATTTCCTATGGC[bhq]		0.1	van Elden et al 2001
Respiratory Syncytial Virus	**RNA**	Respiratory Syncytial Virus A21F	GCTCTTAGCAAAGTCAAGTTGAATGA	26	0.5	Hu et al 2003
N Gene		Respiratory Syncytial Virus A102R	TGCTCCGTTGGATGGTGTATT	21	0.5	Hu et al 2003
Specific for both subtypes		Respiratory Syncytial Virus B17F	GATGGCTCTTAGCAAAGTCAAGTTAA	26	0.5	Hu et al 2003
A and B.		Respiratory Syncytial Virus B120R	TGTCAATATTATCTCCTGTACTACGTTGAA	30	0.5	Hu et al 2003
		Respiratory Syncytial Virus APB48 probe	[fam]ACACTCAACAAAGATCAACTTCTGTCATCCAGC[bhq]		0.2	Hu et al 2003
		Respiratory Syncytial Virus BPB45 probe	[fam]TGATACATTAAATAAGGATCAGCTGCTGTCATCCA[bhq]		0.2	Hu et al 2003
Varicella-Zoster Virus	**DNA**	Varicella-Zoster Virus gene 63F	CGCGTTTTGTACTCCGGG	18	0.2	Cohrs et al 2001
Gene 63		Varicella-Zoster Virus gene 63AR	ACGGTTGATGTCCTCAACGAG	21	0.2	Cohrs et al 2001
Often in latent infections		Varicella-Zoster Virus gene 63A probe	[fam]TGGGAGATCCACCCGGCCAG[bhq]		0.2	Cohrs et al 2001

Table 3.5.1.1-1 Primer Sets Used for Aviation Phase II
PCR product used for clone libraries

Extraction	Sample ID	Primer Set		
		27F 1492R	27f.1 1492R	9bF 1406R
1	1N	+		
	1NR	+		
	BLANK 1	-	-	-
2	2N	+		
	2NR	-	-	-
	3L	-	-	-
	3LR	-	-	-
	BLANK 2/3	+		
3	4S	+		
	4SR	+		
	5N	+		
	5NR	+		
	BLANK 4/5	+		
4	6J	-	+	+
	6JR	-/+	+	+
	6J Seat Sweep	-/+	-/+	+
	6J Gasper Swab	-	-/+	+
	BLANK 6	-	-/+	+
5	7N	+		
	7NR	+		
	BLANK 7	+		
6	8S	-	+	+
	8SR	-	+	+

Table 4.1-1 Viral enumerations of air samples collected from Airline C

Sample	Flight	VZV copies/m^3	EBV copies/m^3	Coronavirus copies/m^3
2-N-1	Los Angeles to New York	0	0	0
2-N-2		0	0	0
2-N-3		218	0	0
2-N-4		0	0	0
2-N-5		0	0	0
2-N-6		0	0	0
2-N-Gasper		39	0	0
2R-N-1	New York to Los Angeles	592	0	68
2R-N-2		682	743	0
2R-N-3		1184	2	0
2R-N-4		553	0	0
2R-N-5		764	792	109
2R-N-6		49	130	0
2R-N-7		180	194	0
2R-N-Gasper		209	732	0
3-L-1	Los Angeles to London	0	0	0
3-L-2		0	0	0
3-L-3		443	0	0
3-L-4		45	0	0
3-L-5		0	0	0
3-L-6		0	0	0
3-L-7		0	0	0
3-L-8		0	0	0
3-L-9		26	0	0
3-L-Gasper		0	0	0
3R-L-1	London to Los Angeles	0	0	0
3R-L-2		0	0	0
3R-L-3		0	0	0
3R-L-4		378	4	0
3R-L-5		0	0	0
3R-L-6		0	99	0
3R-L-7		567	0	0
3R-L-8		94	0	0
3R-L-9		341	1586	0

Table 4.1-1 Viral enumerations of air samples collected from Airline C

Sample	Flight	VZV copies/m^3	EBV copies/m^3	Coronavirus copies/m^3
4-S-2	Los Angeles to Sydney	1550	175	23
4-S-3		342	709	0
4-S-4		688	0	148
4-S-5		920	536	1150
4-S-6		404	240	0
4-S-7		7373	403	14204
4-S-8		184	0	0
4-S-9		715	1506	0
4R-S-1	Sydney to Los Angeles	757	0	0
4R-S-2		791	0	0
4R-S-3		947	817	0
4R-S-4		888	0	0
4R-S-5		298	0	0
4R-S-6		612	280	0
4R-S-7		1818	537	0
4R-S-10		0	0	0
5-N-1	Los Angeles to New York	82	0	0
5-N-2		203	0	0
5-N-3		278	0	24
5-N-4		267	0	832
5-N-5		413	546	3665
5-N-coach1		502	639	0
5-N-coach2		8211	6	29205
5R-N-1	New York to Los Angeles	7290	455	6869
5R-N-2		7839	216	12710
5R-N-3		21466	1288	23897
5R-N-4		6766	1954	5076
5R-N-5		15664	1046	20372
5R-N-Coach		5775	0	19766

Table 4.1-1 Viral enumerations of air samples collected from Airline C

Sample	Flight	VZV copies/m^3	EBV copies/m^3	Coronavirus copies/m^3
6-J-1	Los Angeles to Tokyo	0	0	0
6-J-2		0	0	0
6-J-3		0	0	0
6-J-4		0	325	0
6-J-5		0	0	0
6-J-6		7644	0	0
6-J-7		31531	16	0
6-J-8		3303	0	0
6-J-Gasper		9801	0	0
6R-J-1	Tokyo to Los Angeles	806	0	0
6R-J-2		1810	162	0
6R-J-3		18091	92	14796
6R-J-4		58125	1269	6821
6R-J-5		4459	0	0
6R-J-6		5088	305	15549
6R-J-7		9764	14	47589
6R-J-Coach		0	0	0
6R-J-Gasper		19469	0	198975
7-N-1	Los Angeles to New York	2738	4	0
7-N-2		336	0	0
7-N-3		224	110	0
7-N-4		322	0	0
7-N-5		184	0	0
7-N-6		0	0	0
7-N-Coach		0	0	0

Table 4.1-1 Viral enumerations of air samples collected from Airline C

Sample	Flight	VZV copies/m^3	EBV copies/m^3	Coronavirus copies/m^3
7R-N-1	New York to Los Angeles	27	0	0
7R-N-2		43	0	0
7R-N-3		85	0	0
7R-N-4		0	0	0
7R-N-5		0	0	0
7R-N-6		0	0	0
7R-N-Coach1		0	0	0
7R-N-Coach2		0	0	0
8-S-1	Los Angeles to Sydney	0	0	0
8-S-2		0	0	0
8-S-3		0	0	0
8-S-4		0	0	0
8-S-5		0	0	0
8-S-6		0	0	0
8-S-7		0	0	0
8-S-8		0	0	0
8-S-9		0	0	0
8-S-10		0	0	0
8R-S-1	Sydney to Los Angeles	0	0	0
8R-S-2		8012	1124	0
8R-S-3		13836	0	0
8R-S-4		0	0	0
8R-S-5		0	0	0
8R-S-6		0	0	0
8R-S-7		0	0	0
8R-S-8		0	0	0
8R-S-9		0	0	0
8R-S-10		0	0	0
8R-S-Coach		0	0	0

Table 4.2.1-1 Culture dependent and independent microbial quantification, Chicago to Seattle (4-13-04)

Sample*	Time (PST)	Note	Total heterotrophs CFU/m^3	Intracellular ATP RLU/m^3	Total ATP RLU/m^3	QPCR copies/m^3
1-G-PB	10:02 AM	Gasper	ND	0	2.19E+05	5.24E+04
1-G-CA	11:00 AM	Gasper	ND	0	1.05E+05	
1-G-M	11:50 AM	Gasper	ND	0	9.84E+04	
1-G-PD	1:53 PM	Gasper	ND	0	8.60E+04	
1-G-L	2:29 PM	Gasper	ND	0	0	
1-L-PB	9:43 AM	Lavatory	ND	1.51E+06	2.08E+06	
1-L-CA	10:50 AM	Lavatory	ND	1.14E+06	2.00E+06	
1-L-M	12:15 PM	Lavatory	ND	0	1.78E+05	
1-L-PD	1:38 PM	Lavatory	ND	0	2.19E+05	
1-L-L	2:42 PM	Lavatory	ND	0	0	
1-SH-PB	9:55 AM	Seat height	ND	2.38E+03	5.22E+05	
1-SH-CA	11:10 AM	Seat height	ND	0	1.14E+05	
1-SH-M	11:55 AM	Seat height	ND	0	3.20E+05	
1-SH-PD	2:05 PM	Seat height	ND	0	2.05E+05	
1-SH-L	2:23 PM	Seat height	ND	0	1.20E+05	
1-SF-PB	10:10 AM	Seat floor level	ND	0	3.21E+05	
1-SF-CA	11:20 AM	Seat floor level	ND	0	9.94E+04	
1-SF-M	12:05 PM	Seat floor level	ND	0	9.45E+04	
1-SF-PD	1:45 PM	Seat floor level	ND	0	1.52E+05	
1-SF-L	2:35 PM	Seat floor level	ND	0	7.28E+04	

*Suffixes: PB; preboard, M; midflight, CA; cruise altitude, PD; predescent, L;after landing

Table 4.2.1-2 Culture dependent and independent microbial quantification, Seattle to Dallas 4-14-04

Sample*	Time (PST)	Note	Total heterotrophs CFU/m^3	Intracellular ATP RLU/m^3	Total ATP RLU/m^3	QPCR copies/m^3
2-G-PB	6:00 PM	Gasper	ND	0	8.00E+03	1.20E+07
2-G-CA	7:04 PM	Gasper	ND	0	1.32E+05	
2-G-M	8:14 PM	Gasper	ND	0	5.08E+04	
2-G-PD	8:50 PM	Gasper	ND	0	2.11E+04	
2-G-2-L	9:51 PM	Gasper	ND	0	1.00E-22	
2-L-PB	5:40 PM	Lavatory	ND	0	2.42E+05	
2-L-CA	6:47 PM	Lavatory	ND	0	5.92E+04	
2-L-M	8:21 PM	Lavatory	ND	0	5.83E+04	
2-L-PD	9:20 PM	Lavatory	ND	0	1.18E+05	
2-L-L	10:00 PM	Lavatory	ND	0	5.41E+04	
2-SH-PB	5:50 PM	Seat height	ND	0	7.19E+04	
2-SH-CA	6:40 PM	Seat height	ND	0	2.24E+05	
2-SH-M	8:07 PM	Seat height	ND	0	4.75E+04	
2-SH-PD	9:00 PM	Seat height	ND	0	4.52E+05	
2-SH-2-L	9:45 PM	Seat height	ND	0	5.98E+04	
2-SF-PB	6:07 PM	Seat floor level	ND	0	2.25E+05	
2-SF-CA	7:12 PM	Seat floor level	ND	1.42E+06	2.10E+06	
2-SF-M	8:00 PM	Seat floor level	ND	0	2.10E+04	
2-SF-PD	9:08 PM	Seat floor level	ND	0	1.00E-22	
2-SF-2-L	10:08 PM	Seat floor level	ND	0	2.37E+05	

*PB: preboard, M: midflight, CA: cruise altitude, PD: predescent, L: after landing

Table 4.2.1-3 Culture dependent and independent microbial quantification, Salt Lake City to Cincinnati 4-21-04

Sample*	Time (PST)	Note	Total heterotrophs CFU/m^3	Intracellular ATP RLU/m^3	Total ATP RLU/m^3	QPCR copies/m^3
3-G-PB	1:40 PM	Gasper	55000	3.87E+04	2.37E+05	
3-G-M	6:00 PM	Gasper	183333	2.99E+05	4.54E+05	
3-L-PB	1:26 PM	Lavatory	1967	0	1.61E+05	
3-L-M	3:00 PM	Lavatory	8667	3.63E+05	6.45E+05	
3-SH-PB	1:35 PM	Seat height	600	2.53E+03	6.13E+04	
3-SH-CA	2:20 PM	Seat height	7833	5.00E+03	1.14E+05	
3-SH-M (2:45)	2:45 PM	Seat height	276667	1.91E+05	3.86E+05	
3-SH-M (3:05)	3:05 PM	Seat height	6833	6.00E+04	2.17E+05	3.16E+08
3-SH-M (3:25)	3:25 PM	Seat height	500	0	5.85E+04	
3-SH-M (3:45)	3:45 PM	Seat height	4533	4.30E+03	3.35E+04	
3-SH-M (4:10)	4:10 PM	Seat height	567	0	6.15E+04	
3-SH-M (4:30)	4:30 PM	Seat height	33	0	5.00E+04	
3-SH-PD	4:50 PM	Seat height	0	0	5.76E+04	
3-SH-L	5:15 PM	Seat height	67	0	1.91E+05	
3-SF-PB	1:50 PM	Seat floor level	16000	3.44E+05	6.89E+05	

*PB: preboard, M: midflight, CA: cruise altitude, PD: predescent, L: after landing

Table 4.2.1-4 Culture dependent and independent microbial quantification, Cincinnati to Salt Lake City 4-21-04

Sample*	Time (PST)	Note	Total heterotrophs CFU/m^3	Intracellular ATP RLU/m^3	Total ATP RLU/m^3	QPCR copies/m^3
4-G-PB	5:13 AM	Gasper	32000	4.06E+04	2.76E+05	4.82E+06
4-G-CA	6:14 AM	Gasper	423333	3.29E+05	7.03E+05	
4-L-PB	4:50 AM	Lavatory	300	0	5.91E+04	
4-L-CA	6:05 AM	Lavatory	13667	6.42E+04	2.85E+05	
4-L-M	7:25 AM	Seat height	16833	1.40E+05	3.21E+05	
4-L-PD	8:26 AM	Seat height	933	0	1.75E+05	
4-L-L	9:27 AM	Seat height	19333	2.55E+04	3.44E+05	
4-SH-PB	5:07 AM	Seat height	64167	9.80E+04	2.58E+05	
4-SH-CA	6:21 AM	Seat height	12000	9.53E+03	8.89E+04	
4-SH-M	7:32 AM	Seat height	**433**	**0**	**6.03E+04**	
4-SH-PD	8:37 AM	Seat height	12667	8.04E+04	3.02E+05	
4-SH-L	9:20 AM	Seat height	12667	8.20E+05	9.53E+05	
4-SF-PB	4:58 AM	Seat height	1533	0	4.88E+04	
4-SF-CA	6:30 AM	Seat height	156667	2.44E+05	9.38E+05	

*PB: preboard, M: midflight, CA: cruise altitude, PD: predescent, L: after landing

Table 4.2.1-5 Average total viable (intracellular ATP) versus cultivable (heterotrophic plate count) bioburden of phase I samples

Locations	Viable microbial population as estimated by intracellular ATP for Airline # - flight #:				Cultivable counts as estimated by R2A for Airline # - flight #:		% cultivable microbes for Airline # - flight #:	
	A-1	A-2	B-3	B-4	B-3	B-4	B-3	B-4
Gasper	BDL [n=5]	BDL [n=5]	$1.7x10^5$ [n=2]	$1.2x10^5$ [n=3]	$1.2x10^5$ [n=2]	$1.5x10^5$ [n=3]	70.6	125
Lavatory	$5.2x10^5$ [n=5]	BDL [n=5]	$2.1x10^6$ [n=2]	$4.6x10^4$ [n=5]	$3.2x10^4$ [n=2]	$1.0x10^4$ [n=5]	1.52	21.7
Seat Floor	BDL [n=5]	$2.8x10^5$ [n=5]	$3.4x10^5$ [n=1]	$1.2x10^5$ [n=2]	$1.6x10^4$ [n=1]	$8.0x10^4$ [n=2]	5.0	75.0
Seat Height	BDL [n=5]	BDL [n=5]	$2.4x10^4$ [n=10]	$2.0x10^5$ [n=5]	$2.8x10^4$ [n=10]	$2x10^4$ [n=5]	117	10.0
Total # of samples	20	20	15	15	15	15		

Table 4.2.1-5a. Percentage of viable microbial population[1] of various air samples collected from several locations of a commercial airlines (Chicago to Seattle; April 13 2004)

Description of the samples	Time collected (Pacific Standard Time)	Location of sampling	Percentage of microbial population that are: viable (B/A*100)
Pre-Board			
	10:02 AM	Gasper	-
	9:43 AM	Lavatory	71.4
	9:55 AM	Seat height	0.5
	10:10 AM	Seat Floor	-
Upon reaching cruise altitude			
	11:00 AM	Gasper	-
	10:50 AM	Lavatory	55.0
	11:10 AM	Seat height	-
	11:20 AM	Seat Floor	-
Half-way through flight			
	11:50 AM	Gasper	-
	12:15 PM	Lavatory	-
	11:55 AM	Seat height	-
	12:05 PM	Seat Floor	-
Just before beginning descent			
	1:53 PM	Gasper	-
	1:38 PM	Lavatory	-
	2:05 PM	Seat height	-
	1:45 PM	Seat Floor	-
After landing			
	2:29 PM	Gasper	ND
	2:42 PM	Lavatory	-
	2:23 PM	Seat height	-
	2:35 PM	Seat Floor	-

[1]All values are per 100 L of air

Table 4.2.1-5b. Percentage of viable microbial population[1] of various air samples collected from several locations of a commercial airlines (Seattle to Dallas FW; April 13 2004)

Description of the samples	Time collected (Pacific Standard Time)	Location of sampling	Percentage of microbial population that are: viable (B/A*100)
Pre-Board			
	6:00 PM	Gasper	-
	5:40 PM	Lavatory	-
	5:50 PM	Seat height	-
	6:07 PM	Seat Floor	-
Upon reaching cruise altitude			
	7:04 PM	Gasper	-
	6:47 PM	Lavatory	-
	6:40 PM	Seat height	-
	7:12 PM	Seat Floor	66.7
Half-way through flight			
	8:14 PM	Gasper	-
	8:21 PM	Lavatory	-
	8:07 PM	Seat height	-
	8:00 PM	Seat Floor	-
Just before beginning descent			
	8:50 PM	Gasper	-
	9:20 PM	Lavatory	-
	9:00 PM	Seat height	-
	9:08 PM	Seat Floor	-
After landing			
	9:51 PM	Gasper	ND
	10:00 PM	Lavatory	-
	9:45 PM	Seat height	-
	10:08 PM	Seat Floor	-

[1]All values are per 100 L of air

Table 4.2.1-5c. Percentage of viable and cultivable microbial population[1] of various air samples collected from several locations of commercial airlines (Salt Lake City to Cincinnati; April 21st 2004)

Description of the samples	Time collected (Pacific Standard Time)	Location of sampling	Percentage of microbial population that are:	
			viable (B/A*100)	cultivable (C/B*100)
Pre-Board				
	1:40 PM	Gasper	16.3	142.1
	1:26 PM	Lavatory	-	>CFU
	1:35 PM	Seat height	4.1	23.7
	1:50 PM	Seat Floor	49.9	4.7
Upon reaching cruise altitude				
	2:20 PM	Seat height	4.4	156.7
Half-way through flight				
	6:00 PM	Gasper	65.8	61.4
	3:00 PM	Lavatory	56.3	2.4
	2:45 PM	Seat height	49.5	144.7
	3:05 PM	Seat height	27.6	11.4
	3:25 PM	Seat height	-	>CFU
	3:45 PM	Seat height	12.8	105.4
	4:10 PM	Seat height	-	>CFU
	4:30 PM	Seat height	-	>CFU
Just before beginning descent				
	4:50 PM	Seat height	-	-
After landing				
	5:15 PM	Seat height	-	>CFU

Table 4.2.1-5d. Percentage of viable and cultivable microbial population[1] of various air samples collected from several locations of commercial airlines (Cincinnati to Salt Lake City; April 22nd 2004)

Description of the samples	Time collected (Pacific Standard Time)	Location of sampling	Percentage of microbial population that are:	
			viable (B/A*100)	cultivable (C/B*100)
Pre-Board				
	5:13 AM	Gasper	14.8	80.0
	4:50 AM	Lavatory	-	>CFU
	5:07 AM	Seat height	37.7	65.3
	6:30 AM	Seat Floor	-	>CFU
Upon reaching cruise altitude				
	6:14 AM	Gasper	47.1	127.3
	6:05 AM	Lavatory	22.1	21.9
	6:21 AM	Seat height	10.7	126.3
	6:30 AM	Seat Floor	25.8	66.7
Half-way through flight				
	7:00 PM	Gasper	68.2	60.0
	7:25 AM	Lavatory	43.8	12.1
	7:32 AM	Seat height	-	>CFU
Just before beginning descent				
	8:26 AM	Lavatory	-	>CFU
	8:37 AM	Seat height	25.0	16.3
After landing				
	9:27 AM	Lavatory	73.5	76.0
	9:20 AM	Seat height	86.3	1.6

[1]All values are per 100 L of air

Table 4.2.1-6 Average Phase II culture dependent and independent microbial quantification values [chronological]

Flight	Route	Date	Total heterotrophs CFU/m^3	Intracellular ATP RLU/m^3	Total ATP RLU/m^3	QPCR copies/m^3
1-N	Los Angeles/New York	11/14/05	43	3.33E+02	2.07E+04	2.06E+07
1R-N	New York/Los Angeles	11/17/05	248	1.28E+02	2.80E+04	3.95E+07
2-N	Los Angeles/New York	12/05/05	40	2.47E+03	7.70E+04	1.70E+07
2R-N	New York/Los Angeles	12/08/05	0	3.29E+02	3.73E+04	2.66E+06
3-L	Los Angeles/London	01/15/06	91	2.56E+02	3.88E+04	9.43E+03
3R-L	London/Los Angeles	01/20/06	0	1.64E+03	2.16E+04	8.40E+03
4-S	Los Angeles/Sydney	02/12/06	ND	ND	ND	ND
4R-S	Sydney/Los Angeles	02/17/06	53	3.67E+01	1.11E+05	1.02E+05
5-N	Los Angeles/New York	3/7/06	50	1.64E+02	5.47E+04	5.88E+04
5R-N	New York/Los Angeles	3/10/06	27	0.00E+00	2.89E+04	1.19E+06
6-J	Los Angeles/Tokyo	4/3/06	67	9.31E+01	5.90E+04	1.16E+05
6R-J	Tokyo/Los Angeles	4/7/06	72	3.38E+02	9.28E+04	1.87E+06
7-N	Los Angeles/New York	4/24/06	6	5.61E+02	4.07E+04	4.99E+06
7R-N	New York/Los Angeles	4/27/06	53	2.46E+03	2.45E+04	8.04E+04
8-S	Los Angeles/Sydney	5/7/06	363	1.27E+03	5.10E+04	3.76E+06
8R-S	Sydney/Los Angeles	5/12/06	103	9.06E+02	7.75E+04	1.46E+06

Table 4.2.1-7 Phase II culture dependent and independent microbial quantification ranges [chronological]

Flight	Route	Date	Total heterotrophs CFU/m^3	Intracellular ATP RLU/m^3	Total ATP RLU/m^3	QPCR copies/m^3
1-N	Los Angeles/New York	11/14/05	0 - 233	0 - 4.86×10^2	8.74×10^3 - 6.06×10^4	ND
1R-N	New York/Los Angeles	11/17/05	0 - 1200	0 - 4.33×10^2	1.48×10^4 - 8.09×10^4	ND
2-N	Los Angeles/New York	12/05/05	0 - 100	0 - 8×10^3	1.33×10^4 - 1.32×10^5	ND
2R-N	New York/Los Angeles	12/08/05	0	0 - 5×10^1	1.32×10^4 - 6.48×10^4	ND
3-L	Los Angeles/London	01/15/06	0 - 833	6.67×10^1 - 2.33×10^2	9.93×10^3 - 7.79×10^4	ND
3R-L	London/Los Angeles	01/20/06	0	5.11×10^2 - 6.72×10^2	8.72×10^3 - 4.33×10^4	ND
4-S	Los Angeles/Sydney	02/12/06	ND	ND	ND	ND
4R-S	Sydney/Los Angeles	02/17/06	0 - 233	0 - 3.67×10^2	2.66×10^4 - 3.67×10^5	ND
5-N	Los Angeles/New York	3/7/06	0 - 67	2.22×10^1 - 2.89×10^2	4.89×10^4 - 6.12×10^4	ND
5R-N	New York/Los Angeles	3/10/06	0 - 67	0	1.42×10^4 - 5.07×10^4	ND
6-J	Los Angeles/Tokyo	4/3/06	0 - 400	0 - 3.78×10^2	3.43×10^4 - 9.66×10^4	ND
6R-J	Tokyo/Los Angeles	4/7/06	0 - 367	0 - 2.37×10^3	4.58×10^4 - 1.78×10^5	ND
7-N	Los Angeles/New York	4/24/06	0 - 33	2.22×10^2 - 1.32×10^3	2.88×10^4 - 5.88×10^4	ND
7R-N	New York/Los Angeles	4/27/06	0 - 100	0 - 1.23×10^4	7.31×10^3 - 4.64×10^4	ND
8-S	Los Angeles/Sydney	5/7/06	0 - 2700	6.67×10^1 - 3.24×10^3	1.47×10^4 - 9.96×10^4	ND
8R-S	Sydney/Los Angeles	5/12/06	0 - 367	1.33×10^2 - 2.03×10^3	1.24×10^4 - 1.34×10^5	ND

Table 4.2.1-8 Phase II culture dependent and independent microbial quantification ranges [Domestic vs International]

Flight	Route	Date	Total heterotrophs	Intracellular ATP	Total ATP	QPCR
			CFU/m^3	RLU/m^3	RLU/m^3	copies/m^3
1-N	Los Angeles/New York	11/14/05	43	3.33E+02	2.07E+04	2.06E+07
1R-N	New York/Los Angeles	11/17/05	248	1.28E+02	2.80E+04	3.95E+07
2-N	Los Angeles/New York	12/05/05	40	2.47E+03	7.70E+04	1.70E+07
2R-N	New York/Los Angeles	12/08/05	0	3.29E+02	3.73E+04	2.66E+06
5-N	Los Angeles/New York	03/07/06	50	1.64E+02	5.47E+04	5.88E+04
5R-N	New York/Los Angeles	03/10/06	27	0	2.89E+04	1.19E+06
7-N	Los Angeles/New York	04/24/06	6	5.61E+02	4.07E+04	4.99E+06
7R-N	New York/Los Angeles	04/27/06	53	2.46E+03	2.45E+04	8.04E+04
3-L	Los Angeles/London	01/15/06	91	2.56E+02	3.88E+04	9.43E+03
3R-L	London/Los Angeles	01/20/06	0	1.64E+03	2.16E+04	8.40E+03
4-S	Los Angeles/Sydney	02/12/06	ND	ND	ND	ND
4R-S	Sydney/Los Angeles	02/17/06	53	3.67E+01	1.11E+05	1.02E+05
6-J	Los Angeles/Tokyo	04/03/06	67	9.31E+01	5.90E+04	1.16E+05
6R-J	Tokyo/Los Angeles	04/07/06	72	3.38E+02	9.28E+04	1.87E+06
8-S	Los Angeles/Sydney	05/07/06	363	1.27E+03	5.10E+04	3.76E+06
8R-S	Sydney/Los Angeles	05/12/06	103	9.06E+02	7.75E+04	1.46E+06

Table 4.2.1-9 Phase II culture dependent and independent microbial quantification ranges [Domestic vs International]

Flight	Route	Date	Total heterotrophs	Intracellular ATP	Total ATP	QPCR
			CFU/m^3	RLU/m^3	RLU/m^3	copies/m^3
1-N	Los Angeles/New York	11/14/05	0 - 233	0 - $4.86x10^2$	$8.74x10^3$ - $6.06x10^4$	ND
1R-N	New York/Los Angeles	11/17/05	0 - 1200	0 - $4.33x10^2$	$1.48x10^4$ - $8.09x10^4$	ND
2-N	Los Angeles/New York	12/05/05	0 - 100	0 - $8x10^3$	$1.33x10^4$ - $1.32x10^5$	ND
2R-N	New York/Los Angeles	12/08/05	0	0 - $5x10^1$	$1.32x10^4$ - $6.48x10^4$	ND
5-N	Los Angeles/New York	03/07/06	0 - 67	$2.22x10^1$ - $2.89x10^2$	$4.89x10^4$ - $6.12x10^4$	ND
5R-N	New York/Los Angeles	03/10/06	0 - 67	0	$1.42x10^4$ - $5.07x10^4$	ND
7-N	Los Angeles/New York	04/24/06	0 - 33	$2.22x10^2$ - $1.32x10^3$	$2.88x10^4$ - $5.88x10^4$	ND
7R-N	New York/Los Angeles	04/27/06	0 - 100	0 - $1.23x10^4$	$7.31x10^3$ - $4.64x10^4$	ND
3-L	Los Angeles/London	01/15/06	0 - 833	$6.67x10^1$ - $2.33x10^2$	$9.93x10^3$ - $7.79x10^4$	ND
3R-L	London/Los Angeles	01/20/06	0	$5.11x10^2$ - $6.72x10^2$	$8.72x10^3$ - $4.33x10^4$	ND
4-S	Los Angeles/Sydney	02/12/06	ND	ND	ND	ND
4R-S	Sydney/Los Angeles	02/17/06	0 - 233	0 - $3.67x10^2$	$2.66x10^4$ - $3.67x10^5$	ND
6-J	Los Angeles/Tokyo	04/03/06	0 - 400	0 - $3.78x10^2$	$3.43x10^4$ - $9.66x10^4$	ND
6R-J	Tokyo/Los Angeles	04/07/06	0 - 367	0 - $2.37x10^3$	$4.58x10^4$ - $1.78x10^5$	ND
8-S	Los Angeles/Sydney	05/07/06	0 - 2700	$6.67x10^1$ - $3.24x10^3$	$1.47x10^4$ - $9.96x10^4$	ND
8R-S	Sydney/Los Angeles	05/12/06	0 - 367	$1.33x10^2$ - $2.03x10^3$	$1.24x10^4$ - $1.34x10^5$	ND

Table 4.2.1-10 Culture dependent and independent microbial quantification, Los Angeles to New York 11/14/05

Sample	Time (PST)	Note	Total heterotrophs CFU/m^3	Intracellular ATP RLU/m^3	Total ATP RLU/m^3	QPCR copies/m^3
1-N-1	11:25 AM	boarding	233	2.19E+02	6.06E+04	
1-N-2	12:07 AM		0	0.00E+00	3.08E+04	
1-N-3	12:47 AM		33	3.69E+02	1.19E+04	
1-N-4	1:27 PM		0	4.36E+02	1.27E+04	2.06E+07
1-N-5	2:07 PM		0	4.03E+02	9.58E+03	
1-N-6	2:47 PM		33	4.19E+02	1.04E+04	
1-N-7	3:27 PM		0	4.86E+02	8.74E+03	
1-N-8	4:20 PM	de-planing, virus				
AVERAGE*			**43**	**3.33E+02**	**2.07E+04**	

*Business class samples only

Table 4.2.1-11 Culture dependent and independent microbial quantification, New York to Los Angeles 11/17/05

Sample	Time (PST)	Note	Total heterotrophs CFU/m^3	Intracellular ATP RLU/m^3	Total ATP RLU/m^3	QPCR copies/m^3
1-N-1	4:05 PM	boarding	ND	1.00E+02	4.24E+04	
1-N-2	5:21 PM		67	3.22E+02	1.48E+04	
1-N-3	6:07 PM		1200	0.00E+00	8.09E+04	
1-N-4	6:47 PM		33	3.30E+01	1.92E+04	
1-N-5	7:27 PM		400	0.00E+00	1.67E+04	3.95E+07
1-N-6	8:07 PM		33	1.83E+02	2.13E+04	
1-N-7	8:47 PM		0	ND	ND	
1-N-8	10:07 PM		0	0.00E+00	3.06E+04	
1-N-9	11:00 PM	de-planing, virus				
1st Class	5:50 PM		0	4.33E+02	2.72E+04	
Gasper	9:20 PM		67	3.33E+02	3.82E+04	
AVERAGE*			**248**	**1.28E+02**	**2.80E+04**	

*Business class samples only

ND: Not measured due to low sample volume

Table 4.2.1-12 Culture dependent and independent microbial quantification, Los Angeles to New York 12/5/05

Sample	Time (PST)	Note	Total heterotrophs CFU/m^3	Intracellular ATP RLU/m^3	Total ATP RLU/m^3	QPCR copies/m^3
2-N-1	11:10 AM	Boarding, virus				
2-N-2	1:05 PM		33	0.00E+00	1.05E+05	1.70E+07
2-N-3	1:50 PM		67	8.07E+03	7.18E+04	
2-N-4	2:30 PM		0	1.89E+03	1.32E+05	
2-N-5	3:10 PM		100	2.39E+03	3.81E+04	
2-N-6	3:45 PM		0	0.00E+00	3.78E+04	
Gasper	2:55 PM		33	0.00E+00	1.33E+04	
AVERAGE*			**40**	**2.47E+03**	**7.70E+04**	

*Business class samples only

Table 4.2.1-13 Culture dependent and independent microbial quantification, New York to Los Angeles 12/8/05

Sample	Time (PST)	Note	Total heterotrophs CFU/m^3	Intracellular ATP RLU/m^3	Total ATP RLU/m^3	QPCR copies/m^3
5:15 PM	2R-N-1		0	1.94E+03	5.06E+04	2.66E+06
5:45 PM	2R-N-2		0	5.00E+01	6.48E+04	
6:30 PM	2R-N-3		0	0.00E+00	3.90E+04	
7:10 PM	2R-N-4		0	2.83E+02	5.00E+04	
8:15 PM	2R-N-5		0	0.00E+00	1.32E+04	
8:40 PM	2R-N-6		0	0.00E+00	1.66E+04	
9:15 PM	2R-N-7		0	2.78E+01	2.68E+04	
AVERAGE*			**0**	**3.29E+02**	**3.73E+04**	

*Business class samples only

Table 4.2.1-14 Culture dependent and independent microbial quantification, Los Angeles to London 1/15/06

Sample	Time (PST)	Note	Total heterotrophs CFU/m^3	Intracellular ATP RLU/m^3	Total ATP RLU/m^3	QPCR copies/m^3
3-L-1	4:50 PM	boarding	0	7.56E+02	5.36E+04	9.43E+03
3-L-2	6:10 PM		0	3.33E+02	4.70E+04	
3-L-3	7:00 PM	Dinner	0	2.33E+02	7.79E+04	
3-L-4	8:00 PM		0	0.00E+00	2.40E+04	
3-L-5	9:00 PM		33	8.89E+01	2.66E+04	
3-L-6	10:00 PM		0	6.67E+01	4.20E+04	
3-L-7	11:00 PM		33	2.67E+02	2.17E+04	
3-L-8	12:00 AM		0	6.67E+01	9.93E+03	
3-L-9	1:00 AM		833	6.00E+02	7.13E+04	
3-L-10	2:00 AM		0	1.33E+02	1.10E+04	
Gasper	1:30 AM		100	2.68E+02	4.16E+04	
AVERAGE*			**91**	**2.56E+02**	**3.88E+04**	

*Business class samples only

Table 4.2.1-15 Culture dependent and independent microbial quantification, London to Los Angeles 1/20/06

Sample	Time (PST)	Note	Total heterotrophs CFU/m^3	Intracellular ATP RLU/m^3	Total ATP RLU/m^3	QPCR copies/m^3
3R-L-1	3:30 AM	boarding				
3R-L-2	4:00 AM		0	8.89E+03	3.13E+04	8400.7
3R-L-3	5:00 AM	lunch	0	1.09E+03	2.14E+04	
3R-L-4	6:00 AM		0	4.39E+02	1.54E+04	
3R-L-5	7:00 AM		0	2.89E+02	1.92E+04	
3R-L-6	8:00 AM		0	5.11E+02	8.72E+03	
3R-L-7	9:00 AM		0	1.07E+03	2.24E+04	
3R-L-8	10:00 AM		0	2.00E+02	1.12E+04	
3R-L-9	2:00 PM		0	6.72E+02	4.33E+04	
AVERAGE*			**0**	**1.64E+03**	**2.16E+04**	

*Business class samples only

Table 4.2.1-16 Culture dependent and independent microbial quantification, Los Angeles to Sydney 2/12/06

Sample	Time (PST)	Note	Total heterotrophs CFU/m^3	Intracellular ATP RLU/m^3	Total ATP RLU/m^3	QPCR copies/m^3
4-S-1	9:30 PM	boarding	ND	ND	ND	ND
4-S-2	11:30 PM		ND	ND	ND	
4-S-3	12:40 PM	Dinner	ND	ND	ND	
4-S-4	1:38 AM		ND	ND	ND	
4-S-5	2:30 AM		ND	ND	ND	
4-S-6	8:00 AM		ND	ND	ND	
4-S-7	11:00 AM		ND	ND	ND	
4-S-8	12:10 PM	Breakfast	ND	ND	ND	
4-S-9	1:25 PM		ND	ND	ND	
4-S-10	2:10 PM		ND	ND	ND	

Table 4.2.1-17 Culture dependent and independent microbial quantification, Sydney to Los Angeles 2/17/06

Sample	Time (PST)	Note	Total heterotrophs CFU/m^3	Intracellular ATP RLU/m^3	Total ATP RLU/m^3	QPCR copies/m^3
4R-S-1	8:45 PM	boarding	33	0.00E+00	1.33E+05	1.02E+05
4R-S-2	10:30 PM		0	0.00E+00	1.32E+05	
4R-S-3	12:05 AM		33	0.00E+00	1.29E+05	
4R-S-4	1:00 AM		67	0.00E+00	1.02E+05	
4R-S-5	6:00 AM		233	0.00E+00	6.52E+04	
4R-S-6	7:00 AM		0	0.00E+00	3.86E+04	
4R-S-7	8:00 AM		33	3.67E+02	2.66E+04	
4R-S-8	8:45 AM		0	0.00E+00	3.67E+05	
4R-S-9	9:30 AM		133	0.00E+00	6.83E+04	
4R-S-10			0	0.00E+00	5.35E+04	
AVERAGE*			**53**	**3.67E+01**	**1.11E+05**	

*Business class samples only

Table 4.2.1-18 Culture dependent and independent microbial quantification, Los Angeles to New York 3/7/06

Sample	Time (PST)	Note	Total heterotrophs CFU/m^3	Intracellular ATP RLU/m^3	Total ATP RLU/m^3	QPCR copies/m^3
5-N-1	11:00 AM	Boarding viral only				
5-N-2	11:54 AM		0	2.22E+01	5.95E+04	5.88E+04
5-N-3	12:50 PM	Before lunch	67	2.89E+02	6.12E+04	
5-N-4	2:00 PM	After lunch	67	2.22E+02	4.93E+04	
5-N-5	3:00 PM		67	1.22E+02	4.89E+04	
Coach	2:30 PM	swabs also perforr	133	3.33E+02	1.23E+05	
AVERAGE*			**50**	**1.64E+02**	**5.47E+04**	

*Business class samples only

Table 4.2.1-19 Culture dependent and independent microbial quantification, New York to Los Angeles 3/10/06

Sample	Time (PST)	Note	Total heterotrophs CFU/m^3	Intracellular ATP RLU/m^3	Total ATP RLU/m^3	QPCR copies/m^3
5R-N-1	2:00 PM	Before lunch	67	0.00E+00	5.07E+04	1.19E+06
5R-N-2	3:15 PM	After lunch	67	0.00E+00	3.77E+04	
5R-N-3	4:30 PM		0	0.00E+00	1.42E+04	
5R-N-4	5:30 PM		0	0.00E+00	2.58E+04	
5R-N-5	6:30 PM		0	0.00E+00	1.63E+04	
Coach 1	3:45 PM		67	0.00E+00	9.08E+04	
Coach 2	6:00 PM		67	0.00E+00	6.11E+04	
AVERAGE*			**27**	**0.00E+00**	**28944.44**	

*Business class samples only

Table 4.2.1-20 Culture dependent and independent microbial quantification, Los Angeles to Tokyo 4/3/06

Sample	Time (PST)	Note	Total heterotrophs CFU/m^3	Intracellular ATP RLU/m^3	Total ATP RLU/m^3	QPCR copies/m^3
6-J-1	12:00 PM	Boarding	33	3.78E+02	9.66E+04	1.16E+05
6-J-2	1:05 PM		33	0.00E+00	8.92E+04	
6-J-3	2:00 PM	Lunch	33	2.33E+02	5.08E+04	
6-J-4	3:00 PM		400	6.67E+01	4.30E+04	
6-J-5	4:25 PM		0	6.67E+01	3.53E+04	
6-J-6	7:00 PM		0	0.00E+00	3.43E+04	
6-J-7	8:00 PM		0	0.00E+00	6.88E+04	
6-J-8	10:00 PM		33	0.00E+00	5.41E+04	
Coach	4:00 PM		233	2.96E+03	7.57E+04	
Gasper	7:30 PM		ND	3.33E+02	5.25E+04	
Seat sweep	10:30 PM	vacuumed seat	233	1.18E+05	5.79E+06	
AVERAGE*			**67**	**9.31E+01**	**5.90E+04**	

*Business class samples only

Table 4.2.1-21 Culture dependent and independent microbial quantification, Tokyo to Los Angeles 4/7/06

Sample	Time (PST)	Note	Total heterotrophs CFU/m^3	Intracellular ATP RLU/m^3	Total ATP RLU/m^3	QPCR copies/m^3
6R-J-1	1:30 AM		0	0.00E+00	1.78E+05	1.87E+06
6R-J-2	2:15 AM	Dinner	0	0.00E+00	1.05E+05	
6R-J-3	3:00 AM		0	0.00E+00	5.34E+04	
6R-J-4	3:45 AM		33	2.37E+03	6.48E+04	
6R-J-5	7:45 AM		33	0.00E+00	4.58E+04	
6R-J-6	8:45 AM		ND	0.00E+00	5.91E+04	
6R-J-7	9:30 AM	Breakfast	367	0.00E+00	1.43E+05	
6R-J-Coach	8:10 AM		300	ND	ND	
AVERAGE*			**72**	**3.38E+02**	**9.28E+04**	

*Business class samples only

Table 4.2.1-22 Culture dependent and independent microbial quantification, Los Angeles to New York 4/24/06

Sample	Time (PST)	Note	Total heterotrophs CFU/m^3	Intracellular ATP RLU/m^3	Total ATP RLU/m^3	QPCR copies/m^3
7-N-1	9:15 AM		33	5.39E+02	5.88E+04	4.99E+06
7-N-2	10:17 AM		0	1.32E+03	5.29E+04	
7-N-3	11:15 AM	Breakfast	0	6.56E+02	3.75E+04	
7-N-4	12:15 PM		0	2.22E+02	2.88E+04	
7-N-5	1:10 PM		0	3.39E+02	3.54E+04	
7-N-6	10:47 AM		0	2.89E+02	3.10E+04	
Coach	---		TNTC**	6.89E+02	9.71E+04	
AVERAGE*			**6**	**5.61E+02**	**4.07E+04**	

*Business class samples only
**Excluded from average

Table 4.2.1-23 Culture dependent and independent microbial quantification, New York to Los Angeles 4/27/06

Sample	Time (PST)	Note	Total heterotrophs CFU/m^3	Intracellular ATP RLU/m^3	Total ATP RLU/m^3	QPCR copies/m^3
7R-N-1	7:52					
7R-N-2	8:50		100	0.00E+00	4.64E+04	8.04E+04
7R-N-3	9:55	lunch	67	0.00E+00	2.71E+04	
7R-N-4	10:55		67	0.00E+00	3.36E+04	
7R-N-5	11:55		33	0.00E+00	8.00E+03	
7R-N-6	12:50		0	1.23E+04	7.31E+03	
Coach 1	10:25		300	7.44E+03	1.27E+05	
Coach 2	11:20		233	3.04E+03	1.23E+05	
AVERAGE*			**53**	**2.46E+03**	**2.45E+04**	

*Business class samples only

Table 4.2.1-24 Culture dependent and independent microbial quantification, Los Angeles to Sydney 5/7/06

Sample	Time (PST)	Note	Total heterotrophs CFU/m^3	Intracellular ATP RLU/m^3	Total ATP RLU/m^3	QPCR copies/m^3
8-S-1	9:36 PM	boarding	33	1.20E+03	4.55E+04	
8-S-2	11:15 PM		2700	3.24E+03	6.84E+04	
8-S-3	12:00 AM		233	1.98E+03	2.38E+04	
8-S-4	12:45 AM		167	6.67E+01	9.96E+04	
8-S-5	1:30 AM		167	2.38E+03	3.81E+04	
8-S-6	7:15 AM		100	1.78E+02	6.02E+04	3.76E+06
8-S-7	8:00 AM		0	7.11E+02	1.47E+04	
8-S-8	8:45 AM		233	1.64E+03	4.07E+04	
8-S-9	9:45 AM		0	1.22E+03	5.94E+04	
8-S-10	10:30 AM		0	1.11E+02	5.99E+04	
AVERAGE*			**363**	**1.27E+03**	**5.10E+04**	

*Business class samples only

Table 4.2.1-25 Culture dependent and independent microbial quantification, Sydney to Los Angeles 5/12/06

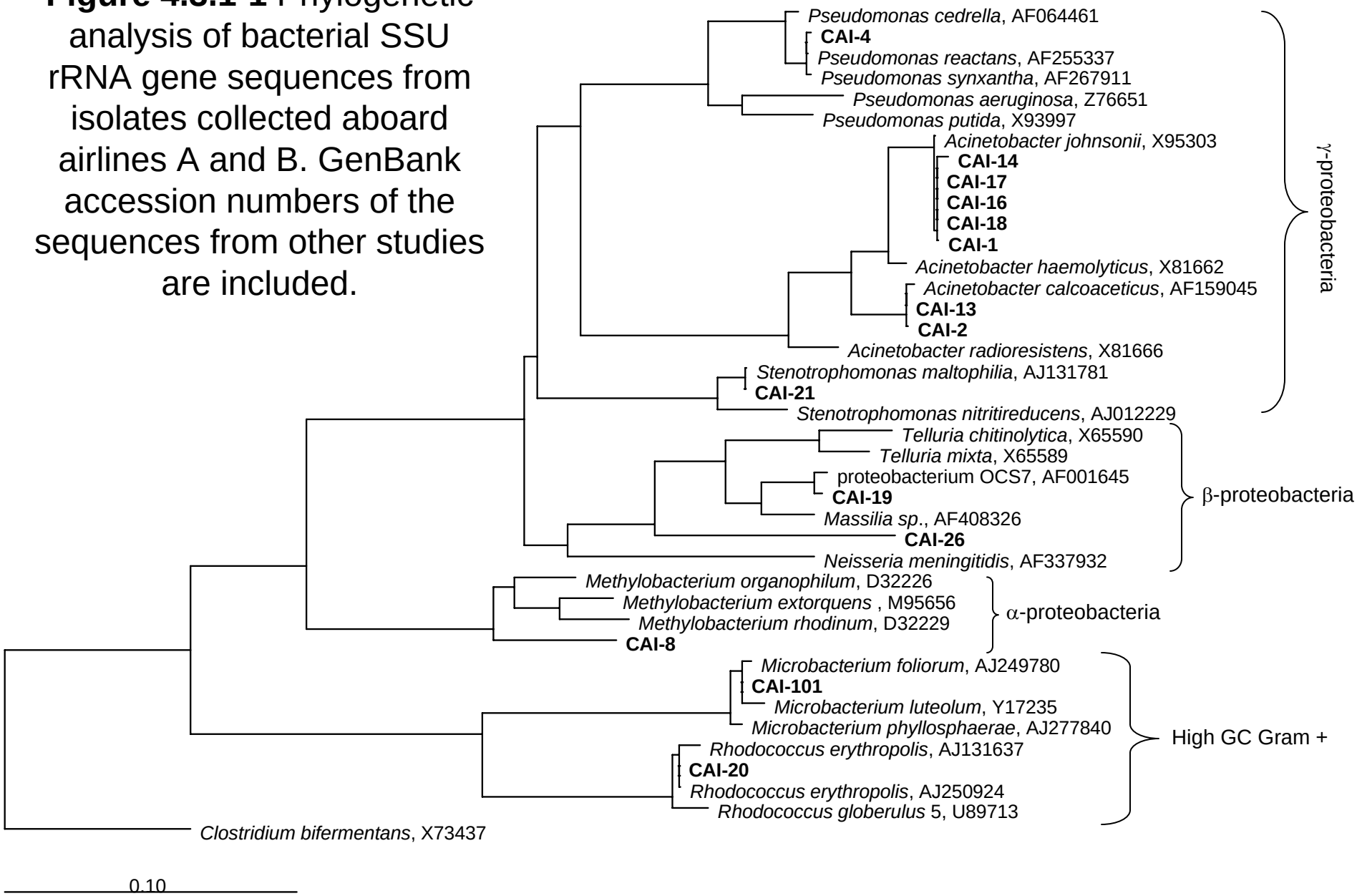
Sample	Time (PST)	Note	Total heterotrophs CFU/m^3	Intracellular ATP RLU/m^3	Total ATP RLU/m^3	QPCR copies/m^3
8R-S-1	8:15 PM		0	1.28E+03	1.34E+05	
8R-S-2	9:45 PM		167	2.03E+03	9.17E+04	
8R-S-3	10:20 PM		33	4.67E+02	1.05E+05	
8R-S-4	11:00 PM		367	7.50E+02	1.06E+05	
8R-S-5	11:45 PM		200	4.06E+02	7.33E+04	
8R-S-6	5:30 AM		67	1.55E+03	6.59E+04	1.46E+06
8R-S-7	6:15 AM		67	1.33E+02	1.24E+04	
8R-S-8	7:00 AM		33	6.33E+02	4.40E+04	
8R-S-9	7:45 AM		100	2.50E+02	3.61E+04	
8R-S-10	8:30 AM		0	1.55E+03	1.07E+05	
AVERAGE*			**103**	**9.06E+02**	**7.75E+04**	

*Business class samples only

Table 4.2.1-26 Percentage cultivable and viable microbes associated with various flights during Phase II

Flight	Route	Date	Total heterotrophs CFU/m^3 A	Intracellular ATP RLU/m^3 B	Total ATP RLU/m^3 C	% of population that are: cultivable (A/B)*100	viable (B/C)*100
1-N	Los Angeles/New York	11/14/05	43	3.33E+02	2.07E+04	12.9	1.6
1R-N	New York/Los Angeles	11/17/05	248	1.28E+02	2.80E+04	>CFU	0.5
2-N	Los Angeles/New York	12/05/05	40	2.47E+03	7.70E+04	1.6	3.2
2R-N	New York/Los Angeles	12/08/05	0	3.29E+02	3.73E+04	no CFU	0.9
3-L	Los Angeles/London	01/15/06	91	2.56E+02	3.88E+04	35.6	0.7
3R-L	London/Los Angeles	01/20/06	0	1.64E+03	2.16E+04	no CFU	7.6
4R-S	Sydney/Los Angeles	02/17/06	53	3.67E+01	1.11E+05	>CFU	0.0
5-N	Los Angeles/New York	3/7/06	50	1.64E+02	5.47E+04	30.5	0.3
5R-N	New York/Los Angeles	3/10/06	27	0	2.89E+04	>CFU	0.0
6-J	Los Angeles/Tokyo	4/3/06	67	9.31E+01	5.90E+04	71.6	0.2
6R-J	Tokyo/Los Angeles	4/7/06	72	3.38E+02	9.28E+04	21.4	0.4
7-N	Los Angeles/New York	4/24/06	6	5.61E+02	4.07E+04	1.0	1.4
7R-N	New York/Los Angeles	4/27/06	53	2.46E+03	2.45E+04	2.2	10.1
8-S	Los Angeles/Sydney	5/7/06	363	1.27E+03	5.10E+04	28.5	2.5
8R-S	Sydney/Los Angeles	5/12/06	103	9.06E+02	7.75E+04	11.4	1.2
					Average	21.7	2.0
					>CFU in ratio	3	
					no CFU value	2	
					Range	1.0 to 71.6	0 to 10

Figure 4.3.1-1 Phylogenetic analysis of bacterial SSU rRNA gene sequences from isolates collected aboard airlines A and B. GenBank accession numbers of the sequences from other studies are included.



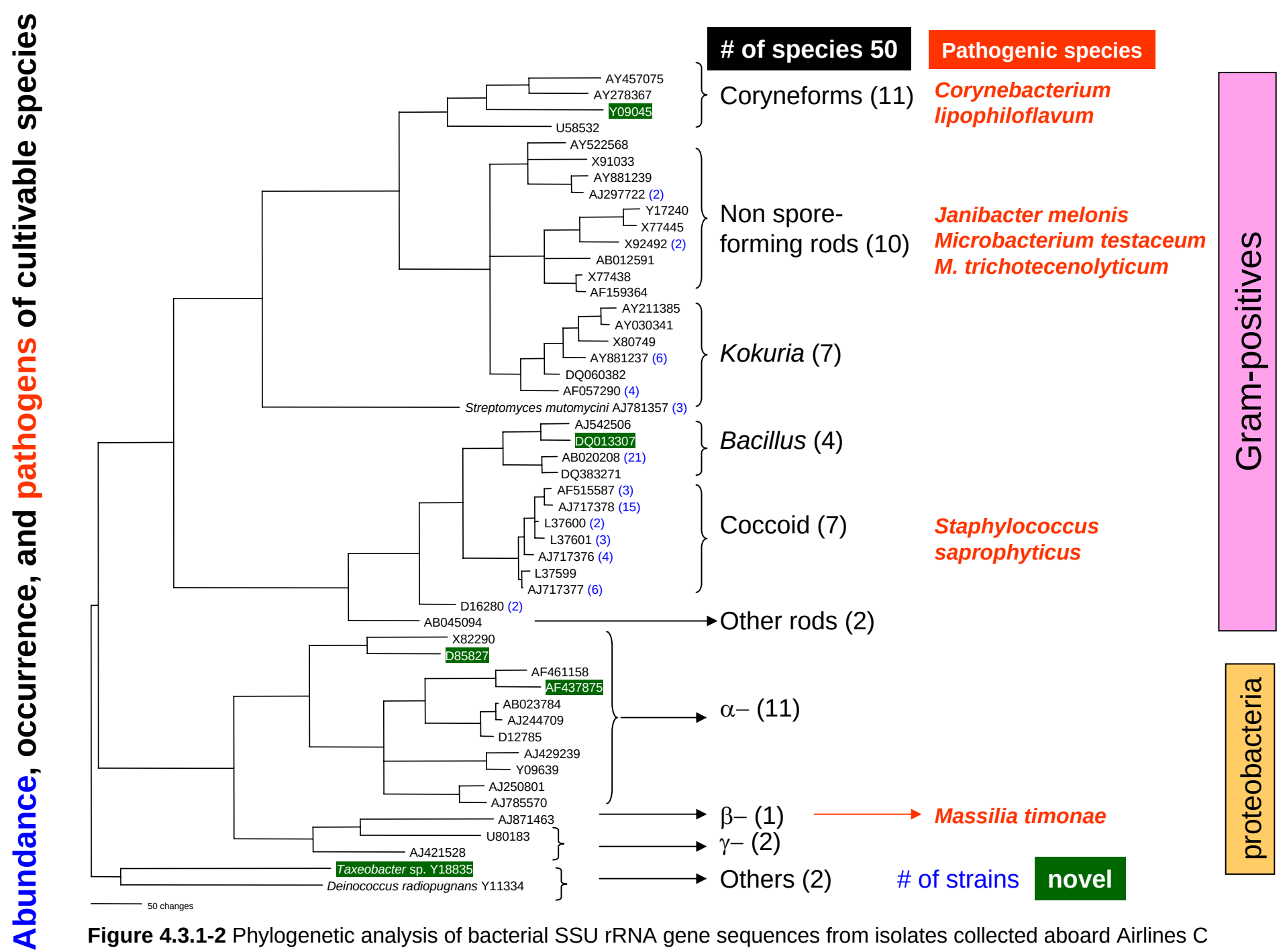


Figure 4.3.1-2 Phylogenetic analysis of bacterial SSU rRNA gene sequences from isolates collected aboard Airlines C

Table 4.3.1-1 Percentage of various kinds of microbes and cultivable microbial diversity of various air samples collected from several locations of commercial airlines during Phase I

Description of the samples	Percentage of microbial population that are:		Bacterial species isolated and identified
	viable (Intra ATP/Total ATP*100)	cultivable (CFU/Intra ATP*100)	
Phase I: Flight #3			
Pre-Board	33.3	22.1	*A. johnsonii*
Upon reaching cruise altitude	4.5	160.0	*A. johnsonii; Oxalobacter* sp.; *Acinetobacter* sp.
Half-way through flight	48.5	52.6	*A. johnsonii*
Just before beginning descent	Undetectable	Uncultivable	
After landing	Undetectable	N/A[1]	*A. johnsonii; Rhodococcus erythropolis*; *Variovorax* sp.
Phase I: Flight #4			
Pre-Board	11.8	70.9	*Microbacterium testaceum*
Upon reaching cruise altitude	32.0	71.8	*A. johnsonii; Acinetobacter* sp.
Half-way through flight	53.5	44.9	*Pseudomonas synxantha; Methylobacterium* sp.
Just before beginning descent	47.5	13.9	*Pseudomonas synxantha; Methylobacterium* sp.
After landing	65.5	3.8	*Methylobacterium* sp.

[1]Since some of the test conducted showed below detectable level, ratio was not obtained

Table 4.3.1-2 Diversity of cultivable bacteria isolated from various flights of Airliner C that are grouped to their nearest neighbours

Nearest neighbour	% Homology	Gene assession number	Class	# of isolates	Isolation source of nearest neighbor or comments
Agrococcus jenensis	99.1	X92492	Actinomycetales	2	Diaminobutyric acid producing actinomycetes
Brachybacterium nesterenkovii	98.0	X91033	Actinomycetales	1	isolated from milk product
Gordonia polyisoprenivorans	98.9	AY278367	Actinomycetales	1	Rubber degrading actinomycetes
Janibacter melonis	99.4	AY522568	Actinomycetales	1	bacteremia causing bacterium
Microbacterium testaceum	98.4	X77445	Actinomycetales	1	isolated from ticks, lice and fleas
Microbacterium trichotecenolyticum	99.0	Y17240	Actinomycetales	1	isolated from a neutropenic patient with catheter-related bacteremia
Rathayibacter tritici	99.0	X77438	Actinomycetales	1	Ultramicrobacteria isolated from freshwater habitats
Zimmermannella faecalis	99.1	AB012591	Actinomycetales	1	Isolated from cow faeces
Brevundimonas intermedia	99.2	AB023784	a-proteo	1	freshwater oligotrophs
Brevundimonas mediterranea	99.8	AJ244709	a-proteo	1	non-stalked species from the Mediterranean Sea
Gluconobacter frateurii	98.5	X82290	a-proteo	1	alcohol dehydrogenase-producing bacterium
Methylobacterium fujisawaense	99.1	AJ250801	a-proteo	1	isolated from drinking water
Methylobacterium hispanicum	99.6	AJ785570	a-proteo	1	Isolated from drinking water
Mycoplana bullata	98.9	D12785	a-proteo	1	freshwater associated microbe
Paracoccus versutus	96.5	AF437875	a-proteo	1	chemolithotrophic thiocyanate-degrading bacterium
Paracoccus zeaxanthinifaciens	96.0	AF461158	a-proteo	1	zeaxanthin-producing bacterium
Paracraurococcus ruber	95.3	D85827	a-proteo	1	Aerobic bacteriochlorophyll a containing bacteria

Table 4.3.1-2 Diversity of cultivable bacteria isolated from various flights of Airliner C that are grouped to their nearest neighbours

Nearest neighbour	% Homology	Gene assession number	Class	# of isolates	Isolation source of nearest neighbor or comments
Sphingomonas asaccharolytica	97.4	Y09639	a-proteo	1	oligotroph
Sphingomonas faenia	98.0	AJ429239	a-proteo	1	air- and dustborne and Antarctic, orange-pigmented, psychrotolerant bacteria
Massilia timonae	98.6	AJ871463	b-proteo	1	Isolated from Blood of an Immunocompromised Patient with Cerebellar Lesions
Taxeobacter ocellatus	91.3	Y18835	CFTB	1	Commonly found oligotrophic environment
Pantoea agglomerans	97.3	U80183	g-proteo	1	Dissimilatory metal reducing bacteria
Psychrobacter faecalis	99.0	AJ421528	g-proteo	1	a new species from a bioaerosol originating from pigeon faeces
Deinococcus radiopugnans	99.6	Y11334	Gram pos. deinococci	1	gamma-radiation resistant bacteria
Kocuria kristinae	99.2	X80749	Gram pos. high GC	1	isolated from the rhizoplane of the narrow-leaved cattail
Kocuria marina	99.0	AY211385	Gram pos. high GC	2	Isolated from marine sediment from the Troitsa Bay of the Gulf of Peter the Great, East Siberian Sea
Kocuria palustris	99.0	AY881237	Gram pos. high GC	6	marine bacteria from East (Aayajin) and South (Busan) coast of South Korea
Kocuria rhizophila	100.0	AY030341	Gram pos. high GC	1	Isolated from the rhizoplane of the narrow-leaved cattail (*Typha anguistifolia*)
Kocuria rosea	99.0	DQ060382	Gram pos. high GC	1	Arctic Ocean Marine Sediments
Kytococcus schroeteri	99.4	AJ297722	Gram pos. high GC	2	Actinobacterium isolated from a human clinical source
Kytococcus sedentarius	98.0	AY881239	Gram pos. high GC	1	marine bacteria from East (Aayajin) and South (Busan) coast of South Korea
Micrococcus luteus	99.0	AJ717378	Gram pos. high GC	15	Common skin microflora of human
Micrococcus lylae	99.5	AF057290	Gram pos. high GC	4	Common skin microflora of human
Rathayibacter caricis	98.4	AF159364	Gram pos. high GC	1	Isolated from the phyllosphere of Carex sp.

Table 4.3.1-2 Diversity of cultivable bacteria isolated from various flights of Airliner C that are grouped to their nearest neighbours

Nearest neighbour	% Homology	Gene assession number	Class	# of isolates	Isolation source of nearest neighbor or comments
Staphylococcus capitis	99.9	L37599	Gram pos. high GC	1	Common skin microflora of human
Staphylococcus epidermidis	100.0	AJ717377	Gram pos. high GC	6	Common skin microflora of human
Staphylococcus haemolyticus	99.6	L37600	Gram pos. high GC	2	Common skin microflora of human
Staphylococcus hominis	98.9	L37601	Gram pos. high GC	3	Common skin microflora of human
Staphylococcus pasteuri	99.9	AJ717376	Gram pos. high GC	4	Isolated from human, animal, and food specimens
Staphylococcus saprophyticus	99.8	AF515587	Gram pos. high GC	3	Urinary tract infection causing bacterium
Streptomyces mutomycini	99.6	AJ781357	Gram pos. high GC	3	soil microflora
Corynebacterium lipophiloflavum	96.5	Y09045	Gram pos. non sporeformer	1	Isolated from a patient with bacterial vaginosis
Bacillus chandigarhensis	95.0	DQ013307	Gram pos. spore-former	1	Novel species
Bacillus drentensis	98.7	AJ542506	Gram pos. spore-former	1	isolated from the soil disused hay fields
Bacillus pumilus	100.0	AB020208	Gram pos. spore-former	21	UV, H2O2-resistant bacteria found clean rooms
Bacillus sphaericus	98.7	D16280	Gram pos. spore-former	2	round spore-forming soil bacterium
Bacillus subtilis	99.0	DQ383271	Gram pos. spore-former	1	soil bacterium
Micromonospora echinospora	99.0	U58532	Gram pos. spore-former	1	antibiotics producing round spore-former
Paenibacillus pabuli	98.0	AB045094	Gram pos. spore-former	1	soil bacterium
Mycobacterium mucogenicum	98.4	AY457075	Non-tuberculosis	1	Non-pigmented mycobacteria

Table 4.3.1-3 Diversity of cultivable bacteria isolated from various flights during Airline C and the percent similarities of 16S rDNA sequences

Aviation #	Nearest neighbour	% Homology	Gene assession #	Class	Isolation source of nearest neighbor or comments
1R-3D2	*Agrococcus jenensis*	99.1	X92492	Actinomycetales	Diaminobutyric acid producing actinomycetes
1R-3F2	*Agrococcus jenensis*	99.1	X92492	Actinomycetales	Diaminobutyric acid producing actinomycetes
4RS-Bb	*Brachybacterium nesterenkovii*	98.0	X91033	Actinomycetales	isolated from milk product
1F-61	*Gordonia polyisoprenivorans*	98.9	AY278367	Actinomycetales	Rubber degrading actinomycetes
6J seat c	*Janibacter melonis*	99.4	AY522568	Actinomycetales	bacteremia causing bacterium
6J coach b	*Microbacterium testaceum*	98.4	X77445	Actinomycetales	isolated from ticks, lice and fleas
5R-vent D	*Microbacterium trichotecenolyticum*	99.0	Y17240	Actinomycetales	isolated from a neutropenic patient with catheter-related bacteremia
6J seat a	*Rathayibacter tritici*	99.0	X77438	Actinomycetales	Ultramicrobacteria isolated from freshwater habitats
6J coach c	*Zimmermannella faecalis*	99.1	AB012591	Actinomycetales	Isolated from cow faeces
6RJ 7d	*Brevundimonas intermedia*	99.2	AB023784	a-proteo	freshwater oligotrophs
1R-3D1	*Brevundimonas mediterranea*	99.8	AJ244709	a-proteo	non-stalked species from the Mediterranean Sea
6RJ Coach d	*Gluconobacter frateurii*	98.5	X82290	a-proteo	alcohol dehydrogenase-producing bacterium
7RN-5A	*Methylobacterium fujisawaense*	99.1	AJ250801	a-proteo	isolated from drinking water
2F-NA	*Methylobacterium hispanicum*	99.6	AJ785570	a-proteo	Isolated from drinking water

Table 4.3.1-3 Diversity of cultivable bacteria isolated from various flights during Airline C and the percent similarities of 16S rDNA sequences

Aviation #	Nearest neighbour	% Homology	Gene assession #	Class	Isolation source of nearest neighbor or comments
7RN-CC	*Mycoplana bullata*	98.9	D12785	a-proteo	freshwater associated microbe
2F-3B	*Paracoccus versutus*	96.5	AF437875	a-proteo	chemolithotrophic thiocyanate-degrading bacterium
6RJ coach c	*Paracoccus zeaxanthinifaciens*	96.0	AF461158	a-proteo	zeaxanthin-producing bacterium
1F-11	*Paracraurococcus ruber*	95.3	D85827	a-proteo	Aerobic bacteriochlorophyll a containing bacteria
7RN-C2D	*Sphingomonas asaccharolytica*	97.4	Y09639	a-proteo	oligotroph
5N-Coach 1a	*Sphingomonas faenia*	98.0	AJ429239	a-proteo	air- and dustborne and Antarctic, orange-pigmented, psychrotolerant bacteria
1F-31	*Massilia timonae*	98.6	AJ871463	b-proteo	Isolated from Blood of an Immunocompromised Patient with Cerebellar Lesions
1F-12	*Taxeobacter ocellatus*	91.3	Y18835	CFTB	Commonly found oligotrophic environment
6RJ 7b	*Pantoea agglomerans*	97.3	U80183	g-proteo	Dissimilatory metal reducing bacteria
5R-vent A	*Psychrobacter faecalis*	99.0	AJ421528	g-proteo	a new species from a bioaerosol originating from pigeon faeces
5RN2	*Deinococcus radiopugnans*	99.6	Y11334	Gram pos. deinococci	gamma-radiation resistant bacteria
6J seat b	*Kocuria kristinae*	99.2	X80749	Gram pos. high GC	isolated from the rhizoplane of the narrow-leaved cattail
1R-3F1	*Kocuria marina*	99.0	AY211385	Gram pos. high GC	Isolated from marine sediment from the Troitsa Bay of the Gulf of Peter the Great, East Siberian Sea
4RS-4b	*Kocuria marina*	98.0	AY211385	Gram pos. high GC	a novel actinobacterium isolated from marine sediment

Table 4.3.1-3 Diversity of cultivable bacteria isolated from various flights during Airline C and the percent similarities of 16S rDNA sequences

Aviation #	Nearest neighbour	% Homology	Gene assession #	Class	Isolation source of nearest neighbor or comments
5NY-4	*Kocuria palustris*	99.0	AY881237	Gram pos. high GC	marine bacteria from East (Aayajin) and South (Busan) coast of South Korea
5R-vent E	*Kocuria palustris*	99.0	AY881237	Gram pos. high GC	isolated from the rhizoplane of the narrow-leaved cattail
7RN-CF	*Kocuria palustris*	99.9	Y16263	Gram pos. high GC	Common skin microflora of human
6J 1	*Kocuria palustris*	99.6	Y16263	Gram pos. high GC	isolated from the rhizoplane of the narrow-leaved cattail
6J coach a	*Kocuria palustris*	98.8	Y16263	Gram pos. high GC	isolated from the rhizoplane of the narrow-leaved cattail
6J coach e	*Kocuria palustris*	99.4	Y16263	Gram pos. high GC	isolated from the rhizoplane of the narrow-leaved cattail
1R-FB	*Kocuria rhizophila*	100.0	AY030341	Gram pos. high GC	Isolated from the rhizoplane of the narrow-leaved cattail (*Typha anguistifolia*)
5R-vent C	*Kocuria rosea*	99.0	DQ060382	Gram pos. high GC	Arctic Ocean Marine Sediments
1R-3A1	*Kytococcus schroeteri*	99.4	AJ297722	Gram pos. high GC	Actinobacterium isolated from a human clinical source
1R-3H	*Kytococcus schroeteri*	99.6	AJ297722	Gram pos. high GC	Actinobacterium isolated from a human clinical source
5NY-2	*Kytococcus sedentarius*	98.0	AY881239	Gram pos. high GC	marine bacteria from East (Aayajin) and South (Busan) coast of South Korea
1R-6A	*Micrococcus luteus*	99.0	AJ717378	Gram pos. high GC	Common skin microflora of human
4RS-9b	*Micrococcus luteus*	99.0	AJ717368	Gram pos. high GC	Common skin microflora of human
4RS-9c	*Micrococcus luteus*	99.0	AJ717367	Gram pos. high GC	Common skin microflora of human

Table 4.3.1-3 Diversity of cultivable bacteria isolated from various flights during Airline C and the percent similarities of 16S rDNA sequences

Aviation #	Nearest neighbour	% Homology	Gene assession #	Class	Isolation source of nearest neighbor or comments
4RS-9d	*Micrococcus luteus*	99.0	AJ717367	Gram pos. high GC	Common skin microflora of human
5NY-5	*Micrococcus luteus*	99.0	AJ717369	Gram pos. high GC	Common skin microflora of human
5R-vent B	*Micrococcus luteus*	99.0	AJ717368	Gram pos. high GC	Common skin microflora of human
6J 3	*Micrococcus luteus*	99.3	AJ536198	Gram pos. high GC	Common skin microflora of human
7RN-C2B	*Micrococcus luteus*	98.7	AJ536198	Gram pos. high GC	Common skin microflora of human
7RN-C2F	*Micrococcus luteus*	96.4	AJ536198	Gram pos. high GC	Common skin microflora of human
4RS-9a	*Micrococcus luteus*	99.0	AJ409096	Gram pos. high GC	Common skin microflora of human
6J 4a	*Micrococcus luteus*	99.3	AJ536198	Gram pos. high GC	Common skin microflora of human
6J seat e	*Micrococcus luteus*	99.3	AJ536198	Gram pos. high GC	Common skin microflora of human
6RJ coach a	*Micrococcus luteus*	99.6	AJ536198	Gram pos. high GC	Common skin microflora of human
7RN-C2C	*Micrococcus luteus*	99.4	AJ536198	Gram pos. high GC	Common skin microflora of human
7RN-CE	*Micrococcus luteus*	99.5	AJ536198	Gram pos. high GC	Common skin microflora of human
1R-5A	*Micrococcus lylae*	99.5	AF057290	Gram pos. high GC	Common skin microflora of human
1R-5B	*Micrococcus lylae*	99.5	AF057290	Gram pos. high GC	Common skin microflora of human

Table 4.3.1-3 Diversity of cultivable bacteria isolated from various flights during Airline C and the percent similarities of 16S rDNA sequences

Aviation #	Nearest neighbour	% Homology	Gene assession #	Class	Isolation source of nearest neighbor or comments
1R-5D	*Micrococcus lylae*	99.6	AF057290	Gram pos. high GC	Common skin microflora of human
1R-5E	*Micrococcus lylae*	99.0	AF057290	Gram pos. high GC	Common skin microflora of human
1R-3C1	*Rathayibacter caricis*	98.4	AF159364	Gram pos. high GC	Isolated from the phyllosphere of Carex sp.
7RN-C2E	*Staphylococcus capitis*	99.9	L37599	Gram pos. high GC	Common skin microflora of human
1R-2A1	*Staphylococcus epidermidis*	100.0	AJ717377	Gram pos. high GC	Common skin microflora of human
4RS-Ba	*Staphylococcus epidermidis*	98.0	AF397060	Gram pos. high GC	Common skin microflora of human
4RS-Bc	*Staphylococcus epidermidis*	99.0	D83362	Gram pos. high GC	clinical isolates from animals
7RN-3B	*Staphylococcus epidermidis*	99.9	L37605	Gram pos. high GC	Common skin microflora of human
7N-A1	*Staphylococcus epidermidis*	99.6	L37605	Gram pos. high GC	Common skin microflora of human
3L 10A	*Staphylococcus epidermis*	99.4	AE015929	Gram pos. high GC	Common human skin microflora
6RJ 5	*Staphylococcus haemolyticus*	99.6	L37600	Gram pos. high GC	Common skin microflora of human
6J 8	*Staphylococcus haemolyticus*	99.4	L37600	Gram pos. high GC	nosocomial bloodstream infections
6J 4b	*Staphylococcus hominis*	98.9	L37601	Gram pos. high GC	Common skin microflora of human
6RJ 7c	*Staphylococcus hominis*	99.6	L37601	Gram pos. high GC	Common skin microflora of human

Table 4.3.1-3 Diversity of cultivable bacteria isolated from various flights during Airline C and the percent similarities of 16S rDNA sequences

Aviation #	Nearest neighbour	% Homology	Gene assession #	Class	Isolation source of nearest neighbor or comments
7RN-3A	*Staphylococcus hominis*	99.6	L37601	Gram pos. high GC	Common skin microflora of human
1R-4A	*Staphylococcus pasteuri*	99.9	AJ717376	Gram pos. high GC	Isolated from human, animal, and food specimens
1R-5C	*Staphylococcus pasteuri*	99.9	AJ717376	Gram pos. high GC	Isolated from human, animal, and food specimens
2F-5A	*Staphylococcus pasteuri*	100.0	AJ717376	Gram pos. high GC	Isolated from human, animal, and food specimens
5NY-3	*Staphylococcus pasteuri*	99.0	AJ717376	Gram pos. high GC	Isolated from human, animal, and food specimens
1R-FA	*Staphylococcus saprophyticus*	99.8	AF515587	Gram pos. high GC	Urinary tract infection causing bacterium
5R-coach 1	*Staphylococcus saprophyticus*	99.0	AP008934	Gram pos. high GC	Whole genome sequence of Staphylococcus saprophyticus reveals the pathogenesis of uncomplicated urinary tract infection
6RJ coachb	*Staphylococcus saprophyticus*	98.8	D83371	Gram pos. high GC	Common skin microflora of human
3L 10G	*Streptomyces mutomycini*	99.6	AJ781357	Gram pos. high GC	soil microflora
3L 10H	*Streptomyces mutomycini*	99.8	AJ781359	Gram pos. high GC	soil microflora
3L 10I	*Streptomyces mutomycini*	99.5	AJ781358	Gram pos. high GC	soil microflora
1R-3G	*Corynebacterium lipophiloflavum*	96.5	Y09045	Gram pos. non sporeformer	Isolated from a patient with bacterial vaginosis
1R-3C2	*Bacillus chandigarhensis*	95.0	DQ013307	Gram pos. spore-former	Novel species
4RS 4a	*Bacillus drentensis*	98.7	AJ542506	Gram pos. spore-former	isolated from the soil disused hay fields

Table 4.3.1-3 Diversity of cultivable bacteria isolated from various flights during Airline C and the percent similarities of 16S rDNA sequences

Aviation #	Nearest neighbour	% Homology	Gene assession #	Class	Isolation source of nearest neighbor or comments
1R-3B	*Bacillus pumilus*	100.0	AB020208	Gram pos. spore-former	UV, H2O2-resistant bacteria found clean rooms
1R-3E	*Bacillus pumilus*	100.0	AB020208	Gram pos. spore-former	UV, H2O2-resistant bacteria found clean rooms
2F-GA	*Bacillus pumilus*	100.0	AB020208	Gram pos. spore-former	UV, H2O2-resistant bacteria found clean rooms
3L 10B	*Bacillus pumilus*	99.5	AB098579	Gram pos. spore-former	UV, H2O2-resistant bacteria found clean rooms
3L 10C	*Bacillus pumilus*	99.3	AB098580	Gram pos. spore-former	UV, H2O2-resistant bacteria found clean rooms
3L 10E	*Bacillus pumilus*	96.9	AB098581	Gram pos. spore-former	UV, H2O2-resistant bacteria found clean rooms
3L 10F	*Bacillus pumilus*	97.2	AB098582	Gram pos. spore-former	UV, H2O2-resistant bacteria found clean rooms
3L 6A	*Bacillus pumilus*	99.6	AB098578	Gram pos. spore-former	UV, H2O2-resistant bacteria found clean rooms
4RS-5a	*Bacillus pumilus*	99.0	AB098578	Gram pos. spore-former	UV, H2O2-resistant bacteria found clean rooms
4RS-5c	*Bacillus pumilus*	99.0	AB098578	Gram pos. spore-former	UV, H2O2-resistant bacteria found clean rooms
4RS-5e	*Bacillus pumilus*	99.0	AB098578	Gram pos. spore-former	UV, H2O2-resistant bacteria found clean rooms
4RS-5f	*Bacillus pumilus*	99.0	AF526907	Gram pos. spore-former	UV, H2O2-resistant bacteria found clean rooms
4RS seat e	*Bacillus pumilus*	99.5	AY456263	Gram pos. spore-former	UV, H2O2-resistant bacteria found clean rooms
5R-2	*Bacillus pumilus*	98.0	AY647298	Gram pos. spore-former	UV, H2O2-resistant bacteria found clean rooms

Table 4.3.1-3 Diversity of cultivable bacteria isolated from various flights during Airline C and the percent similarities of 16S rDNA sequences

Aviation #	Nearest neighbour	% Homology	Gene assession #	Class	Isolation source of nearest neighbor or comments
5R-2	*Bacillus pumilus*	98.0	AB098578	Gram pos. spore-former	UV, H2O2-resistant bacteria found clean rooms
4RS 5g	*Bacillus pumilus*	99.0	AY456263	Gram pos. spore-former	UV, H2O2-resistant bacteria found clean rooms
4RS-5b	*Bacillus pumilus*	99.0	AB098578	Gram pos. spore-former	UV, H2O2-resistant bacteria found clean rooms
4RS-5d	*Bacillus pumilus*	99.0	AY462205	Gram pos. spore-former	UV, H2O2-resistant bacteria found clean rooms
6J 4c	*Bacillus pumilus*	99.5	AY456263	Gram pos. spore-former	UV, H2O2-resistant bacteria found clean rooms
6J 4d	*Bacillus pumilus*	99.7	AY456263	Gram pos. spore-former	UV, H2O2-resistant bacteria found clean rooms
6RJ coach e	*Bacillus pumilus*	99.2	AY456263	Gram pos. spore-former	UV, H2O2-resistant bacteria found clean rooms
7N-CB	*Bacillus sphaericus*	98.7	D16280	Gram pos. spore-former	round spore-forming soil bacterium
7N-CA	*Bacillus sphaericus*	99.3	D16280	Gram pos. spore-former	round spore-forming soil bacterium
6RJ coach e	*Bacillus subtilis*	99.0	DQ383271	Gram pos. spore-former	soil bacterium
4RS-3a	*Micromonospora echinospora*	99.0	U58532	Gram pos. spore-former	antibiotics producing round spore-former
4RS-1a	*Paenibacillus pabuli*	98.0	AB045094	Gram pos. spore-former	soil bacterium
1R-2A2	*Mycobacterium mucogenicum*	98.4	AY457075	Non-tuberculosis	Non-pigmented mycobacteria

Table 4.3.1-4 Diversity of cultivable bacteria isolated from Flight 1

Aviation #	Route	Nearest neighbour	% Homology	Gene assession number	Isolation source of nearest neighbor or comments	Class
1F-61	Los Angeles to New York	*Gordonia polyisoprenivorans*	98.9	AY278367	Rubber degrading actinomycetes	Actinomycetales
1F-11		*Paracraurococcus ruber*	95.3	D85827	Aerobic bacteriochlorophyll a containing bacteria	a-proteo
1F-31		*Massilia timonae*	98.6	AJ871463	Isolated from Blood of an Immunocompromised Patient with Cerebellar Lesions	b-proteo
1F-12		*Taxeobacter ocellatus*	91.3	Y18835	Commonly found oligotrophic environment	CFTB
1R-3D2	New York to Los Angeles	*Agrococcus jenensis*	99.1	X92492	Diaminobutyric acid producing actinomycetes	Actinomycetales
1R-3F2		*Agrococcus jenensis*	99.1	X92492	Diaminobutyric acid producing actinomycetes	Actinomycetales
1R-3D1		*Brevundimonas mediterranea*	99.8	AJ244709	non-stalked species from the Mediterranean Sea	a-proteo
1R-3C2		*Bacillus chandigarhensis*	95.0	DQ013307	Novel species	Gram pos. spore-former
1R-3B		*Bacillus pumilus*	100.0	AB020208	UV, H2O2-resistant bacteria found clean rooms	Gram pos. spore-former
1R-3E		*Bacillus pumilus*	100.0	AB020208	UV, H2O2-resistant bacteria found clean rooms	Gram pos. spore-former
1R-3F1		*Kocuria marina*	99.0	AY211385	Isolated from marine sediment from the Troitsa Bay of the Gulf of Peter the Great, East Siberian Sea	Gram pos. high GC
1R-FB		*Kocuria rhizophila*	100.0	AY030341	Isolated from the rhizoplane of the narrow-leaved cattail (*Typha anguistifolia*)	Gram pos. high GC
1R-3A1		*Kytococcus schroeteri*	99.4	AJ297722	Actinobacterium isolated from a human clinical source	Gram pos. high GC
1R-3H		*Kytococcus schroeteri*	99.6	AJ297722	Actinobacterium isolated from a human clinical source	Gram pos. high GC
1R-6A		*Micrococcus luteus*	99.0	AJ717378	Common skin microflora of human	Gram pos. high GC
1R-5A		*Micrococcus lylae*	99.5	AF057290	Common skin microflora of human	Gram pos. high GC
1R-5B		*Micrococcus lylae*	99.5	AF057290	Common skin microflora of human	Gram pos. high GC
1R-5D		*Micrococcus lylae*	99.6	AF057290	Common skin microflora of human	Gram pos. high GC
1R-5E		*Micrococcus lylae*	99.0	AF057290	Common skin microflora of human	Gram pos. high GC
1R-3C1		*Rathayibacter caricis*	98.4	AF159364	Isolated from the phyllosphere of Carex sp.	Gram pos. high GC
1R-2A1		*Staphylococcus epidermidis*	100.0	AJ717377	Common skin microflora of human	Gram pos. high GC
1R-4A		*Staphylococcus pasteuri*	99.9	AJ717376	Isolated from human, animal, and food specimens	Gram pos. high GC
1R-5C		*Staphylococcus pasteuri*	99.9	AJ717376	Isolated from human, animal, and food specimens	Gram pos. high GC
1R-FA		*Staphylococcus saprophyticus*	99.8	AF515587	Urinary tract infection causing bacterium	Gram pos. high GC
1R-3G		*Corynebacterium lipophiloflavum*	96.5	Y09045	Isolated from a patient with bacterial vaginosis	Gram pos. non sporeformer
1R-2A2		*Mycobacterium mucogenicum*	98.4	AY457075	Non-pigmented mycobacteria	Non-tuberculosis

Table 4.3.1-5 Diversity of cultivable bacteria isolated from Flight 2

Aviation #	Route	Nearest neighbour	% Homology	Gene assession number	Isolation source of nearest neighbor or comments	Class
2F-NA	Los Angeles to New York	*Methylobacterium hispanicum*	99.6	AJ785570	Isolated from drinking water	a-proteo
2F-3B		*Paracoccus versutus*	96.5	AF437875	chemolithotrophic thiocyanate-degrading bacterium	a-proteo
2F-GA		*Bacillus pumilus*	100.0	AB020208	UV, H2O2-resistant bacteria found clean rooms	Gram pos. spore-former
2F-5A		*Staphylococcus pasteuri*	100.0	AJ717376	Isolated from human, animal, and food specimens	Gram pos. high GC

Table 4.3.1-6 Diversity of cultivable bacteria isolated from Flight 3

Aviation #	Route	Nearest neighbour	% Homology	Gene assession number	Isolation source of nearest neighbor or comments	Class
3L 10B	Los Angeles to London	*Bacillus pumilus*	99.5	AB098579	UV, H2O2-resistant bacteria found clean rooms	Gram pos. spore-former
3L 10C		*Bacillus pumilus*	99.3	AB098580	UV, H2O2-resistant bacteria found clean rooms	Gram pos. spore-former
3L 10E		*Bacillus pumilus*	96.9	AB098581	UV, H2O2-resistant bacteria found clean rooms	Gram pos. spore-former
3L 10F		*Bacillus pumilus*	97.2	AB098582	UV, H2O2-resistant bacteria found clean rooms	Gram pos. spore-former
3L 6A		*Bacillus pumilus*	99.6	AB098578	UV, H2O2-resistant bacteria found clean rooms	Gram pos. spore-former
3L 10A		*Staphylococcus epidermis*	99.4	AE015929	Common human skin microflora	Gram pos. high GC
3L 10G		*Streptomyces mutomycini*	99.6	AJ781357	soil microflora	Gram pos. high GC
3L 10H		*Streptomyces mutomycini*	99.8	AJ781359	soil microflora	Gram pos. high GC
3L 10I		*Streptomyces mutomycini*	99.5	AJ781358	soil microflora	Gram pos. high GC

Table 4.3.1-7 Diversity of cultivable bacteria isolated from Flight 4

Aviation #	Route	Nearest neighbour	% Homology	Gene assession number	Isolation source of nearest neighbor or comments	Class
4RS-Bb	Sydney to Los Angeles	*Brachybacterium nesterenkovii*	98.0	X91033	isolated from milk product	Actinomycetales
4RS-4b		*Kocuria marina*	98.0	AY211385	a novel actinobacterium isolated from marine sediment	Gram pos. high GC
4RS-9b		*Micrococcus luteus*	99.0	AJ717368	Common skin microflora of human	Gram pos. high GC
4RS-9c		*Micrococcus luteus*	99.0	AJ717367	Common skin microflora of human	Gram pos. high GC
4RS-9d		*Micrococcus luteus*	99.0	AJ717367	Common skin microflora of human	Gram pos. high GC
4RS-9a		*Micrococcus luteus*	99.0	AJ409096	Common skin microflora of human	Gram pos. high GC
4RS-Ba		*Staphylococcus epidermidis*	98.0	AF397060	Common skin microflora of human	Gram pos. high GC
4RS-Bc		*Staphylococcus epidermidis*	99.0	D83362	clinical isolates from animals	Gram pos. high GC
4RS 4a		*Bacillus drentensis*	98.7	AJ542506	isolated from the soil disused hay fields	Gram pos. spore-former
4RS-5a		*Bacillus pumilus*	99.0	AB098578	UV, H2O2-resistant bacteria found clean rooms	Gram pos. spore-former
4RS-5c		*Bacillus pumilus*	99.0	AB098578	UV, H2O2-resistant bacteria found clean rooms	Gram pos. spore-former
4RS-5e		*Bacillus pumilus*	99.0	AB098578	UV, H2O2-resistant bacteria found clean rooms	Gram pos. spore-former
4RS-5f		*Bacillus pumilus*	99.0	AF526907	UV, H2O2-resistant bacteria found clean rooms	Gram pos. spore-former
4RS 5g		*Bacillus pumilus*	99.0	AY456263	UV, H2O2-resistant bacteria found clean rooms	Gram pos. spore-former
4RS-5b		*Bacillus pumilus*	99.0	AB098578	UV, H2O2-resistant bacteria found clean rooms	Gram pos. spore-former
4RS-5d		*Bacillus pumilus*	99.0	AY462205	UV, H2O2-resistant bacteria found clean rooms	Gram pos. spore-former
4RS seat e		*Bacillus pumilus*	99.5	AY456263	UV, H2O2-resistant bacteria found clean rooms	Gram pos. spore-former
4RS-3a		*Micromonospora echinospora*	99.0	U58532	antibiotics producing round spore-former	Gram pos. spore-former
4RS-1a		*Paenibacillus pabuli*	98.0	AB045094	soil bacterium	Gram pos. spore-former

Table 4.3.1-8 Diversity of cultivable bacteria isolated from Flight 5

Aviation #	Route	Nearest neighbour	% Homology	Gene assession number	Isolation source of nearest neighbor or comments	Class
5N-Coach 1a	Los Angeles to New York	*Sphingomonas faenia*	98.0	AJ429239	air- and dustborne and Antarctic, orange-pigmented, psychrotolerant bacteria	a-proteo
5NY-4		*Kocuria palustris*	99.0	AY881237	marine bacteria from East (Aayajin) and South (Busan) coast of South Korea	Gram pos. high GC
5NY-2		*Kytococcus sedentarius*	98.0	AY881239	marine bacteria from East (Aayajin) and South (Busan) coast of South Korea	Gram pos. high GC
5NY-5		*Micrococcus luteus*	99.0	AJ717369	Common skin microflora of human	Gram pos. high GC
5NY-3		*Staphylococcus pasteuri*	99.0	AJ717376	Isolated from human, animal, and food specimens	Gram pos. high GC
5R-vent D	New York to Los Angeles	*Microbacterium trichotecenolyticum*	99.0	Y17240	isolated from a neutropenic patient with catheter-related bacteremia	Actinomycetales
5R-vent A		*Psychrobacter faecalis*	99.0	AJ421528	a new species from a bioaerosol originating from pigeon faeces	g-proteo
5RN2		*Deinococcus radiopugnans*	99.6	Y11334	gamma-radiation resistant bacteria	Gram pos. deinococci
5R-vent E		*Kocuria palustris*	99.0	AY881237	isolated from the rhizoplane of the narrow-leaved cattail	Gram pos. high GC
5R-vent C		*Kocuria rosea*	99.0	DQ060382	Arctic Ocean Marine Sediments	Gram pos. high GC
5R-vent B		*Micrococcus luteus*	99.0	AJ717368	Common skin microflora of human	Gram pos. high GC
5R-coach 1		*Staphylococcus saprophyticus*	99.0	AP008934	Whole genome sequence of Staphylococcus saprophyticus reveals the pathogenesis of uncomplicated urinary tract infection	Gram pos. high GC
5R-2		*Bacillus pumilus*	98.0	AY647298	UV, H2O2-resistant bacteria found clean rooms	Gram pos. spore-former
5R-2		*Bacillus pumilus*	98.0	AB098578	UV, H2O2-resistant bacteria found clean rooms	Gram pos. spore-former

Table 4.3.1-9 Diversity of cultivable bacteria isolated from Flight 6

Aviation #	Route	Nearest neighbour	% Homology	Gene assession number	Isolation source of nearest neighbor or comments	Class
6J seat c	Los Angeles to Tokyo	*Janibacter melonis*	99.4	AY522568	bacteremia causing bacterium	Actinomycetales
6J coach b		*Microbacterium testaceum*	98.4	X77445	isolated from ticks, lice and fleas	Actinomycetales
6J seat a		*Rathayibacter tritici*	99.0	X77438	Ultramicrobacteria isolated from freshwater habitats	Actinomycetales
6J coach c		*Zimmermannella faecalis*	99.1	AB012591	Isolated from cow faeces	Actinomycetales
6J seat b		*Kocuria kristinae*	99.2	X80749	isolated from the rhizoplane of the narrow-leaved cattail	Gram pos. high GC
6J 1		*Kocuria palustris*	99.6	Y16263	isolated from the rhizoplane of the narrow-leaved cattail	Gram pos. high GC
6J coach a		*Kocuria palustris*	98.8	Y16263	isolated from the rhizoplane of the narrow-leaved cattail	Gram pos. high GC
6J coach e		*Kocuria palustris*	99.4	Y16263	isolated from the rhizoplane of the narrow-leaved cattail	Gram pos. high GC
6J 3		*Micrococcus luteus*	99.3	AJ536198	Common skin microflora of human	Gram pos. high GC
6J 4a		*Micrococcus luteus*	99.3	AJ536198	Common skin microflora of human	Gram pos. high GC
6J seat e		*Micrococcus luteus*	99.3	AJ536198	Common skin microflora of human	Gram pos. high GC
6J 8		*Staphylococcus haemolyticus*	99.4	L37600	nosocomial bloodstream infections	Gram pos. high GC
6J 4b		*Staphylococcus hominis*	98.9	L37601	Common skin microflora of human	Gram pos. high GC
6J 4c		*Bacillus pumilus*	99.5	AY456263	UV, H2O2-resistant bacteria found clean rooms	Gram pos. spore-former
6J 4d		*Bacillus pumilus*	99.7	AY456263	UV, H2O2-resistant bacteria found clean rooms	Gram pos. spore-former

Table 4.3.1-9 Diversity of cultivable bacteria isolated from Flight 6

Aviation #	Route	Nearest neighbour	% Homology	Gene assession number	Isolation source of nearest neighbor or comments	Class
6RJ 7d	Tokyo to Los Angeles	*Brevundimonas intermedia*	99.2	AB023784	freshwater oligotrophs	a-proteo
6RJ Coach d		*Gluconobacter frateurii*	98.5	X82290	alcohol dehydrogenase-producing bacterium	a-proteo
6RJ coach c		*Paracoccus zeaxanthinifaciens*	96.0	AF461158	zeaxanthin-producing bacterium	a-proteo
6RJ 7b		*Pantoea agglomerans*	97.3	U80183	Dissimilatory metal reducing bacteria	g-proteo
6RJ coach a		*Micrococcus luteus*	99.6	AJ536198	Common skin microflora of human	Gram pos. high GC
6RJ 5		*Staphylococcus haemolyticus*	99.6	L37600	Common skin microflora of human	Gram pos. high GC
6RJ 7c		*Staphylococcus hominis*	99.6	L37601	Common skin microflora of human	Gram pos. high GC
6RJ coachb		*Staphylococcus saprophyticus*	98.8	D83371	Common skin microflora of human	Gram pos. high GC
6RJ coach e		*Bacillus pumilus*	99.2	AY456263	UV, H2O2-resistant bacteria found clean rooms	Gram pos. spore-former
6RJ coach e		*Bacillus subtilis*	99.0	DQ383271	soil bacterium	Gram pos. spore-former

Table 4.3.1-10 Diversity of cultivable bacteria isolated from Flight 7

Aviation #	Route	Nearest neighbour	% Homology	Gene assession number	Isolation source of nearest neighbor or comments	Class
7N-A1	Los Angeles to New York	*Staphylococcus epidermidis*	99.6	L37605	Common skin microflora of human	Gram pos. high GC
7N-CA		*Bacillus sphaericus*	99.3	D16280	round spore-forming soil bacterium	Gram pos. spore-former
7N-CB		*Bacillus sphaericus*	98.7	D16280	round spore-forming soil bacterium	Gram pos. spore-former
7RN-3A	New York to Los Angeles	*Staphylococcus hominis*	99.6	L37601	Common skin microflora of human	Gram pos. high GC
7RN-3B		*Staphylococcus epidermidis*	99.9	L37605	Common skin microflora of human	Gram pos. high GC
7RN-5A		*Methylobacterium fujisawaense*	99.1	AJ250801	isolated from drinking water	a-proteo
7RN-C2B		*Micrococcus luteus*	98.7	AJ536198	Common skin microflora of human	Gram pos. high GC
7RN-C2C		*Micrococcus luteus*	99.4	AJ536198	Common skin microflora of human	Gram pos. high GC
7RN-C2D		*Sphingomonas asaccharolytica*	97.4	Y09639	oligotroph	a-proteo
7RN-C2E		*Staphylococcus capitis*	99.9	L37599	Common skin microflora of human	Gram pos. high GC
7RN-C2F		*Micrococcus luteus*	96.4	AJ536198	Common skin microflora of human	Gram pos. high GC
7RN-CC		*Mycoplana bullata*	98.9	D12785	freshwater associated microbe	a-proteo
7RN-CE		*Micrococcus luteus*	99.5	AJ536198	Common skin microflora of human	Gram pos. high GC
7RN-CF		*Kocuria palustris*	99.9	Y16263	Common skin microflora of human	Gram pos. high GC

Table 4.3.2-1 Sample description pooled for DNA extraction and eubacterial burden estimated by Q-PCR analysis

Sample number	Airline	# of samples pooled	Times[a]	Locations[b]	Q-PCR based 16S rRNA gene copy numbers (per m^3 of air)		
					Average		SD[c]
Flight #1							
XXIV	A	3	CA, M, PD	Gasper	1.56×10^5	±	5.43×10^3
XXI	A	3	CA, M, PD	Lavatory	BDL		BDL
XXV	A	3	CA, M, PD	Seat, Floor	BDL		BDL
Flight #2							
LXIX	A	5	All	Lavatory	1.02×10^7	±	2.34×10^5
XXII	A	2	Pre-Board	Seat, Floor	BDL		BDL
ML	A	3	CA, M, PD	Seat, Floor	3.90×10^7	±	1.84×10^6
XXX	A	2	Post-Landing	Seat, Floor	1.01×10^5	±	7.41×10^3
Flight #3							
XX	B	6	Mid-Flight	Seat	3.16×10^6	±	3.36×10^6
Flight #4							
XXIII	B	2	CA, M, PD	Seat, Floor	3.22×10^6	±	8.23×10^4
XXVI	B	5	All	Lavatory	6.42×10^6	±	1.41×10^5

[a]Abbreviations: PB, pre-board; CA: cruise altitude; M, mid-way through; PD, pre-descent; L, after landing

[b]Samples from identical locations on distinct flights were pooled to facilitate effective DNA extraction for PCR and clone library construction.

[c]SD: standard deviation was calculated based on 3 individual measurement of each sample.

Table 4.3.2-2 Identification of clones based on sequences of the 16S rRNA gene

RFLP pattern	Nearest Neighbor[a]	% Similarity	% occurrence of clones found in:		Ecological and medical significance
			Airline A	Airline B	
1	*Abiotrophia paraadiacens*	99	3.6	0.7	Oral cavity, endocarditis [31, 32]
2	*Acinetobacter radioresistens*	99	0.3		Widespread about the skin of healthy humans [33]
3	*Capnocytophaga sputigena*	99	0.5		Oral cavity, periodontitis, gingivitis [34]
4	*Fusobacterium nucleatum*	98	1.1	0.7	Very common in periodontal infections [35]
5	*Gemella haemolysans*	99	4.4	0.4	Oral cavity, endocarditis, meningitis [32]
6	*Haemophilus parainfluenzae*	97	1.1		Causes chronic obstructive pulmonary disease [36]
7	*Massilia timonae*	98	0.6	1.1	Associated with post-surgical infections and cerebellar lesions [37]
8	*Neisseria meningitidis*	98	62.3	13.2	Causative agent of bacterial meningitis and other human diseases [29]
9	*Peptostreptococcus anaerobius*	98	0.8		Causes prosthetic valve endocarditis [38]
10	*Porphyromonas* sp. oral clone	99	0.6		Associated with chronic periodontal disease and dentoalveolar abscesses [39]
11	*Prevotella melaninogenica*	99	1.5	1.5	Putative etiologic agent of periodontal disease [40]
12	*Pseudomonas synxantha*	99		69.2	Worldwide distribution [3]
13	*Rothia mucilaginosa*	99	0.8		Oral cavity, endocarditis, meningitis, periodontitis [32]
14	*Streptococcus oralis/mitis*	98	18.9	9.2	Endocarditis [32]
15	*Terrahaemophilus aromaticivorans*	98	2.6	4.0	Present in T-RFLP collected from subgingival plaque [41]
16	*Veillonella parvula*	99	1.1		Periodontopathic bacteria [42]

[a]Sequences of clone inserts were compared to that of known microbes via NCBI blast.

Table 4.3.2-3 Microbial diversity fluctuations at various locations of commercial airliner cabin air

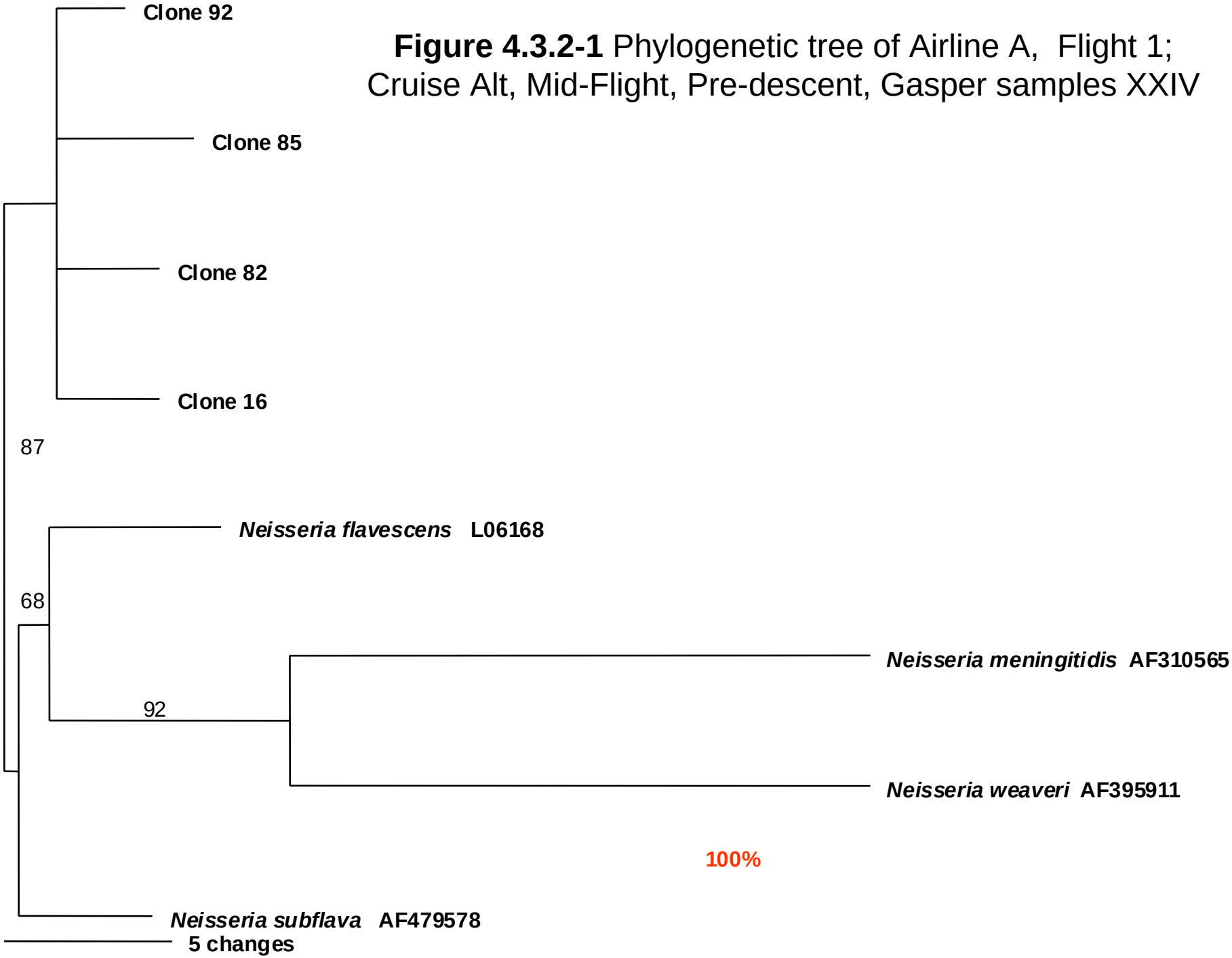
Times[a]	Sample #	Locations	Flight #	Airliner	# of OTUs[b]	Nearest neighbors of the clones sequences [% of occurrence]
Category A:						
CA, M, PD	XXIV	Gasper	1	A	1	*Neisseria meningitidis* [100]
CA, M, PD	XXI	Lavatory	1	A	6	*Neisseria meningitidis* [55], *Streptococcus oralis/mitis* [23], *Gemella haemolysans* [9], *Terrahaemophilus aromaticivorans* [6], *Prevotella melaninogenica* [5], *Abiotrophia paraadiacens* [2]
CA, M, PD	XXV	Seat, Floor	1	A	6	*Neisseria meningitidis* [52], *Streptococcus oralis/mitis* [23], *Gemella haemolysans* [10], *Haemophilus parainfluenzae* [8], *Porphyromonas catoniae* [3], *Peptostreptococcus anaerobius* [2]
Category B:						
PB	XXII	Seat, Floor	2	A	6	*Neisseria meningitidis* [74], *Abiotrophia paraadiacens* [8], *Rothia mucilaginosa* [7], *Fusobacterium nucleatum* [3], *Gemella haemolysans* [5], *Terrahaemophilus aromaticivorans* [4]
CA, M, PD	ML	Seat, Floor	2	A	9	*Neisseria meningitides* [41], *Streptococcus oralis/mitis* [39], *Gemella haemolysans* [7], *Veillonella parvula* [3], *Abiotrophia paraadiacens* [2], *Haemophilus parainfluenzae* [1]
L	XXX	Seat, Floor	2	A	3	*Neisseria meningitides* [98], *Haemophilus parainfluenzae* [1], *Massilia timonae* [1]
Category C:						
All	LXIX	Lavatory	2	A	6	*Streptococcus oralis/mitis* [51], *Neisseria meningitidis* [32], *Terrahaemophilus*

						aromaticivorans [6], *Abiotrophia paraadiacens* [4], *Peptostreptococcus anaerobius* [4], *Fusobacterium nucleatum* [3]
All	XXVI	Lavatory	4	B	2	*Massilia timonae* [97], *Pseudomonas synxantha* [3]
Category D:						
M	XX	Seat	3	B	5	*Neisseria meningitidis* [47], *Streptococcus oralis/mitis* [32], *Terrahaemophilus aromaticivorans* [14], *Prevotella melaninogenica* [5], *Gemella haemolysans* [1]
CA, M, PD	XXIII	Seat, Floor	4	B	1	*Pseudomonas synxantha* [100]

[a]Abbreviations: PB, pre-board; CA: cruise altitude; M, mid-way through; PD, pre-descent; L, after landing; Refer to the text for categorical description.

[b]OTU: Operational taxonomic units

Figure 4.3.2-1 Phylogenetic tree of Airline A, Flight 1; Cruise Alt, Mid-Flight, Pre-descent, Gasper samples XXIV



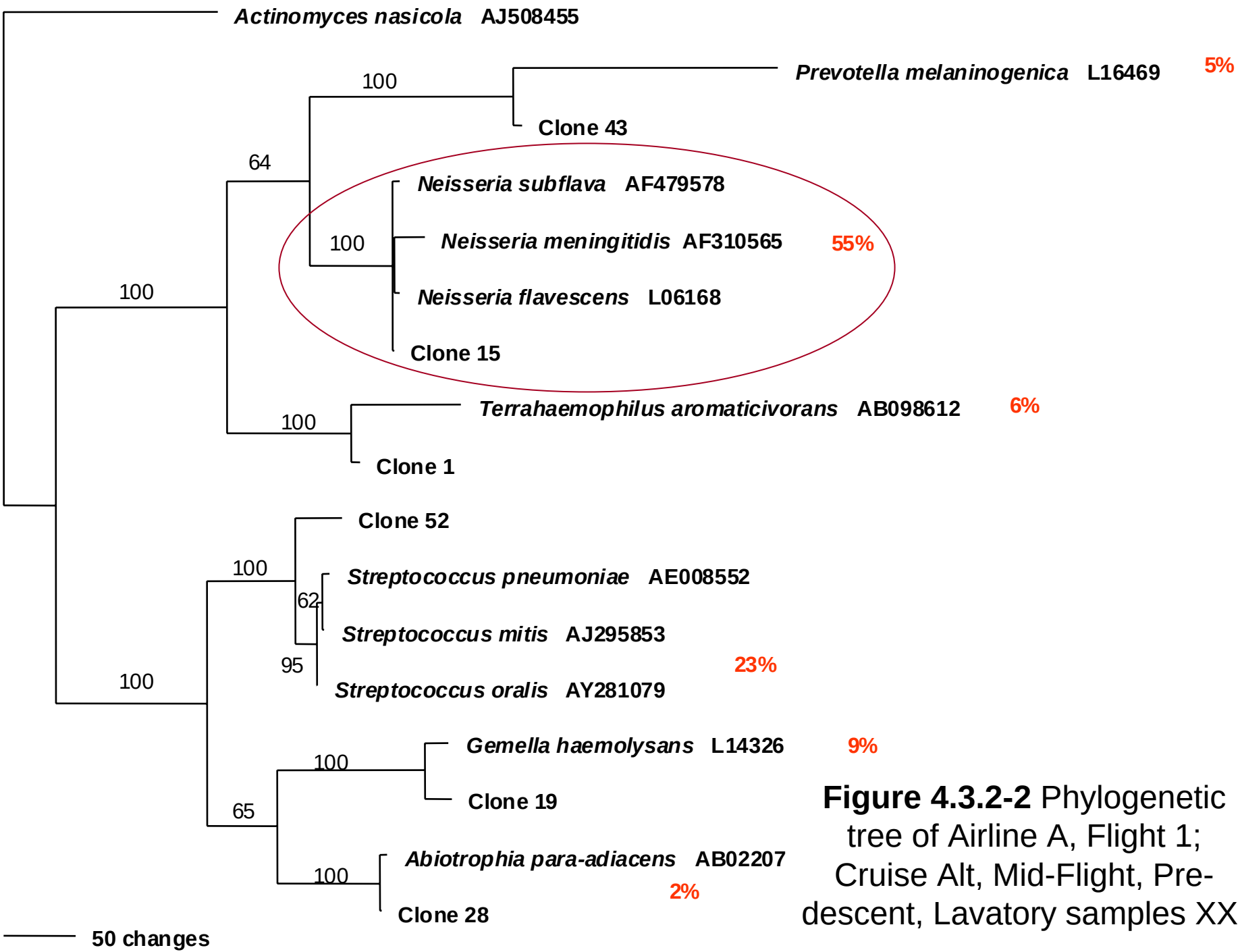


Figure 4.3.2-2 Phylogenetic tree of Airline A, Flight 1; Cruise Alt, Mid-Flight, Pre-descent, Lavatory samples XXI

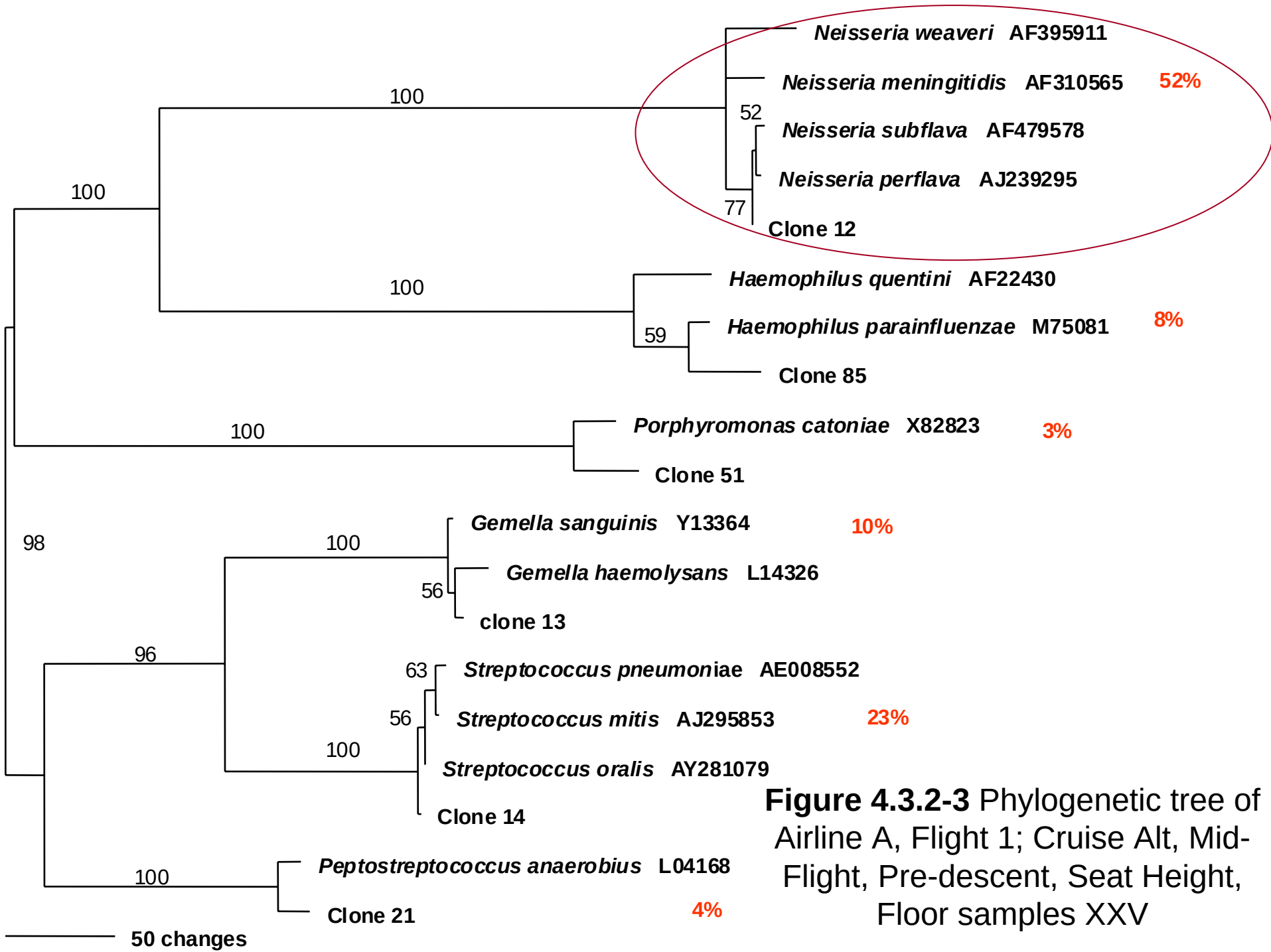


Figure 4.3.2-3 Phylogenetic tree of Airline A, Flight 1; Cruise Alt, Mid-Flight, Pre-descent, Seat Height, Floor samples XXV

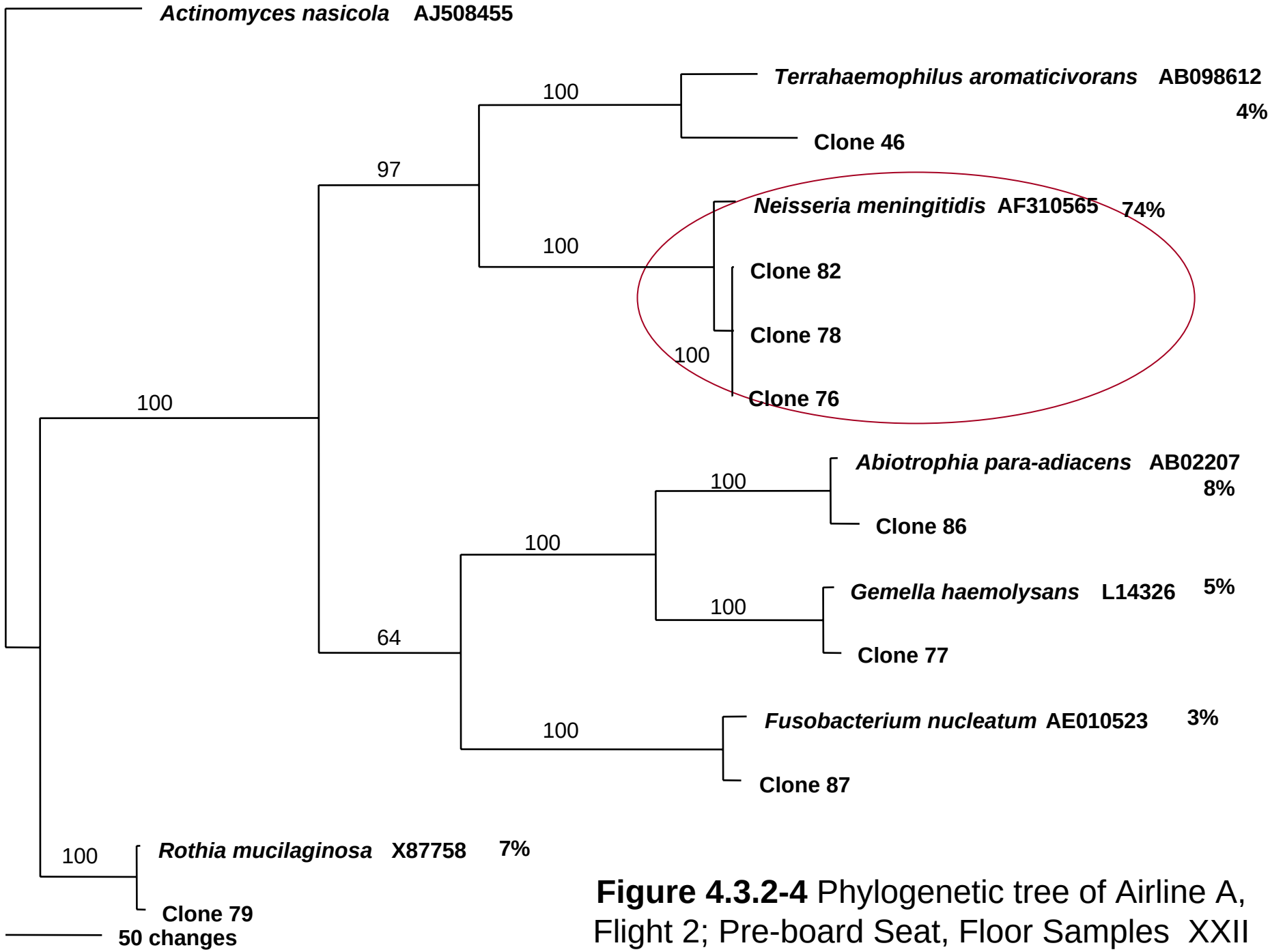


Figure 4.3.2-4 Phylogenetic tree of Airline A, Flight 2; Pre-board Seat, Floor Samples XXII

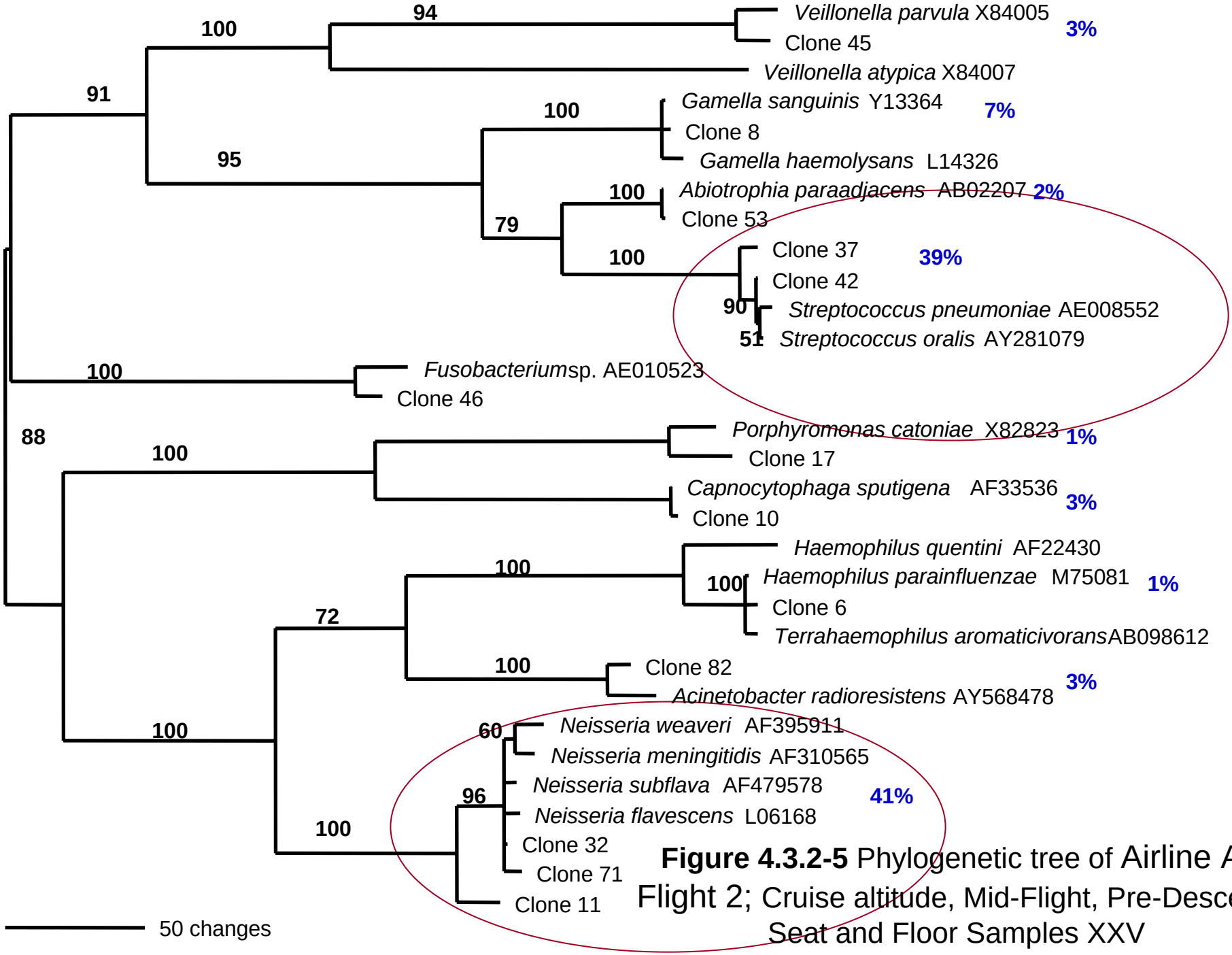
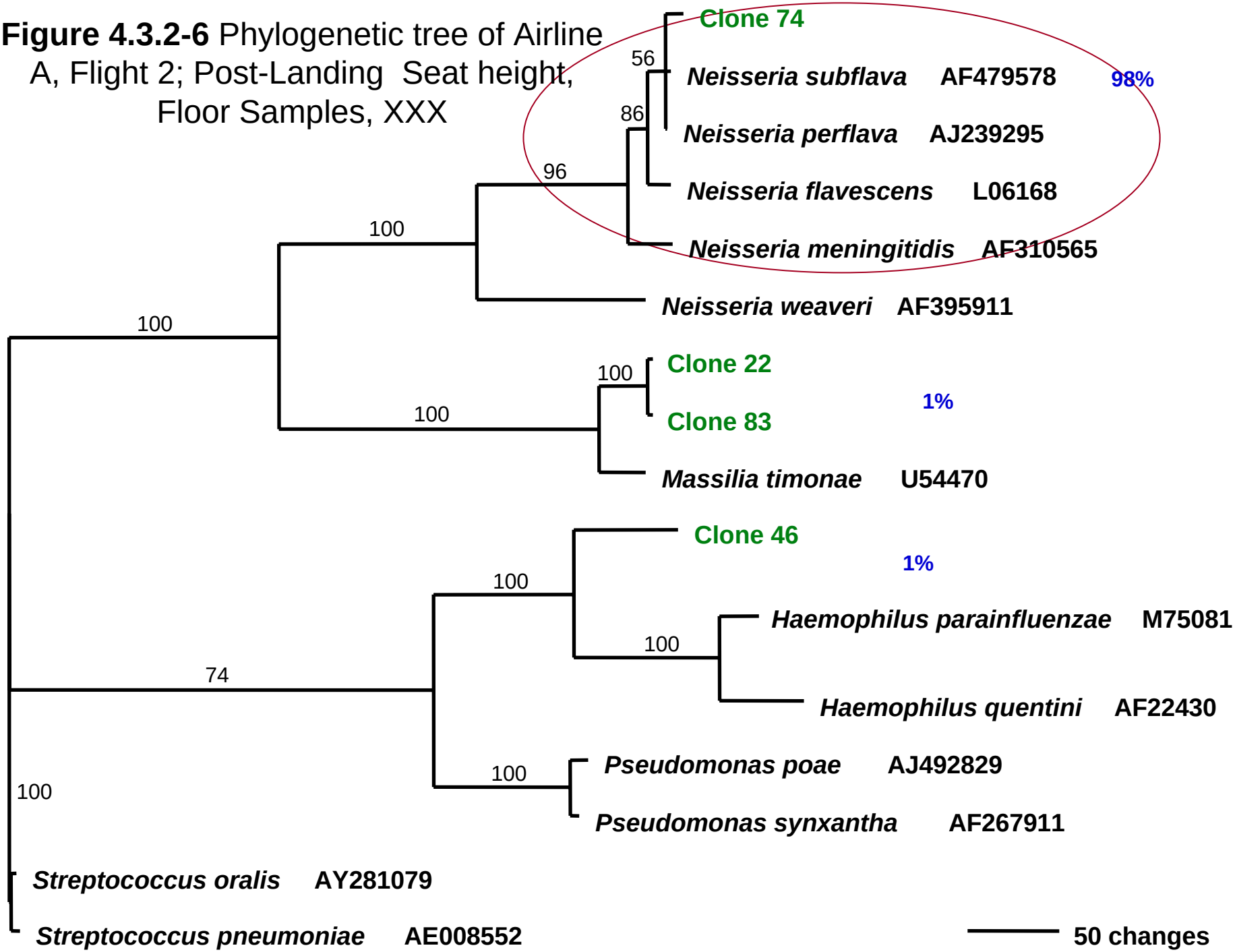


Figure 4.3.2-5 Phylogenetic tree of Airline A, Flight 2; Cruise altitude, Mid-Flight, Pre-Descent, Seat and Floor Samples XXV

Figure 4.3.2-6 Phylogenetic tree of Airline A, Flight 2; Post-Landing Seat height, Floor Samples, XXX



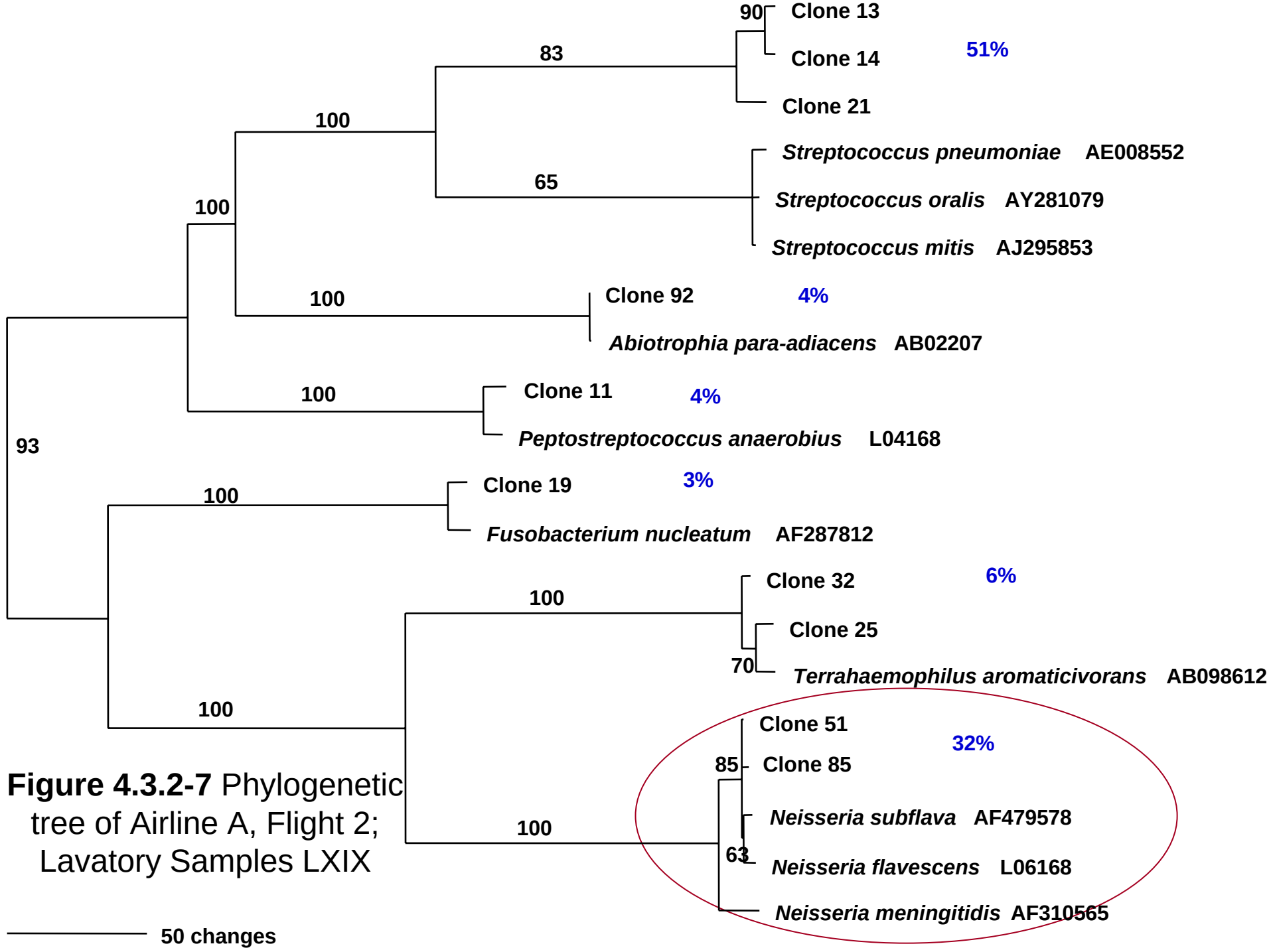


Figure 4.3.2-7 Phylogenetic tree of Airline A, Flight 2; Lavatory Samples LXIX

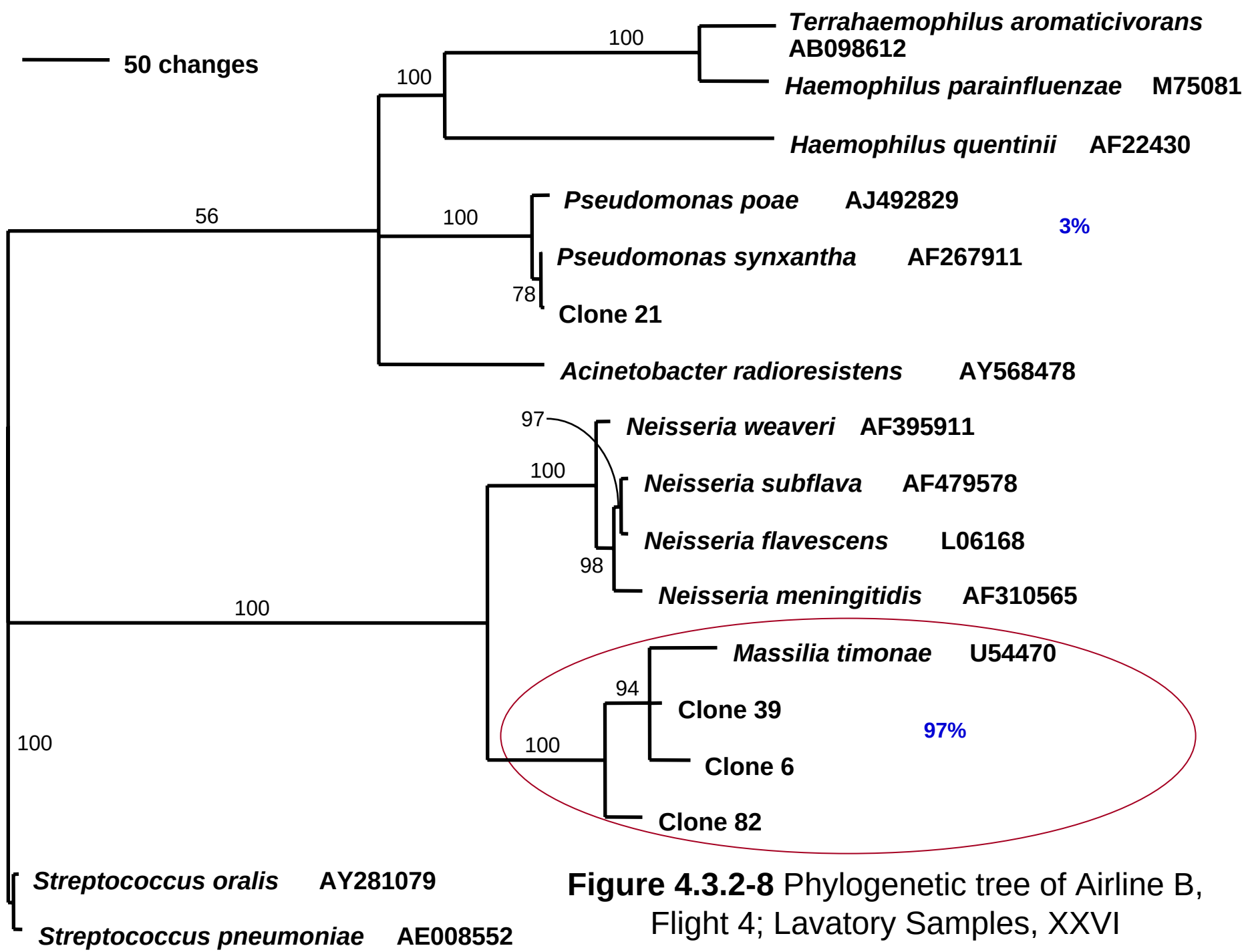


Figure 4.3.2-8 Phylogenetic tree of Airline B, Flight 4; Lavatory Samples, XXVI

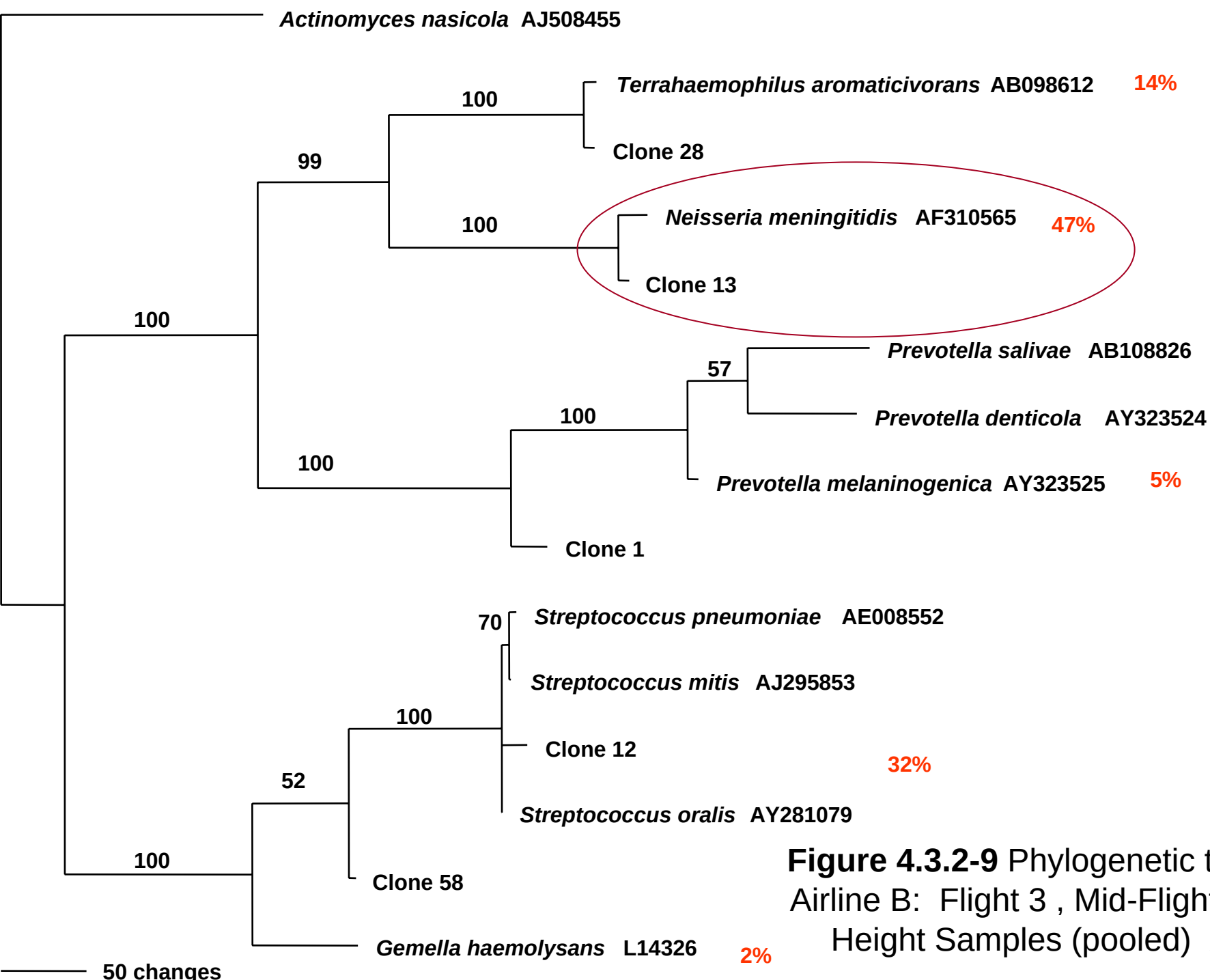


Figure 4.3.2-9 Phylogenetic tree of Airline B: Flight 3 , Mid-Flight Seat Height Samples (pooled) XX

Figure 4.3.2-10 Phylogenetic tree of Airline B, Flight 4; Cruise Alt, Mid-Flight, Pre-Descent Seat, Floor Samples XXIII

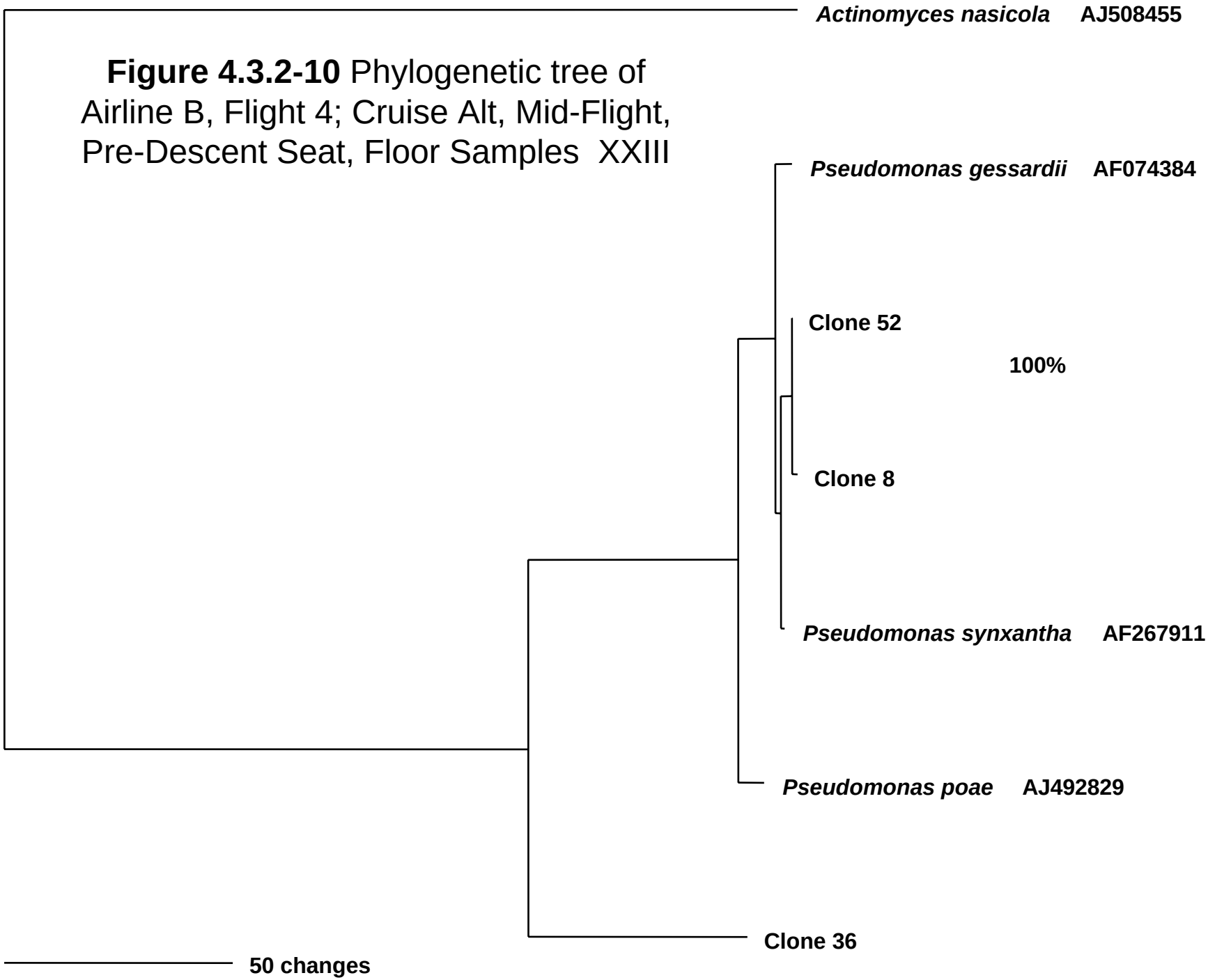


Table 4.3.2-4 Overview of molecular bacterial diversity in Phase 2 air samples (all flights)

Class of microbe	Percent incidence of clones retrieved from:															
	1.0	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
	1N	1R-N	2N	2R-N	3-L	3R-L	4-S	4R-S	5N	5R-N	6J	6R-J	7N	7R-N	8S	8S-R
	LAX to JFK	JFK to LAX	LAX to JFK	JFK to LAX	LAX to London	London to LAX	LAX to Sydney	Sydney to LAX	LAX to JFK	JFK to LAX	LAX to NRT	NRT to LAX	LAX to JFK	JFK to LAX	LAX to Sydney	Sydney to LAX
α–proteobacteria:		**100.0**		No PCR product amplified and subsequently cloning process was not carried	No PCR product amplified and subsequently cloning process was not carried	No PCR product amplified and subsequently cloning process was not carried	**19.2**		**15.9**	**27.3**	**10.0**	**85.7**		**26.8**	**59.9**	**45.0**
β– proteobacteria:	**3.6**		**33.5**					**3.2**	**5.3**		**15.0**		**30.8**	**6.7**		**2.5**
γ–proteobacteria:	**85.5**		**40.2**				**6.4**	**12.8**	**37.1**		**10.0**		**53.9**	**13.4**		
δ–proteobacteria:								**3.2**						**6.7**		
Actinobacteria	**1.2**		**13.4**				**16.0**	**19.2**	**10.6**	**45.5**	**15.0**			**6.7**		**5.0**
Firmicutes			**13.4**				**44.8**	**38.4**	**31.8**	**27.3**	**45.0**	**8.6**		**40.2**	**13.4**	**7.5**
Fusobacteria							**3.2**									
Deinococcus													**7.7**		**66.9**	**35.0**
Spirochaetes																
Bacterioidetes												**2.9**				
Cyanobacteria							**9.6**	**22.6**				**2.9**	**7.7**			**2.5**
Planktomycetes														**6.7**		
# of clones sequenced in specified flight	83	49	15				31	31	19	11	20	35	13	15	28	40
# of bacterial species in specified flight	5	2	9				26	17	17	5	13	4	7	10	8	14

Table 4.3.2-5 Detailed summary of molecular bacterial diversity in Phase 2 air samples (all flights)

Nearest neighbor (GenBank accession #)			Percent incidence of clones retrieved specified flight NOTE: >10% of clone library																	
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
			1N	1R-N	2N	2R-N	3-L	3R-L	4-S	4R-S	5N	5R-N	6J	6R-J	7N	7R-N	8S	8S-R	6J-G	6R-SS
			LAX to JFK	JFK to LAX	LAX to JFK	JFK to LAX	LAX to London	London to LAX	LAX to Sydney	Sydney to LAX	LAX to JFK	JFK to LAX	LAX to NRT	NRT to LAX	LAX to JFK	JFK to LAX	LAX to Sydney	Sydney to LAX	LAX to NRT	NRT to LAX
α–proteobacteria:				**100.0**					**19.2**		**15.9**	**27.3**	**10.0**	**85.7**		**26.8**	**59.9**	**45.0**	**14.4**	**5.8**
	Acetobacter aceti	X74066							3.2											
	Agrobacterium sanguineum	AB021493																2.5		
	Agrobacterium tumefaciens	M11223											5.0						1.6	
	Bradyrhizobium betae	AY372184									5.3									
	Caedibacter caryophilus	X71837																	1.6	
	Caulobacter henricii	AJ227758															13.3			
	Caulobacter vibrioides	AJ009957															20.0	37.5		
	Erythrobacter litoralis	AB013354		98.0																
	Methylobacterium hispanicum	AJ635304												85.7			13.3			
	Methylobacterium organophilum	D32226							3.2											
	Novosphingobium hassiacum	AJ416411		2.0							5.3									
	Novosphingobium pentaromativorans	AF502400							3.2							20.1				
	Novosphingobium subarcticum	X94102														6.7				
	Paracoccus alcaliphilus	D32238							3.2											
	Paracoccus carotinifaciens	AB006899							3.2											
	Paracoccus denitrificans	X69159																		2.9
	Phenylobacterium immobile	Y18216																	1.6	
	Rhizobium leguminosarum	AY509899															13.3	5.0		
	Rhodobacter blasticus	D16429																	1.6	
	Rhodopseudomonas palustris	D25312							3.2			27.3							6.4	2.9
	Skermanella parooensis	X90760																	1.6	
	Sphingomonas faeni	AJ429239											5.0							
	Stella vacuolata	AJ535711									5.3									
β– proteobacteria:			**3.6**		**33.5**					**3.2**	**5.3**		**15.0**		**30.8**	**6.7**		**2.5**	**22.4**	**5.8**
	Aquabacterium commune	AF035054																	3.2	
	Burkholderia andropogonis	AB021422																		2.9
	Burkholderia cepacia	AB114607	1.2										10.0						1.6	

Table 4.3.2-5 Detailed summary of molecular bacterial diversity in Phase 2 air samples (all flights)

Nearest neighbor (GenBank accession #)			Percent incidence of clones retrieved specified flight NOTE: >10% of clone library																	
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
			1N	1R-N	2N	2R-N	3-L	3R-L	4-S	4R-S	5N	5R-N	6J	6R-J	7N	7R-N	8S	8S-R	6J-G	6R-SS
			LAX to JFK	JFK to LAX	LAX to JFK	JFK to LAX	LAX to London	London to LAX	LAX to Sydney	Sydney to LAX	LAX to JFK	JFK to LAX	LAX to NRT	NRT to LAX	LAX to JFK	JFK to LAX	LAX to Sydney	Sydney to LAX	LAX to NRT	NRT to LAX
	Burkholderia vietnamiensis	U96929	2.4																	
	Curvibacter gracilis	AB109889													7.7					
	Dechloromonas agitata	AF047462																	6.4	
	Diaphorobacter nitroreducens	AB076856			6.7															
	Duganella violaceinigra	AY376163																	1.6	
	Duganella zoogloeoides	D14256																	8.0	
	Herbosprillum seropedicae	Y10146																2.5		
	Hydrogenophaga defluvii	AJ585993																	1.6	
	Imtechium assamiensis	AY544767			13.4															
	Janthinobacterium lividum	Y08846											5.0							
	Leptothrix mobilis	X97071														6.7				
	Limnobacter thiooxidans	AJ289885																		2.9
	Massilia timonae	U54470			13.4						5.3									
	Neisseria flavescens	L06168																		
	Neisseria weaveri	L10738								3.2										
	Wautersia paucula	AF085226													23.1					
γ–proteobacteria:			**85.5**		**40.2**				**6.4**	**12.8**	**37.1**		**10.0**		**53.9**	**13.4**			**19.2**	
	Acinetobacter calcoaceticus	Z93434								3.2										
	Acinetobacter junii	X81658			20.1						5.3									
	Acinetobacter johnsonii	X81663							3.2	3.2										
	Acinetobacter lwoffii	X81665																	1.6	
	Citrobacter murliniae	AF025369								3.2										
	Escherichia coli	X80729			6.7															
	Haemophilus haemolyticus	M75045									5.3									
	Haemophilus paraphrohaemolyticus	M75076									5.3									
	Pseudomonas hibiscicola	AB021405								3.2	10.6					6.7				
	Pseudomonas lanceolata	AB021390							3.2											
	Pseudomonas putida	D85994			6.7						5.3									

Table 4.3.2-5 Detailed summary of molecular bacterial diversity in Phase 2 air samples (all flights)

Nearest neighbor (GenBank accession #)		Percent incidence of clones retrieved specified flight NOTE: >10% of clone library																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
		1N	1R-N	2N	2R-N	3-L	3R-L	4-S	4R-S	5N	5R-N	6J	6R-J	7N	7R-N	8S	8S-R	6J-G	6R-SS
		LAX to JFK	JFK to LAX	LAX to JFK	JFK to LAX	LAX to London	London to LAX	LAX to Sydney	Sydney to LAX	LAX to JFK	JFK to LAX	LAX to NRT	NRT to LAX	LAX to JFK	JFK to LAX	LAX to Sydney	Sydney to LAX	LAX to NRT	NRT to LAX
Pseudomonas saccharophila	AB021407													23.1					
Pseudomonas trivialis	AJ492831														6.7				
Psychrobacter marinicola	AY292940	1.2																	
Psychrobacter meningitidis	AY057116	84.3																	
Salmonella typhi	Z47544									5.3				7.7					
Shigella flexneri	X96963																	3.2	
Stenotrophomonas maltophilia	AB008509											10.0		23.1				12.8	
Thiothrix eikelboomii	AB042819																	1.6	
Serratia ficaria	AJ279054			6.7															
δ–proteobacteria:									**3.2**						**6.7**				
Bacteriovorax stolpii	M34125								3.2						6.7				
Actinobacteria		**1.2**		**13.4**				**16.0**	**19.2**	**10.6**	**45.5**	**15.0**			**6.7**		**5.0**	**9.6**	**20.3**
Aerococcus viridans	M58797																2.5		
Arsenicicoccus bolidensis	AJ558133								3.2										
Actinomyces viscosus	X82453							3.2											
Corynebacterium lipophiloflavum	Y09045																		2.9
Corynebacterium tuberculostearicum	AJ438050								16.0										11.6
Curtobacterium citreum	X77436																	1.6	
Dermatophilus chelonae	AJ243919																		2.9
Facklamia hominis	Y10772																2.5		
Gordonia sinesedis	AF380834	1.2																	
Granulicatella adiacens	D50540									5.3									
Kocuria rhizophila	Y16264											5.0							
Microbacterium hominis	AB004727							3.2											
Microbacterium kitamiense	AB013907											5.0							
Microbacterium phyllosphaerae	AJ277840																	1.6	
Micrococcus luteus	AJ536198																	1.6	2.9

Table 4.3.2-5 Detailed summary of molecular bacterial diversity in Phase 2 air samples (all flights)

Nearest neighbor (GenBank accession #)			Percent incidence of clones retrieved specified flight NOTE: >10% of clone library																	
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
			1N	1R-N	2N	2R-N	3-L	3R-L	4-S	4R-S	5N	5R-N	6J	6R-J	7N	7R-N	8S	8S-R	6J-G	6R-SS
			LAX to JFK	JFK to LAX	LAX to JFK	JFK to LAX	LAX to London	London to LAX	LAX to Sydney	Sydney to LAX	LAX to JFK	JFK to LAX	LAX to NRT	NRT to LAX	LAX to JFK	JFK to LAX	LAX to Sydney	Sydney to LAX	LAX to NRT	NRT to LAX
	Mycobacterium chitae	M29560							6.4										1.6	
	Propionibacterium acnes	AB042287			13.4															
	Propionibacterium propionicum	AJ003058							3.2		5.3									
	Rubrobacter radiotolerans	U65647											5.0						3.2	
	Williamsia maris	AB010909										45.5				6.7				
Firmicutes					**13.4**				**44.8**	**38.4**	**31.8**	**27.3**	**45.0**	**8.6**		**40.2**	**13.4**	**7.5**	**17.6**	**64.1**
	Anaerococcus octavius	Y07841																		2.9
	Bacillus flexus	AB021185							6.4											
	Bacillus licheniformis	X68416							3.2											
	Bacillus pallidus	Z26930														20.1				
	Bacillus pumilus	AY456263										9.1							1.6	
	Bacillus muralis	AJ628748																2.5		
	Bacillus vallismortis	AB021198								3.2										
	Bacillus weihenstephanensis	AB021199							3.2											
	Brevibacillus choshinensis	D78459							3.2											
	Enterococcus durans	AJ276354																		8.8
	Exiguobacterium oxidotolerans	AB105164								3.2										
	Gemella haemolysans	L14326								3.2										
	Jeotgalicoccus halotolerans	AY028925																	1.6	
	Lactococcus lactis subsp. cremoris	M58836									5.3		5.0							
	Sporanaerobacter acetigenes	AF358114							3.2											
	Staphylococcus arlettae	AB009933							3.2											
	Staphylococcus aureus	L37597																	1.6	
	Staphylococcus caprae	AB009935							3.2	6.4										
	Staphylococcus cohnii	AB009936									5.3									
	Staphylococcus epidermidis	L37605									5.3	9.1	15.0						4.8	
	Staphylococcus haemolyticus	X66100								6.4										
	Staphylococcus hominis	X66101							3.2		10.6	9.1	10.0	8.6						17.4

Table 4.3.2-5 Detailed summary of molecular bacterial diversity in Phase 2 air samples (all flights)

Nearest neighbor (GenBank accession #)			Percent incidence of clones retrieved specified flight NOTE: >10% of clone library																	
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
			1N	1R-N	2N	2R-N	3-L	3R-L	4-S	4R-S	5N	5R-N	6J	6R-J	7N	7R-N	8S	8S-R	6J-G	6R-SS
			LAX to JFK	JFK to LAX	LAX to JFK	JFK to LAX	LAX to London	London to LAX	LAX to Sydney	Sydney to LAX	LAX to JFK	JFK to LAX	LAX to NRT	NRT to LAX	LAX to JFK	JFK to LAX	LAX to Sydney	Sydney to LAX	LAX to NRT	NRT to LAX
	Staphylococcus pasteuri	AB009944															6.7			2.9
	Staphylococcus saccharolyticus	L37602																		2.9
	Staphylococcus sanguinis	AF003928															6.7			
	Staphylococcus succinus	AF004220																		2.9
	Staphylococcus warneri	L37603							3.2	3.2										2.9
	Streptococcus australis	AY485604											10.0							
	Streptococcus iniae	AF335572																		2.9
	Streptococcus mitis	AF003929							3.2	6.4			5.0			6.7			3.2	
	Streptococcus mutans	X58303																		8.8
	Streptococcus parasanguinis	AF003933							6.4											
	Streptococcus pneumoniae	AF003930														13.4				
	Streptococcus pseudopneumoniae	AY612844																2.5		
	Streptococcus thermophilus	X68418							3.2	6.4	5.3							2.5	3.2	2.9
	Streptococcus sanguinis	AY281085			13.4															8.8
	Weissella cibaria	AJ295989																	1.6	
Fusobacteria									**3.2**											
	Fusobacterium canifelinum	AY162220							3.2											
Deinococcus															**7.7**		**66.9**	**35.0**	**1.6**	
	Deinococcus geothermalis	Y13038																2.5		
	Deinococcus grandis	Y11329															13.3	2.5		
	Deinococcus murrayi	Y13041															53.6	30.0	1.6	
	Meiothermus silvanus	X84211													7.7					
Spirochaetes																			**1.6**	
	Leptospira parva	Z21636																	1.6	
Bacterioidetes														**2.9**					**6.4**	

Table 4.3.2-5 Detailed summary of molecular bacterial diversity in Phase 2 air samples (all flights)

Nearest neighbor (GenBank accession #)			Percent incidence of clones retrieved specified flight NOTE: >10% of clone library																	
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
			1N	1R-N	2N	2R-N	3-L	3R-L	4-S	4R-S	5N	5R-N	6J	6R-J	7N	7R-N	8S	8S-R	6J-G	6R-SS
			LAX to JFK	JFK to LAX	LAX to JFK	JFK to LAX	LAX to London	London to LAX	LAX to Sydney	Sydney to LAX	LAX to JFK	JFK to LAX	LAX to NRT	NRT to LAX	LAX to JFK	JFK to LAX	LAX to Sydney	Sydney to LAX	LAX to NRT	NRT to LAX
	Bacteriodes vulgatus													2.9						
	Chryseobacterium scophthalmum	AJ271009																	3.2	
	Flavobacterium ferrugineum	M62798																	3.2	
Cyanobacteria									**9.6**	**22.6**				**2.9**	**7.7**			**2.5**	**6.4**	**8.8**
	Oscillatoria sancta	AF132933							9.6	22.6								2.5	3.2	8.8
	Oscillatoria sp.	AB074509												2.9						
	Microcoleus chthonoplastes	X70770													7.7					
	Microcystis aeruginosa	AB035549																	3.2	
Planctomycetes																**6.7**				
	Gemmata obscuriglobus	X56305														6.7		2.5		
	# of clones sequenced		83	49	15				31	31	19	11	20	35	13	15	28	40	62	34
	# of bacterial species		5	2	9				26	17	17	5	13	4	7	10	8	14	34	20
	# of dominant species (>10%)		2	1	5				0	2	2	2	5	1	3	3	6	2	1	2

Table 4.3.2-6 Molecular bacterial diversity of Los Angeles/New York round trip (1N and 1R-N)

Nearest neighbor (GenBank accession #)			% clones		Significance (given in color are human pathogens or isolated from patient)
			1	2	
			1N	1R-N	
			Los Angeles/New York	New York/Los Angeles	
α–proteobacteria:				**100.0**	
	Erythrobacter litoralis	AB013354		98.0	Obligate aerobic phototrophs
	Novosphingobium hassiacum	AJ416411		2.0	Isolated from a well aerated sewage pond
β– proteobacteria:			**3.6**		
	Burkholderia cepacia	AB114607	1.2		Opportunistic human pathogens, able to cause devastating infections in patients with cystic fibrosis
	Burkholderia vietnamiensis	U96929	2.4		Not easily distinguished from the more problematic *B. cepacia*
γ–proteobacteria:			**85.5**		
	Psychrobacter marinicola	AY292940	1.2		Psychrophilic halophiles isolated from marine environments
	Psychrobacter meningitidis	AY057116	84.3		Causes meningitis in infants
Actinobacteria			**1.2**		
	Gordonia sinesedis	AF380834	1.2		Soil bacteria

# of clones sequenced in clone library		83	49	
# of bacterial species in clone library		5	2	
# of dominant species (>10% of clone library)		1	1	

Table 4.3.2-7 Molecular bacterial diversity of Los Angeles/New York round trip (2N and 2R-N)

Nearest neighbor (GenBank accession #)			% clones		Significance (given in color are human pathogens or isolated from patient)
			3	4	
			2N	2R-N	
			Los Angeles/New York	New York/Los Angeles	
β– proteobacteria:			**33.5**		
	Diaphorobacter nitroreducens	AB076856	6.7		Denitrifying bacterium capable of degrading polyhydroxybutyrate. Isolated from activated sludge
	Imtechium assamiensis	AY544767	13.4		isolated from a warm spring of Assam, India
	Massilia timonae	U54470	13.4		Isolated from the blood of a meningoencephalitis patient
γ–proteobacteria:			**40.2**		
	Acinetobacter junii	X81658	20.1		Associated with bacteremia in preterm infants and pediatric oncologic patients
	Escherichia coli	X80729	6.7		Commensal human gut microflora; opportunistic pathogen
	Pseudomonas putida	D85994	6.7		Non-pathogenic environmental isolate able to degrade a variety of aromatic hydrocarbons
	Serratia ficaria	AJ279054	6.7		patients with gallbladder empyemas and one patient with septicemia originating from the gut
Actinobacteria			**13.4**		
	Propionibacterium acnes	AB042287	13.4		Frequently recovered cause of anaerobic arthritis in association with prosthetic joints and, rarely, has been implicated in cases of osteomyelitis
Firmicutes			**13.4**		
	Streptococcus sanguinis	AY281085	13.4		Indigenous human oral microflora, recognized as a key player in the bacterial colonization of the mouth
# of clones sequenced in clone library			15		
# of bacterial species in clone library			9		
# of dominant species (>10% of clone library)			5		

Table 4.3.2-8 Molecular bacterial diversity of Los Angeles/Sydney round trip (4S and 4R-S)

Nearest neighbor (GenBank accession #)			% clones		Significance (given in color are human pathogens or isolated from patient)
			7	8	
			4-S	4R-S	
			Los Angeles/Sydney	Sydney/Los Angeles	
α–proteobacteria:			**19.2**		
	Acetobacter aceti	X74066	3.2		Soil microflora
	Methylobacterium organophilum	D32226	3.2		Oxidizes methanol
	Novosphingobium hassiacum	AJ416411			Isolated from a well aerated sewage pond
	Novosphingobium pentaromativorans	AF502400	3.2		Polycyclic aromatic hydrocarbon-degrading bacterium isolated from estuarine sediment
	Paracoccus alcaliphilus	D32238	3.2		Alkaliphilic and facultatively methylotrophic bacterium
	Paracoccus carotinifaciens	AB006899	3.2		Aerobic, Gram-negative astaxanthin-producing bacterium
	Rhodopseudomonas palustris	D25312	3.2		Common in soil and water, makes its living by converting sunlight to cellular energy and by absorbing atmospheric carbon dioxide and converting it to biomass
β– proteobacteria:				**3.2**	
	Neisseria weaveri	L10738		3.2	Normal canine oral flora. Infections in humans are usually associated with dog bite wounds
γ–proteobacteria:			**6.4**	**12.8**	
	Acinetobacter calcoaceticus	Z93434		3.2	Oil degrading bacterium
	Acinetobacter junii	X81658			Associated with bacteremia in preterm infants and pediatric oncologic patients
	Acinetobacter johnsonii	X81663	3.2	3.2	Catheter related infections; Clin Infect Dis. 1993 Oct;17(4):632-6
	Citrobacter murliniae	AF025369		3.2	Common coliforms associated with human gut
	Pseudomonas hibiscicola	AB021405		3.2	Bacteria from ticks, lice and fleas
	Pseudomonas lanceolata	AB021390	3.2		Aquatic microflora
δ–proteobacteria:				**3.2**	
	Bacteriovorax stolpii	M34125		3.2	Bdellovibrio-and-like organisms that are Gram-negative, predatory bacteria that inhabit terrestrial, freshwater and salt-water environments.
Actinobacteria			**16.0**	**19.2**	
	Arsenicicoccus bolidensis	AJ558133		3.2	Facultatively anaerobic, non-spore-forming, coccus-shaped bacterium from sediment

Table 4.3.2-8 Molecular bacterial diversity of Los Angeles/Sydney round trip (4S and 4R-S)

Nearest neighbor (GenBank accession #)			% clones		Significance (given in color are human pathogens or isolated from patient)
			7	8	
			4-S	4R-S	
			Los Angeles/Sydney	Sydney/Los Angeles	
	Actinomyces viscosus	X82453	3.2		Can cause Primary Actinomycosis. Breast Journal, Vol 11, pp. 57-59
	Corynebacterium tuberculostearicum	AJ438050		16.0	A leprosy-derived Corynebacterium
	Microbacterium hominis	AB004727	3.2		Soil microflora
	Mycobacterium chitae	M29560	6.4		Nonpathogenic, rapidly growing mycobacterial species found in the environment
	Propionibacterium propionicum	AJ003058	3.2		Involved with infections of the lacrimal apparatus. J. Clin. Microbiol. 1997, 35: 2464-2471
Firmicutes			**44.8**	**38.4**	
	Bacillus flexus	AB021185	6.4		Soil microflora
	Bacillus licheniformis	X68416	3.2		Can be found in soils and plant material, used in the biotechnology industry to manufacture enzymes, antibiotics, biochemicals and consumer products
	Bacillus pumilus	AY456263			Nonpathogenic, used for alkaline protease production, in environmental decontamination of dioxins, and in the baking industry.
	Bacillus vallismortis	AB021198		3.2	Isolated from soil in Death Valley, California
	Bacillus weihenstephanensis	AB021199	3.2		Psychrotolerant, nonpathogenic member of the B. cereus group
	Brevibacillus choshinensis	D78459	3.2		Protein-hyperproducing bacterium - has been used for commercial protein production
	Exiguobacterium oxidotolerans	AB105164		3.2	An alkaliphile was isolated from a drain of a fish processing plant
	Gemella haemolysans	L14326		3.2	a coccoid bacterium was isolated from the blood of a patient with endocarditis
	Lactococcus lactis subsp. cremoris	M58836			Widely used in the manufacture of fermented milk products
	Sporanaerobacter acetigenes	AF358114	3.2		Anaerobic, moderately thermophilic, sporulating rod isolated from an anaerobic sludge blanket
	Staphylococcus arlettae	AB009933	3.2		Coagulase-negative, novobiocin-resistant species from isolated from animals
	Staphylococcus caprae	AB009935	3.2	6.4	Commensal on human skin, implicated in infections of the bloodstream, urinary tract, bones and joints
	Staphylococcus haemolyticus	X66100		6.4	Commensal on human & animal skin, may occasionally cause infection in immunocompromised patients

Table 4.3.2-8 Molecular bacterial diversity of Los Angeles/Sydney round trip (4S and 4R-S)

Nearest neighbor (GenBank accession #)			% clones		Significance (given in color are human pathogens or isolated from patient)
			7	8	
			4-S	4R-S	
			Los Angeles/Sydney	Sydney/Los Angeles	
	Staphylococcus hominis	X66101	3.2		Commensal on human & animal skin, may occasionally cause infection in immunocompromised patients
	Staphylococcus warneri	L37603	3.2	3.2	Commensal on human & animal skin, may occasionally cause infection in immunocompromised patients
	Streptococcus mitis	AF003929	3.2	6.4	Commensal in the human respiratory tract
	Streptococcus parasanguinis	AF003933	6.4		New pathogen associated with asymptomatic mastitis in sheep
	Streptococcus thermophilus	X68418	3.2	6.4	One of the most commercially important of all lactic acid bacteria; yogurt, cheese, etc.
Fusobacteria			**3.2**		
	Fusobacterium canifelinum	AY162220	3.2		Isolated from the oral cavity of cats and dogs
Cyanobacteria			**9.6**	**22.6**	
	Oscillatoria sancta	AF132933	9.6	22.6	Marine cyanobacterium.
# of clones sequenced in clone library			31	31	
# of bacterial species in clone library			26	17	
# of dominant species (>10% of clone library)			0	2	

Table 4.3.2-9 Molecular bacterial diversity of Los Angeles/New York round trip (5N and 5R-N)

Nearest neighbor (GenBank accession #)			% clones		Significance (given in color are human pathogens or isolated from patient)
			9	10	
			5N	5R-N	
			Los Angeles/New York	New York/Los Angeles	
α–proteobacteria:			**15.9**	**27.3**	
	Bradyrhizobium betae	AY372184	5.3		Symbiont of many different plants, such as sugarcane and coffee plants
	Novosphingobium hassiacum	AJ416411	5.3		Isolated from a well aerated sewage pond
	Rhodopseudomonas palustris	D25312		27.3	Common in soil and water, makes its living by converting sunlight to cellular energy and by absorbing atmospheric carbon dioxide and converting it to biomass
	Stella vacuolata	AJ535711	5.3		Soil prosthecobacteria
β– proteobacteria:			**5.3**		
	Massilia timonae	U54470	5.3		Isolated from the blood of a meningoencephalitis patient
γ–proteobacteria:			**37.1**		
	Acinetobacter junii	X81658	5.3		Associated with bacteremia in preterm infants and pediatric oncologic patients
	Haemophilus haemolyticus	M75045	5.3		Pharyngeal microflora of human
	Haemophilus paraphrohaemolyticus	M75076	5.3		Pharyngeal microflora of human
	Pseudomonas hibiscicola	AB021405	10.6		Bacteria from ticks, lice and fleas
	Pseudomonas putida	D85994	5.3		Non-pathogenic environmental isolate able to degrade a variety of aromatic hydrocarbons
	Salmonella typhi	Z47544	5.3		Human pathogen, causuative agent of typhoid disease
Actinobacteria			**10.6**	**45.5**	
	Granulicatella adiacens	D50540	5.3		Associated with haematological malignancies, neutropenic fever, and primary bacteraemia
	Propionibacterium propionicum	AJ003058	5.3		Involved with infections of the lacrimal apparatus. J. Clin. Microbiol. 1997, 35: 2464-2471
	Williamsia maris	AB010909		45.5	Soil actinomycetes
Firmicutes			**31.8**	**27.3**	
	Bacillus pumilus	AY456263		9.1	Nonpathogenic, used for alkaline protease production, in environmental decontamination of dioxins, and in the baking industry.
	Lactococcus lactis subsp. cremoris	M58836	5.3		Widely used in the manufacture of fermented milk products

Table 4.3.2-9 Molecular bacterial diversity of Los Angeles/New York round trip (5N and 5R-N)

Nearest neighbor (GenBank accession #)			% clones		Significance (given in color are human pathogens or isolated from patient)
			9	10	
			5N	5R-N	
			Los Angeles/New York	New York/Los Angeles	
	Sporanaerobacter acetigenes	AF358114			Anaerobic, moderately thermophilic, sporulating rod isolated from an anaerobic sludge blanket
	Staphylococcus cohnii	AB009936	5.3		Commensal on human & animal skin, may occasionally cause infection in immunocompromised patients
	Staphylococcus epidermidis	L37605	5.3	9.1	Commensal on human & animal skin, may occasionally cause infection in immunocompromised patients
	Staphylococcus hominis	X66101	10.6	9.1	Commensal on human & animal skin, may occasionally cause infection in immunocompromised patients
	Streptococcus thermophilus	X68418	5.3		One of the most commercially important of all lactic acid bacteria; yogurt, cheese, etc.
# of clones sequenced in clone library			19	11	
# of bacterial species in clone library			17	5	
# of dominant species (>10% of clone library)			2	2	

Table 4.3.2-10 Molecular bacterial diversity of Los Angeles/Japan (Narita, Tokyo) round trip (6J and 6R-J)

Nearest neighbor (GenBank accession #)			% clones				Significance (given in color are human pathogens or isolated from patient)
			11	12	17	18	
			6J	6R-J	6J-G	6R-SS	
			Los Angeles/Japan	Japan/Los Angeles	Los Angeles/Japan	Japan/Los Angeles	
α–proteobacteria:			**10.0**	**85.7**	**14.4**	**5.8**	
	Agrobacterium tumefaciens	M11223	5.0		1.6		Plant pathogen
	Caedibacter caryophilus	X71837			1.6		Endosymbionts of *Acanthamoeba* spp, an amoeba
	Methylobacterium hispanicum	AJ635304		85.7			Oligotroph associated with fresh water
	Paracoccus denitrificans	X69159				2.9	A denitrifying soil microbe
	Phenylobacterium immobile	Y18216			1.6		Bacterium that degrades hebicide
	Rhizobium leguminosarum	AY509899					Rhizosphere microflora
	Rhodobacter blasticus	D16429			1.6		Soil microflora
	Rhodopseudomonas palustris	D25312			6.4	2.9	Common in soil and water, makes its living by converting sunlight to cellular energy and by absorbing atmospheric carbon dioxide and converting it to biomass
	Skermanella parooensis	X90760			1.6		Soil microflora
	Sphingomonas faeni	AJ429239	5.0				Oligotrophic microflora
β– proteobacteria:			**15.0**		**22.4**	**5.8**	
	Aquabacterium commune	AF035054			3.2		Drinking water biofilm communities
	Burkholderia andropogonis	AB021422				2.9	Nitrogen-fixing strains that were isolated from rhizosphere macerates of rice
	Burkholderia cepacia	AB114607	10.0		1.6		Opportunistic human pathogens, able to cause devastating infections in patients with cystic fibrosis
	Dechloromonas agitata	AF047462			6.4		Perchlorate reducing bacterium
	Duganella violaceinigra	AY376163			1.6		Mesophilic bacterium isolated from a forest soil
	Duganella zoogloeoides	D14256			8.0		Rhizosphere bacterial communities
	Hydrogenophaga defluvii	AJ585993			1.6		Isolated from wastewater
	Janthinobacterium lividum	Y08846	5.0				Metallo- -lactamase producing bacterium
	Limnobacter thiooxidans	AJ289885				2.9	Thiosulfate-oxidizing strains isolated from sediment of the littoral zone of a freshwater lake
γ–proteobacteria:			**10.0**		**19.2**		
	Acinetobacter lwoffii	X81665			1.6		Bacteremia causing bacteria
	Shigella flexneri	X96963			3.2		Human enteric pathogen
	Stenotrophomonas maltophilia	AB008509	10.0		12.8		Opportunistic human pathogen causing nosocomial infection

Table 4.3.2-10 Molecular bacterial diversity of Los Angeles/Japan (Narita, Tokyo) round trip (6J and 6R-J)

Nearest neighbor (GenBank accession #)			% clones				Significance (given in color are human pathogens or isolated from patient)
			11	12	17	18	
			6J	6R-J	6J-G	6R-SS	
			Los Angeles/Japan	Japan/Los Angeles	Los Angeles/Japan	Japan/Los Angeles	
	Thiothrix eikelboomii	AB042819			1.6		Bacterium isolated from bulking activated sludges
Actinobacteria			**15.0**		**9.6**	**20.3**	
	Corynebacterium lipophiloflavum	Y09045				2.9	Coryneform bacterium isolated from a patient with bacterial vaginosis
	Corynebacterium tuberculostearicum	AJ438050				11.6	A leprosy-derived Corynebacterium
	Curtobacterium citreum	X77436			1.6		Bacteria associated with clover and potatoes
	Dermatophilus chelonae	AJ243919				2.9	Isolated from skin lesions in farmed saltwater crocodiles and king cobra
	Kocuria rhizophila	Y16264	5.0				Isolated from the rhizoplane of the narrow-leaved cattail (*Typha angustifolia*)
	Microbacterium kitamiense	AB013907	5.0				Polysaccharide-producing bacterium isolated from the wastewater of a sugar-beet factory
	Microbacterium phyllosphaerae	AJ277840			1.6		Soil microflora
	Micrococcus luteus	AJ536198			1.6	2.9	Found in soil as well as on human skin, as a normal member of our flora
	Mycobacterium chitae	M29560			1.6		Nonpathogenic, rapidly growing mycobacterial species found in the environment
	Rubrobacter radiotolerans	U65647	5.0		3.2		Highly radiation resistant bacterium
Firmicutes			**45.0**	**8.6**	**17.6**	**64.1**	
	Anaerococcus octavius	Y07841				2.9	Soil microflora
	Bacillus pumilus	AY456263			1.6		Nonpathogenic, used for alkaline protease production, in environmental decontamination of dioxins, and in the baking industry.
	Enterococcus durans	AJ276354				8.8	Nosocomial enterococcal infections causing bacterium
	Jeotgalicoccus halotolerans	AY028925			1.6		Isolated from the traditional Korean fermented seafood jeotgal
	Lactococcus lactis subsp. cremoris	M58836	5.0				Widely used in the manufacture of fermented milk products
	Staphylococcus aureus	L37597			1.6		Bacteremea causing human pathogen
	Staphylococcus epidermidis	L37605	15.0		4.8		Commensal on human & animal skin, may occasionally cause infection in immunocompromised patients
	Staphylococcus hominis	X66101	10.0	8.6		17.4	Commensal on human & animal skin, may occasionally cause infection in immunocompromised patients
	Staphylococcus pasteuri	AB009944				2.9	Commensal on human & animal skin, may occasionally cause infection in immunocompromised patients
	Staphylococcus saccharolyticus	L37602				2.9	Prosthetic valve endocarditis causing bacterium

Table 4.3.2-10 Molecular bacterial diversity of Los Angeles/Japan (Narita, Tokyo) round trip (6J and 6R-J)

Nearest neighbor (GenBank accession #)			% clones				Significance (given in color are human pathogens or isolated from patient)
			11	12	17	18	
			6J	6R-J	6J-G	6R-SS	
			Los Angeles/Japan	Japan/Los Angeles	Los Angeles/Japan	Japan/Los Angeles	
	Staphylococcus succinus	AF004220				2.9	Recovered from 25-35-million-year-old Dominican amber
	Staphylococcus warneri	L37603				2.9	Commensal on human & animal skin, may occasionally cause infection in immunocompromised patients
	Streptococcus australis	AY485604	10.0				Isolated from the mouths of children
	Streptococcus iniae	AF335572				2.9	Bacterial agent causing septicemic infection in fish
	Streptococcus mitis	AF003929	5.0		3.2		Commensal in the human respiratory tract
	Streptococcus mutans	X58303				8.8	Dental infections such as tooth decay and periodontal disease
	Streptococcus thermophilus	X68418			3.2	2.9	One of the most commercially important of all lactic acid bacteria; yogurt, cheese, etc.
	Streptococcus sanguinis	AY281085				8.8	Indigenous human oral microflora, recognized as a key player in the bacterial colonization of the mouth
	Weissella cibaria	AJ295989			1.6		Isolated from Malaysian food
Deinococcus					**1.6**		
	Deinococcus murrayi	Y13041			1.6		Extremely radiation-resistant and slightly thermophilic species from hot springs
Spirochaetes					**1.6**		
	Leptospira parva	Z21636			1.6		Human pathogen
Bacterioidetes				**2.9**	**6.4**		
	Bacteriodes vulgatus			2.9			Common microflora associated with animal guts
	Chryseobacterium scophthalmum	AJ271009			3.2		Isolated from wastewater
	Flavobacterium ferrugineum	M62798			3.2		Isoated from cold temperate places
Cyanobacteria				**2.9**	**6.4**	**8.8**	
	Oscillatoria sancta	AF132933			3.2	8.8	Marine cyanobacterium
	Oscillatoria sp.	AB074509		2.9			Marine cyanobacterium
	Microcystis aeruginosa	AB035549			3.2		Blue green alga
# of clones sequenced in clone library			20	35	62	34	
# of bacterial species in clone library			13	4	34	20	
# of dominant species (>10% of clone library)			5	1	1	2	

Table 4.3.2-11 Molecular bacterial diversity of Los Angeles/New York round trip (7N and 7R-N)

Nearest neighbor (GenBank accession #)			% clones		Significance (given in color are human pathogens or isolated from patient)
			13	14	
			7N	7R-N	
			Los Angeles/New York	New York/Los Angeles	
α–proteobacteria:				**26.8**	
	Novosphingobium pentaromativorans	AF502400		20.1	Polycyclic aromatic hydrocarbon-degrading bacterium isolated from estuarine sediment
	Novosphingobium subarcticum	X94102		6.7	Yellow-pigmented, halophilic bacterium isolated from Antactia
β– proteobacteria:			**30.8**	**6.7**	
	Curvibacter gracilis	AB109889	7.7		Soil microflora
	Leptothrix mobilis	X97071		6.7	Isolated from the sediment of a big freshwater lake (Chiemsee) in Upper Bavaria, Germany
	Wautersia paucula	AF085226	23.1		Bacteria exhibiting heavy metal resistance
γ–proteobacteria:			**53.9**	**13.4**	
	Pseudomonas hibiscicola	AB021405		6.7	Bacteria from ticks, lice and fleas
	Pseudomonas saccharophila	AB021407	23.1		Soil microflora
	Pseudomonas trivialis	AJ492831		6.7	Fluorescent pseudomonads associated with the phyllosphere of grasses
	Salmonella typhi	Z47544	7.7		Human pathogen, causuative agent of typhoid disease
	Stenotrophomonas maltophilia	AB008509	23.1		Opportunistic human pathogen causing nosocomial infection
δ–proteobacteria:				**6.7**	
	Bacteriovorax stolpii	M34125		6.7	Bdellovibrio-and-like organisms that are Gram-negative, predatory bacteria that inhabit terrestrial, freshwater and salt-water environments.
Actinobacteria				**6.7**	
	Williamsia maris	AB010909		6.7	Soil actinomycetes
Firmicutes				**40.2**	
	Bacillus pallidus	Z26930		20.1	A thermophilic species from sewage
	Streptococcus mitis	AF003929		6.7	Commensal in the human respiratory tract
	Streptococcus pneumoniae	AF003930		13.4	Human pathogen that causes pneumonia, bacteremia, meningitis, and otitis media
Deinococcus			**7.7**		

Table 4.3.2-11 Molecular bacterial diversity of Los Angeles/New York round trip (7N and 7R-N)

Nearest neighbor (GenBank accession #)			% clones		Significance (given in color are human pathogens or isolated from patient)
			13	14	
			7N	7R-N	
			Los Angeles/New York	New York/Los Angeles	
	Meiothermus silvanus	X84211	7.7		A filamentous, thermophilic species
Cyanobacteria			**7.7**		
	Microcoleus chthonoplastes	X70770	7.7		Microbial mat building cyanobacterium
Planktomycetes				**6.7**	
	Gemmata obscuriglobus	X56305		6.7	Budding bacteria
# of clones sequenced in clone library			13	15	
# of bacterial species in clone library			7	10	
# of dominant species (>10% of clone library)			3	3	

Table 4.3.2-12 Molecular bacterial diversity of Los Angeles/Sydney round trip (8S and 8R-S)

Nearest neighbor (GenBank accession #)			% clones		Significance (given in color are human pathogens or isolated from patient)
			15	16	
			8S	8S-R	
			Los Angeles/Sydney	Sydney/Los Angeles	
α–proteobacteria:			**59.9**	**45.0**	
	Agrobacterium sanguineum	AB021493		2.5	Aerobic marine bacteria
	Caulobacter henricii	AJ227758	13.3		Oligotrophic drinking water associated bacteria
	Caulobacter vibrioides	AJ009957	20.0	37.5	Oligotrophic drinking water associated bacteria
	Methylobacterium hispanicum	AJ635304	13.3		Oligotrophic drinking water associated bacteria
	Rhizobium leguminosarum	AY509899	13.3	5.0	Soil microflora associated with rhizosphere of plant roots
β– proteobacteria:				**2.5**	
	Herbasprillum seropedicae	Y10146		2.5	A root-associated nitrogen-fixing bacterium
Actinobacteria				**5.0**	
	Aerococcus viridans	M58797		2.5	Septic arthritis causing bacterium
	Facklamia hominis	Y10772		2.5	Anaerobic coccus isolated from human
Firmicutes			**13.4**	**7.5**	
	Bacillus muralis	AJ628748		2.5	Isolated from deteriorated mural paintings situated in Spain (necropolis of Carmona) and Germany (church of Greene-Kreiensen)
	Staphylococcus pasteuri	AB009944	6.7		Commensal on human & animal skin, may occasionally cause infection in immunocompromised patients
	Streptococcus sanguinis	AF003928	6.7		Indigenous human oral microflora, recognized as a key player in the bacterial colonization of the mouth
	Streptococcus pseudopneumoniae	AY612844		2.5	Human pathogen that causes pneumonia, bacteremia, meningitis, and otitis media
	Streptococcus thermophilus	X68418		2.5	One of the most commercially important of all lactic acid bacteria; yogurt, cheese, etc.
Deinococcus			**66.9**	**35.0**	
	Deinococcus geothermalis	Y13038		2.5	Isolated from the hot spring and runoff at Agnano, Naples, Italy
	Deinococcus grandis	Y11329	13.3	2.5	A radiation resistant rod-shaped bacterium
	Deinococcus murrayi	Y13041	53.6	30.0	Isolated from the hot spring and runoff at Agnano, Naples, Italy
Cyanobacteria				**2.5**	
	Oscillatoria sancta	AF132933		2.5	Marine cyanobacterium.
Planktomycetes				**2.5**	
	Gemmata obscuriglobus	X56305		2.5	Budding bacteria
# of clones sequenced in clone library			28	40	
# of bacterial species in clone library			8	14	
# of dominant species (>10% of clone library)			6	2	

Table 4.3.2-13 Detailed summary of molecular bacterial diversity in Phase 2 air samples (domestic flights)

Nearest neighbor (GenBank accession #)		% clones per flight NOTE: >10% of clone library							
		1	2	3	4	9	10	13	14
		1N	1R-N	2N	2R-N	5N	5R-N	7N	7R-N
		Los Angeles/New York	New York/Los Angeles	Los Angeles/New York	New York/Los Angeles	Los Angeles/New York	New York/Los Angeles	Los Angeles/New York	New York/Los Angeles
α–proteobacteria:			**100.0**		nplified and subsequently cloning process was not carried	**15.9**	**27.3**		**26.8**
Bradyrhizobium betae	AY372184					5.3			
Erythrobacter litoralis	AB013354		98.0						
Novosphingobium hassiacum	AJ416411		2.0			5.3			
Novosphingobium pentaromativorans	AF502400								20.1
Novosphingobium subarcticum	X94102								6.7
Rhodopseudomonas palustris	D25312						27.3		
Stella vacuolata	AJ535711					5.3			
β– proteobacteria:		**3.6**		**33.5**		**5.3**		**30.8**	**6.7**
Burkholderia cepacia	AB114607	1.2							
Burkholderia vietnamiensis	U96929	2.4							
Curvibacter gracilis	AB109889							7.7	
Diaphorobacter nitroreducens	AB076856			6.7					
Imtechium assamiensis	AY544767			13.4					
Leptothrix mobilis	X97071								6.7
Massilia timonae	U54470			13.4		5.3			
Wautersia paucula	AF085226							23.1	
γ–proteobacteria:		**85.5**		**40.2**		**37.1**		**53.9**	**13.4**
Acinetobacter junii	X81658			20.1		5.3			
Escherichia coli	X80729			6.7					
Haemophilus haemolyticus	M75045					5.3			
Haemophilus paraphrohaemolyticus	M75076					5.3			
Pseudomonas hibiscicola	AB021405					10.6			6.7
Pseudomonas putida	D85994			6.7		5.3			
Pseudomonas saccharophila	AB021407							23.1	
Pseudomonas trivialis	AJ492831								6.7
Psychrobacter marinicola	AY292940	1.2							
Psychrobacter meningitidis	AY057116	84.3							
Salmonella typhi	Z47544					5.3		7.7	
Stenotrophomonas maltophilia	AB008509							23.1	
Serratia ficaria	AJ279054			6.7					
δ–proteobacteria:									**6.7**
Bacteriovorax stolpii	M34125								6.7
Actinobacteria		**1.2**		**13.4**		**10.6**	**45.5**		**6.7**
Gordonia sinesedis	AF380834	1.2							
Granulicatella adiacens	D50540					5.3			

Table 4.3.2-13 Detailed summary of molecular bacterial diversity in Phase 2 air samples (domestic flights)

Nearest neighbor (GenBank accession #)			% clones per flight NOTE: >10% of clone library							
			1	2	3	4	9	10	13	14
			1N	1R-N	2N	2R-N	5N	5R-N	7N	7R-N
			Los Angeles/New York	New York/Los Angeles	Los Angeles/New York	New York/Los Angeles	Los Angeles/New York	New York/Los Angeles	Los Angeles/New York	New York/Los Angeles
	Propionibacterium acnes	AB042287			13.4	No PCR product an				
	Propionibacterium propionicum	AJ003058					5.3			
	Williamsia maris	AB010909						45.5		6.7
Firmicutes					**13.4**		**31.8**	**27.3**		**40.2**
	Bacillus pallidus	Z26930								20.1
	Bacillus pumilus	AY456263						9.1		
	Lactococcus lactis subsp. cremoris	M58836					5.3			
	Staphylococcus cohnii	AB009936					5.3			
	Staphylococcus epidermidis	L37605					5.3	9.1		
	Staphylococcus hominis	X66101					10.6	9.1		
	Streptococcus mitis	AF003929								6.7
	Streptococcus pneumoniae	AF003930								13.4
	Streptococcus thermophilus	X68418					5.3			
	Streptococcus sanguinis	AY281085			13.4					
Deinococcus									**7.7**	
	Meiothermus silvanus	X84211							7.7	
Cyanobacteria									**7.7**	
	Microcoleus chthonoplastes	X70770							7.7	
Planktomycetes										**6.7**
	Gemmata obscuriglobus	X56305								6.7
# of clones sequenced in clone library			83	49	15		19	11	13	15
# of bacterial species in clone library			5	2	9		17	5	7	10
# of dominant species (>10% of clone library)			2	1	5		2	2	3	3

Table 4.3.2-14 Detailed summary of molecular bacterial diversity in Phase 2 air samples (international flights)

Nearest neighbor (GenBank accession #)		% clones per flight NOTE: >10% of clone library									
		5	6	7	8	11	12	15	16	17	18
		3-L	3R-L	4-S	4R-S	6J	6R-J	8S	8S-R	6J-G	6R-SS
		Los Angeles/London	London/Los Angeles	Los Angeles/Sydney	Sydney/Los Angeles	Los Angeles/Japan	Japan/Los Angeles	Los Angeles/Sydney	Sydney/Los Angeles	Los Angeles/Japan	Japan/Los Angeles
α–proteobacteria:				**19.2**		**10.0**	**85.7**	**59.9**	**45.0**	**14.4**	**5.8**
Acetobacter aceti	X74066			3.2							
Agrobacterium sanguineum	AB021493								2.5		
Agrobacterium tumefaciens	M11223					5.0				1.6	
Caedibacter caryophilus	X71837									1.6	
Caulobacter henricii	AJ227758							13.3			
Caulobacter vibrioides	AJ009957							20.0	37.5		
Methylobacterium hispanicum	AJ635304						85.7	13.3			
Methylobacterium organophilum	D32226			3.2							
Novosphingobium pentaromativorans	AF502400			3.2							
Paracoccus alcaliphilus	D32238			3.2							
Paracoccus carotinifaciens	AB006899			3.2							
Paracoccus denitrificans	X69159										2.9
Phenylobacterium immobile	Y182216									1.6	
Rhizobium leguminosarum	AY509899							13.3	5.0		
Rhodobacter blasticus	D16429									1.6	
Rhodopseudomonas palustris	D25312			3.2						6.4	2.9
Skermanella parooensis	X90760									1.6	
Sphingomonas faeni	AJ429239					5.0					
β– proteobacteria:					**3.2**	**15.0**			**2.5**	**22.4**	**5.8**
Aquabacterium commune	AF035054									3.2	
Burkholderia andropogonis	AB021422										2.9
Burkholderia cepacia	AB114607					10.0				1.6	
Dechloromonas agitata	AF047462									6.4	
Duganella violaceinigra	AY376163									1.6	
Duganella zoogloeoides	D14256									8.0	
Herbosprillum seropedicae	Y10772								2.5		
Hydrogenophaga defluvii	AJ585993									1.6	
Janthinobacterium lividum	Y08846					5.0					
Limnobacter thiooxidans	AJ289885										2.9
Neisseria weaveri	L10738				3.2						
γ–proteobacteria:				**6.4**	**12.8**	**10.0**				**19.2**	
Acinetobacter calcoaceticus	Z93434				3.2						
Acinetobacter johnsonii	X81663			3.2	3.2						
Acinetobacter lwoffii	X81665									1.6	
Citrobacter murliniae	AF025369				3.2						
Pseudomonas hibiscicola	AB021405				3.2						
Pseudomonas lanceolata	AB021390			3.2							
Shigella flexneri	X96963									3.2	
Stenotrophomonas maltophilia	AB008509					10.0				12.8	

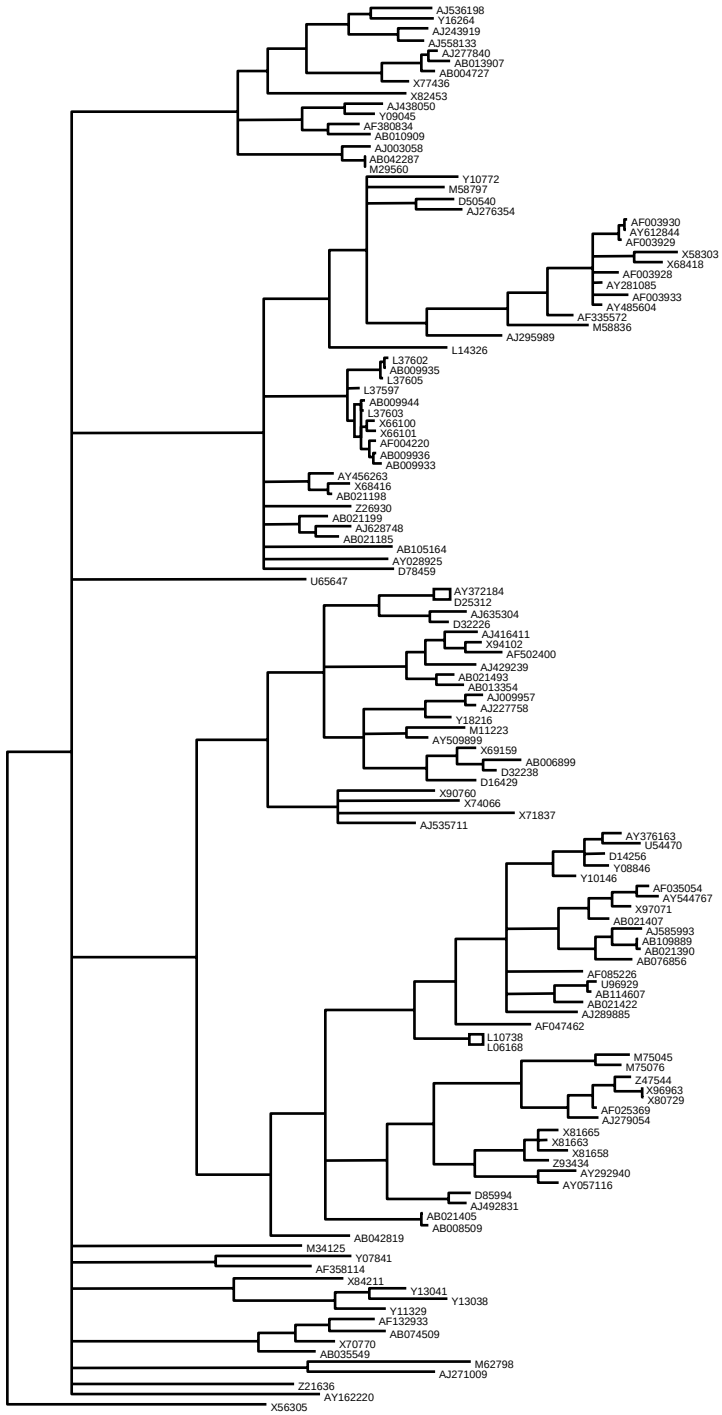
Table 4.3.2-14 Detailed summary of molecular bacterial diversity in Phase 2 air samples (international flights)

Nearest neighbor (GenBank accession #)			% clones per flight NOTE: >10% of clone library									
			5	6	7	8	11	12	15	16	17	18
			3-L	3R-L	4-S	4R-S	6J	6R-J	8S	8S-R	6J-G	6R-SS
			Los Angeles/London	London/Los Angeles	Los Angeles/Sydney	Sydney/Los Angeles	Los Angeles/Japan	Japan/Los Angeles	Los Angeles/Sydney	Sydney/Los Angeles	Los Angeles/Japan	Japan/Los Angeles
	Thiothrix eikelboomii	AB042819	No PCR product amplified and subsequently cloning process was not carried	No PCR product amplified and subsequently cloning process was not carried							1.6	
δ–proteobacteria:						**3.2**						
	Bacteriovorax stolpii	M34125				3.2						
Actinobacteria					**16.0**	**19.2**	**15.0**			**5.0**	**9.6**	**20.3**
	Aerococcus viridans	M58797								2.5		
	Arsenicicoccus bolidensis	AJ558133				3.2						
	Actinomyces viscosus	X82453			3.2							
	Corynebacterium lipophiloflavum	Y09045										2.9
	Corynebacterium tuberculostearicum	AJ438050				16.0						11.6
	Curtobacterium citreum	X77436									1.6	
	Dermatophilus chelonae	AJ243919										2.9
	Facklamia hominis	Y10772								2.5		
	Kocuria rhizophila	Y16264					5.0					
	Microbacterium hominis	AB004727			3.2							
	Microbacterium kitamiense	AB013907					5.0					
	Microbacterium phyllosphaerae	AJ277840									1.6	
	Micrococcus luteus	AJ536198									1.6	2.9
	Mycobacterium chitae	M29560			6.4						1.6	
	Propionibacterium acnes	AB042287										
	Propionibacterium propionicum	AJ003058			3.2							
	Rubrobacter radiotolerans	U65647					5.0				3.2	
Firmicutes					**44.8**	**38.4**	**45.0**	**8.6**	**13.4**	**7.5**	**17.6**	**64.1**
	Anaerococcus octavius											2.9
	Bacillus flexus	AB021185			6.4							
	Bacillus licheniformis	X68416			3.2							
	Bacillus pumilus	AY456263									1.6	
	Bacillus muralis	AJ628748								2.5		
	Bacillus vallismortis	AB021198				3.2						
	Bacillus weihenstephanensis	AB021199			3.2							
	Brevibacillus choshinensis	D78459			3.2							
	Enterococcus durans	AJ276354										8.8
	Exiguobacterium oxidotolerans	AB105164				3.2						
	Gemella haemolysans	L14326				3.2						
	Jeotgalicoccus halotolerans	AY028925									1.6	
	Lactococcus lactis subsp. cremoris	M58836					5.0					
	Sporanaerobacter acetigenes	AF358114			3.2							
	Staphylococcus arlettae	AB009933			3.2							
	Staphylococcus aureus	L37597									1.6	
	Staphylococcus caprae	AB009935			3.2	6.4						
	Staphylococcus epidermidis	L37605					15.0				4.8	

Table 4.3.2-14 Detailed summary of molecular bacterial diversity in Phase 2 air samples (international flights)

Nearest neighbor (GenBank accession #)		% clones per flight NOTE: >10% of clone library									
		5	6	7	8	11	12	15	16	17	18
		3-L	3R-L	4-S	4R-S	6J	6R-J	8S	8S-R	6J-G	6R-SS
		Los Angeles/London	London/Los Angeles	Los Angeles/Sydney	Sydney/Los Angeles	Los Angeles/Japan	Japan/Los Angeles	Los Angeles/Sydney	Sydney/Los Angeles	Los Angeles/Japan	Japan/Los Angeles
Staphylococcus haemolyticus	X66100				6.4						
Staphylococcus hominis	X66101			3.2		10.0	8.6				17.4
Staphylococcus pasteuri	AB009944							6.7			2.9
Staphylococcus saccharolyticus	L37602										2.9
Staphylococcus sanguinis	AF003928							6.7			
Staphylococcus succinus	AF004220										2.9
Staphylococcus warneri	L37603			3.2	3.2						2.9
Streptococcus australis	AY485604					10.0					
Streptococcus iniae	AF335572										2.9
Streptococcus mitis	AF003929			3.2	6.4	5.0				3.2	
Streptococcus mutans	X58303										8.8
Streptococcus parasanguinis	AF003933			6.4							
Streptococcus pseudopneumoniae	AY612844								2.5		
Streptococcus thermophilus	X68418			3.2	6.4				2.5	3.2	2.9
Streptococcus sanguinis	AY281085										8.8
Weissella cibaria	AJ295989									1.6	
Fusobacteria				**3.2**							
Fusobacterium canifelinum	AY162220			3.2							
Deinococcus								**66.9**	**35.0**	**1.6**	
Deinococcus geothermalis	Y13038								2.5		
Deinococcus grandis	Y11329							13.3	2.5		
Deinococcus murrayi	Y13041							53.6	30.0	1.6	
Spirochaetes										**1.6**	
Leptospira parva	Z21636									1.6	
Bacterioidetes							**2.9**			**6.4**	
Bacteriodes vulgatus							2.9				
Chryseobacterium scophthalmum	AJ271009									3.2	
Flavobacterium ferrugineum	M62798									3.2	
Cyanobacteria				**9.6**	**22.6**		**2.9**		**2.5**	**6.4**	**8.8**
Oscillatoria sancta	AF132933			9.6	22.6				2.5	3.2	8.8
Oscillatoria sp.	AB074509						2.9				
Microcystis aeruginosa	AB035549									3.2	
Planktomycetes											
Gemmata obscuriglobus	X56305								2.5		
# of clones sequenced in clone library				31	31	20	35	28	40	62	34
# of bacterial species in clone library				26	17	13	4	8	14	34	20
# of dominant species (>10% of clone library)				0	2	5	1	6	2	1	2

Abundance, occurrence, and pathogens of bacterial species



	>10% abundance	#	pathogen
Gram-positive	*Corynebacterium tuberculostearicum* *Propionibacterium acnes* *Williamsia maris* *Bacillus pallidus* *Staphylococcus epidermidis* *Staphylococcus hominis* *Streptococcus australis* *Streptococcus pneumoniae* *Streptococcus sanguinis*	113 57	29 12
α–proteo	*Caulobacter henricii* *Caulobacter vibrioides* *Erythrobacter litoralis* *Methylobacterium hispanicum* *Novosphingobium pentaromativorans* *Rhizobium leguminosarum* *Rhodopseudomonas palustris*	23	1
β	*Burkholderia cepacia* *Imtechium assamiensis* *Massilia timonae* *Wautersia paucula*	19	5
γ	*Acinetobacter junii* *Pseudomonas hibiscicola* *Pseudomonas saccharophila* *Psychrobacter meningitidis* *Stenotrophomonas maltophilia*	20	10
others	*Deinococcus grandis* *Deinococcus murrayi* *Oscillatoria sancta*	14	1

Figure 4.3.2-11 Overview of Airline C microbial diversity

Figure 4.3.2-12 Actinobacterial diversity of Airline C

Sample	%
1-N:	1.2
2-N:	13.4
5-N:	10.6
5R-N:	45.5
7R-N:	6.7
4-S:	16.0
4R-S:	19.2
6-J:	15.0
8R-S:	5.0

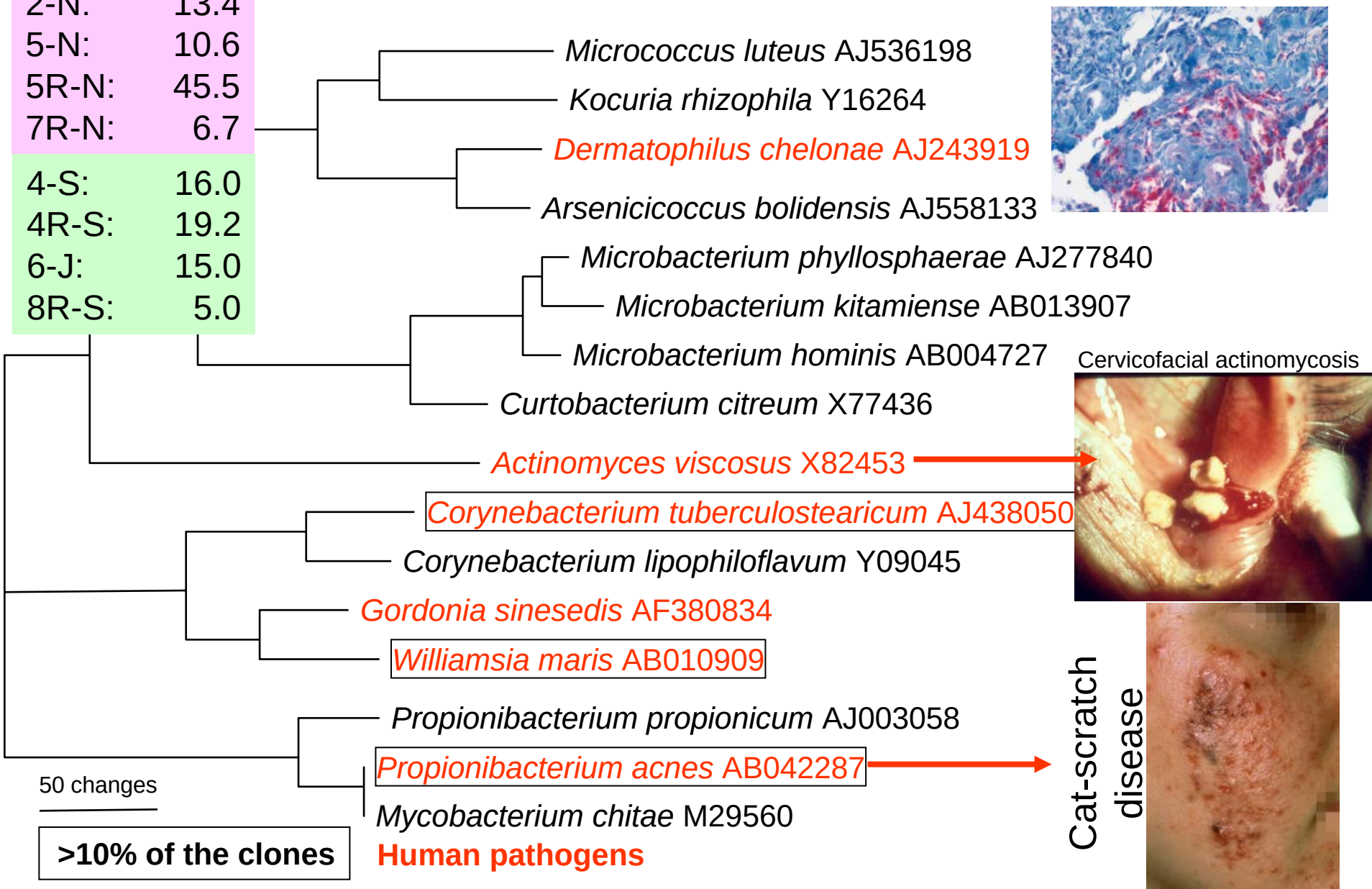
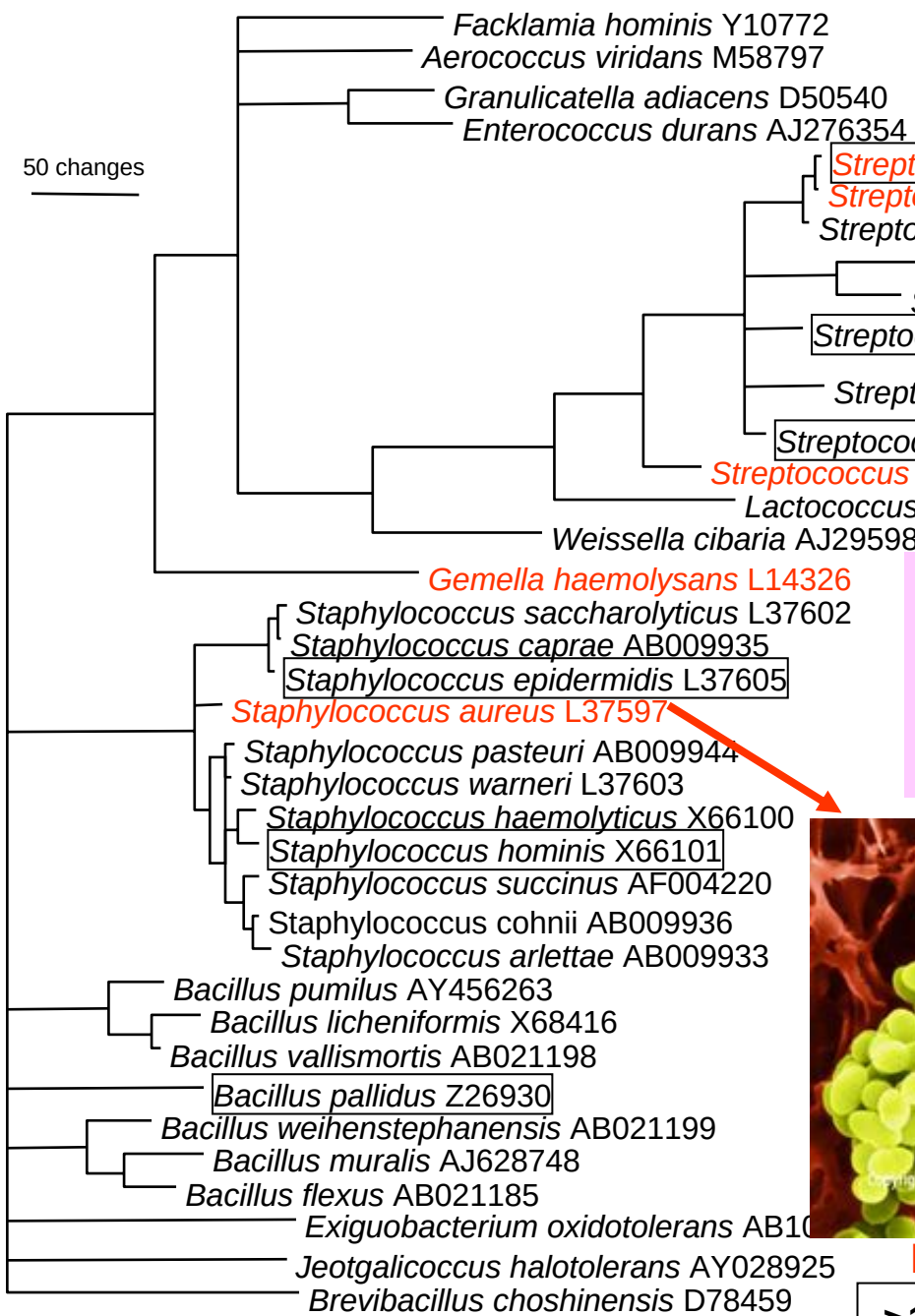
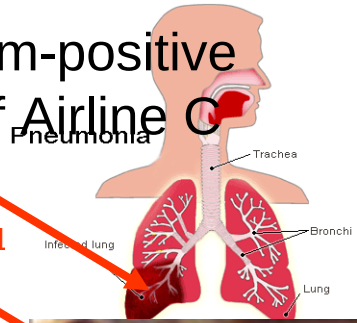
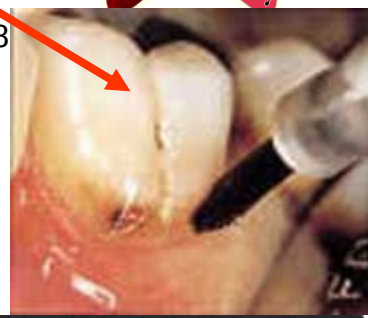


Figure 4.3.2-13 Gram-positive bacterial diversity of Airline C







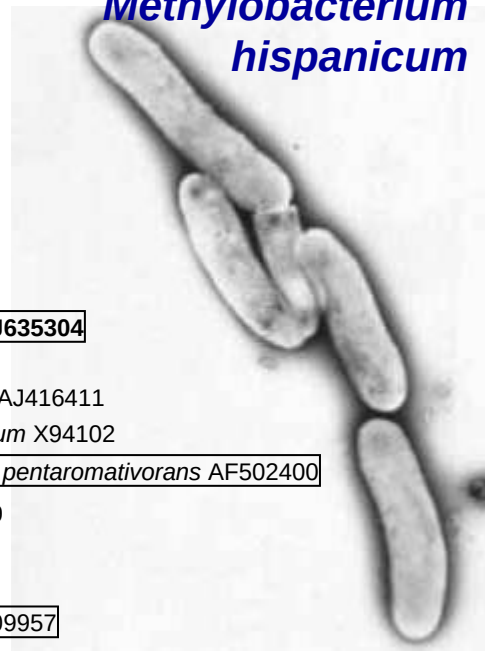
2-N:	13.4
5-N:	31.8
5R-N:	27.3
7R-N:	40.2

4-S:	44.8
4R-S:	38.4
6-J:	45.0
6R-J:	8.6
8-S:	13.4
8R-S:	7.5

Human pathogens

>10% of the clones

Figure 4.3.2-14 α-proteobacterial diversity of Airline C



50 changes

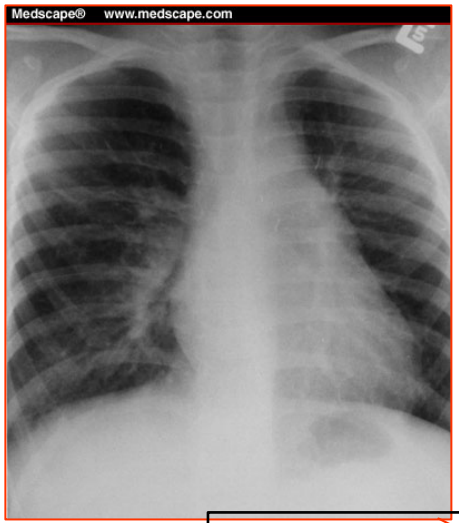
Bradyrhizobium betae AY372184
Rhodopseudomonas palustris D25312
***Methylobacterium hispanicum* AJ635304**
Methylobacterium organophilum D32226
Novosphingobium hassiacum AJ416411
Novosphingobium subarcticum X94102
Novosphingobium pentaromativorans AF502400
Sphingomonas faeni AJ429239
Agrobacterium sanguineum AB021493
***Erythrobacter litoralis* AB013354**
Caulobacter vibrioides AJ009957
Caulobacter henricii AJ227758
Phenylobacterium immobile Y18216
***Agrobacterium tumefaciens* M11223**
Rhizobium leguminosarum AY509899
Paracoccus denitrificans X69159
Paracoccus carotinifac
Paracoccus alcaliphilus D32238
Rhodobacter blasticus D16429
Skermanella parooensis X90760
Acetobacter aceti X74066
Caedibacter caryophilus X
Stella vacuolata AJ535711

Plant pathogens

>10% of the clones

Sample	%
1R-N:	100.0
5-N:	15.9
5R-N:	27.3
7R-N:	26.8
4-S:	19.2
6-J:	10.0
6R-J:	85.7
8-S:	59.9
8R-S:	45.0

Figure 4.3.2-15 β-proteobacterial diversity of Airline C



Duganella violaceinigra AY376163

Massilia timonae U54470

Duganella zoogloeoides D14256

Janthinobacterium lividum Y08846

Herbosprillum seropedicae Y10146

Aquabacterium commune AF035054

Imtechium assamiensis AY544767

Leptothrix mobilis X97071

Pseudomonas saccharophila AB021407

Hydrogenophaga defluvii AJ585993

Curvibacter gracilis AB109889

Pseudomonas lanceolata AB021390

Diaphorobacter nitroreducens AB076856

Wautersia paucula AF085226

Burkholderia vietnamiensis U96929

Burkholderia cepacia AB114607

Burkholderia andropogonis AB021422

Limnobacter thiooxidans AJ289885

Dechloromonas agitata AF047462

Neisseria weaveri L10738

Neisseria flavescens L06168

50 changes

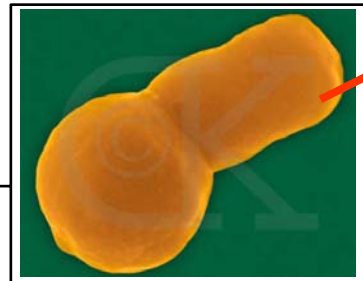
Human pathogens

>10% of the clones

Sample	%
1R-N:	3.6
2-N:	33.5
5-N:	5.3
7-N:	30.8
7R-N:	6.7
4R-S:	3.2
6-J:	15.0
8R-S:	2.5

Figure 4.3.2-16

γ-proteobacterial diversity of Airline C



Haemophilus haemolyticus M75045

Haemophilus paraphrohaemolyticus M75076

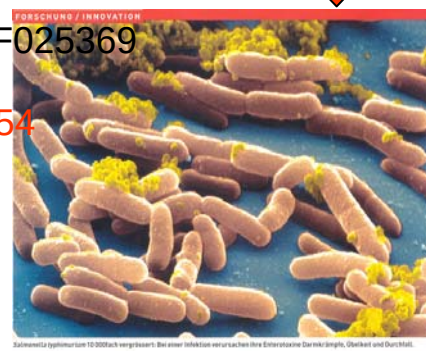
Salmonella typhi Z47544

Shigella flexneri X96963

Escherichia coli X80729

Citrobacter murliniae AF025369

Serratia ficaria AJ279054



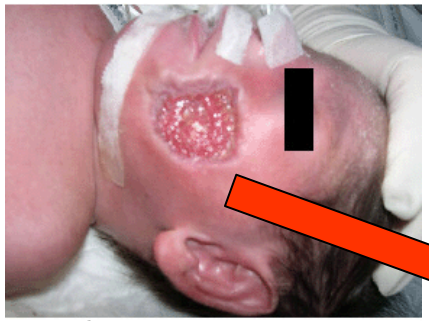


Figura 4. Área lesionada con tejido de granulación.

Acinetobacter lwoffii X81665

Acinetobacter johnsonii X81663

Acinetobacter junii X81658

Acinetobacter calcoaceticus Z93434

Psychrobacter marinicola AY292940

Psychrobacter meningitidis AY057116

Pseudomonas putida D85994

Pseudomonas trivialis AJ492831

50 changes

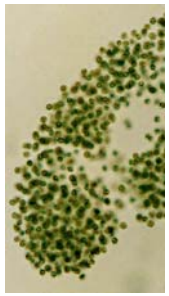
Human pathogens

>10% of the clones

Sample	%
1-N:	85.5
2-N:	33.5
5-N:	5.3
7-N:	30.8
7R-N:	6.7
4-S:	3.2
4R-S:	3.2
6-J:	15.0

Figure 4.3.2-17 Other classes of bacteria from Airline C

- Cyanobacteria:
  Microcystis aeruginosa AB035549
  Microcoleus chthonoplastes X70770
  Oscillatoria sancta AF132933
  Oscillatoria sp. AB074509



- Fusobacteria:
  Fusobacterium canifelinum AY162220

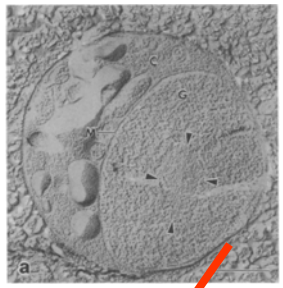
- Bacterioidetes:
  Flavobacterium ferrugineum M62798
  Meiothermus silvanus X84211
  Chryseobacterium scophthalmum AJ271009

- Fusobacteria:
  Anaerococcus octavius Y07841
  Sporanaerobacter acetigenes AF358114

δ–proteobacteria:

- *Bacteriovorax stolpii* M34125

- Deinococcales:
  Meiothermus silvanus X84211
  Deinococcus murrayi Y13041
  Deinococcus geothermalis Y13038
  Deinococcus grandis Y11329



Planctomycetes:

- *Gemmata obscuriglobus* X56305

Spirochaetes:

- *Leptospira parva* Z21636

>10% of the clones

Human pathogens

A Comprehensive Assessment of Biologicals Contained Within Commercial Airliner Cabin Air

Biotechnology and Planetary Protection Group

NASA-Jet Propulsion Laboratory

California Institute of Technology

Participants

- Task members:
  - *Jet Propulsion Laboratory*:
    - Myron La Duc, Shariff Osman, Anne Dekas, Tara Stuecker, David Newcombe, and Kasthuri Venkateswaran (Sampling, processing, microbiology)
  - *Lawrence Berkley National Lab*:
    - Yvette Piceno, Todd DeSantis, Gary Andersen (DNA Microarray)
  - *University of Southern California*:
    - Jed Fuhrman (Viral diversity)
- Program manager:
  - Greg Bearman
- Non-participant facilitators:
  - Judith Pillinger, Charlie Cockell, Open University, UK
  - Masataka Satomi, The National Research Institute of Fisheries Science, Japan
  - Carola Holmstrom, Staffan Kjelleberg, The University of New South Wales, Australia
  - Jeff Steiner, City College of New York
  - Howard Shuman, Columbia University

Limitations of the study

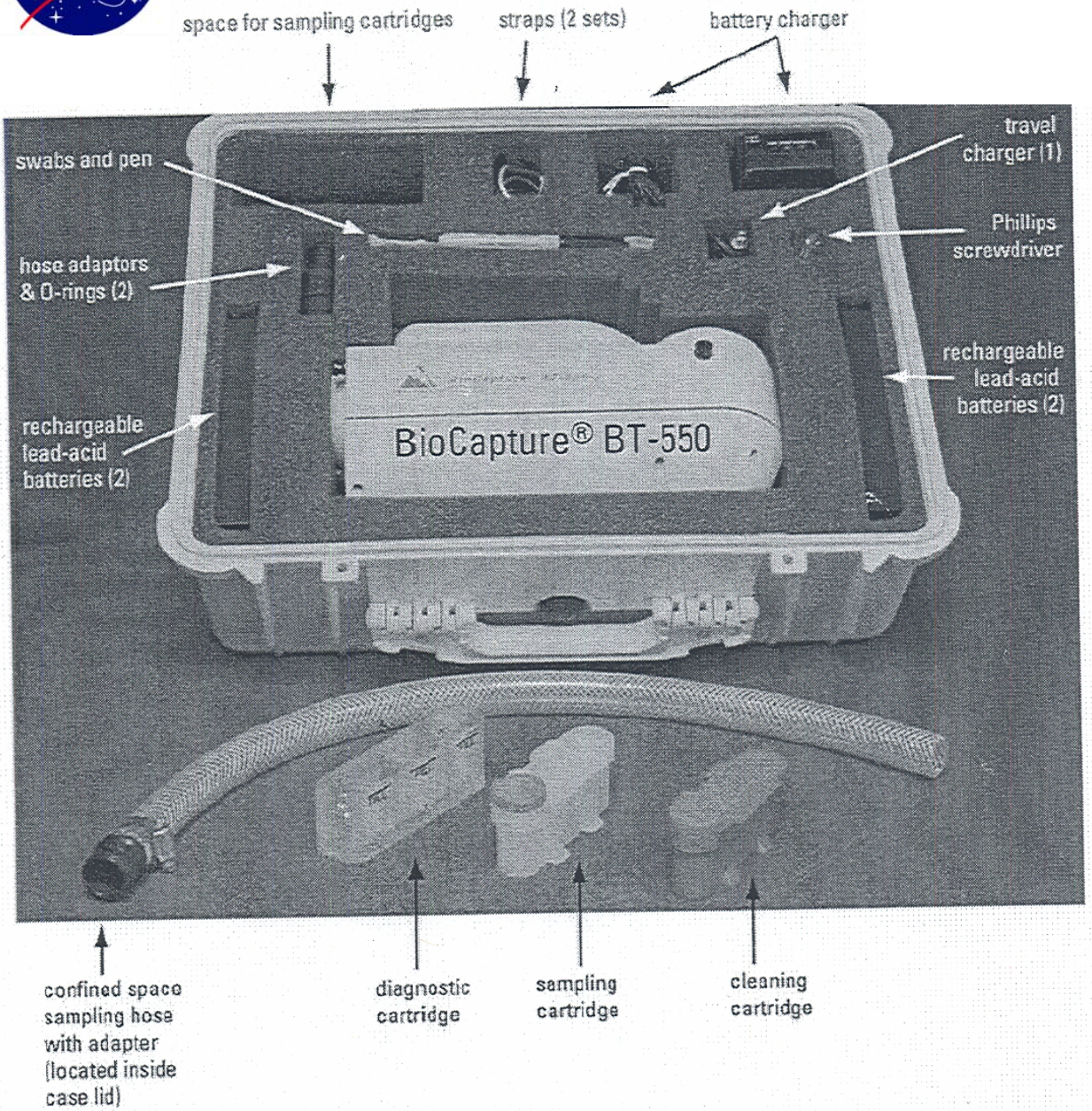
- Information not available on:
  - aircraft make and model
  - age of aircraft
  - passenger load per flight
  - periodicity of the aircraft used
- Airflow:
  - timing of ventilation/bleeding events
  - treatment and processing of the incoming airflow
  - filtration systems in place and the maintenance of such systems
  - schedules of routine and/or rigorous cleaning of the cabin
- Other limitations:
  - airline policy
  - limitations arising from “premature” sampling technology
  - detection limitations of the biological assays used

Issues associated with sampling

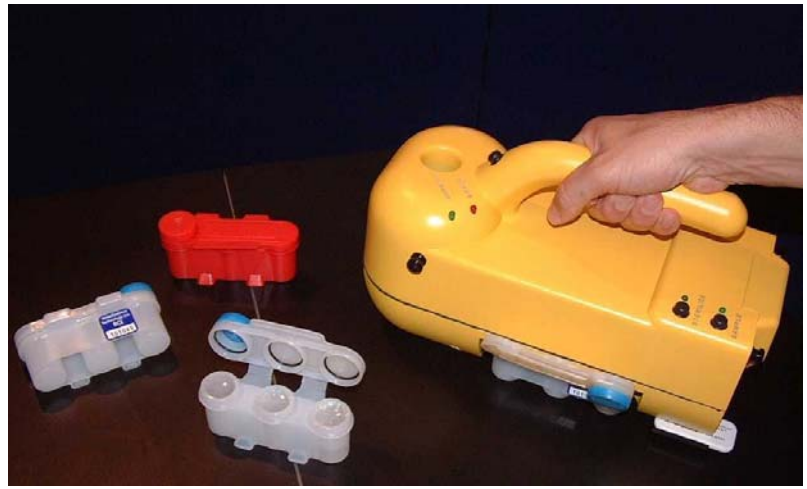
Bioaerosol collection technology

- *Impingement*:
  - forced deposition of particles into a liquid substrate, buffer that can sustain its bio- load for future culture, microscopic, and molecular methods
  - samplers are commercially available, and depending on the manufacturer, readily tailored to meet the needs
  - use of self-contained, commercially available sample cartridges ensures consistent buffer composition and eliminates additional sample handling
- Merits of Biocapture BT-550:
  - Simple, field deployable, light weight, easy to operate, collects large volume of air (150 mL per minute), battery operable, can be modified to AC, disposable are contained, sterile buffers
- Demerits:
  - Sterilization treatment did not remove biological molecules, not DNA-free, pyrogen-free etc.
  - Problem for low biomass samples since buffer associated DNA (negative control) are just above the instrument detection limit

Field-deployable Biocapture BT-550 unit



Quality control of buffer



- Proprietary
- Phosphate-saline buffer (5 mL)
- Free from cultivable microbes
- Free from ATP
- Not-free from DNA molecules
  - ~ 10^3 copies per 5 mL buffer
  - Not good for low biomass samples
  - Most of the airline samples possesses ~10^4 copies of rRNA gene
- Not-free from pyrogens
- DNA signatures of predominant bacterial groups in buffer
  - *Rhodopseudomonas rhenobacensis*
  - *Sphingomonas melonis*

Air Sampling Logistics: Phase I

- Commercial airlines
  - Airline A
    - Chicago to Seattle (Flight 1)
      - April 13, 2004
      - 20 Samples
    - Seattle to Dallas / Fort Worth (Flight 2)
      - April 13, 2004
      - 20 Samples
  - Airline B
    - Cincinnati to Salt Lake City (Flight 3)
      - April 21, 2004
      - 14 Samples
    - Salt Lake City to Cincinnati (Flight 4)
      - April 21, 2004
      - 15 Samples

- Total: 69 samples

Air Sampling Logistics: Phase II

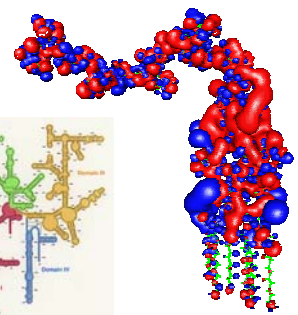
- Commercial airlines (Airline C)
- Nov 2005 to July 2006
  - 8 flights samples for biological analysis
  - Domestic (Los Angeles to New York (4 flights)
  - International (Los Angeles to London (1) Tokyo (1) to Sydney (2)
- Locations
  - Seat Height (SH), Gasper (G), Coach and business class
- Time of sampling
  - Pre-board (PB), Cruise altitude (CA), Mid flight (M), Pre-descent (PD), After landing (L)
- Periodicity
  - All flights were sampled exclusively during “Mid-Air” at 60 minutes intervals
- Total: 125 samples

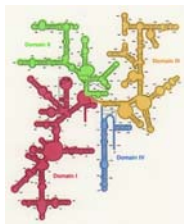
Methodologies Assessing Total BioBurden of Commercial Aircraft Cabin Air

Microbial Detection & Enumeration Strategies

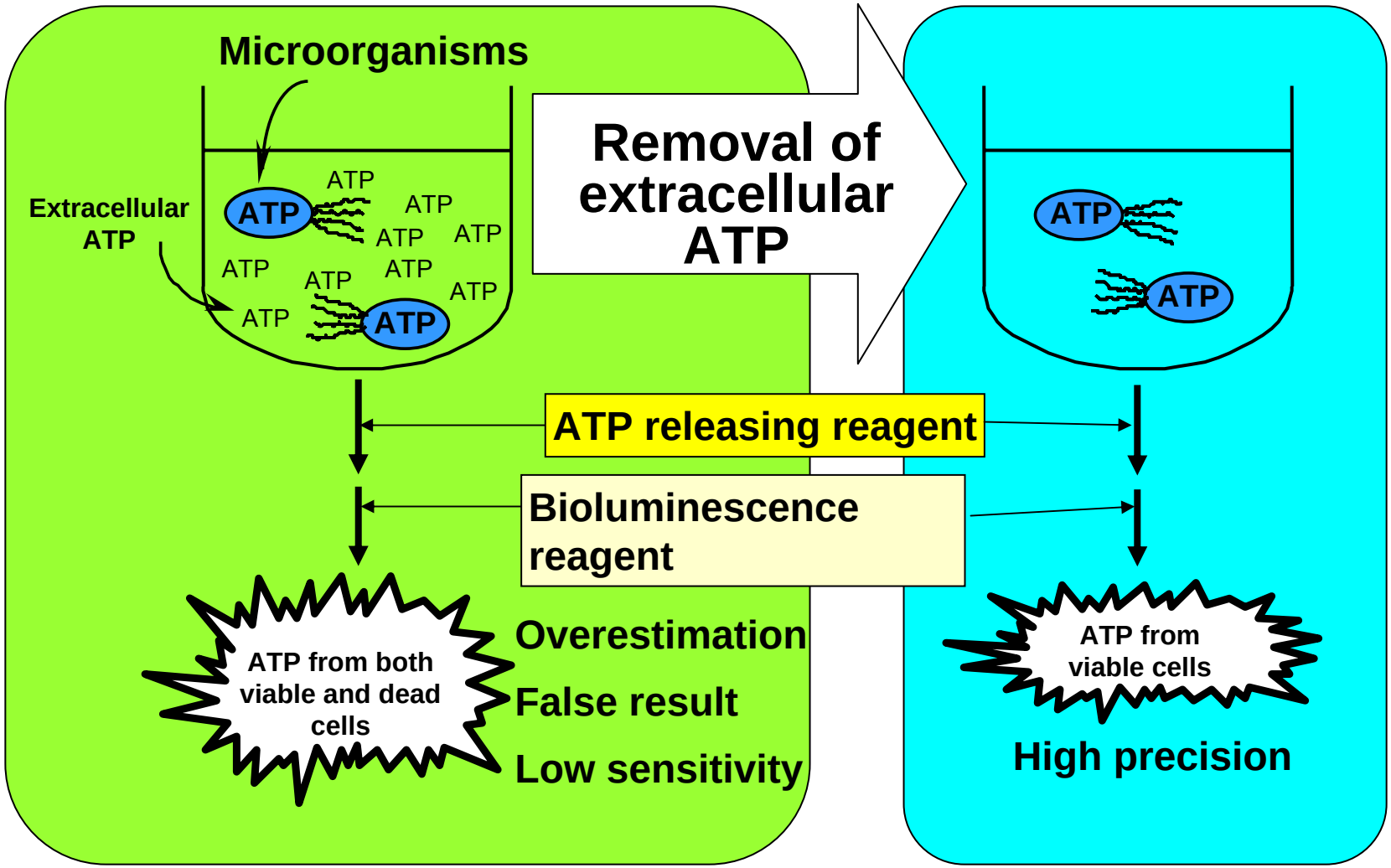
- **Culture-dependent**
  - Conventional plate count assays
    - R2A agar plate enumeration (25°C incubation, 7 days)

- **Culture-independent**
  - Enzymatic, Biomarker-targeted approaches
    - Adenosine TriPhosphate (ATP) Assay
      - » Quantifies total microbial burden
      - » Differentiates viable vs. dead portions of microbial population
    - Endotoxin (lipopolysaccharide (LPS)) Assay
      - » Detects and quantitates total Gram-negative microbial burden
  - Nucleic acid-based approaches
    - Qualitative: Clone Library Analyses
      - Direct DNA extraction
      - 16S ribosomal DNA PCR amplification
      - Cloning, RFLP discrimination, sequencing
      - Phylogenetic analyses
    - Quantitative
      - TaqMan® Q-PCR
        - » estimates total gene (16S rRNA) copy numbers
        - » Eubacterial





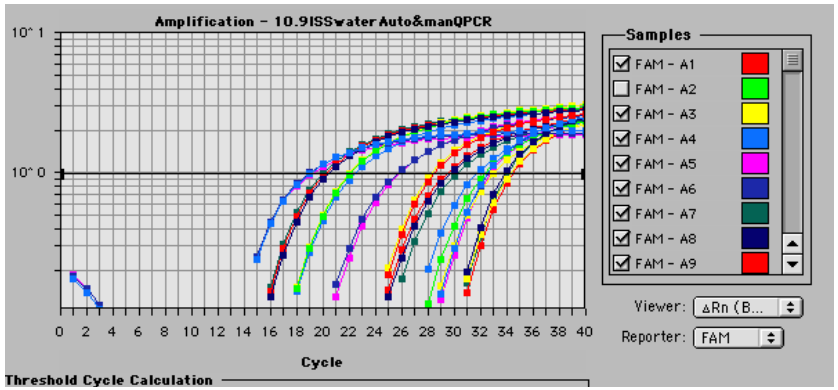
Extracellular vs Viable cells by ATP analysis

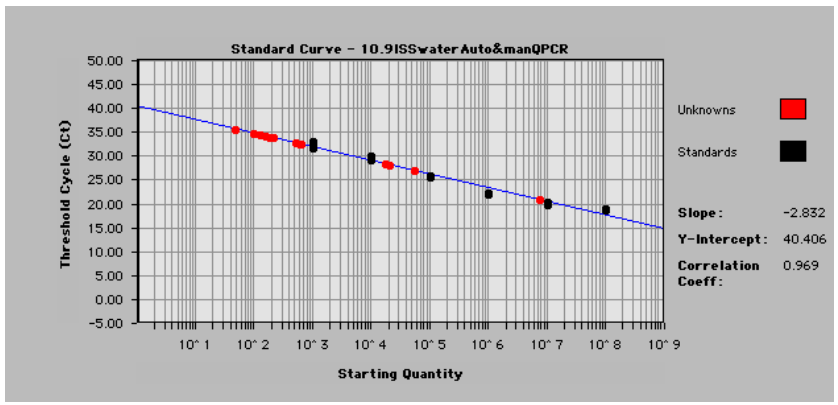


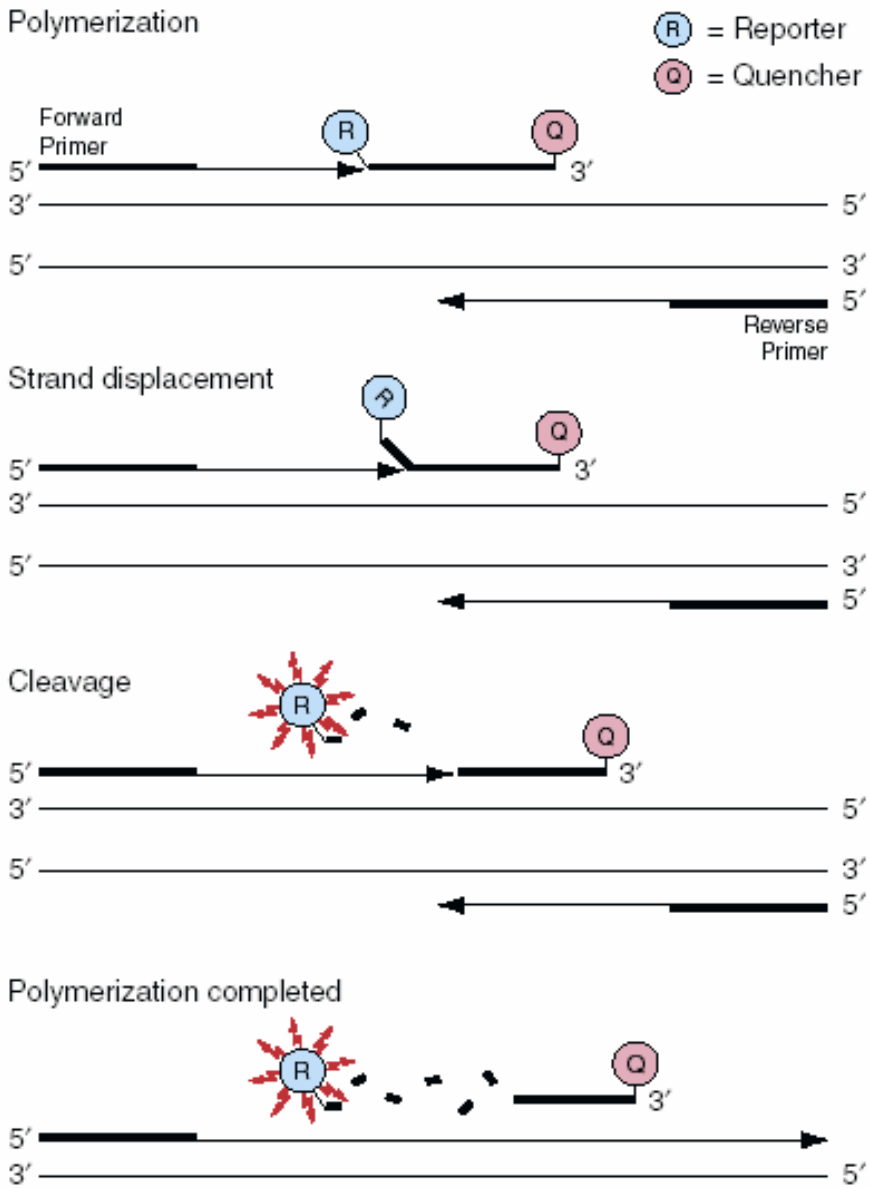
Quantitative and Rapid; 45-min; Start to End

Collaboration with Kikkoman, Japan

TaqMan® Quantitative PCR





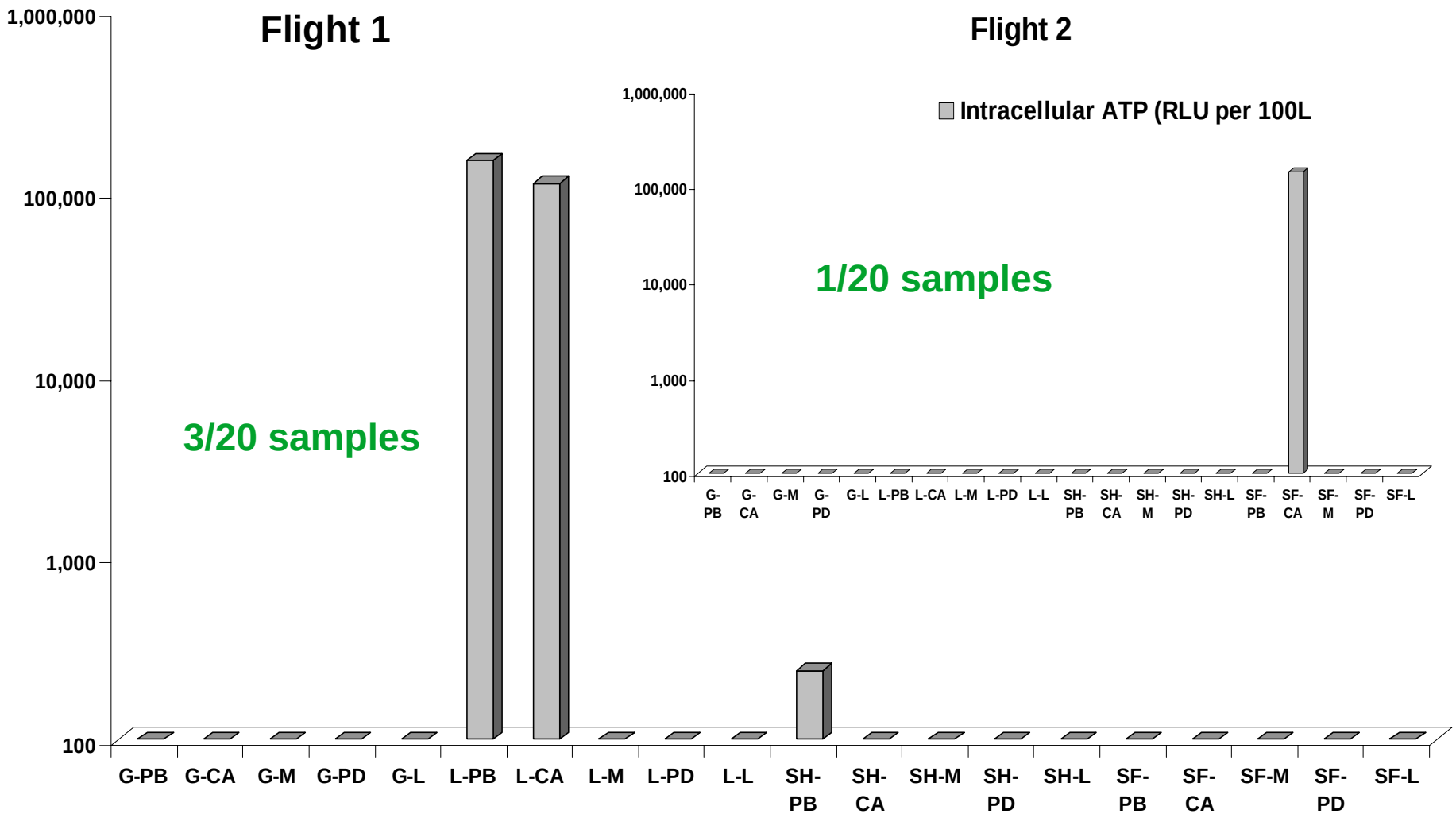


Microbial Burden Estimated by Several Molecular Assays

Viable Microbes in Cabin Air

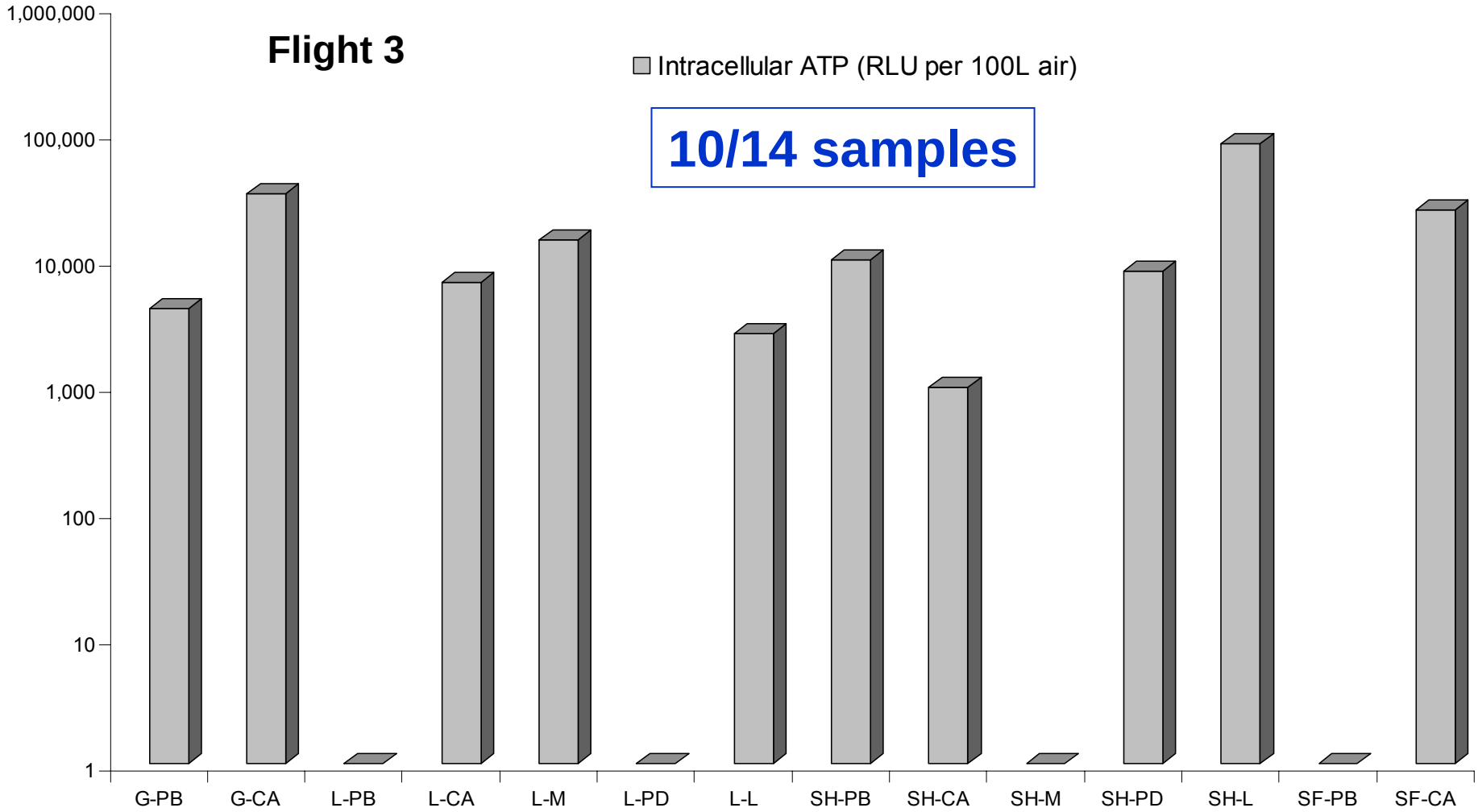
- **Among the two airlines analyzed, a <u>greater</u> viable microbial burden (via plate counts and intracellular ATP assay) was observed in Airline B.**
  - **Airline A (5 to 15% contaminated above threshold levels)**
    - **15% (3/20) for Flight 1**
      - **>10^5 cells per 100 liters of air in 2 samples**
    - **5% (1/20) for Flight 2**
      - **>10^5 cells per 100 liters of air in 1 sample**
  - **Airline B (~ 65% contaminated above threshold)**
    - **71% (10/14) for Flight 3**
      - **>10^4 cells per 100 liters of air in 4 samples**
    - **60% (9/15) for Flight 4**
      - **>10^4 cells per 100 liters of air in 4 samples**

Viable Microbes in Cabin Air



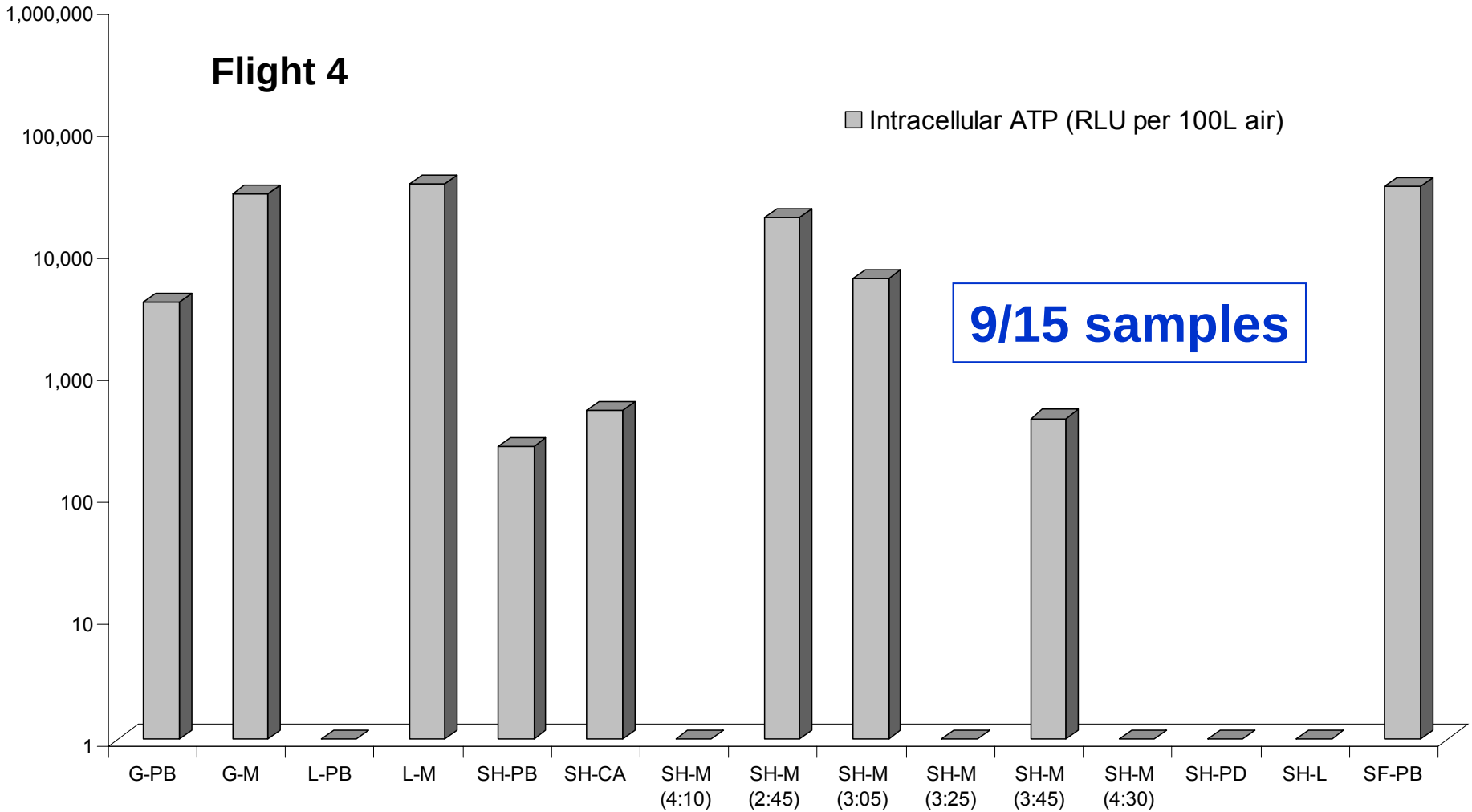
5 to 15% of samples from Airline A were contaminated.

Viable Microbes in Cabin Air



~ 65% of samples from Airline B were contaminated.

Viable Microbes in Cabin Air



~ 65% of samples from Airline B were contaminated.

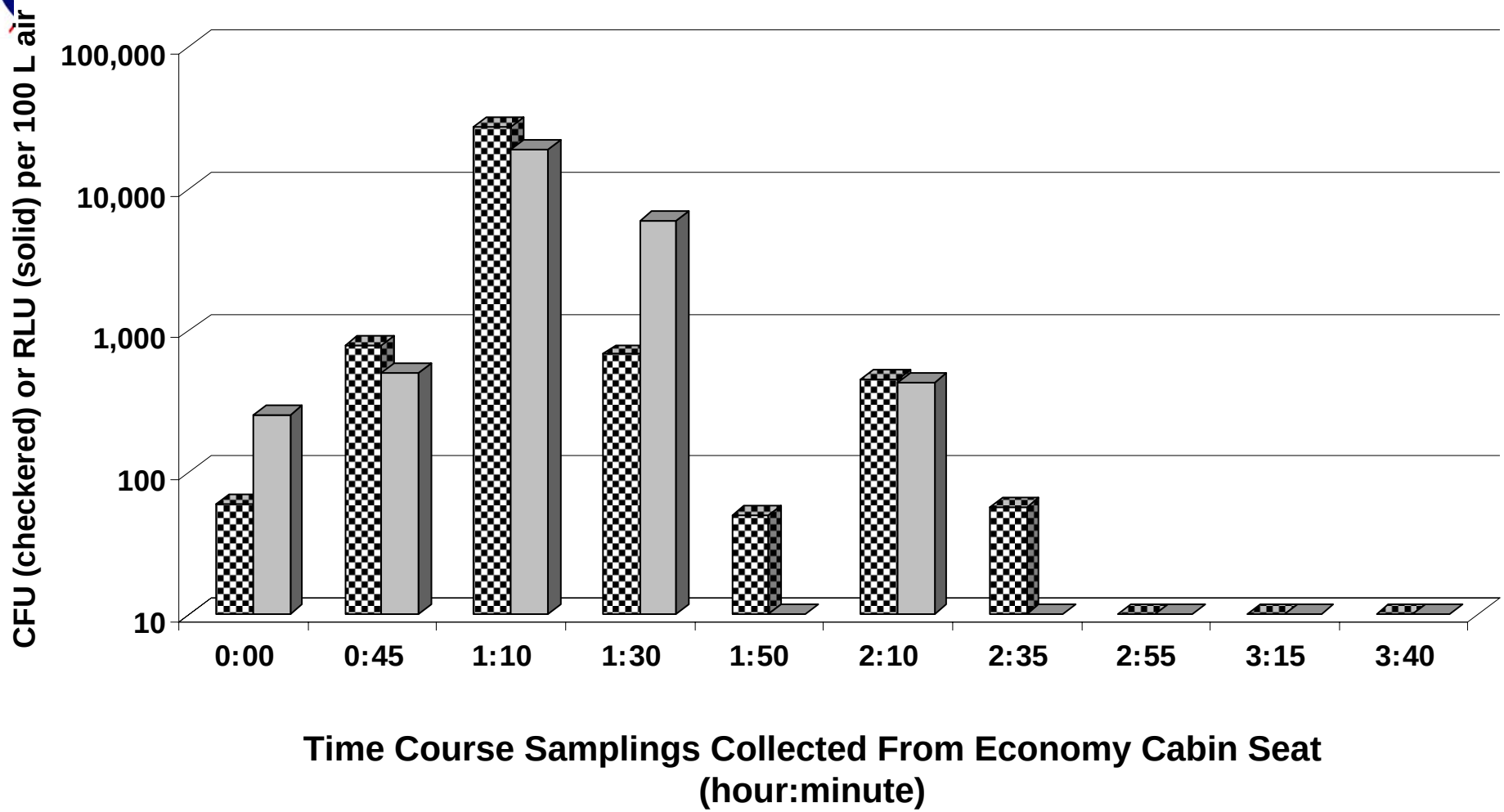


Figure 1. Changes in microbial burden of cabin air collected at seat height of commercial airline B; flight #3 for the Salt Lake City to Cincinnati segment. Air samples were collected at about 20 to 25 minutes of interval for a period of 3h 40 minutes flight duration at the seat height. Systematic air sampling was performed during Pre-Board (0:00), upon reaching cruise altitude (0:45), half-way through the flight (1:10 to 2:55), just before beginning to descent (3:15), and after landing (3:40).

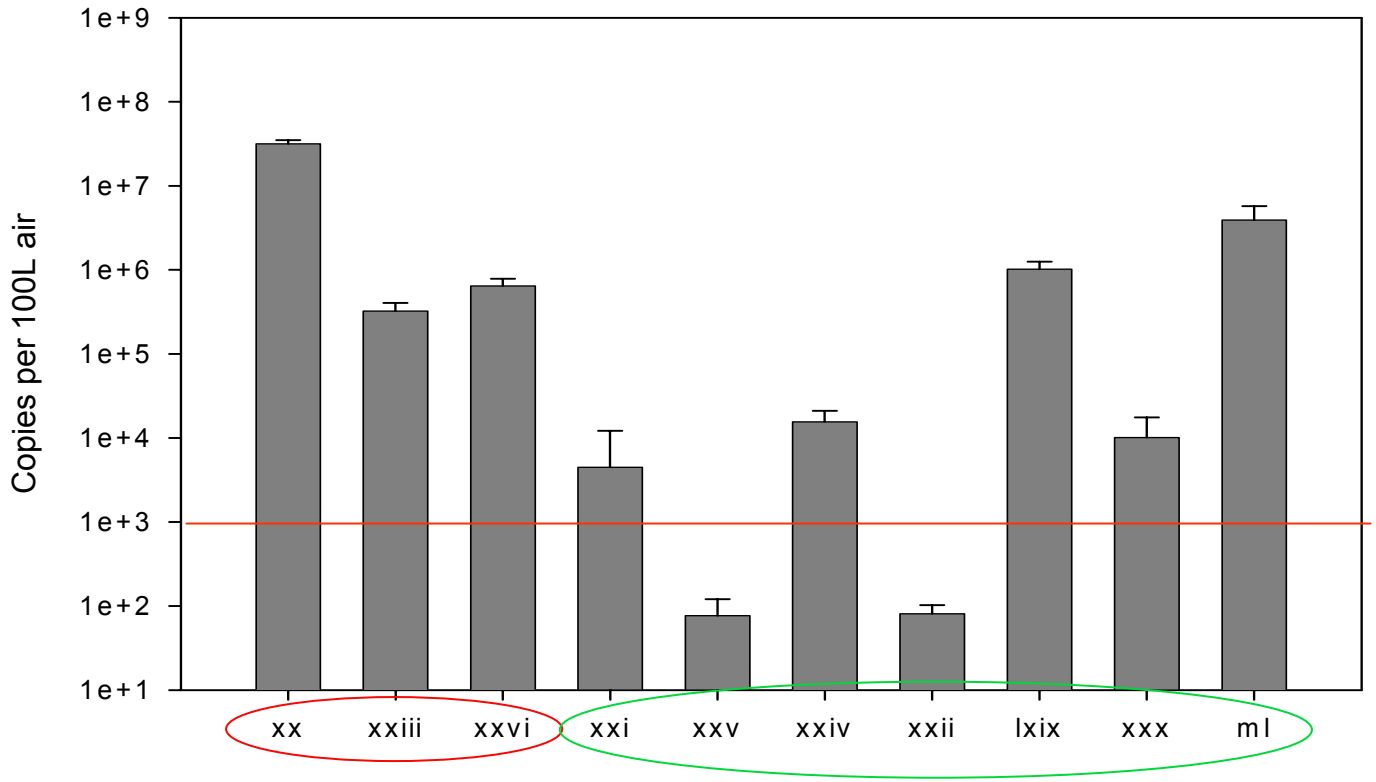
Cultivable counts and viable (intracellular-ATP) microbial population per cubic meter of air collected from various locations of two commercial airliners

Locations	Viable microbial population as estimated by intracellular ATP for Airline # - flight #:				Cultivable counts as estimated by R2A for Airline # - flight #:		% cultivable microbes for Airline # - flight #:	
	A-1	A-2	B-3	B-4	B-3	B-4	B-3	B-4
Gasper	BDL [n=5]	BDL [n=5]	3.4×10^5 [n=2]	3.7×10^5 [n=3]	2.4×10^5 [n=2]	4.6×10^5 [n=3]	70.5	124.0
Lavatory	2.6×10^6 [n=5]	BDL [n=5]	4.1×10^6 [n=2]	2.3×10^5 [n=5]	6.4×10^4 [n=2]	5.1×10^4 [n=5]	1.5	22.2
Seat Floor	BDL [n=5]	1.4×10^6 [n=5]	3.4×10^5 [n=1]	2.4×10^5 [n=2]	1.6×10^4 [n=1]	1.6×10^5 [n=2]	4.7	66.7
Seat Height	BDL [n=5]	BDL [n=5]	2.4×10^5 [n=10]	1.0×10^6 [n=5]	2.8×10^5 [n=10]	1.0×10^5 [n=5]	116.0	10.0
Total # of samples	20	20	15	15	15	15		

Percentage cultivable and viable microbes associated with various flights during Phase II

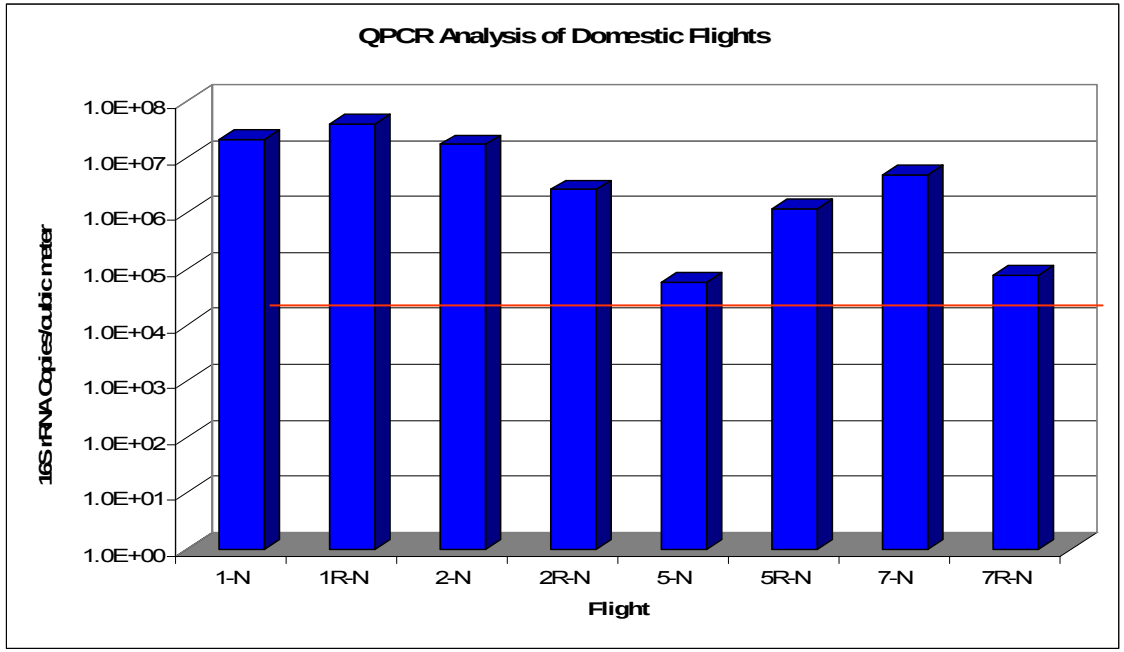
Flight	Route	Date	Total heterotrophs CFU/m^3 A	Intracellular ATP RLU/m^3 B	Total ATP RLU/m^3 C	% of population that are: cultivable (A/B)*100	viable (B/C)*100
1-N	Los Angeles/New York	11/14/05	43	3.33E+02	2.07E+04	12.9	1.6
1R-N	New York/Los Angeles	11/17/05	248	1.28E+02	2.80E+04	>CFU	0.5
2-N	Los Angeles/New York	12/05/05	40	2.47E+03	7.70E+04	1.6	3.2
2R-N	New York/Los Angeles	12/08/05	0	3.29E+02	3.73E+04	no CFU	0.9
3-L	Los Angeles/London	01/15/06	91	2.56E+02	3.88E+04	35.6	0.7
3R-L	London/Los Angeles	01/20/06	0	1.64E+03	2.16E+04	no CFU	7.6
4R-S	Sydney/Los Angeles	02/17/06	53	3.67E+01	1.11E+05	>CFU	0.0
5-N	Los Angeles/New York	3/7/06	50	1.64E+02	5.47E+04	30.5	0.3
5R-N	New York/Los Angeles	3/10/06	27	0	2.89E+04	>CFU	0.0
6-J	Los Angeles/Tokyo	4/3/06	67	9.31E+01	5.90E+04	71.6	0.2
6R-J	Tokyo/Los Angeles	4/7/06	72	3.38E+02	9.28E+04	21.4	0.4
7-N	Los Angeles/New York	4/24/06	6	5.61E+02	4.07E+04	1.0	1.4
7R-N	New York/Los Angeles	4/27/06	53	2.46E+03	2.45E+04	2.2	10.1
8-S	Los Angeles/Sydney	5/7/06	363	1.27E+03	5.10E+04	28.5	2.5
8R-S	Sydney/Los Angeles	5/12/06	103	9.06E+02	7.75E+04	11.4	1.2

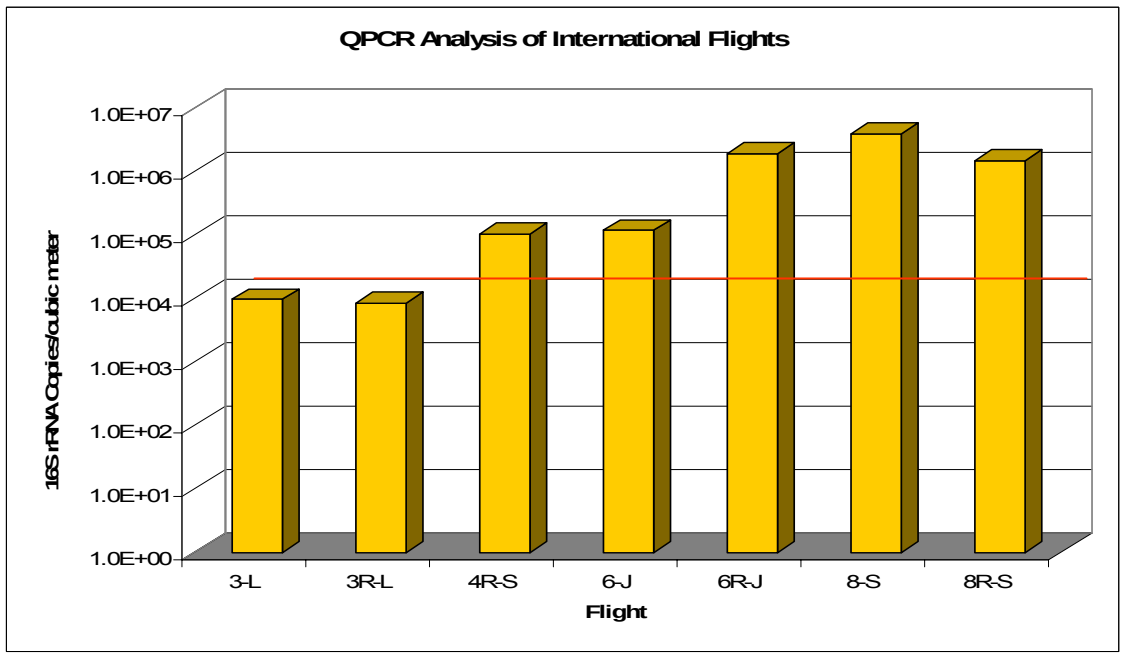
Total bacterial burden as seen by Q-PCR for Phase I



XX	Flt 3	Mid-Flight Seat Samples	B
XXIII	Flt 4	Cruise Alt, Mid-Flight, Pre-Descent Seat, Floor Samples	B
XXVI	Flt 4	Lavatory Samples	B
XXI	Flt 1	Cruise Alt, Mid-Flight, Pre-Descent Lavatory Samples	A
XXV	Flt 1	Cruise Alt, Mid-Flight, Pre-Descent Seat, Floor Samples	A
XXIV	Flt 1	Cruise Alt, Mid-Flight, Pre-Descent Gasper Samples	A
XXII	Flt 2	Pre-Flight Seat, Floor Samples	A
LXIX	Flt 2	Lavatory Samples	A
XXX	Flt 2	Post-Landing Seat, Floor Samples	A
ML	Flt 2	Cruise Alt, Mid-Flight, Pre-Descent Seat, Floor Samples	A

Total bacterial burden as seen by Q-PCR for Phase II



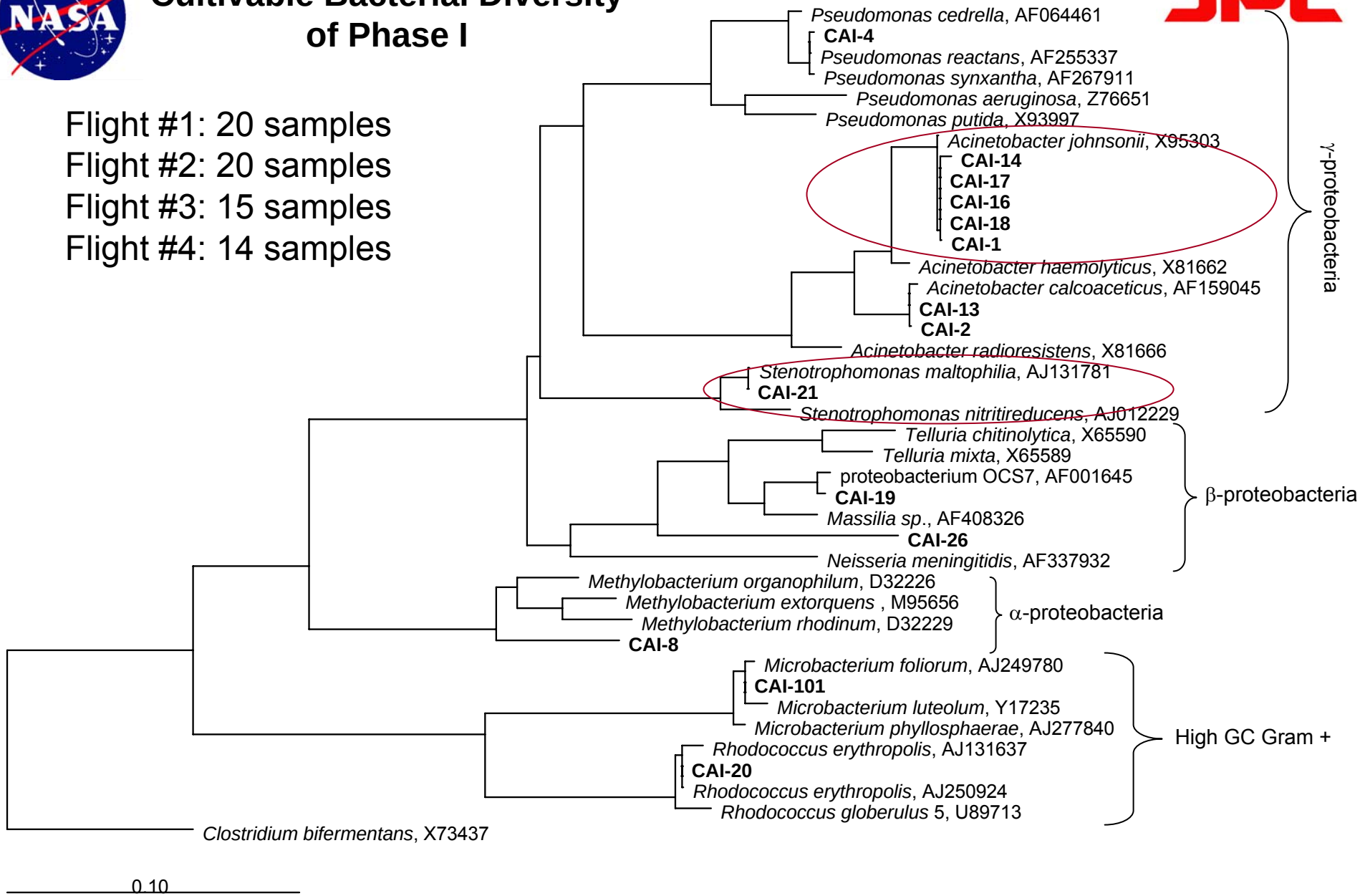


Bacterial Diversity of Commercial Air Cabin

- Cultivable
- Molecular bacterial community analyses
  - Conventional cloning and sequencing

Cultivable Bacterial Diversity of Phase I

Flight #1: 20 samples
Flight #2: 20 samples
Flight #3: 15 samples
Flight #4: 14 samples



Cultivable bacteria in Phase I

- Representatives of the α-, β-, and γ-proteobacteria, as well as high G+C Gram-positive bacteria, were identified in varying abundance.
- Cells of *Acinetobacter johnsonii* (36%) dominated samples retrieved from Flight #3, while Flight #4 contained equal allotments of *Pseudomonas reactans, Methylobacterium* spp., and *Microbacterium phyllosphaerae*, based on morphometric comparison between colonies and sequence identification data.
- No discernable phylotypic correlations were evident between the time and/or location of sampling and the types of microbes detected.

Abundance, occurrence, and pathogens of cultivable species

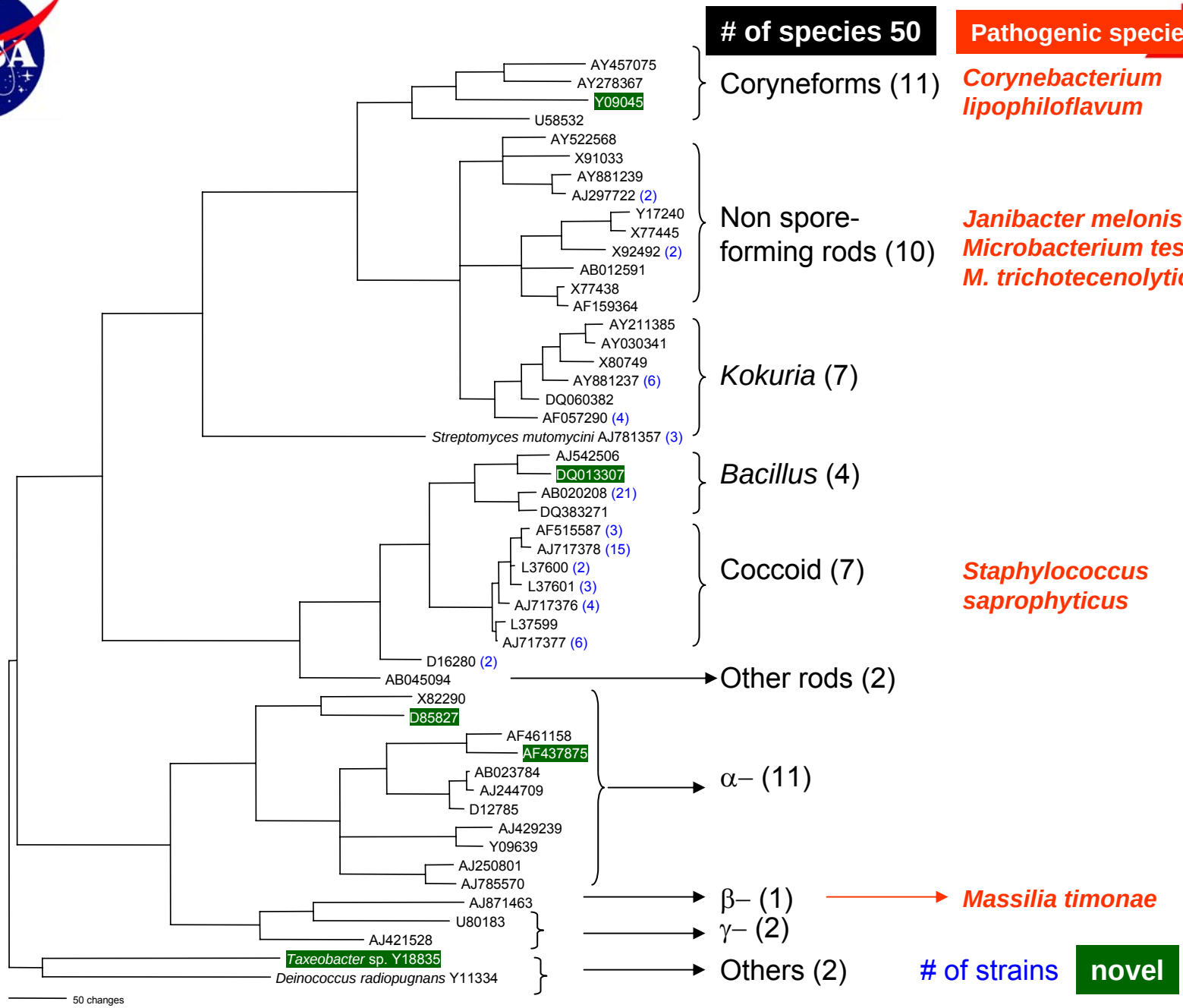


Figure 4.3.1-2 Phylogenetic analysis of bacterial SSU rRNA gene sequences from isolates collected aboard Airlines C

Cultivable bacteria in Phase II

- 86% were Gram-positive, and 60% of these were of high G/C content, including *Micrococcus*, *Kytococcus*, and *Staphylococcus*.
- As one would expect a significant abundance of Staphylococci and Micrococci were isolated (38 of 57 High G/C non-spore-forming isolates).
- Spore-forming *Bacillus* species were also quite prevalent, as 19 of the 57 high G/C Gram-positive cultured cell lines arose from this lineage.
- Members of the α-proteobacteria, such as *Methylobacterium* spp., comprised 10% of all bacterial strains isolated, complementary to results obtained from 16S rDNA-based clone library analyses.
- Over the course of the Phase II study, five novel bacterial lineages were uncovered about the cabin air samples analyzed: two members of the α-proteobacteria (*Paracoccus* sp. and *Paracraurococcus* sp.), one CFTB (Taxeobacter sp.), and 2 Gram-positive bacteria (*Corynebacterium* sp. and *Bacillus* sp.).

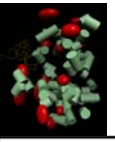
Cultivable bacteria in Phase II

- Several bacteria implicated in the cause of human illness were also isolated, including *Janibacter melonis* – the causative agent of bacteremia, *Microbacterium trichotecenolyticum* – the causative agent of neutropenia, *Massilia timonae* – a cause of cerebellar lesions, *Staphylococcus saprophyticus* – a cause of urinary tract infections, and *Corynebacterium lipophiloflavum* – the cause of bacterial vaginosis.
- The 16S rDNA sequences of most of these isolates were also retrieved and observed in clone libraries, lending further support to the conclusion that these species are indeed prevalent about these cabin air parcels. These isolations are sporadic and cannot be linked to any given time points or locations about any specific aircraft.

Molecular Bacterial Community Analyses

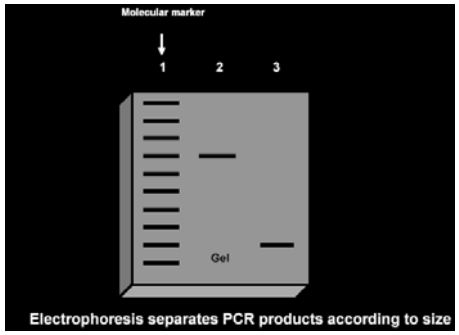
16S rDNA phylotypic discrimination

DNA Extraction

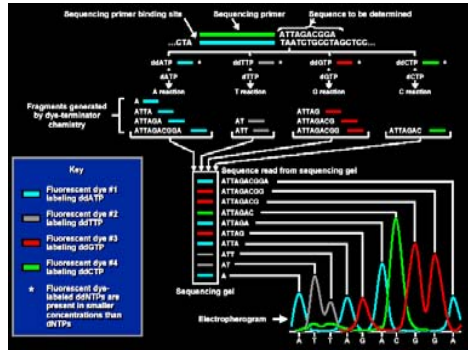


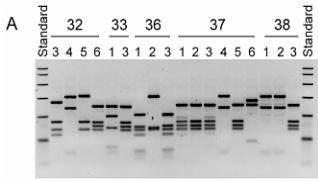
PCR

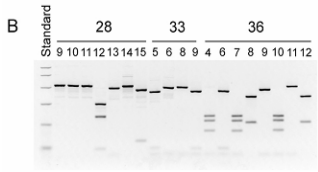
Amplicon excision



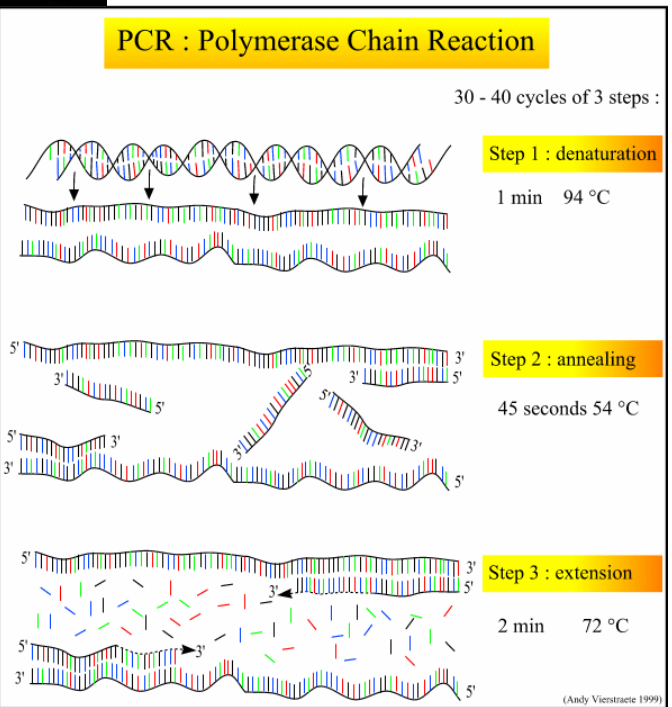
Licor® Sequencing



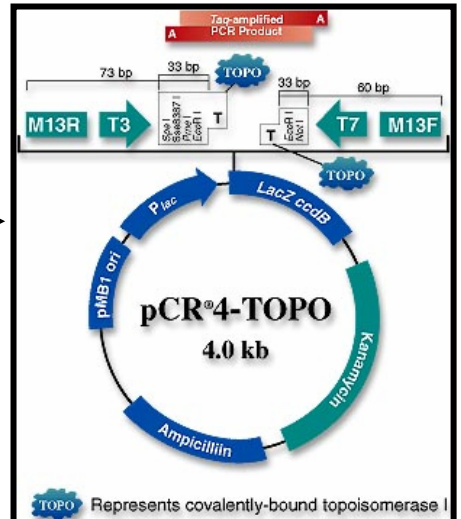




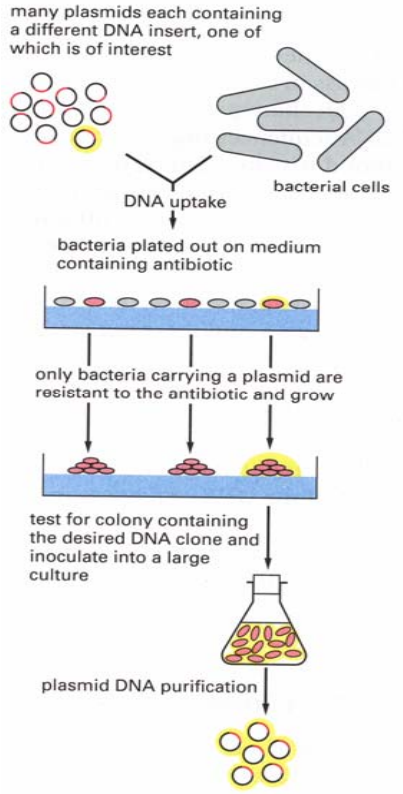
RFLP's



Cloning



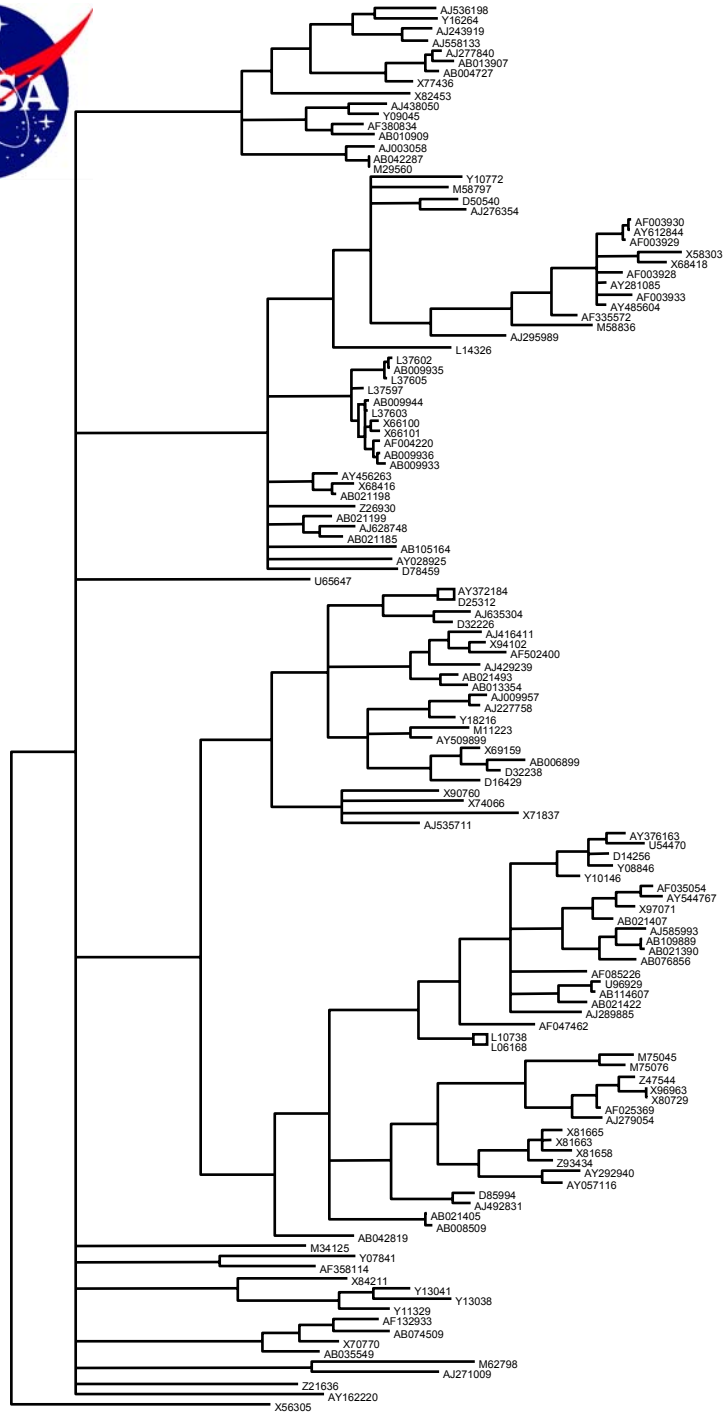
Clone Library



Overview of molecular bacterial diversity in Phase 2

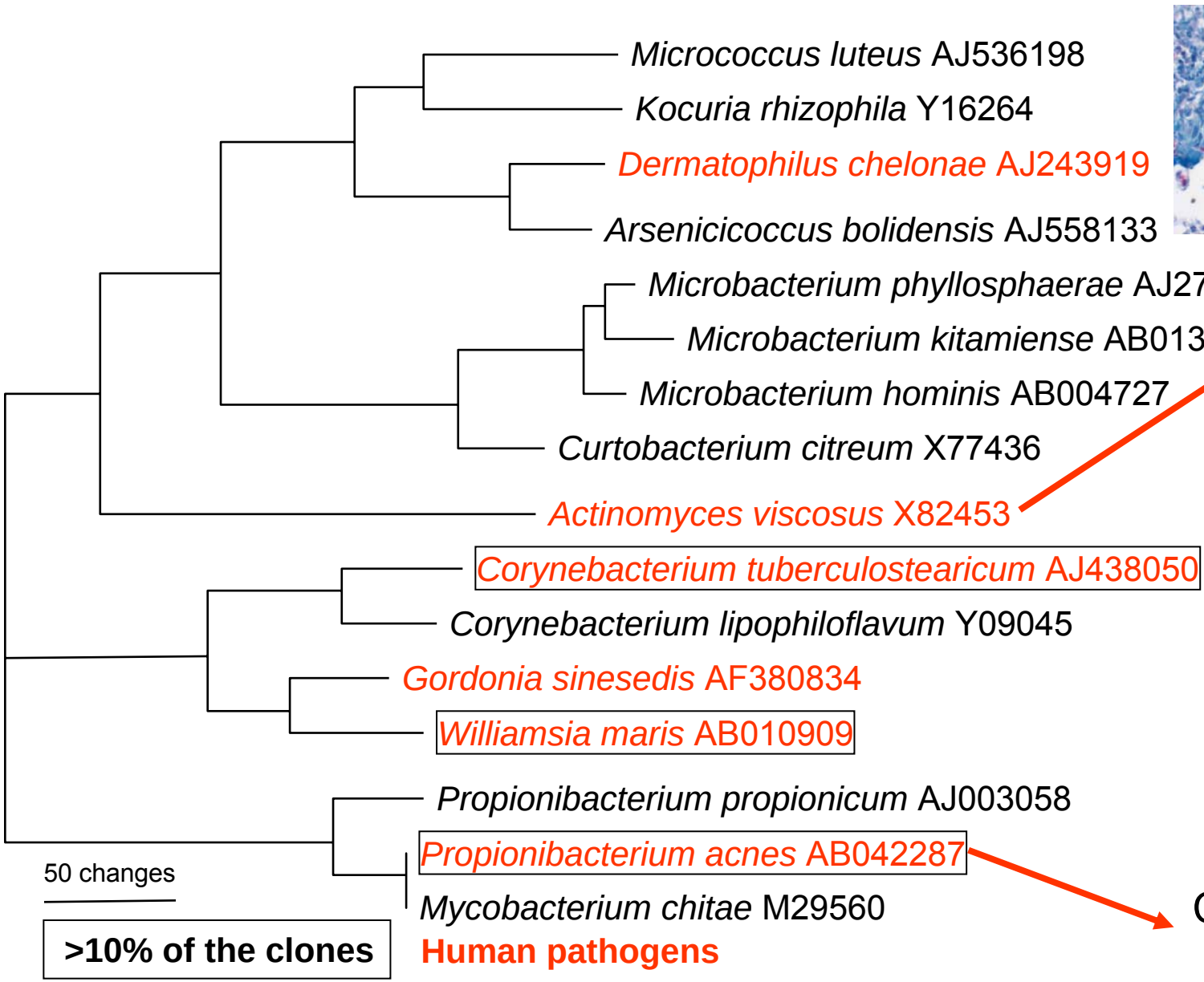
Class of microbe	Percent incidence of clones retrieved from:															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
	1N	1R-N	2N	2R-N	3-L	3R-L	4-S	4R-S	5N	5R-N	6J	6R-J	7N	7R-N	8S	8S-R
	LAX to JFK	JFK to LAX	LAX to JFK	JFK to LAX	LAX to London	London to LAX	LAX to Sydney	Sydney to LAX	LAX to JFK	JFK to LAX	LAX to NRT	NRT to LAX	LAX to JFK	JFK to LAX	LAX to Sydney	Sydney to LAX
α−proteobacteria:		100		No PCR product amplified and subsequently cloning process was not carried			19.2		15.9	27.3	10.0	85.7		26.8	59.9	45.0
β−proteobacteria:	3.6		33.5					3.2	5.3		15.0		30.8	6.7		2.5
γ−proteobacteria:	85.5		40.2				6.4	12.8	37.1		10.0		53.9	13.4		
δ−proteobacteria:								3.2						6.7		
Actinobacteria	1.2		13.4				16.0	19.2	10.6	45.5	15.0			6.7		5.0
Firmicutes			13.4				44.8	38.4	31.8	27.3	45.0	8.6		40.2	13.4	7.5
Fusobacteria							3.2									
Deinococcus													7.7		66.9	35.0
Spirochaetes																
Bacterioidetes												2.9				
Cyanobacteria							9.6	22.6				2.9	7.7			2.5
Planktomycetes														6.7		
# of bacterial species	**5**	**2**	**9**				**26**	**17**	**17**	**5**	**13**	**4**	**7**	**10**	**8**	**14**

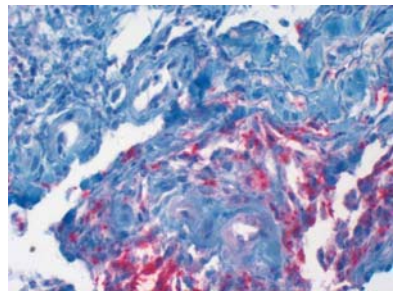
Abundance, occurrence, and pathogens of bacterial species



	>10% abundance	#	pathogen
Gram-positive	*Corynebacterium tuberculostearicum* *Propionibacterium acnes* *Williamsia maris* *Bacillus pallidus* *Staphylococcus epidermidis* *Staphylococcus hominis* *Streptococcus australis* *Streptococcus pneumoniae* *Streptococcus sanguinis*	113 57	29 12
α–proteo	*Caulobacter henricii* *Caulobacter vibrioides* *Erythrobacter litoralis* *Methylobacterium hispanicum* *Novosphingobium pentaromativorans* *Rhizobium leguminosarum* *Rhodopseudomonas palustris*	23	1
β	*Burkholderia cepacia* *Imtechium assamiensis* *Massilia timonae* *Wautersia paucula*	19	5
γ	*Acinetobacter junii* *Pseudomonas hibiscicola* *Pseudomonas saccharophila* *Psychrobacter meningitidis* *Stenotrophomonas maltophilia*	20	10
others	*Deinococcus grandis* *Deinococcus murrayi* *Oscillatoria sancta*	14	1

Actinobacteria



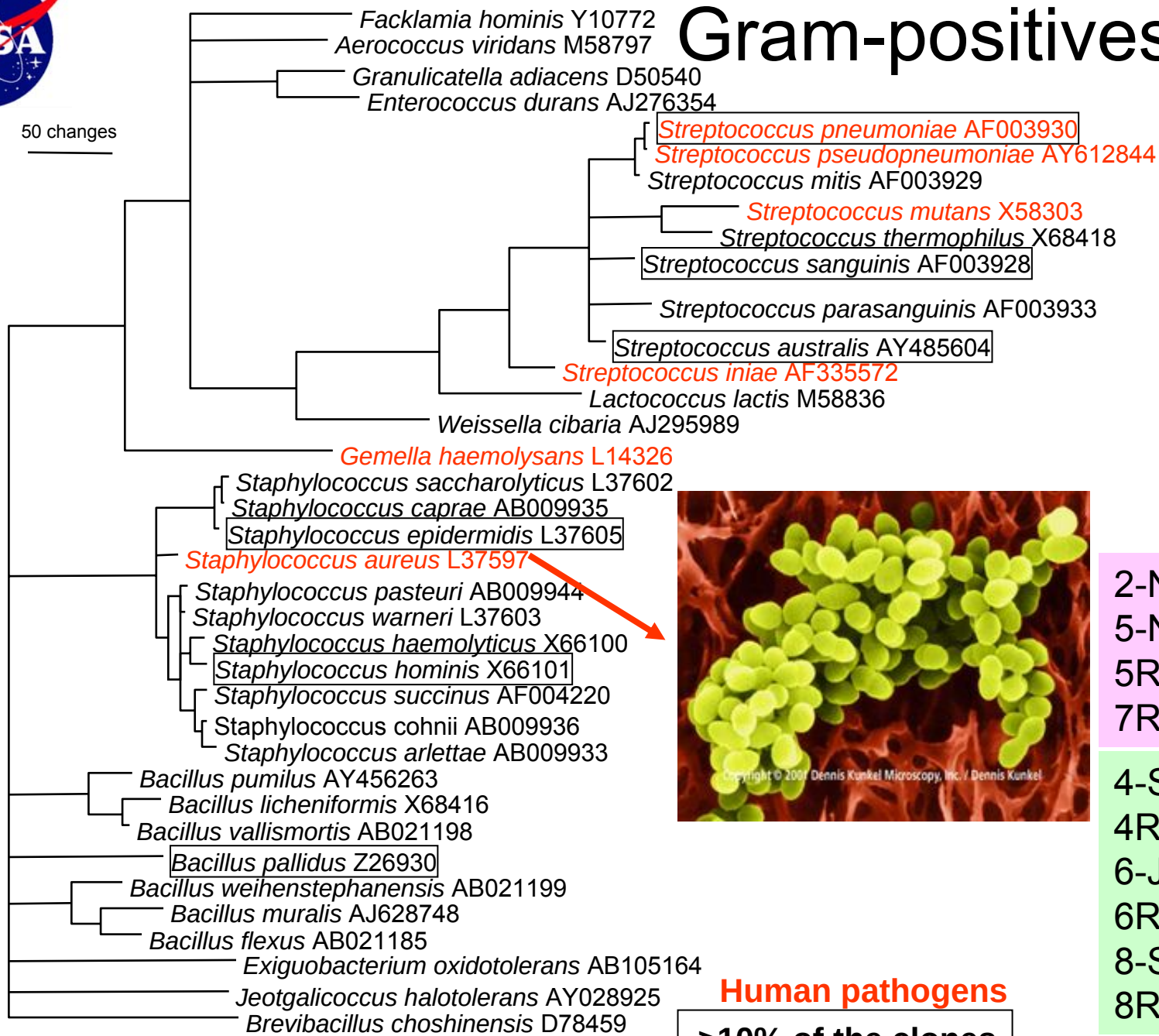


Cervicofacial actinomycosis

Sample	%
1-N:	1.2
2-N:	13.4
5-N:	10.6
5R-N:	45.5
7R-N:	6.7
4-S:	16.0
4R-S:	19.2
6-J:	15.0
8R-S:	5.0

Cat-scratch disease

Gram-positives

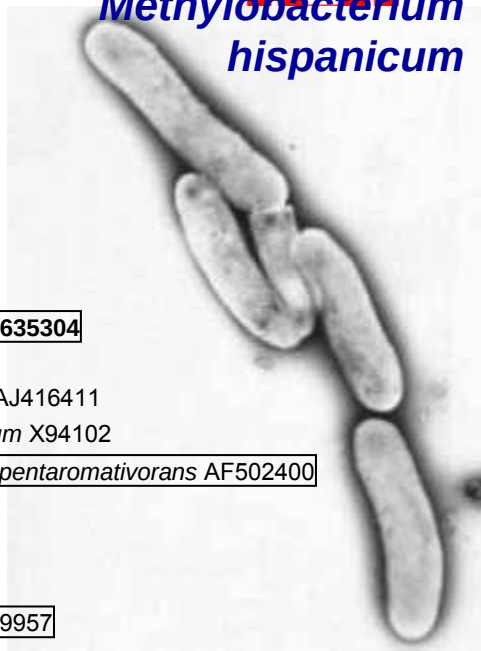


2-N:	13.4
5-N:	31.8
5R-N:	27.3
7R-N:	40.2
4-S:	44.8
4R-S:	38.4
6-J:	45.0
6R-J:	8.6
8-S:	13.4
8R-S:	7.5

Human pathogens

>10% of the clones

α–proteobacteria



50 changes

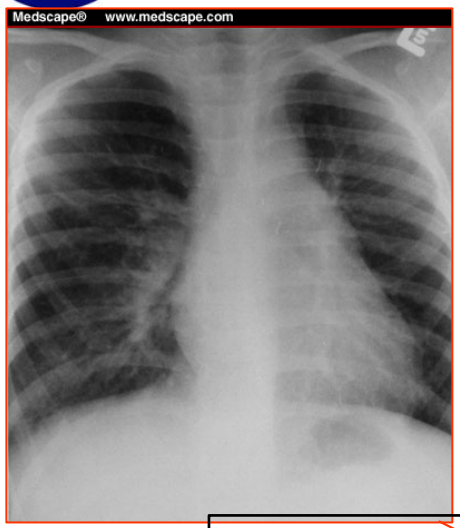
Bradyrhizobium betae AY372184
Rhodopseudomonas palustris D25312
***Methylobacterium hispanicum* AJ635304**
Methylobacterium organophilum D32226
Novosphingobium hassiacum AJ416411
Novosphingobium subarcticum X94102
Novosphingobium pentaromativorans AF502400
Sphingomonas faeni AJ429239
Agrobacterium sanguineum AB021493
***Erythrobacter litoralis* AB013354**
Caulobacter vibrioides AJ009957
Caulobacter henricii AJ227758
Phenylobacterium immobile Y18216
***Agrobacterium tumefaciens* M11223**
Rhizobium leguminosarum AY509899
Paracoccus denitrificans X69159
Paracoccus carotinifac
Paracoccus alcaliphilus D32238
Rhodobacter blasticus D16429
Skermanella parooensis X90760
Acetobacter aceti X74066
Caedibacter caryophilus X
Stella vacuolata AJ535711

Plant pathogens

>10% of the clones

Sample	%
1R-N:	100.0
5-N:	15.9
5R-N:	27.3
7R-N:	26.8
4-S:	19.2
6-J:	10.0
6R-J:	85.7
8-S:	59.9
8R-S:	45.0

β–proteobacteria



Duganella violaceinigra AY376163

Massilia timonae U54470

Duganella zoogloeoides D14256

Janthinobacterium lividum Y08846

Herbosprillum seropedicae Y10146

Aquabacterium commune AF035054

Imtechium assamiensis AY544767

Leptothrix mobilis X97071

Pseudomonas saccharophila AB021407

Hydrogenophaga defluvii AJ585993

Curvibacter gracilis AB109889

Pseudomonas lanceolata AB021390

Diaphorobacter nitroreducens AB076856

Wautersia paucula AF085226

Burkholderia vietnamiensis U96929

Burkholderia cepacia AB114607

Burkholderia andropogonis AB021422

Limnobacter thiooxidans AJ289885

Dechloromonas agitata AF047462

Neisseria weaveri L10738

Neisseria flavescens L06168

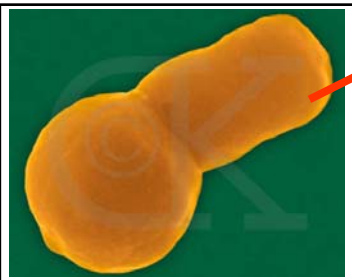
50 changes

Human pathogens

>10% of the clones

Sample	%
1R-N:	3.6
2-N:	33.5
5-N:	5.3
7-N:	30.8
7R-N:	6.7
4R-S:	3.2
6-J:	15.0
8R-S:	2.5

γ–proteobacteria



Haemophilus haemolyticus M75045

Haemophilus paraphrohaemolyticus M75076

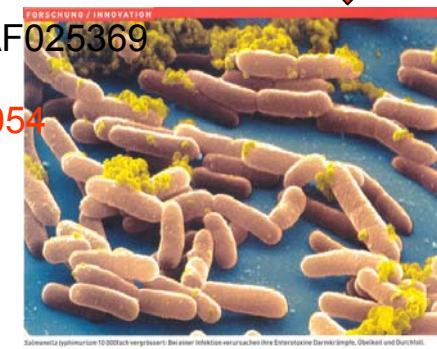
Salmonella typhi Z47544

Shigella flexneri X96963

Escherichia coli X80729

Citrobacter murliniae AF025369

Serratia ficaria AJ279054



Acinetobacter lwoffii X81665

Acinetobacter johnsonii X81663

Acinetobacter junii X81658

Acinetobacter calcoaceticus Z93434

Psychrobacter marinicola AY292940

Psychrobacter meningitidis AY057116

Pseudomonas putida D85994

Pseudomonas trivialis AJ492831

50 changes

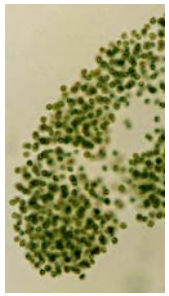
Human pathogens

>10% of the clones

Sample	%
1-N:	85.5
2-N:	33.5
5-N:	5.3
7-N:	30.8
7R-N:	6.7
4-S:	3.2
4R-S:	3.2
6-J:	15.0

Other classes of bacteria

- Cyanobacteria:
  Microcystis aeruginosa AB035549
  Microcoleus chthonoplastes X70770
  Oscillatoria sancta AF132933
  Oscillatoria sp. AB074509



- Fusobacteria:
  Fusobacterium canifelinum AY162220

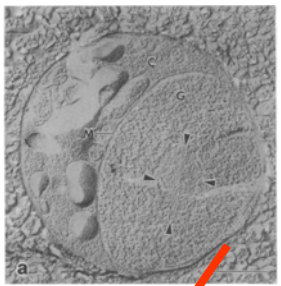
- Bacterioidetes:
  Flavobacterium ferrugineum M62798
  Meiothermus silvanus X84211
  Chryseobacterium scophthalmum AJ271009

- Fusobacteria:
  Anaerococcus octavius Y07841
  Sporanaerobacter acetigenes AF358114

δ–proteobacteria:

- *Bacteriovorax stolpii* M34125

- Deinococcales:
  Meiothermus silvanus X84211
  Deinococcus murrayi Y13041
  Deinococcus geothermalis Y13038
  Deinococcus grandis Y11329



Planctomycetes:

- *Gemmata obscuriglobus* X56305

Spirochaetes:

- *Leptospira parva* Z21636

>10% of the clones

Human pathogens

Quantitation of Select Viruses Within Commercial Airliner Cabin Air

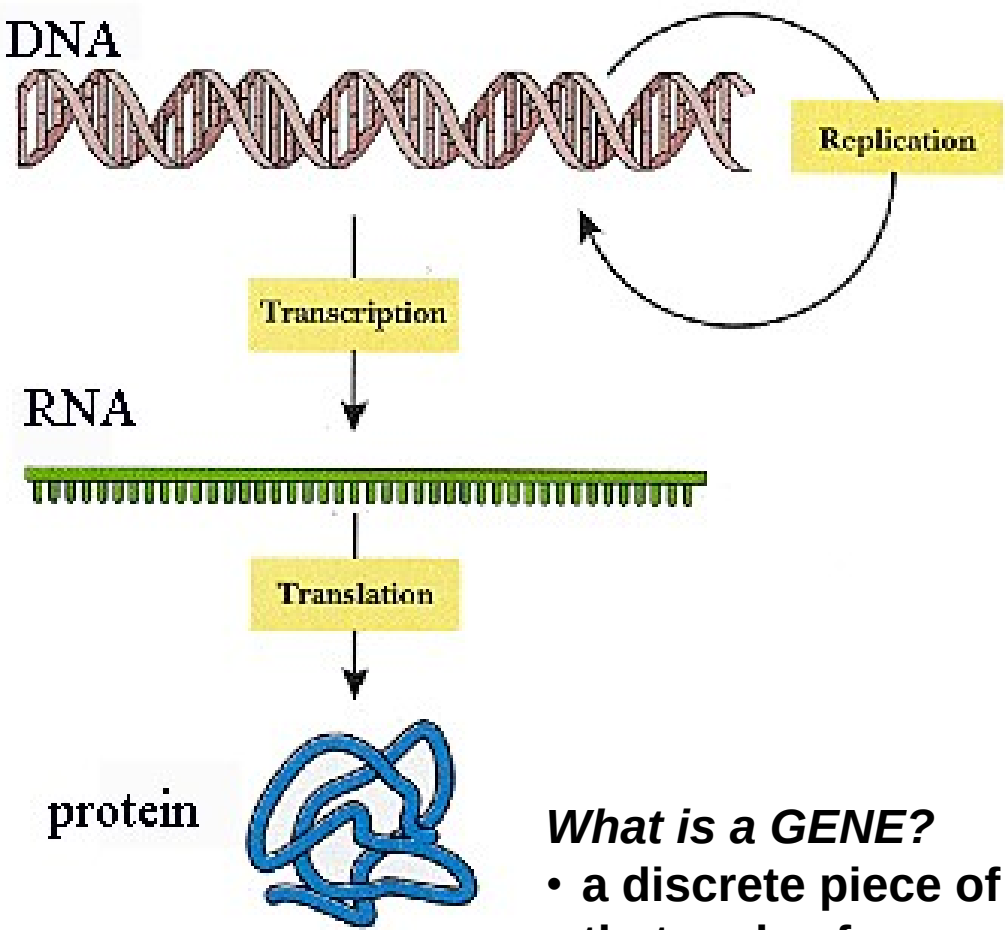
M.T. La Duc, S. Osman, A. Dekas, J. Fuhrman,* and K. Venkateswaran

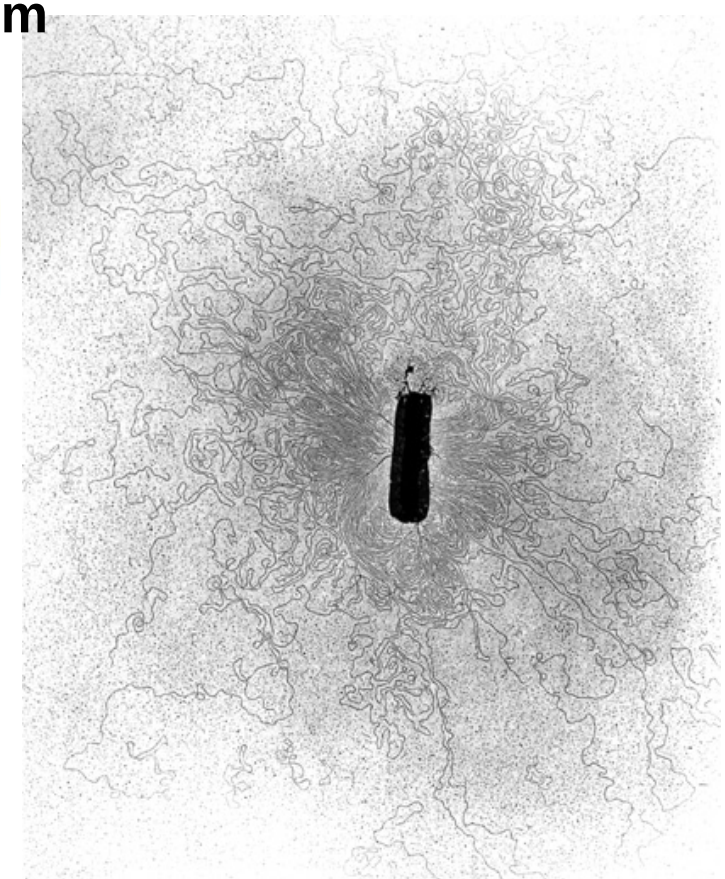
****Lead virologist***

contact: fuhrman@usc.edu

Mol Bio Refresher: The Central Dogma

Gene → Enzyme → → → Metabolism





What is a GENE?

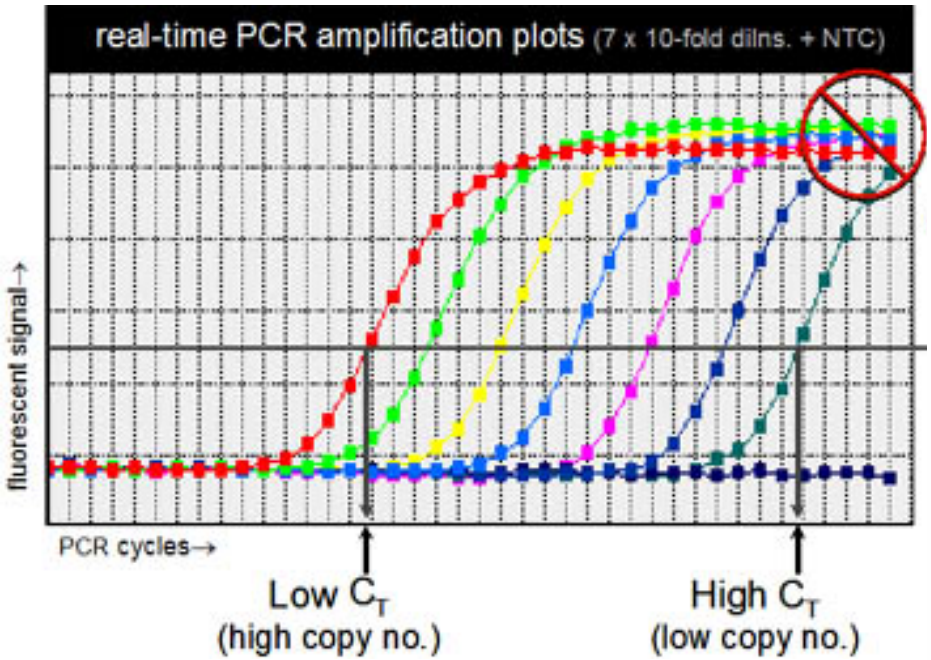
- **a discrete piece of deoxyribonucleic acid that codes for a particular protein/enzyme**
- **linear polymer of repeating nucleotide monomers A = adenine, C = cytosine, T = thymidine, G = guanine**

Methods

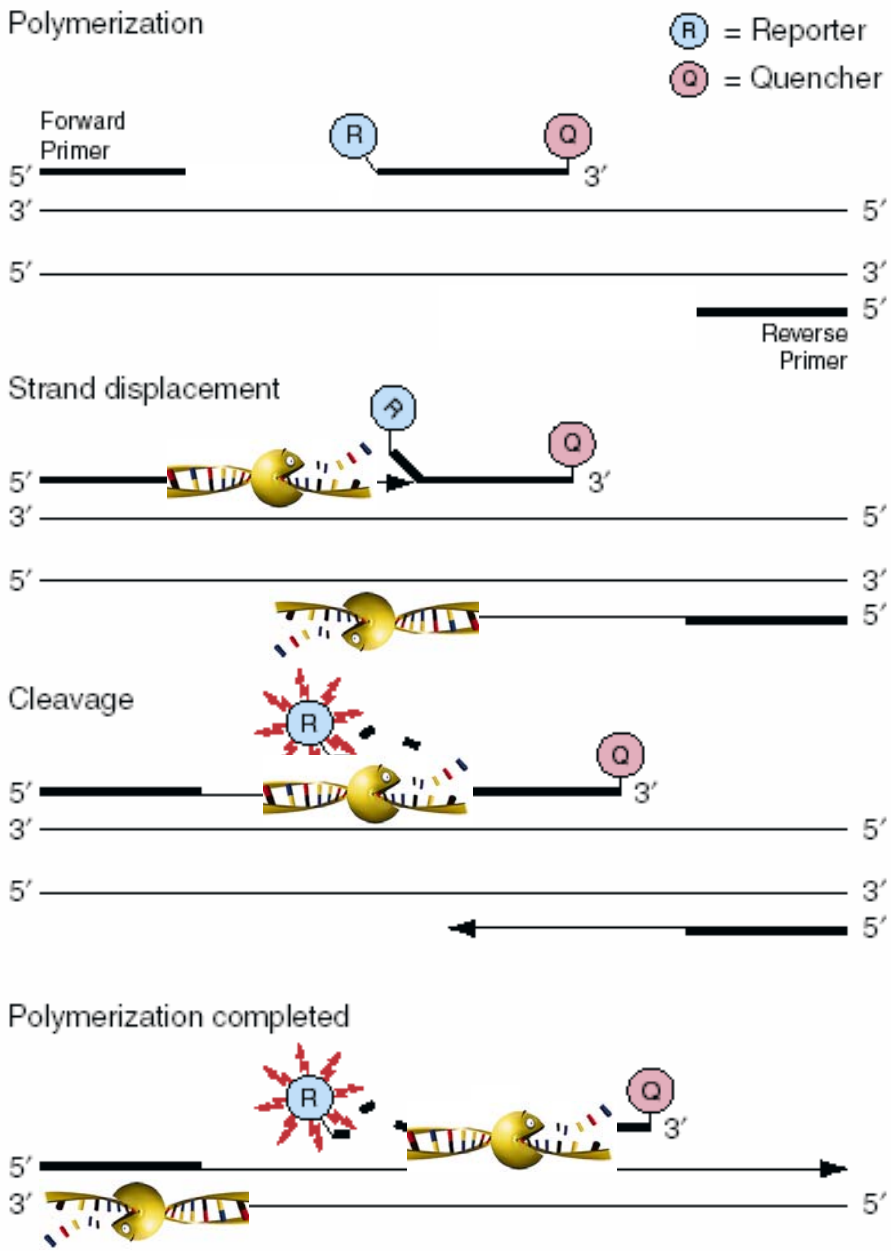
Sampling procedure: Mesosystems BT-550, as described earlier

- **Viral nucleic acid isolation from samples and blank cartridges**:
  - QiaAmp Minelute Virus Spin Kit (Qiagen)
  - Three 200 µl subsamples were extracted from each cartridge, and eluted with 35 µl buffer
  - The three subsamples were mixed, divided into three aliquots, and frozen at -80ºC
- **RT-QPCR and Q-PCR of viral nucleic acids (+ controls)**:
  - Each assay was initially verified with a dilution series of viral nucleic acids using previously published Taqman-based QPCR assays
  - All amplification and detection were performed by the Stratagene MX3000P QPCR System. All samples, PCR standards, and negative controls were run in duplicate.
- **Generation of viral DNA PCR standards and standard curves**:
  - Target genes for were PCR amplified and amplicons were cloned with a Qiagen cloning kit. Plasmids containing the target gene were purified with the QiaPrep Spin Miniprep Kit (Qiagen), eluted into 50 ul EB buffer, and quantified with a PicoGreen dsDNA Quantitation Kit (Molecular Probes). A dilution series from 5 x 10^5 to 5 copies per microliter served as a standard curve in each QPCR assay.
- **RT-QPCR and Q-PCR of flight samples and blank cartridges**:
  - Each assay was conducted on nucleic acid subsamples that had not been thawed. Standard curves were not run with each 96-well plate, in order to reduce the risk of contamination by multiple positive controls, but a single low-level positive control (in duplicate) was run on each plate.

TaqMan® Quantitative PCR



Result: Gene Copy number / volume of sample

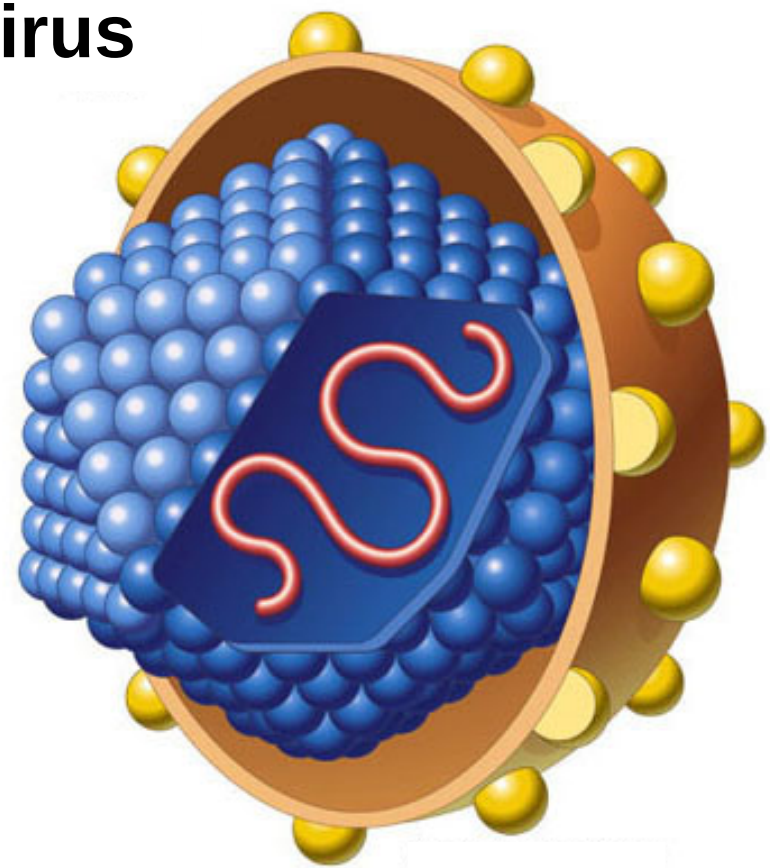


Viral Q-PCR Oligonucleotide Primers and Probes

Virus and Target Gene	Primer name	Sequence (5'-3')	bp	PCR Concentration (µM)	Reference
Adenovirus *Hexon Gene*	Adenovirus AQ1F	GCCACGGTGGGGTTTCTAAACTT	23	0.5	Heim et al 2003
Generic for all five fully	Adenovirus AQ2R	GCCCCAGTGGTCTTACATGCACATC	25	0.5	Heim et al 2003
sequenced types.	Adenovirus probe	[fam]TGCACCAGACCCGGGCTCAGGTACTCCGA[bhq]		0.4	Heim et al 2003
Coronavirus	Coronavirus N229E-1F	CAGTCAAATGGGCTGATGCA	20	0.15	van Elden et al 2004
N gene	Coronavirus N229E-2R	AAAGGGCTATAAAGAGAATAAGGTATTCT	29	0.15	van Elden et al 2004
Specific for human strains	Coronavirus N229E-p probe	[fam]CCCTGACGACCACGTTGTGGTTCA[bhq]		0.05	van Elden et al 2004
229E and OC43	Coronavirus NOC43-1F	CGATGAGGCTATTCCGACTAGGT	23	0.45	van Elden et al 2004
	Coronavirus NOC43-2R	CCTTCCTGAGCCTTCAATATAGTAACC	27	0.45	van Elden et al 2004
	Coronavirus NOC43-p probe	[fam]TCCGCCTGGCACGGTACTCCCT[bhq]		0.1	van Elden et al 2004
Epstein-Barr Virus	Epstein-Barr Virus F	CCCTGTTTATCCGATGGAATG	21	0.2	Kimura et al 1999
BALF-5 Viral DNA	Epstein-Barr Virus R	CGGAAGCCCTCTGGACTTC	19	0.2	Kimura et al 1999
Polymerase Gene	Epstein-Barr virus probe	[fam]TGTACACGCACGAGAAATGCGCC[bhq]		0.1	Kimura et al 1999
Influenza A and B	Influenza A INFA-1F	GGACTGCAGCGTAGACGCTT	20	0.9	van Elden et al 2001
INF-A: *Matrix Protein Gene*	Influenza A INFA-2R	CATCCTGTTGTATATGAGGCCCAT	24	0.9	van Elden et al 2001
INF-B: *Hemagglutinin Gene*	Influenza A INFA-3R	CATTCTGTTGTATATGAGGCCCAT	24	0.9	van Elden et al 2001
	Influenza A INFA-probe	[fam]CTCAGTTATTCTGCTGGTGCACTTGCCA[bhq]		0.1	van Elden et al 2001
	Influenza B INFB-1F	AAATACGGTGGATTAAATAAAAGCAA	26	0.3	van Elden et al 2001
	Influenza B INFB-2R	CCAGCAATAGCTCCGAAGAAA	21	0.3	van Elden et al 2001
	Influenza B INFB-probe	[fam]CACCCATATTGGGCAATTTCCTATGGC[bhq]		0.1	van Elden et al 2001
Respiratory Syncytial Virus	Respiratory Syncytial Virus A21F	GCTCTTAGCAAAGTCAAGTTGAATGA	26	0.5	Hu et al 2003
N Gene	Respiratory Syncytial Virus A102R	TGCTCCGTTGGATGGTGTATT	21	0.5	Hu et al 2003
Specific for both subtypes	Respiratory Syncytial Virus B17F	GATGGCTCTTAGCAAAGTCAAGTTAA	26	0.5	Hu et al 2003
A and B.	Respiratory Syncytial Virus B120R	TGTCAATATTATCTCCTGTACTACGTTGAA	30	0.5	Hu et al 2003
	Respiratory Syncytial Virus APB48 probe	[fam]ACACTCAACAAAGATCAACTTCTGTCATCCAGC[bhq]		0.2	Hu et al 2003
	Respiratory Syncytial Virus BPB45 probe	[fam]TGATACATTAAATAAGGATCAGCTGCTGTCATCCA[bhq]		0.2	Hu et al 2003
Varicella-Zoster Virus	Varicella-Zoster Virus gene 63F	CGCGTTTTGTACTCCGGG	18	0.2	Cohrs et al 2001
Gene 63	Varicella-Zoster Virus gene 63AR	ACGGTTGATGTCCTCAACGAG	21	0.2	Cohrs et al 2001
Often in latent infections	Varicella-Zoster Virus gene 63A probe	[fam]TGGGAGATCCACCCGGCCAG[bhq]		0.2	Cohrs et al 2001

RNA Viruses

- Respiratory Syncytial Virus
- Human Coronavirus
- Influenza A/B



Respiratory Syncytial Virus - B (RSV-B)

- single-stranded RNA virus of the Paramyxoviridae family
- causes respiratory tract infections in patients of all ages
- **most common respiratory disease in infants/children: In US, 60% of infants infected in first RSV season, nearly all children infected by 3rd b-day**
- causes 50% of all bronchiolits
- causes 25% of all pneumonia
- responsible for more than 4,500 deaths per year in the US
- transmission: direct contact and aerosols
- **Highly transmissable!**
- Temperate climates: annual winter epidemic
- Infection with RSV does not induce protective immunity, and thus people can be infected multiple times
- Ubiquitous worldwide, avoidance of infection is impossible
- For most people, RSV produces only mild symptoms, often indistinguishable from common colds and minor illnesses
- Vaccine development has been fraught with failure
- **INFECTIOUS DOSE:** >100-640 infectious organisms when administered intranasally

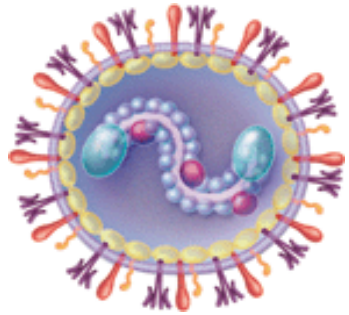


Figure 1. RSV structure

In most children, RSV looks like the common cold.

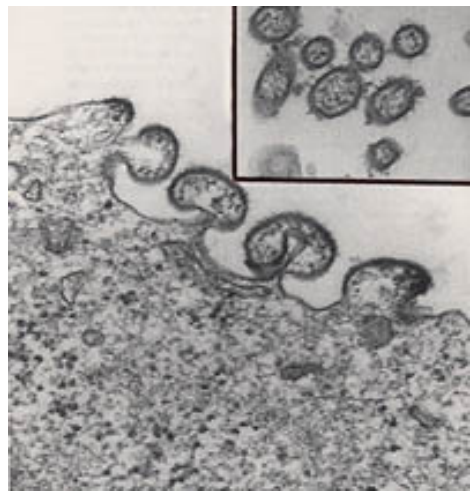


Figure 2. SEM of RSV virions

RSV-B Abundance in Cabin Air

- Below detection limits
- Not detected in ANY of the 14 flights analyzed

Human Coronavirus

- cause of 30 - 50% of all common colds
- ~15 species, family Coronaviridae
- infect man, cattle, pigs, rodents, cats, dogs, birds
- enveloped, single-stranded RNA genome
- Transmission: respiratory or fomites
- Symptoms: throat discomfort, sneezing, runny nose, nasal congestion, coughing, lethargy
- Infectious dose: unknown

- **Survival outside host:** Survives up to 24 hours on metal surfaces at ambient conditions
- In 2003 the WHO stated that a novel coronavirus identified by a number of laboratories was the causative agent for SARS.
- The virus was officially named the SARS coronavirus (SARS-CoV), causing upper and lower respiratory tract infections and gastroenteritis.
- The SARS epidemic resulted in over 8000 infections, about 10% of which resulted in death.

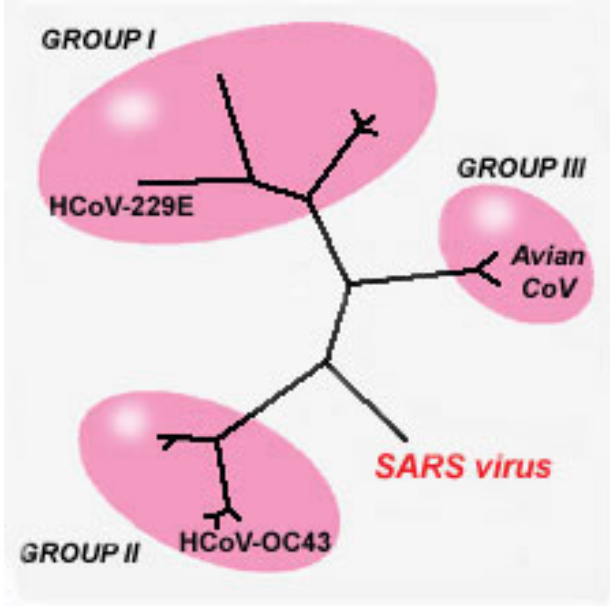


Figure 1. Coronavirus phylogeny

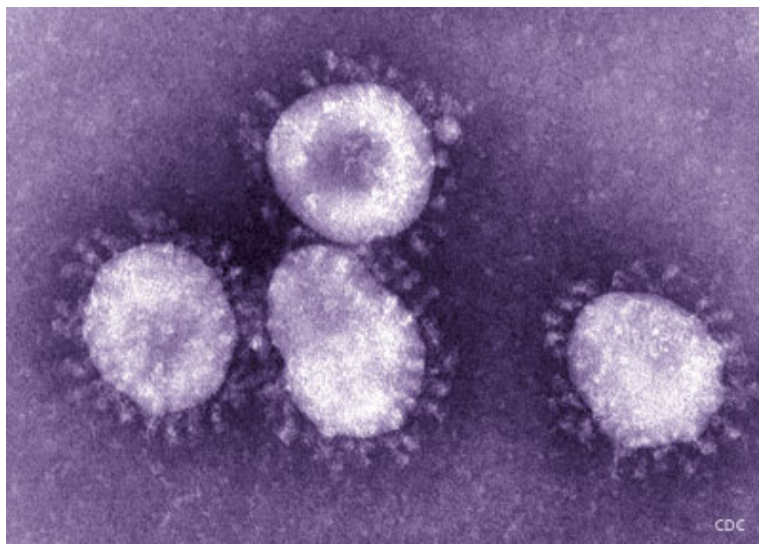


Figure 2. SEM of Coronavirus virions

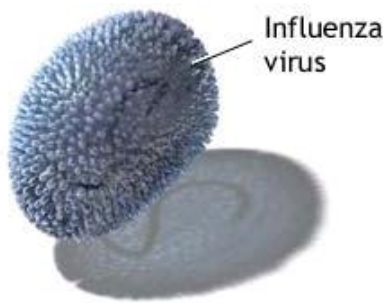
Coronavirus Abundance in Cabin Air

- **Only RNA virus detected**
- **Detected on only 5 flights** (14 total)
  - Flight 2R-N (NY to LA): 22 copies/m^3
  - Flight 5-N (LA to NY): ~ 5 x 10^3 copies/m^3
  - Flight 5R-N (NY to LA): ~ 1.4 x 10^4 copies/m^3
  - Flight 6R-J (Tokyo to LA): ~ 3.2 x 10^4 copies/m^3
  - Flight 4-S (LA to Sydney): ~ 1.9 x 10^3 copies/m^3

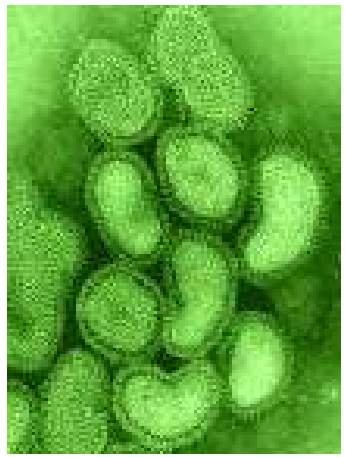
  Flight averages

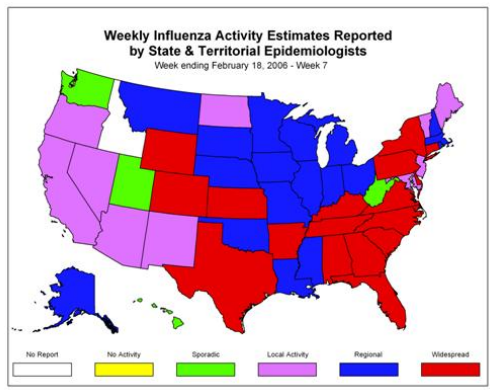
  - **Overall, concentrations varied immensely among samples, and ranged from BDL to 2 x 10^5 copies/m^3**

Influenza A / B



- nonpandemic years: 10,000 to 20,000 deaths in US
- pandemic years: US deaths can exceed 100,000
- resistance to antiviral drugs rising, from 1% in 1994 to 91% in 2005
- RNA viruses designated as types A, B, and C
- Influenza A virus causes pandemics:
    - "avian influenza" (a.k.a. bird flu, avian flu, type A flu)
    - undergoes antigenic shifts in two outer membrane proteins, hemagglutinin and neuraminidase, thus introducing a new virus into a population lacking protective serum antibody
- Influenza B virus usually causes a minor illness
    - No different subtypes of H and N have been identified for influenza B
- Influenza virus infection causes three syndromes:
    - uncomplicated rhinotracheitis
    - respiratory viral infection followed by bacterial pneumonia
    - viral pneumonia
- Infection typified by rapid onset of profound malaise
- Infectious dose: >800 plaque forming units via nasopharyngeal inoculation



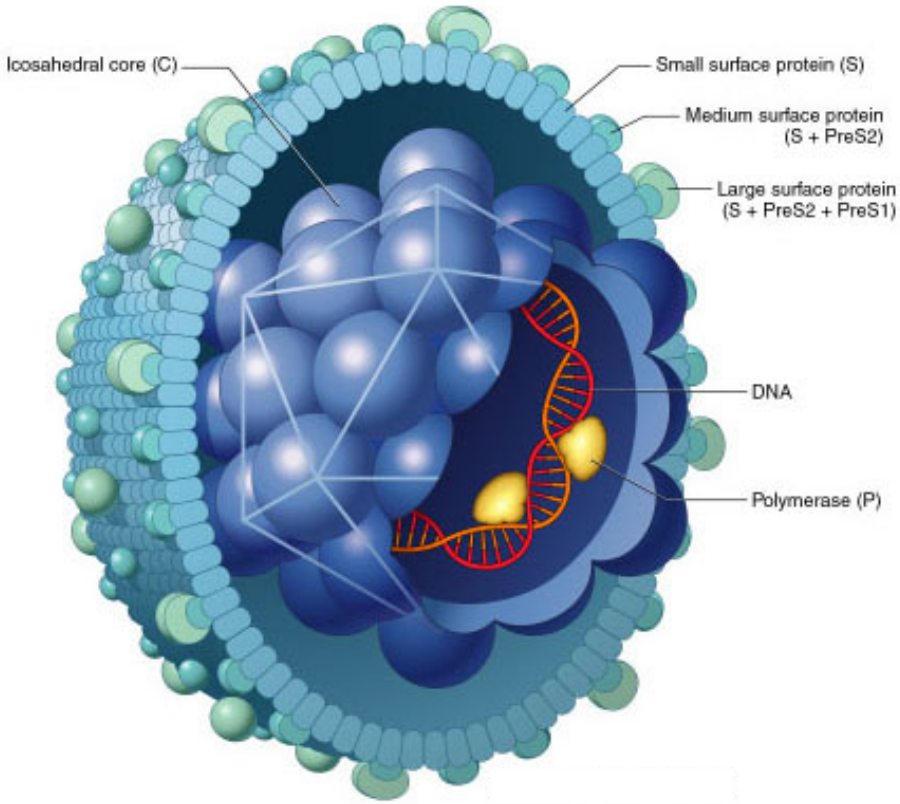


Influenza Abundance in Cabin Air

- Below detection limits
- Not detected in ANY of the 14 flights analyzed

DNA Viruses

- **Epstein-Barr Virus**
- **Varicella-Zoster Virus**
- **Adenovirus**



Epstein Barr Virus (EBV)

- member of the herpesviridae family
- one of the most common human viruses
- occurs worldwide
- most people become infected sometime in their life
- In the US, 95% of adults between 35 and 40 years of age are commonly infected
- Infants become susceptible to EBV as soon as maternal antibody protection disappears
- Many children become infected with EBV, most infections cause no symptoms or are indistinguishable from the other mild, brief illnesses of childhood
- When infection with EBV occurs during adolescence or young adulthood, it causes infectious mononucleosis 35% to 50% of the time
- Outbreaks triggered by stress
- EBV plays an oncogenic role in the onset of several tumors (Burkitt's lymphoma, B-cell lymphomas, etc.)
- Infectious dose: unknown

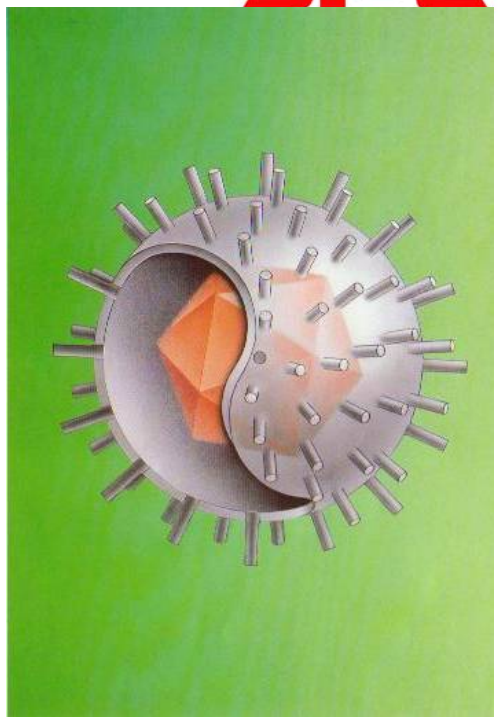


Figure 1. EBV structure

Figure 2. SEM of EBV virions

EBV Abundance in Cabin Air

- **Detected on 10 flights** (14 total)
  - Flight 2R-N (NY to LA): 324 copies/m^3
  - Flight 5-N (LA to NY): 170 copies/m^3
  - Flight 5R-N (NY to LA): 827 copies/m^3
  - Flight 7-N (LA to NY): 16 copies/m^3
  - Flight 3R-L (London to LA): 188 copies/m^3
  - Flight 6-J (LA to Tokyo): 38 copies/m^3
  - Flight 6R-J (Tokyo to LA): 205 copies/m^3
  - Flight 4-S (LA to Sydney): 446 copies/m^3
  - Flight 4R-S (Sydney to LA): 205 copies/m^3
  - Flight 8R-S (Sydney to LA): 102 copies/m^3

  Average values / flight

  - **Of the 3 viruses detected, EBV yielded the most consistent results. Concentration varied minimally (with respect CV and VZV) across samples, ranging from BDL to 1.9 x 10^3 copies/m^3**

Varicella-Zoster Virus (VZV)

- a.k.a. human herpes virus 3 (HHV-3)
- one of eight herpes viruses known to infect humans
- At any given time, 5 people in 1,000 are presenting symptoms of VZV
- Primary VZV infection results in chickenpox (*Varicella*), which rarely results in encephalitis or pneumonia
- Even when clinical symptoms of *Varicella* have resolved, VZV remains dormant in the nervous system
- 10-20% of the time VZV reoccurs later in life as *Herpes Zoster* (shingles)
- more than 500,000 people develop shingles each year
- **The virus is very susceptible to disinfectants**
- A controversial live attenuated vaccine has been recommended for children in Australia, the United States, and other countries. It is only effective for twenty years, rendering adults vulnerable to the most dangerous forms of infection, whereas acquiring chickenpox naturally as a child typically confers immunity for life.
- **Infectious dose: unknown**

Figure 1. VZV structure

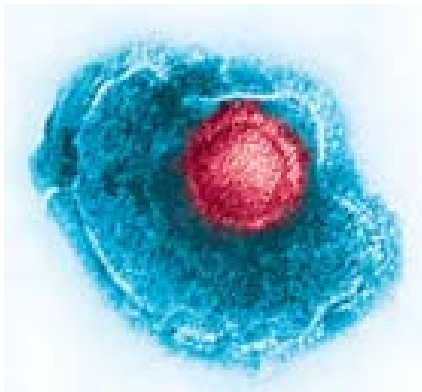


Figure 2. SEM of VZV virion

VZV Abundance in Cabin Air

- **Detected on 13 flights** (of 14 total)
  - Flight 2-N (LA to NY): 37 copies/m^3
  - Flight 2R-N (NY to LA): 527 copies/m^3
  - Flight 5-N (LA to NY): 1.4 x 10^3 copies/m^3
  - Flight 5R-N (NY to LA): 1 x 10^4 copies/m^3
  - Flight 7-N (LA to NY): 543 copies/m^3
  - Flight 7R-N (NY to LA): 19 copies/m^3
  - Flight 3-L (LA to London): 51.4 copies/m^3
  - Flight 3R-L (London to LA): 153.3 copies/m^3
  - Flight 6-J (LA to Tokyo): 5.8 x 10^3 copies/m^3
  - Flight 6R-J (Tokyo to LA): 1.3 x 10^4 copies/m^3
  - Flight 4-S (LA to Sydney): 1.5 x 10^3 copies/m^3
  - Flight 4R-S (Sydney to LA): 764 copies/m^3
  - Flight 8R-S (Sydney to LA): 2 x 10^3 copies/m^3

  Average values / flight

  - **Of the 3 viruses detected, VZV was the most cosmopolitan (13 of 14 flights). Concentrations varied greatly between samples, ranging from BDL to 5.8 x 10^4 copies/m^3**

Adenovirus

- first isolated in human adenoids (tonsils)
- Nonenveloped, icosahedral, double-stranded DNA
- 51 immunologically distinct adenovirus serotypes able to cause human infections
- most commonly cause illness of the respiratory system
- symptoms of respiratory illness: range from the common cold syndrome to pneumonia, croup, and bronchitis
- depending on serotype, may cause various other illnesses (gastroenteritis, conjunctivitis, cystitis, and rash illness)
- cause 5-20% of all gastroenteritis in young children
- By age 4, 85% of all children have developed immunity
- Patients with compromised immune systems are especially susceptible to severe complications
- **Resistant to chemical and physical agents allowing for prolonged survival outside of the body**
- spread via respiratory droplets and less frequently via fecal routes
- **Intranasal infectious dose:** >150 plaque forming units

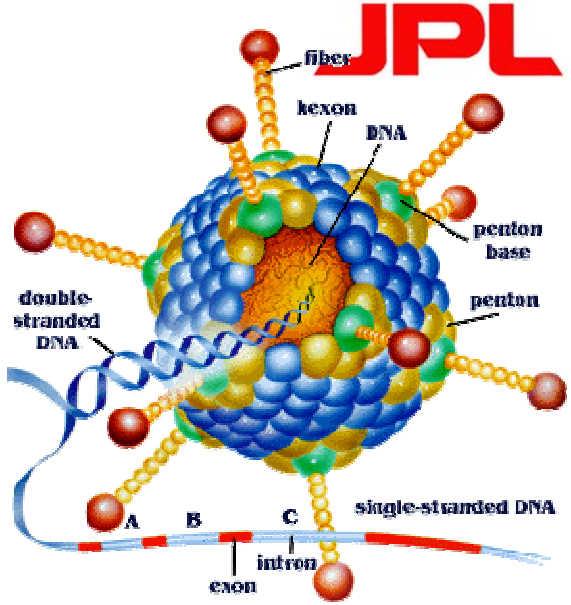


Figure 1. Adenovirus structure

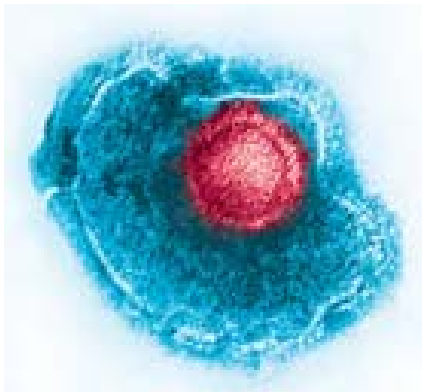
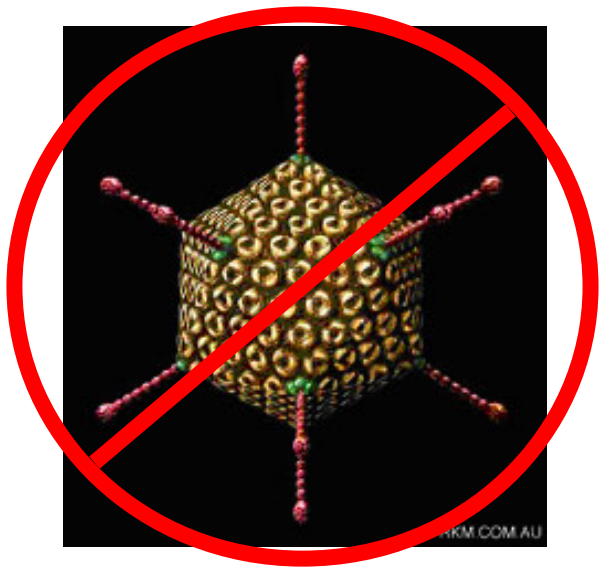


Figure 2. SEM of Adenovirus

Adenovirus Abundance in Cabin Air

- Below detection limits
- Not detected in ANY of the 14 flights analyzed



Summary of Viral Abundance

Table 2. Viral burden of cabin air aboard 6 domestic and 8 international flights on Airline C

Flight		RNA viruses*						DNA viruses*								
		RSV	Coronavirus				Influenza	Epstein-Barr virus				Varicella-Zoster virus				Adenovirus
			n	Mean	s	Range		*n*	Mean	s	Range	*n*	Mean	s	Range	
New York	2-N											7	**36.7**	81.2	0 - 218	
	2R-N		8	**22.1**	42.4	0 - 109		8	**324.1**	364.3	0 - 792	8	**526.6**	371.6	0 - 1184	
	5-N		7	**4818**	10836.4	0 - 29205		7	**170.1**	289.8	0 - 639	7	**1422.3**	2996.6	0 - 8211	
	5R-N		6	**14781.7**	7749.7	5076 - 23897		6	**826.5**	738.4	0 - 1954	6	**10800**	6325.1	5775 - 21466	
	7-N							7	**16.3**	41.4	0 - 110	7	**543.4**	977.2	0 - 2738	
	7R-N											8	**19.4**	31.2	0 - 85	
London	3-L											10	**51.4**	138.5	0 - 443	
	3R-L							9	**187.7**	525.4	0 - 1586	9	**153.3**	217.3	0 - 567	
Tokyo	6-J							9	**37.9**	107.8	0 - 325	9	**5808.8**	10343.6	0 - 31531	
	6R-J		9	**31525.6**	64631.9	0 -198975		9	**204.7**	412.5	0 - 1269	9	**13068**	18347.1	0 - 58125	
Sydney	4-S		8	**1940.5**	4970.5	0 - 14204		8	**446.1**	495.2	0 - 1506	8	**1522**	2401.7	0 - 7373	
	4R-S							8	**204.3**	316.3	0 - 817	8	**763.9**	532.4	0 - 1818	
	8-S															
	8R-S							11	**102.2**	338.9	0 - 1124	11	**1986.2**	4606.9	0 - 13836	

***All values are derived from Q-PCR enumerations of viral genes, expressed in gene copies/m^3 of air. Shading intensity is a function of mean burden.**

Below Detection Limits	Minimal Limit for Detection - 10^3	10^3 - 10^4	> 10^4

Conclusions of Viral Burden Assessment

- This is the **FIRST and ONLY** attempt at assessing viral burden aboard aircraft.
- Current sampling technologies do not appear to be at readiness levels requisite for an investigation of this nature (majority of samples BDL).
- All 6 viruses must certainly exist in greater abundance than reported here (especially Adenovirus, RSV, and Influenza).
- In addition to inefficient sample collection, the degradation of viral nucleic acid upon collection, while in transit, and throughout processing is thought to be largely responsible for the scarcity of viruses detected.
- As several flights occurred in the winter months (2N, 2R-N, 3L, 3R-L, 4S, 4R-S; 6 of 14), it is surprising that we were unable to detect **ANY** Influenza A/B.
- Results are highly variable; it is nearly impossible to conclude on trends based on the stochastic nature of the immense data set collected.
- The 2 most frequently detected viruses, EBV and VZV:
  - members of the Herpesviridae family, **DNA viruses** (more stable?)
  - once contracted, exist in latent state in nervous system
  - subsequent outbreaks triggered by stress (Pierson et al.)

Executive summary-1

- Airline C are far cleaner (3 orders of magnitude), likely a consequence of the different cabin locations: coach vs business.
- Airline B is by far laden with higher microbial burden.
  - ~73% of samples (flight #4; Airline B) contained >10^4 CFU/m^3 of air.
- The total viable microbes, as estimated by assaying ATP, is about one log higher in magnitude than cultivable microbes.
- The total bacterial populations (dead and alive), based on Q-PCR assay, ranged from BDL to 3 x 10^7 gene copies per m^3 of air.
- When samples were collected as a function of time over the course of flights, a gradual accumulation of microbes was observed from the time of passenger boarding through mid-flight, followed by a sharp decline in microbial abundance and viability from the initiation of descent through landing.

Executive summary-2

- Representatives of the α-, β-, γ-, and δ-proteobacteria, as well as Gram-positive bacteria, Fusobacteria, Cyanobacteria, Deinococci, Bacterioidetes, Spirochetes, and Planctomyces were detected in varying abundance.
  - Airline A: *Neisseria meningitidis* followed by *Streptococcus oralis/mitis*.
  - Airline B: *Pseudomonas synxantha* followed by *N. meningitidis* and *S. oralis/mitis*.
  - Airline C: Gram-positive and α-proteobacteria seem to have a higher affinity for international flights, whereas β-and γ-proteobacteria are far more common about domestic cabin air.
  - It is apparent that these cabin air samples housed relatively normal flora (some of which opportunistically pathogenic) of the human upper respiratory tract and oral cavity.
- Viral results are highly variable; it is nearly impossible to conclude on trends based on the stochastic nature of the immense data set collected.
  - The 2 most frequently detected viruses, EBV and VZV:
- The data documented in this study will be useful to generate a baseline microbial population database and can be utilized to develop biosensor instrumentation for monitoring microbial quality of cabin or urban air.