Report Supporting Document

CULTURABLE ENVIRONMENTAL BACTERIA: COUNT AND GRAM STAIN

(Analysis Codes: EBC)

INTRODUCTION

Bacteria are an important component of ecosystems and are closely related to human health. Successful detection of bacteria in various environments is crucial in preventing potential health problems.

Culturable bacteria refers to bacteria that are viable when sampled from the environment and will grow on culture media at laboratory conditions. Environmental bacteria are those that can grow at ambient temperature or 25°C. Bacteria are prevalent in soil, water, and many other environments. Most bacteria cells are about 0.5-1 µm in diameter by 1-2 µm long. Sphere-like shaped bacteria are called cocci, cylinder-like shaped are termed rods, and spiral-like shaped are termed spiral. Actinomycetes are prokaryotic organisms classified as bacteria, but resemble fungal hyphae in shape (about 2 µm diameter). Bacteria have different cell wall structures causing differences in the uptake of dyes, which is a basic principle in differentiating bacteria as gram-positive or gram-negative. Both gram-positive and gram-negative bacteria are abundant in the environment.

Gram stain analysis will generate baseline data on general characterization of bacteria communities on the sample. Combined with comparative sampling, the gram stain analysis results will indicate the type and level of bacteria in the specified environment, possible microbial contamination, and impact on indoor bioaerosols.

The purpose of this report is to present scientific data obtained by the analysis of samples submitted to Aemtek, Inc. for the evaluation of possible microbial contamination.

MATERIALS AND METHODS

As a part of the investigation project, samples were collected and transported to the Aemtek microbiology laboratory for analysis. Data on sample identification, location, and air volume (for air samples) on this report are from the original **Chain of Custody Form.**

Culture preparation

During air sampling, a Petri plate containing culture medium was used to capture aerosols, biological or non-biological, as a measured volume of air is pulled in. The Petri plate with the sample was then incubated after being received by the laboratory. After 3 days incubation, gram stain analyses were performed on each of the bacteria colonies and counted as CFU (=Colony Forming Units). By factoring in

the air volume sample, this sampling analysis generated quantitative data on viable and culturable bacteria.

For swab or bulk samples, the serial dilution method was used to obtain bacteria suspension series after the sample was washed thoroughly with sterile and distilled water. A measured volume from each bacteria suspension was poured and spread onto a pre-selected culture medium and subsequently incubated for 3 days. Plates with an optimal number of accountable colonies were selected for further analysis. Sample weight or area sampled, concentration of bacteria suspension, and number of CFU were used to calculate the final concentration of CFU.

Culture media and growth condition

Typically, a wide spectrum medium, Tryptic Soy Agar (TSA) with 5% sheep blood, was used to grow bacteria. Incubation temperature for detecting environmental bacteria was 25°C and the length was 1 to 3 days.

Gram stain procedure

After sufficient incubation, bacteria colonies were transferred onto microscope slides to prepare fixed smear. A 3-step gram stain procedure was then performed on each of the colonies present on the sample cultures, following reagent manufacturer's instructions (Becton, Dickinson and Company).

Microscopic examination

Microscopic examination was performed using a bright field microscope with 600X – 1500X magnifications. The bacteria colonies were categorized based on gram stain differentiation and bacteria morphology.

RESULTS

Gram stain analysis results are presented as colony forming unit (CFU), as shown on the **Data Sheet**. Results of air sample analysis are shown as CFU/m^3, bulk and dust samples as CFU/g, and swab samples as CFU/Swab or CFU/1000cm^2. The percentage of each of the bacteria categories present in the sample is also given on the Data Sheet.

DISCUSSION

Gram stain is a fundamental classification in bacteriology. The positive or negative staining reaction of a bacterium is still one of the first items mentioned in its description. Gram stains are also important in medicine because generally drugs work optimally either on gram-positive bacteria or gram-negative ones.

Gram stain is also of significance in indoor air investigations. Gram-negative bacteria have been associated with water-damaged buildings. Endotoxins are cell wall component (lipopolysaccharides) of gram-negative bacteria. Exposure to endotoxins is associated with adverse health effects, such as cough, chest tightness, fatigue, and respiratory problems.

Laboratory results must be used in conjunction with sampling method and design. Some basic guidelines on data interpretation of environmental bacteria gram stain analysis may include: 1) The bacteria community in a typical indoor environment is normally dominated by gram-positive cocci and rods. 2) Elevated levels of gram-negative rods may indicate water damage or a condition that requires

further investigation, because gram-negative bacteria are endotoxin producers and are of health concerns. 3) Elevated levels of Actinomycetes may be an indication of microbial contamination and should be investigated thoroughly. Thermophilic Actinomycetes are allergens and potential pathogens. 4) High levels of *Bacillus* species may indicate past water damage.

ABOUT AEMTEK

Aemtek, Inc. is an environmental microbiology laboratory serving the indoor air quality industry. Its mission is to provide accurate, fast, and reliable expert services for detection, identification, analysis of fungi and bacteria in human environments. Aemtek also provides contracted research and expert testimony services. Aemtek is committed to the highest level of quality, scientific integrity, and customer service.

Aemtek, Inc. implements a series of quality assurance procedures. All analytical protocols are developed based on scientific merits and are validated regularly. Aemtek is accredited by the American Industrial Hygiene Association (AIHA) Environmental Microbiology Laboratory Accreditation Program (Lab No.: 167620)

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