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Qualitative and Quantitative Methods for the Detection of Microbes

Mohammad Waseem Mir[1], Anirudh Gupta[2], and Indu Sharma[2]

[1]Department of Microbiology, Nims Institute of Allied Medical Science and Technology, NIMS University, Rajasthan, Jaipur 303121, Rajasthan (India)

[2]Department of Biotechnology, Nims Institute of Allied Medical Science and Technology, NIMS University, Rajasthan, Jaipur 303121, Rajasthan (India)

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Correspondence:
E-mail: endusharma@gmail.com

ABSTRACT

Microorganisms significantly influence human activities, health, and consequently, there is a high demand to develop automated, sensitive, and rapid methods for their detection. These methods might be applicable for clinical, industrial and environmental applications. Although different techniques have been suggested and employed for the detection of microorganisms, most of these methods are not cost-effective and suffer from low sensitivity and low specificity, especially in mixed samples. The next generation microbiological analysis is progressed swiftly due to its numerous advantages such as sensitivity, reproducibility, accuracy, low cost and rapidity. Although, conventional culture and analytical methods are frequently used for microbiological examination in terms of their easily accessible, advanced methods provide improving its selectivity and sensitivity. Different types of qualitative and quantitative methods are used for microbial detection in samples. The most common methods used for the detection of microbes are morphological, chromogenic media, microscopy, biochemical, colorimetric, spectrophotometric analysis, molecular, dd PCR (Digital Droplet Polymerase Chain Reaction), etc. A variety of qualitative and quantitative techniques used for the determination of both commonly and rarely emerged microorganisms such as *Escherichia coli, Staphylococcus aureus, Listeria monocytogenes, Acinetobacter, Rothia, Pantoea, Cryptococcus, Bacillus, Micrococcus* etc. The main results based on some qualitative and quantitative techniques used for microbial detection in different water samples. The application of detection and quantification methods is often hindered by the low pathogen concentrations in natural waters. Rapid and efficient sample concentration methods are urgently needed.

1. INTRODUCTION

Qualitative analysis is a method using the number of unique bacteria that exist in only one sample and not in other samples. Quantitative analysis is the number and percentage of reads (%) of unique bacteria that exist only in one sample and not in other samples and Quantitative

analysis is also defined as the total percentage (%) of 'unique bacteria' matched between the fingertips and the personal belongings of participants.

Based on nineteenth-century techniques, conventional microbiological test methods are time-consuming and labour-intensive, lack sensitivity and are subjective and poorly validated. Bacterial culture and drug susceptibility testing are used to identify pathogen infections. Nevertheless, the process requires several days from collection to the identification of bacterial species and drug-resistance patterns (Alves de Lima et al., 2007).

Molecular methods for pathogenic bacteria detection are biomarkers including nucleic acids, proteins, antigens, adenosine triphosphate (ATP), and metabolic products are employed in the analysis of microorganisms. To differentiate microorganisms within one sample, nucleic acids (DNA/RNA), proteins, and antigens are usually selected as biomarkers because of their special physical and chemical characteristics within different pathogens. The detection of DNA/RNA is based on the specific hybridization and amplification of targets, thus enabling good specificity and accuracy. In the case of pathogenic bacteria in wastewater, the most important biomarker is the pathogenic DNA or RNA residues from these bacteria. The biomarkers include genus/species-specific genes, functional genes, and antimicrobial resistance genes. Moreover, in the analysis of antimicrobial resistance, gene transfer is another significant point. Various mobile genetic elements, including plasmids, transposons, bacteriophages, integrons, and combinations of them, are notable nucleic acid targets for investigating the prevalence and spread of resistance genes in bacteria. Instruments incorporating flow, such as automated cell counters and flow cytometers, provide mechanized methods. These different direct methods will be described in subsequent sections

The digital PCR system is a rapidly developing quantitative detection technology widely applied to molecular diagnosis, including copy number variations, single nucleotide variant analysis, cancer biomarker discovery, and pathogen identification**.** The dd PCR (Digital Droplet Polymerase Chain Reaction), system is highly promising as a qualitatively and quantitatively screening method for rapidly detecting pathogens. The first information about microorganisms started 200 years before Pasteur and Koch with a "micrography" study by a British researcher named "Robert Hooke" in 1665. Anton von Leeuwenhoek developed a new micrography technique that enables 200x magnification with a single lens, which was called Animalcules in the 1670 s. Significant investigations were carried out via this earlier version of the microscope, and in 1857, Pasteur made his first study to eliminate spoilage microorganisms in wine and beer in 1860. This heat-based procedure is called pasteurization which is the most common technique in food preservation, this investigation ended the theory of spontaneous generation (Smith et al., 2014).

In recent years, molecular biology techniques have been applied to detect pathogenic microorganisms. They have attracted attention due to their high specificity, and lower, and

reduced incidence of cross-infection. These techniques include Enzyme-Linked Immunosorbent Assay, Polymerase Chain Reaction and Gene microarray chips. To date, Polymerase Chain Reaction technology has been widely used in many research fields because of its simple, intuitive, economical, and rapid detection characteristics. Digital polymerase chain reaction (d PCR) was first proposed by Kenneth Kinzler and Bert Vogelstein in 1999. This is a PCR technology that truly achieves absolute quantification after qPCR technology. Droplet digital PCR (dd PCR) is a new technology that enables the absolute quantification of nucleic acids with high analytical sensitivity and precision. dd PCR splits PCR reagents into tens of thousands of nanoliter or picolitre partitions by a microfluidic chip so that each droplet contains zero or one DNA template. After PCR amplification and fluorescence detection, the target nucleic acids are calculated from the number of positive and negative droplets by Poisson statistics. Compared with traditional qPCR, digital PCR has higher sensitivity, specificity, and accuracy. It plays an essential role in many fields, including early diagnosis of tumour markers, gene expression product analysis, food safety testing, pathogenic microorganism testing, common genetic disease testing, and non-invasive prenatal diagnosis (Khan et al., 2007; Maheux, et al., 2013).

The generation of airborne microorganisms from concentrated animal-feeding operations (CAFOs) is a human and animal health concern. To better understand the airborne microorganisms found in these environments, several collection and analytical techniques have been utilized. The most commonly used bioaerosol collection method is the liquid impingement format, which is suitable with several culture-based and non-culture molecular-based approaches, such as polymerase chain reaction. The generation of airborne microorganisms from concentrated animal-feeding operations (CAFOs) is a human and animal health concern. To better understand the airborne microorganisms found in these environments, several collection and analytical techniques have been utilized and will be discussed in this review. The most commonly used bioaerosol collection method is the liquid impingement format, which is suitable with several culture-based and non-culture molecular-based approaches, such as polymerase chain reaction. The digital PCR system is a rapidly developing quantitative detection technology widely applied to molecular diagnosis, including copy number variations, single nucleotide variant analysis, cancer biomarker discovery, and pathogen identification.

This study aimed to use a droplet digital PCR system to identify bacteria in blood samples and explore its ability to identify pathogens in bacteremia. The ddPCR system is highly promising as a qualitatively and quantitatively screening method for rapidly detecting pathogens. The most common pathogenic bacteria in general surgery departments *are Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae,* and *Acinetobacter*

*baumannii*4. Traditional bacterial detection and identification methods are based on bacterial culture and biochemical identification, and testing usually requires several days, even one week. As a gold standard, the diagnosis of bacterial infectious diseases often relies on the specific detection of pathogenic bacteria. The use of antibiotics must be based on the bacterial culture results and drug sensitivity test results in clinical diagnosis and treatment.

The most common pathogenic bacteria are *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Acinetobacter baumannii*. Traditional bacterial detection and identification methods are based on bacterial culture and biochemical identification, and testing usually requires several days, even one week. As a gold standard, the diagnosis of bacterial infectious diseases often relies on the specific detection of pathogenic bacteria. The use of antibiotics must be based on the bacterial culture results and drug sensitivity test results in clinical diagnosis and treatment. The journey started with the chemist Pasteur detecting bacteria using a microscope and continued with German physician Koch. He obtained pure culture through the development of bacteria in the solid medium and it was understood that microorganisms were the main cause of many diseases. So far, with the enlightenment of Koch ‘s contribution to the microbiology field, many discoveries and research have been made. Following this investigation, various vaccines against polio, measles, mumps, and smallpox have been developed and through these successful vaccine applications, many dangerous microbial diseases were eradicated.

One of the most basic and most commonly acquired types of bacterial measurements, whether in planktonic or biofilm cultures, is the determination of how much is present. A variety of direct and indirect methods have been used to quantify cells in biofilms. Direct counting methods permit the enumeration of cells that can be cultured, including plate counts, microscopic cell counts, Coulter cell counting, flow cytometry, and fluorescence microscopy. Indirect measurement methods include the determination of dry mass, total organic carbon, microtiter plate assays, ATP bioluminescence, total protein, and quartz crystal microbalance. It should be noted that many methods, both direct and indirect, involve homogenization of the biofilm to disperse cells in a liquid medium before analysis via a commercially available homogenizer and vortex mixing.

Various techniques have been devised to permit the analysis of the structure and function of microorganisms. Some techniques are qualitative in their intent. Other techniques are quantitative in their intent. These techniques provide numerical information about a sample. Assessing the growth of a bacterial sample provides examples of both types of analysis techniques. An example of a qualitative technique would be the growth of a bacterial sample on a solid growth medium, to solely assess whether the bacteria in the sample are living or dead.

An example of a quantitative technique is the use of that solid growth media to calculate the actual number of living bacteria in a sample. Microscopic observation of microorganisms can reveal a wealth of qualitative information. The observation of a suspension of bacteria on a microscope slide (the wet mount) reveals whether the bacteria are capable of self-propelled motion. Microorganisms, particularly bacteria, can be applied to a slide as a so-called smear, which is then allowed to dry on the slide. The dried bacteria can be stained to reveal, for example, whether they retain the primary stain in the Gram stain protocol (Gram-positive) or whether that stain is washed out of the bacteria and a secondary stain retained (Gram-negative). Examination of such smears will also reveal the shape, size, and arrangement (singly, in pairs, in chains, in clusters) of the bacteria. These qualitative attributes are important in categorizing bacteria. Microscopy can be extended to provide qualitative information.

The incorporation of antibodies to specific components of the sample can be used to calculate the proportion of the samples in a population that possess the target of interest. Fluorescent-labelled antibodies, or antibodies combined with a dark-appearing molecule such as ferritin, are useful in such studies. The scanning confocal microscope is proving to be tremendously useful in this regard. The optics of the microscope allows visual data to be obtained at various depths through a sample (typically the sample is an adherent population of microorganisms). These optical thin sections can be reconstructed via computer imaging to produce a three-dimensional image of the specimen. The use of fluorescent-tagged antibodies allows the location of protein within the living biofilm to be assessed. The self-propelled movement of living microorganisms, a behaviour that is termed motility, can also provide quantitative information. For example, recording a moving picture image of the moving cells is used to determine their speed of movement, and whether the presence of a compound acts as an attractant or a repellant to the microbes.

Bacterial growth is another area that can yield qualitative or quantitative information. Water analysis for the bacterium Escherichia coli provides an example. A specialized growth medium allows the growth of only Escherichia coli. Another constituent of the growth medium is utilized by the growing bacteria to produce a by-product that fluoresces when exposed to ultraviolet light. If the medium is dispensed in bottles, the presence of growing Escherichia coli can be detected by the development of fluorescence.

If the medium is dispensed in smaller volumes in a grid-like pattern, then the number of areas of the grid that are positive for growth can be related to a mathematical formula to produce the most probable number of living Escherichia coli in the water sample. Viable bacterial counts can be determined for many other bacteria by several other means. The ability of bacteria to grow or not to grow on a media containing controlled amounts and types of compounds yields quantitative information about the nutritional requirements of the microbes.

The advent of molecular techniques has expanded the repertoire of quantitative information that can be obtained. For example, a technique involving reporter genes can show whether a particular gene is active and can indicate the number of copies of the gene product that is manufactured. Gene probes have also been tagged to fluorescent or radioactive labels to provide information as to where in a population a certain metabolic activity is occurring and the course of the activity over time.

The detection and identification of microbes are crucial in various fields, including clinical diagnostics, food safety, environmental monitoring, and research. Over the years, numerous qualitative and quantitative methods have been developed to detect and quantify microbes, ranging from traditional culture-based techniques to modern molecular methods. This review aims to explore the literature surrounding the qualitative and quantitative methods employed for the detection of microbes, highlighting their strengths, limitations, and applications in different fields. Developing a proper quantification method for textile-adhered bacteria requires a collective understanding of both bacteria and material. Through the experimental validation of different methods, it is anticipated to identify a suitable test method that can efficiently measure the bacterial-adhesive properties for specific surfaces and analytical situations. While this study uses *E. coli* for method optimization, it is thought that the overall methodological procedures and verification processes can be expanded to other bacteria. This study intends to broadly impact the research in the field of anti-bio adhesive surfaces and related product design.

Rapid microbial monitoring (RMM) is the real-time or near-real-time determination of microbial presence in a sample without the need for incubation, laboratory services, or intervention. RMM is a definition, not a single methodology. Regulatory guidelines accept many methods of bioburden detection and measurement.

2. Qualitative and Quantitative Methods

Water testing is a process to identify and determine pathogen or microorganism contamination levels. Quantitative analysis is numbers-based, countable, or measurable. Qualitative analysis is interpretation-based, descriptive, and relating to information. Quantitative analysis tells us how many, how much, or how often in calculations. Qualitative analysis can help us to understand why, how, or what happened behind certain behaviors.

2.1 Morphology based Methods

Microscopy in microbial identification involves the use of microscope to observe the microbial morphology. It reveals among other thing, the cell shape, arrangement, and structural components like flagella, endospore, and capsule as well as the revealing of chemical differences on the components of the cell.

2.2 Culture-based Methods- Culture-based methods have long been the gold standard for microbial detection. These methods involve the growth of microorganisms on selective media

under controlled laboratory conditions. The growth characteristics, morphology, and biochemical properties of the colonies are then analyzed to identify the microorganism. Although culture-based methods are widely used and relatively inexpensive, they are time-consuming and may fail to detect viable but non-culturable or slow-growing microorganisms.

2.3 Spectrophotometer based Methods- In bacterium analysis, 600 nm is commonly utilized, giving rise to the name OD600nm. From the early research, it is considered that OD at 600nm directly corresponds to the cell concentration. Numerous investigations concluded that absorbance at 600nm is highly consistent and reproducible; nevertheless, these results were predicated on tolerating error rates of more than 50 per cent. Bacteria are known to produce pathogenic byproducts as they proliferate. These byproducts have the potential to improve absorption. As a result, these byproducts contribute to the signal in addition to the optical loss caused by scattering. Furthermore, the bacteria's internal and subwavelength components might lead to a rise in optical loss, which in turn distinguishes the structural variability in bacteria. Since the OD measurements are based on the concept that the scattering signal is directly proportional to the concentration of cells, any deviation may jeopardize this connection. Interestingly, few bacteria are smaller than the usual range, making them poor scatterers. When a certain concentration is achieved, bacteria will cluster together or form lengthy chains, depending on the non-growth signal. Given the differences in structure and development patterns across bacteria, a multiwavelength analysis strategy to enhance the signal's accuracy would be helpful. While the OD600 approach is appealing because of its simplicity, single-wavelength measurement intensifies the variables of the recorded signal. As a result, it is vital to thoroughly analyze the usefulness and accuracy of the present OD600 technique, especially considering past work indicating inconsistencies between different spectrophotometers and recent breakthroughs in optical spectroscopy and signal processing.

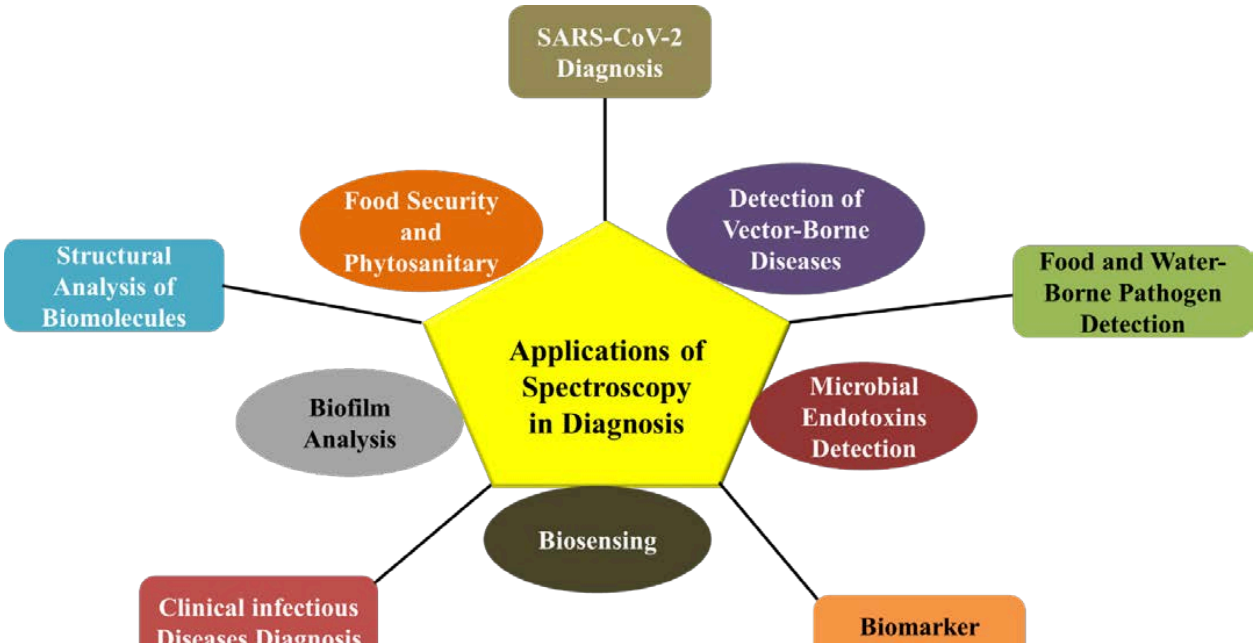

Figure 1: Overview of applications of spectroscopy in diagnosis

2.4 Immunological Methods- Immunological methods, such as enzyme-linked immunosorbent assays (ELISAs) and immunofluorescence assays (IFAs), rely on the specific interaction between antibodies and antigens to detect microbes. These techniques can rapidly identify the presence of target microorganisms based on the recognition of specific epitopes. Immunological methods offer high specificity and sensitivity, allowing for the detection of low levels of microbes. However, they may require prior knowledge of the target antigen or antibody production, limiting their application to known pathogens or organisms with available antibodies.

2.5 Molecular Methods- Molecular methods have revolutionized microbial detection by enabling the direct detection and identification of microorganisms based on their genetic material. Polymerase chain reaction (PCR) is one of the most widely used molecular techniques. It amplifies specific DNA sequences of target microorganisms, allowing for their detection. PCR-based methods provide high sensitivity, specificity, and rapid results, making them invaluable in clinical diagnostics, epidemiology, and environmental monitoring. Further advancements, such as real-time PCR, multiplex PCR, and digital PCR, have enhanced the accuracy and efficiency of microbial detection Maheux et al., 2011).

3. Rapid microbiological methods (RMMs)

Because microbiological testing is a bottleneck in product release, the pharmaceutical industry's interest in rapid microbiological methods (RMMs) has grown considerably during the past ten years. RMMs such as adenosine triphosphate (ATP) bioluminescence and solid-phase laser cytometry are being investigated for their speed, sensitivity, and accuracy advantages. GlaxoSmithKline (GSK) has taken the opportunity provided by the PAT initiative to establish rapid microbiological methods for microbial-limit testing of pharmaceutical-grade waters and product release of selected dosage forms. The current compendial methods for microbial testing of finished drug products or pharmaceutical-grade waters generally use either a membrane filtration-based or pour plate /spread plate-based microbial-limit test (MLT). The MLT defined by the current US Pharmacopeia is a threefold test, entailing.

1. enumeration of microorganisms (quantitative)
2. determination of the presence or absence of pharmacopeial indicator microorganisms
3. identification of recovered microorganisms (qualitative).

Two new technologies for microbial determination, ATP bioluminescence and laser cytometry, are superior to traditional techniques in many respects, including sensitivity, selectivity, and speed. The ATP bioluminescence assay quickly and effectively determines the

presence of viable microorganisms. Bio-luminescence occurs naturally in the firefly (Photinus pyralis) when the enzyme luciferase catalyzes the reaction of luciferin and the nucleotide ATP to produce light. By producing light, luciferase thus can quickly and accurately detect the presence of ATP (found in all living cells, including microbial cells).

In laser cytometry, a fluorescent stain signals viable microbial cell. The stain is converted to a fluorescing state only when esterase enzyme activity and an intact cell membrane are present; nonviable cells thus are not detected. Validation requirements for microbial-limit testing with

RMMs are defined in PDA's Technical Report 33, "Evaluation, Validation and Implementation of New Microbiological Testing Methods". The validation criteria, listed for both qualitative and quantitative methods, are independent of the technological platform. The equivalence of RMMs to conventional methods can be demonstrated through a series of experiments designed to assess the validation criteria, which can be performed by the prospective user or by others.

3.1 Design qualification- The staff developed a validation master plan for RMM evaluation. The plan included both equipment and microbiological-performance evaluation details such as user requirements, performance capabilities, computer hardware and software requirements, supplier audits, costs, and benefits. The flow diagram illustrates a process for assessing the feasibility of any rapid microbiological technology, using ATP bioluminescence as an example.

3.2 Installation qualification- The IQ stage verified and documented that the RMM instrumentation had been supplied, installed, and tested according to the manufacturer's specifications. The installation qualification package included documentation of a visual inspection of all equipment, copies of all operation manuals, and confirmation that all required utilities (*e.g.,* electricity, vacuum, and laminar flow hoods) were installed properly. Copies of protocols and results for all tests performed by the vendor and on-site are maintained in the event of an inspection.

3.3 Operational qualification- The manufacturers of rapid microbiological technologies supply the protocols for the OQ of their instruments. These protocols may be used as is or modified by the end user as appropriate. Typical tests include verifying the interface between the software and the instrument, verifying user access to each input message or command processed by the software, cross-checking each external file or data record referenced by the supplier, and verifying output messages, displays, and recorded data generated by the software. These tests were performed and documented with both compendial microorganisms

and site-specific environmental microorganisms. This OQ was critical at this stage because it ensured the correct operation of the method under working conditions.

3.4 Performance qualification. PQ demonstrated the suitability of the RMMs for microbial-limit testing. Validation experiments were designed to demonstrate and justify the use of the RMMs for testing specific drug products and pharmaceutical-grade waters. PDA Technical was followed in performing the validation experiments. Testing for each criterion (absence of interference, specificity, limit of detection, ruggedness and repeatability, and robustness) was outlined in protocols with specified acceptance criteria.

3.5 Next-Generation Sequencing (NGS)- NGS technologies, including metagenomics and amplicon sequencing, enable the simultaneous identification and characterization of microbial communities present in a sample. These methods sequence and analyze the genetic material extracted from the sample, providing comprehensive information about the diversity and abundance of microbes.

NGS has revolutionized microbial ecology studies and has the potential to uncover previously unknown pathogens. However, NGS techniques are expensive, require bioinformatics expertise for data analysis, and may produce large datasets that can be challenging to interpret.

3.6 Biosensors- Biosensors combine biological components, such as antibodies or nucleic acids, with transducers to detect and quantify microbes. They offer real-time detection, high sensitivity, and portability, making them suitable for on-site monitoring. Biosensors can be designed to detect specific microbial targets, including pathogens, toxins, or specific DNA sequences. Recent advancements in nanotechnology and microfluidics have further improved the performance and miniaturization of biosensor platforms, expanding their potential applications.

The detection of microbes is essential in various fields, and the choice of qualitative or quantitative method depends on the specific requirements of the application. Culture-based methods remain valuable for their simplicity and cost-effectiveness, while immunological methods offer specificity and sensitivity. Molecular methods, particularly PCR and NGS, provide rapid and accurate results, enabling the detection of known and unknown microorganisms. Biosensors offer real-time monitoring and portability. As technology continues to advance, further developments and integration of these methods are expected to enhance the accuracy, sensitivity, and efficiency of microbial detection, contributing to improved diagnostics, public health, and environmental monitoring.

4. Qualitative and Quantitative Analysis of Microbes in water samples

Water testing is a process to identify and determine pathogen or microorganism contamination levels. Quantitative analysis is numbers-based, countable, or measurable. Qualitative analysis is interpretation-based, descriptive, and relating to information. Quantitative analysis tells us how many, how much, or how often in calculations. Qualitative analysis can help us to understand why, how, or what happened behind certain behaviors.

4.1 Qualitative analysis

Qualitative analysis in research is a method to analyze nontangible aspects of a subject to valuate its characteristics.

4.2 Microbiology Testing

Under the microbiological testing of water samples, we examine microscopic organisms that could be a single cell, multiple cells, or without cell. during the manufacturing process and in final consumer products to ensure consumer safety, prevent brand desecration, and minimize costly mitigation following failed inspections.

4.3 Isolation of Discreate Colonies from Mixed Culture

In nature, microbial populations do not segregate themselves by species but exist with a mixture of many other cell types. In the laboratory, these populations can be separated into pure cultures. Isolation of pure culture by using spread-plate or streak-plate and pour plate methods

The culture contains only one type of organism and are suitable for the study of their cultural, morphological, biochemical and molecular characterization.

In this experiment, we were first use one of the techniques designed to produce discrete colonies. Colonies are individual, macroscopically, visible masses of microbial growth on solid medium surface, each representing the multiplication of single organism. Discrete colonies make an aseptic transfer onto nutrient agar slant for isolation of pure cultures.

4.4 Microscopy:

Microscopic examination of stained microbes under different magnification low power 10X, High-power 40X, Oil-immersion 100X respectively. Further microbial identification by different microscope like Light Microscope, Bright-field microscope (Compound microscope), Dark field microscope, Phase-contrast microscope, Differential Interference Contrast (DIC) Microscope, Fluorescence Microscope, Confocal Scanning Laser Microscope, Electron Microscopy, Transmission Electron Microscope (TEM) and Scanning Electron Microscope (SEM), Scanning: Probe Microscopy, Scanning Tunneling Microscope (STM), Atomic Force Microscope (AFM) etc.

5. Preparation of specimen for Light Microscope:

Living microorganism were directly examined with a light microscope by fixed and stained microbes to increase visibility, accurate specific morphological feature and preserve them for future work.

5.1 Staining Methods

Microbes were stain by Simple and Gram Stanning (Differential Stanning) for the study of morphology and identify the Gram-positive and Gram-negative bacteria. Other staining methods such as special staining, negative staining, capsule staining, endospore stanning and flagella staining etc. for study of microbes in details and also study size, shape and arrangement of cells. Microscope examination of living microorganism using a Hanging-Drop preparation or wet mount.

5.2 Quantitative analysis

Quantitative analysis involves looking at the hard data, the actual numbers. Qualitative analysis is less tangible. It concerns subjective characteristics and opinions things that cannot be expressed as a number.

5.3 Microscopic Measurement of Microorganisms

After staining slide were examined under the light microscope for morphological and structural characteristics of the microorganism**.** Besides these, dimension of the microbes in terms of its length, breadth, diameter etc. also necessary for its authentic identification. Appropriate methods have been for the measurements. This the general method of measuring various structures of a microbial cell by micrometry.

5.4 Microbial Growth Measurement

The increase in the cell mass of the organism is measured by using the Spectrophotometer. The Spectrophotometer measures the turbidity or Optical density (600 nm) which is the measure of the amount of light absorbed by a bacterial suspension.

5.5 Measurements of Microbial Growth

There are various ways to measures microbial growth for the determination of growth rates and generation times.

For the measurement of growth either mass or population number is followed because growth leads to increase in both.

5.1.1 Growth can be measured by one of the following types of measurements:

Cell count this method involves the measurement of growth either by microscopy or by using an electronic particle counter or indirectly by a colony count.

Cell mass in this growth can be measured directly by weighing or by a measurement of nitrogen concentration in cells or indirectly by the determination of turbidity using spectrophotometer.

Cell activity in this growth can be measured indirectly by analysis of the degree of biochemical activity to the size of population.

Some Specific Procedure Will Illustrate The Application of Each Type of Measurement

5.1.2 Direct microscopic count

Electronic enumeration of cell numbers

The plate count method

Turbidity estimation of bacterial numbers

Determination of nitrogen content

Determination of dry weight of cells

Filtration method

Most Probable Number (MPN) Method

5.1.2 Direct Microscopic Count

The most obvious way to count microbial numbers is through direct counting.

Petroff-hausser counting is one of the easiest and accurate way to count bacteria.

Side view of the chamber showing the cover glass and the space beneath it that holds a bacterial suspension.

A top view of the chamber. The grid is located in the center of the slide.

An enlarged view of the grid. The bacteria in several of the central squares are counted, usually at different magnification.

6. Rapid Detection Methods

Polyethene terephthalate films (PET-F) were purchased from Goodfellow (Huntingdon, UK) PET spun bond (PET-SB) and polypropylene melt blown (PP-MB) nonwovens were supplied from Korea Institute of Industrial Technology (KITECH, Ansan-si, Gyeonggi-do, Korea). The solidity and porosity of the webs were calculated by Equations (1) and (2). The mean diameter of nonwoven fibres was measured from the scanning electron microscopy (SEM) images. The characteristics of the film and nonwovens used.

Solidity (unitless) = m/(A_t), *E. coli* strain KCTC 1039 was used as the test bacteria. Phosphate buffered saline (PBS) and the TrypLE Express enzyme for cell detachment were purchased from Thermo Fisher Scientific (Waltham, MA, USA). PVDF microfilters in 0.22 μm pore size were purchased from BIOFIL (Guangzhou, China), and dimethyl sulfoxide (DMSO) was purchased from Daihan Scientific (Wonju-si, Gangwon-do, Korea). Sodium

dodecyl sulfate (SDS), iodonitrotetrazolium (INT) chloride culture Luria-Bertani broth (LB), and all other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA). 2.2. Adhering Bacteria to Substrates

Before the test, sample substrates (1 cm _ 1 cm) were cleaned in isopropanol for 5 min, using a sonicator (Digital Ultrasonic Cleaner, WUC-D03H, Diahann Science Co., Gangwon-do, Korea), and were then rinsed with distilled water. For bacterial binding to a sample substrate, the substrate was immersed in 1 mL of bacterial culture in LB broth, with an initial OD600 of 0.5 which corresponds to 3.4 _ 108 cells/mL. The 1 mL of culture and a substrate were put in a 24-well plate and incubated for 1 h at 100 rpm. After incubation, the E. coli-adhered substrate was placed in a new plate; then the weakly adhered bacteria were removed from the sample by rinsing the substrate two times in 1 mL PBS at 100 rpm for 5 min.

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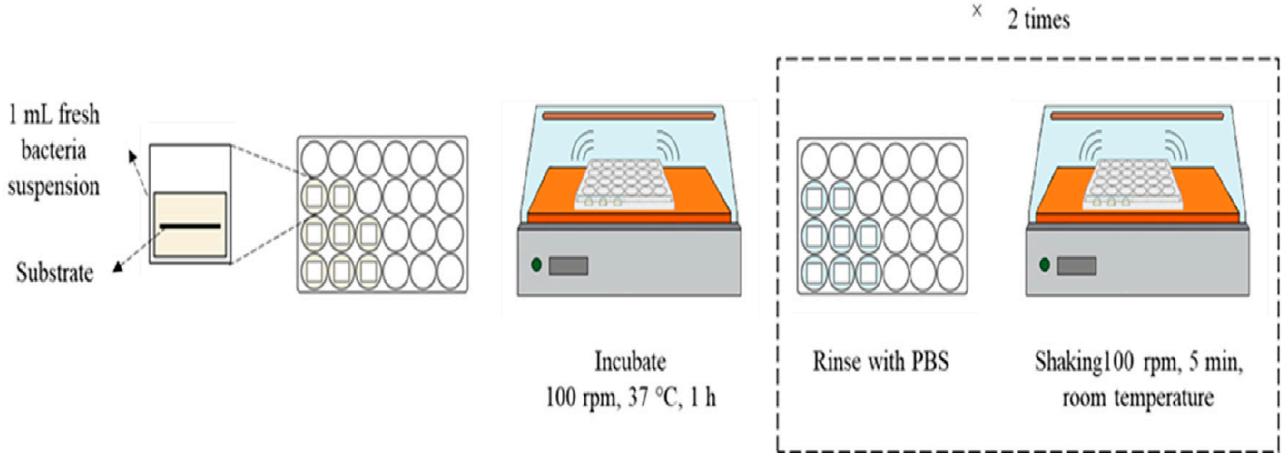

Figure-2 The procedure of adhering bacteria to substrates. PBS = phosphate-buffered saline.

6.2 Evaluation Methods for Bacterial Adhesion on Polymeric Substrates –

For evaluation of surface-adhered bacteria on polymeric substrates, three different methods were tested as follows: Method 1, extraction of surface-adhered bacteria and CFU counting;

Method 2, INT-staining of bacteria and colourimetric analysis; and, Method 3, microscopic analysis.

6.2.1 Method 1: Extraction of Surface-Adhered Bacteria and CFU Counting Standard Curve for Optical Density and Cell Counting:

E. coli in LB broth was incubated at 37ºC and 200 rpm for 4 h; then the bacteria suspension was pelleted by centrifuging at 3000 rpm for 5 min at 4ºC (Multi-Centrifuge-VARISPIN 15R, CRYSTE, Bucheon-si, Gyunggi-do, Korea). The supernatant was removed, and the pellet was resuspended in PBS to the OD600 of approximately 0.5, corresponding to 3.4 _108 cells/mL, from which a series of diluted suspensions were prepared.

The dilution series's optical density at 600 nm wavelength (OD600) was measured using a microplate reader spectrophotometer (SpectraMax 190, Molecular Devices LLC, San Jose, CA, USA). The optical OD600 of the suspension was corrected for a blank PBS solution. A 20L of each dilution was plated on LB agar for cell counting, and the OD600 of dilution series was correlated with the cell counting in cells/mL. Illustrates the procedures for standard curve generation and cell extraction.

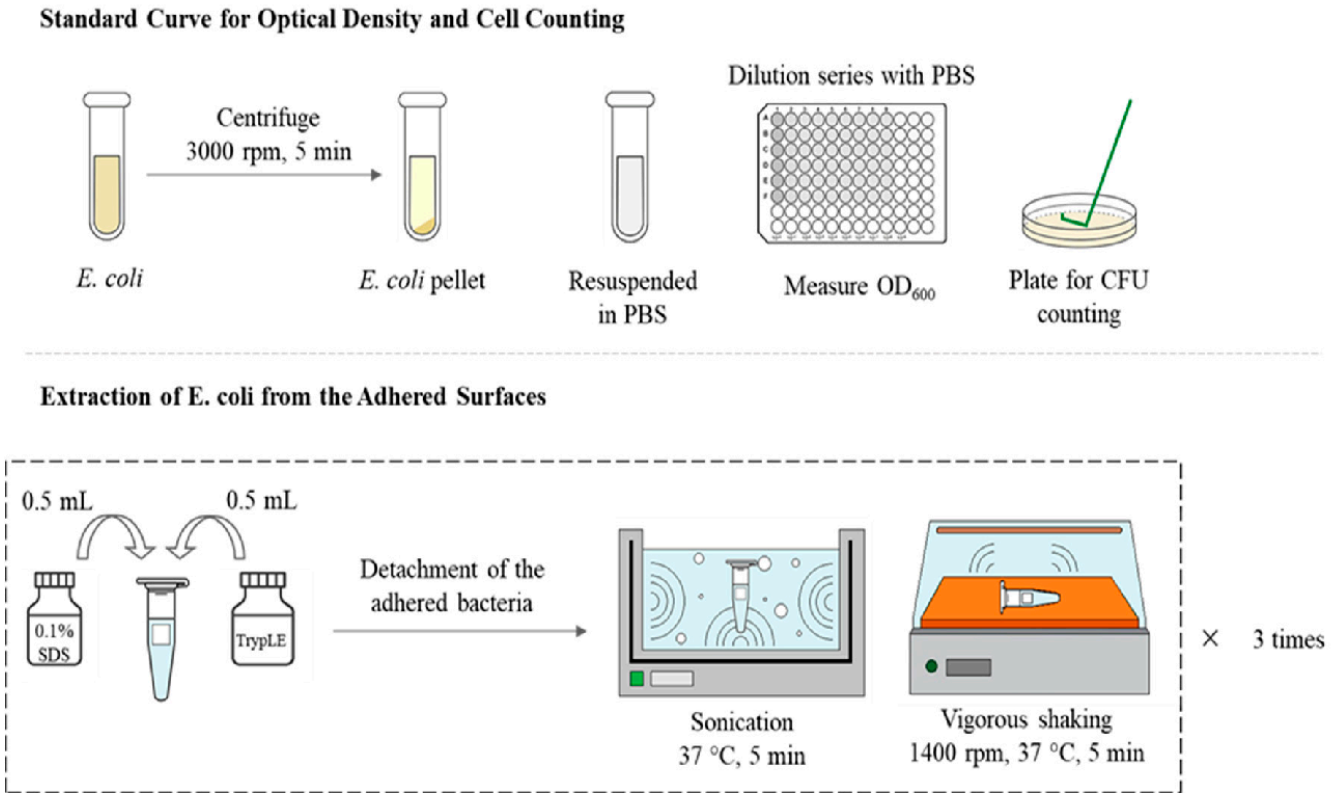

Figure 3- Extraction of surface-adhered bacteria and cell counting. CFU = colony forming units.

Extraction of *E. coli* from the adhered surfaces: for the full extraction of bacteria from the textile materials, the extraction methods used in the previous studies [were modified for centrifugation speed, use of surfactant/enzyme, etc. After removing the weakly adhered bacteria by gentle rinsing procedure, the remaining bacteria on the surface were extracted using a surfactant/enzyme solution, and the OD600 of the extract was measured to estimate the number of released bacteria. For a detachment of bacteria, the bacteria-adhered substrate was placed in a centrifuge tube containing 0.5 mL of 0.1% sodium dodecyl sulfate (SDS)/PBS solution and 0.5 mL of TrypLE Express enzyme; then the solution was sonicated in an ultrasonic bath for 5 min at 37 °C (60 Hz with the power output of 300 W).

After sonication, tubes were shaken at 1400 rpm for 5 min at room temperature, using a micromixer (Thermo micro mixer Mxi4t, FINEPCR, Gunpo-si, Gyeonggi-do, Korea). Each sample underwent extraction procedures three times, using a fresh solution with SDS surfactant and

TrypLE Express enzyme. Three extracts with detached cells were combined for the measurement of OD600. The number of CFU was correlated with the OD600 measurement and expressed as cells/mL solution or] cells/cm2 substrate surface.

6.2.2 Method 2: INT-staining of Bacteria and Colorimetric Analysis:

Colourimetric Measurement: To estimate the number of adhered bacteria that contributed to formazan formation, the bacteria-adhered substrates were incubated at 100 rpm for 4 h at 37ºC, in a 24-well plate containing 200 L (1.98 _ 106 moles) of prepared INT stock solution and 1 mL of PBS. After incubation with INT, samples were moved to a new plate and the formazan was extracted with 2 mL of DMSO; the suspension in DMSO was heated to about 105ºC for 5 min for complete extraction. The suspension was then filtered through a microfilter (0.22 m pore), and the INT formazan/DMSO eluent was measured for its absorbency at the wavelength of 470 nm using a spectrophotometer. The number of adhered bacteria was correlated withOD470 measurement and expressed as CFU/ml solution or CFU/cm^2 substrate surface. Illustrates the procedure for the colourimetric measurement.

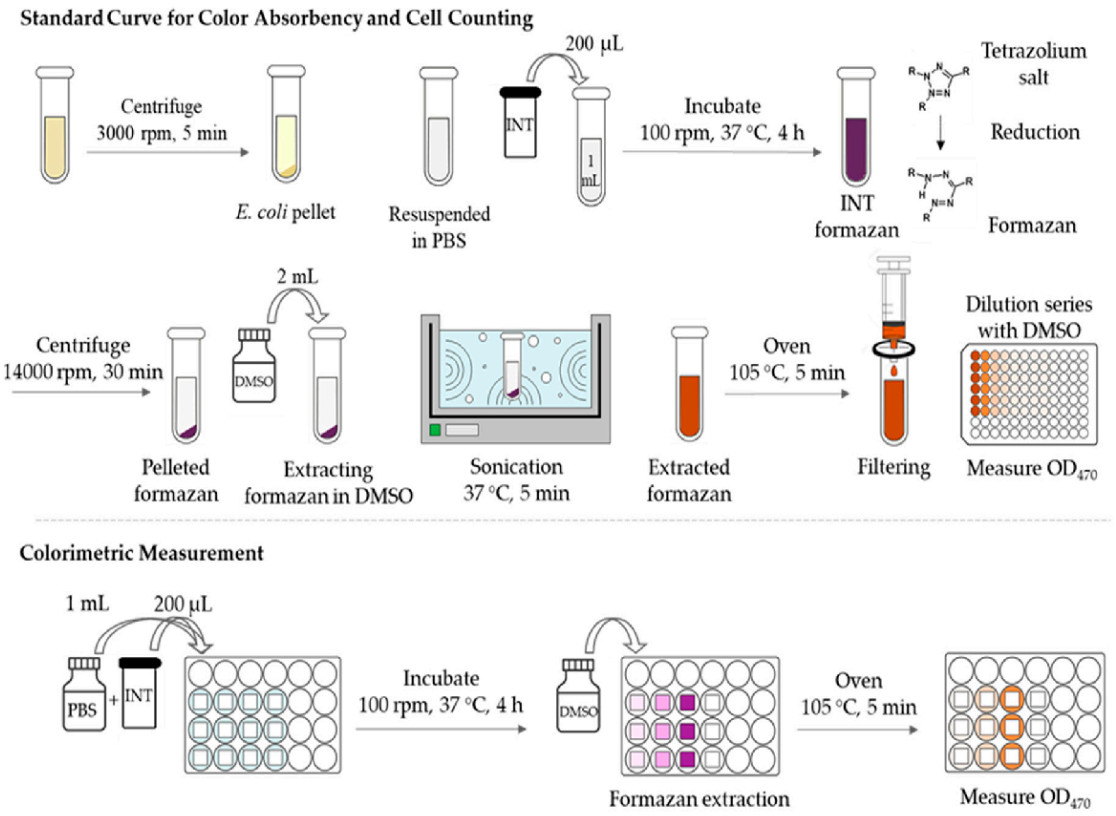

Figure 4 Colourimetric analysis with iodonitrotetrazolium staining of cells. DMSO = dimethyl sulfoxide

6.3 Microscopic Analysis:

Scanning electron microscopy (SEM) imaging of bacteria-adhered substrates on PP-MB, PET-SB, and PET-F was analysed with and without extraction procedure (method 1). The bacteria-adhered substrates were fixed with 2% (v/v) glutaraldehyde in PBS (pH 7.4) at around 23ºC for 2 h, then exposed to the vapour of 2% (w/v) osmium tetroxide for 2 h. The treated samples were sputter-coated with platinum at about 5 nm thickness (20 mA, 180 sec sputter time) before SEM analysis (JSM-5410LV, JEOL Ltd, Tokyo, Japan).

Concentration Methods of Waterborne Pathogens Concentration methods are crucial for detecting pathogens in environmental waters, because the concentrations of pathogens in environmental water samples are usually orders of magnitudes lower than those in clinical samples. Small sample volumes in detection assays make the direct detection of pathogens in

SUMMARY AND CONCLUSION

The detection of microbes is a critical aspect of various fields, including healthcare, environmental monitoring, and food safety. To identify and characterize these microorganisms, researchers employ both qualitative and quantitative methods. Qualitative methods focus on determining the presence or absence of specific microbial species or groups, while quantitative methods provide information on the abundance or concentration of microorganisms. Qualitative methods include traditional culture-based techniques, such as streak plating, selective media, and biochemical tests. These methods rely on the growth and identification of microorganisms based on their unique characteristics. Molecular-based techniques, such as polymerase chain reaction (PCR), DNA sequencing, and fluorescence in situ hybridization (FISH), are also commonly used.

These methods target specific genes or genetic regions to detect and identify microbial species accurately. Quantitative methods, on the other hand, enable researchers to estimate the number of microorganisms present in a given sample. One widely used quantitative technique is the standard plate count, which involves diluting the sample and plating it on agar media to obtain colony-forming units (CFUs). Another approach is the most probable number (MPN) method, which uses a series of dilutions and statistical analysis to estimate the microbial population. Advancements in technology have led to the development of rapid and high-throughput methods for microbial detection. These include automated systems, such as flow cytometry and real-time PCR, which provide quick and reliable results. Additionally, metagenomic sequencing and next-generation sequencing techniques allow for the comprehensive analysis of microbial communities in complex samples. The qualitative and quantitative methods for microbial detection play crucial roles in various scientific disciplines. Qualitative methods provide valuable information on the presence or absence of specific microbes and enable the identification of microbial species. These methods have been enhanced by molecular techniques, which offer higher specificity and sensitivity.

Quantitative methods, on the other hand, allow researchers to estimate the abundance and concentration of microorganisms in a sample. These methods are essential for assessing the microbial load in various environments and monitoring changes over time. Both the standard plate count and MPN methods have been widely employed, providing reliable and reproducible results. Advancements in technology have revolutionized microbial detection, offering faster and more efficient methods. Automated systems and high-throughput techniques enable rapid analysis of large sample sets, making them invaluable for applications where timely results are critical. Metagenomic sequencing and next-generation sequencing have also expanded our understanding of microbial communities by providing comprehensive

genetic information. the combination of qualitative and quantitative methods for microbial detection provides researchers with a robust toolkit to explore and monitor microbial populations. These methods continue to evolve with technological advancements, enabling more accurate, sensitive, and efficient microbial detection in diverse settings.

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