

Novel Methods for Detection of Microbes at Cellular and Molecular Level

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Abstract

Bacterial contamination is a growing global public health threat for individuals, food industry, hospital diagnostics and society. While antibiotics can be used to treat most bacterial infections, they constantly accelerate the emergence and spread of resistance bacteria. Today, many antibiotics are ineffective, but the pace of discovery of new antibiotic has dropped substantially. Conventional methods used to detect and quantify bacteria are plate culturing, polymerase chain reaction (PCR), enzyme linked immunosorbent assay (ELISA) and chemical sensors based detection strategies. Molecular based detection method PCR is considered to be another standard method to detect bacteria. This method can be used for bacteria detection with high sensitivity and specificity, but well-trained personnel and costly instrumentations are indispensable. New colorimetric bacteria detection method based on bacterial inhibition of glucose oxidase-catalyzed reaction. The concentrations of microbes access by following the disc diffusion method that using the antibiotics and also discs to differentiate the different strains of bacteria. Bacterial cultures ability grown in petri plates by using the growth medium such as nutrient agar. Methicillin resistant *Staphylococcus aureus* (MRSA) is detected with excellent specificity and sensitivity by IDI-MRSA via simultaneous targeting of the staphylococcal chromosomal cassettes.

Keywords: Microbes, Enzymes, Cellular biology, Molecular, methods, ELISA, PCR.

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INTRODUCTION

Enzyme-linked immunosorbent assay (ELISA) is the enzyme based detection of the antigen or antibody in the particular samples by assisting the diagnosis of diseases in clinical as basic sciences [1-3]. Through ELISA, a variety of antigens or proteins in pathogens, viruses, bacteria can be tested or detected in order to spearing of infections to other tissues as well as to control the diseases rate. An easy and rapid ELISA system, Filtration ELISA to detect antibodies against bacterial cell surface antigens. The variations in ELISA allow us to detect either antigen or antibody, identifying the different strains of microbes at a time and also in characterization of the epitope distribution on the microbial surface [4, 5].

ELISA is also used for bacteria detection and considered as one of the most popular immunoassay methods. ELISA may provide results slightly faster compared with plating culturing and PCR, but high false positive and complex experimental operation limit its applications. Furthermore, each of those methods mentioned above has its advantages, but all of them are laboratory-based and unable to be tested on site, which limit their utility for bacteria analysis in daily life practice. The need for improved bacteria detection methods has led to the development of chemical sensors, including colorimetric detection. New colorimetric bacteria detection method based on bacterial inhibition of glucose oxidase-catalyzed reaction [5, 6].

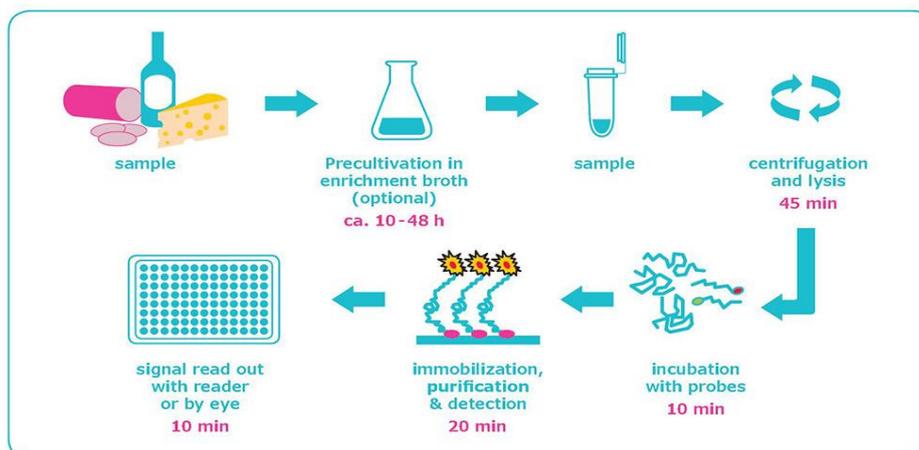


Fig-1: Shows the Quantitative Identification of microorganism in food water

PCR based detection

The PCR is the most sensitive of the existing rapid methods to detect microbial pathogens in clinical specimens. During the course of a bacterial infection, the rapid identification of the causative agent is necessary for the determination of effective treatment options. Different types of bacterial pathogens can be

detected through PCR such as *Enterococcus faecalis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Streptococcus pyogenes*. Real-time PCR technology that can monitor the products by measuring the fluorescent signal continuously is most commonly used as a rapid and reliable tool because of its high sensitivity and specificity [7, 8].

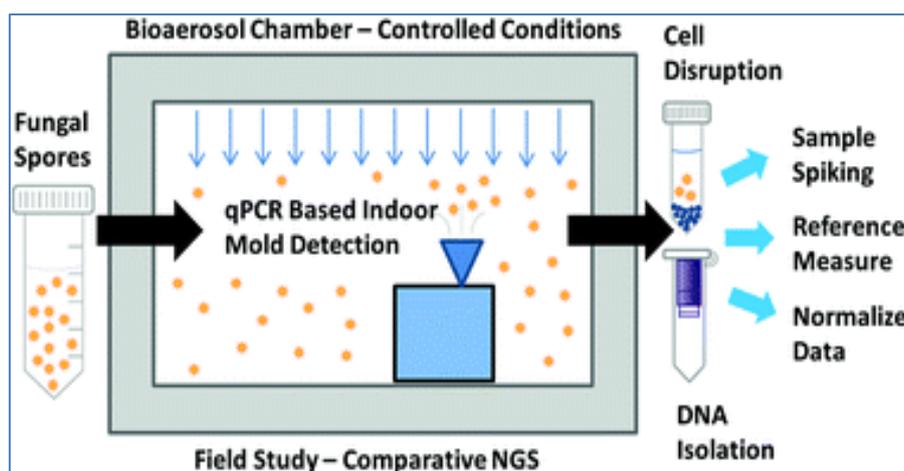


Fig-2: Shows the microbial detection through PCR at molecular level

Colorimetric bacterial detection method

Glucose oxidase as one of the important enzyme is used for the oxidation of glucose to produce hydrogen glucose. Starch reacts with the iodide through chemical combination and then starch-iodide paper as the substrate reacts with hydrogen peroxide to produce a deep blue color. Glucose metabolism is a common phenomenon for bacteria. With the presence of bacteria, the GOX-catalyzed reaction inhibited due to the lack of glucose, which subsequently influenced the oxidation chromogenic reaction of iodide and starch [9, 10].

Disc diffusion method for testing the antimicrobial activity

The concentrations of microbe's ability to determine by following the disc diffusion method that

using the antibiotics and also discs to differentiate the different strains of bacteria. Bacterial cultures in this method grow in petri plates by using the growth medium such as nutrient agar. The protocols of this method as follow. For purpose of inoculation, nutrient broth powder in the amount of 13 g/L catalysed put to the distilled water, and then mixed both them properly for homogenization. Then medium catalysed passed through the autoclave at temperature of 120°C for 20 minutes. Medium catalysed taken from autoclave and suitable bacterial strain catalysed put and mixed properly to medium and placed it to shaker for proper shaking 37°C for 24 hours. Inoculate catalysed and obtained and stored at 5°C [11, 12].

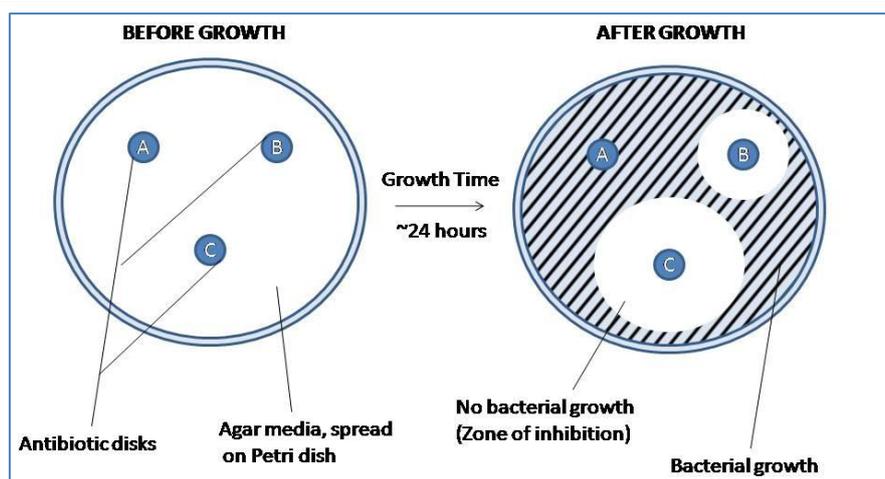


Fig-3: Shows the potential detection of microbes using disc diffusion method

Amplification methods for Bacteria

Amplification methods in staphylococci, the resistance to methicillin and cephalosporins is linked to an altered penicillin-binding protein 2a (PBP2a). Methicillin resistant *Staphylococcus aureus* (MRSA) is detected with excellent specificity and sensitivity by IDI-MRSA via simultaneous targeting of the staphylococcal chromosomal cassettes and a *S. aureus* highly conserved open reading frame, *ofrX*, within 1.5 h from having the specimen in hand. This approach utilizes a specific PCR primer coupled with a molecular beacon probe [12, 13]

Cytotoxicity or pathogenicity detection

Different pathogens contain a variety of compounds that can be tested in order to check the status of disease at the level of infections. When pathogens enter into body, then they attack the body also blood. The specific concentrations of blood can be tested to diagnose the disease caused by specific bacteria [14]. The procedure and protocols of this method as follow by taken the 3 mL of blood that put into the tubes use for blood collection containing heparin to avoid the coagulation. The tubes then mix carefully in order to avoid the destruction of blood cells and finally transfer into falcon tubes that capacity to store 15 mL samples of respective testing. The blood samples then centrifuge for a period of 4 minutes at 900 Xg. The supernatant catalyze by separate and cells with red blood cells primarily with phosphate buffer with a pH around the 7.4 for four times and hemacytometer catalyzed used for counting the RBCs [14-17].

Then samples of respective testing again ability incubate for forty minutes at 37°C and after ten minutes of incubation, tubes ability on the normal ice and again centrifuge at for five minutes at 1400 X g. When incubation by enzymes and complete, falcon tubes containing supernatant catalyzed initially taken

and catalyzed primarily with phosphate buffer. These dilutions ability principally maintain with and epidorff then finally placed on low temperature such as on ice. 100 µL samples that ability taken from epidorff catalyzed put to the well plates for further analysis. The extracts incubated at 37°C for the 30 minutes with dilute blood suspension and centrifuged. The triton on X-100 and phosphate buffer use as positive for complete lysis of RBCs and negative control that catalyze used for zero percent lysates respectively. Then results ability present in the form of percentage in relation to lysis of RBCs. Spectrophotometer use for taken the absorbance principally at 576nm. The hemolytic activity evaluate to determine the cytotoxic behavior of samples. The experiments ability repeate again for three times and results finally ability recorded. The hemolytic inhibition catalyzed basically calculated with chemical formula given below in the form of percentage [18-20]. Lysis of the RBCs (in %) = $(A \text{ sample} - A \text{ negative Control}) / A \text{ positive control} \times 100$

DNA Fragmentation in Microorganisms

It is observed that specific breaks in DNA strands can be visualize with through gel electrophoresis as well as through comic biochemical analysis. Chromosomal DNA fragmentation also leads to breakage of DNA strands under specific conditions of temperature and other specific materials required for throe breakage. Different methods and protocols are used for DNA Fragmentation in microorganisms [21, 22].

In higher eukaryotic cells, this may be a consequence of active programmed cell death. The presence of DNA breakage is usually evaluated using biochemical or molecular procedures such as alkaline unwinding, DNA elution, gel electrophoresis, sucrose gradient sedimentation, melting curve analysis, viscoelastometry, or light scattering[23, 24].

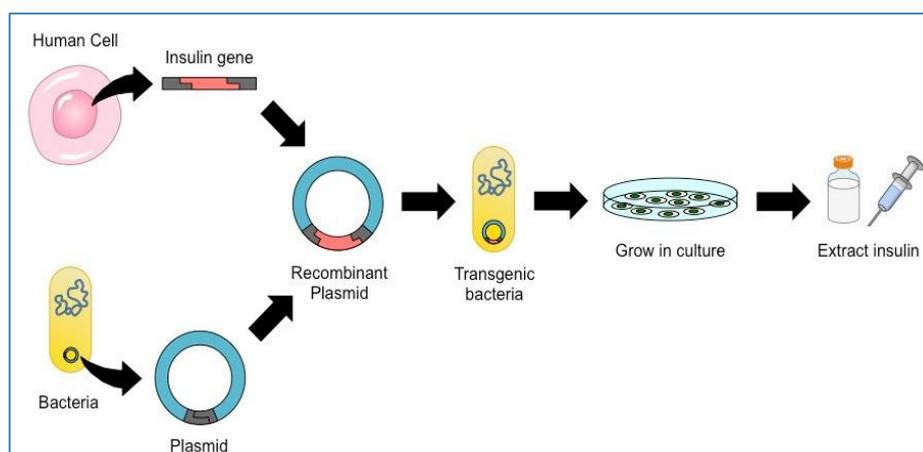


Fig-4: Shows the DNA fragmentation and production transgenic bacteria

The protocol of methods of DNA fragmentation as follows. DNA damage protection assay is used to check the concentration of microbial extract at molecular level. Blood collection tubes normally use and EDTA add in these collection tubes. Plasma the reaction and separated out from remaining samples by the process of centrifugation. DNA that obtain through the process of purification. The preparation of the phosphate buffer 50 Mm involves the 0.18g chemical compound such as NaH_2PO_4 and 0.55 g of another chemical compound such as Na_2HPO_4 catalyzed add to the 100 mL of the distilled water. Then properly catalyze shake this mixture for proper homogenization [25-28].

1X TAE buffer that prepare by mixing the 10 mL of the 50X TAE buffer in the distilled water that add in the amount approximately 490 mL. Then next, 1% of the Agarose gel prepare by mixing the agarose powder of one gram to the 1X TAE buffer in the amount 10 mL. Then properly shake this mixture and put it to oven in such a way that proper homogenization occurs. The mixture then providing the cooling temperature. Ethidium bromide in the amount of 20 μL properly add to freshly prepared Agarose gel. Then agarose gel then pour carefully to gel tank that run supplying the electricity. Agarose gel finally solidify into the gel tank. After that 1X TAE buffer in the proper amount catalysed added to the gel tank so that electrodes of the gel tank system ability sink into the buffer. Loading dye that bromophenol blue appears in blue colour in the amount of 3 mL carefully add to mixture. Then samples ability carefully taken and load to the freshly wells and electricity supply in the 100 volts approximately for 50minutes depends upon the thickness of the gel. The gel finally observe in the gel luminesce system that gives brightness to bands if the gel bright [29-33].

This method is useful to differentiate the different normal bands as compared to other bands that shrink die to oxidative stress. The normal bands in this method clearly appear visible while on the other hand,

shredded bands appears blurry due to more oxidative stress and error during running the experiment [34, 35].

CONCLUSION

Different types of nucleic acid amplification as well as genetic testing methods such as PCR and nucleic acid identification and characterization methods, such as hybridization in the form of gene probes, RFLP analysis, and nucleotide sequencing, have been applied to the detection of microbes both at molecular and cellular level. DNA sequencing that is used to identify bacteria, molds and yeasts.

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