Application of Molecular Biology Technology in Pathogenic Microorganism Testing

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Abstract: Pathogenic Microorganisms Are Very Small in Size and Various in Variety, and Will Undergo Rapid Variation. with the Development of the Current Society, People's Material Living Standards Are Continuously Improving and Antibacterial Drugs Are Not Used in a Standardized Way. Infectious Diseases in the Crowd, Drug Resistance of Common Pathogens, Food-Borne Infectious Diseases, and in Order to Do a Good Job in the Prevention and Treatment of Infectious Diseases, We Must Ensure That Pathogens Can Be Detected Quickly and Accurately. Modern Molecular Biology is a Vast and Profound Discipline, Which is Developing Vigorously. with the Continuous Development of Molecular Biology Techniques, Various Pcr Methods Such as Multiplex Pcr, Labeled Pcr and Asymmetric Pcr Have Been Applied to the Detection of Pathogenic Microorganisms. Their Application Gives Pcr Technology a Higher Sensitivity and Shorter Cycle Times. At This Stage, Our Task is to Strengthen Learning, Pay Attention to New Trends, and Be Prepared in Theory. We Believe That with the Deepening of Research, Molecular Biotechnology Will Certainly Play a More Important Role in the Detection of Pathogenic Microorganisms.

1. Introduction

There Are Many Kinds of Microorganisms, and Various Health Damages Caused by the Respiratory Tract, Digestive Tract, Mucous Membrane, Blood and the Imbalance of Their Own Normal Flora Are Called Pathogenic Microorganisms [1]. Pathogenic Microorganisms Are Very Diverse and Can Rapidly Mutate. after the Occurrence of Most Diseases, Due to the Hysteresis of Pathogen Detection, a Disease Pandemic Will Still Occur, So Accurate Pathogen Detection Must Be Done in the Early Stage of the Disease [2]. with the Advancement of Technology, the Test Methods for Rapid Identification of Pathogenic Microorganisms in the Fields of Medical Treatment, Food Inspection, Disease Prevention and Control Have Also Been Significantly Developed [3].from the Initial Observation Limited to Optical Microscopy, It is Observed That Molecular Biology, Immunological Methods and Metabolic Technology Have Been Widely Used in Medical Treatment, Disease Control, Food Safety Inspection and Other Related Departments [4]. in Actual Work, It is Necessary to Select a Detection Method with Strong Specificity and High Sensitivity for the Specimen to Be Detected According to the Technical Conditions of Laboratory Detection Equipment and the Technical Level of Operating Technicians.

Along with the Development of Economic Technology, the Gradual Progress of Chemistry and Life Sciences, People's Understanding of Biological Related Knowledge Has Gradually Increased to the Micro Level [6]. At Present, It is Possible to Detect the Linear Structure of Molecules, So That Different Substances Can Be Laterally Compared, Reflecting Differences between Different Cells of Different Individuals or Different Physiological States and Individuals of the Same Species [7]. the Vastness and Breadth of Modern Molecular Biology is a New Subject That is Booming [8].It is Mainly a Science That Studies the Structure, Function, Heredity, Regulation, Interrelation and Interaction of Biological Macromolecules-Nucleic Acids and Their Expression Products-Proteins. Molecular Biology Tests Must Have Basic Theoretical Knowledge of Molecular Biology and Trained Professionals [9]. At This Stage, Our Task is to Strengthen Our Study, Pay Attention to New Trends, and Be Prepared in Theory [10]. Because from the Perspective of Development,

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Molecular Biology Methods Will Be More and More Popularized in Clinical Tests, Which is Inevitable.

2. Traditional Methods for Detecting Pathogenic Microorganisms

2.1 Tissue Cell Culture and Biochemistry Experiment

For pathogens living in tissue cells, it is more suitable to select tissue cell culture for detection, specifically chlamydia, rickettsia, virus, etc. Different pathogens have different tissue sensitive cells. Live cells are extracted from pathogen-sensitive animal tissues, and primary culture can be carried out in vitro, or pathogen-sensitive cell lines can be selected for subculture. Then the pathogen is inoculated with the corresponding tissue cells, and then the pathogen will multiply and grow in it, resulting in specific cytopathic response. Pathogens can also be directly inoculated in sensitive animals, resulting in specific pathological changes in the corresponding tissues and organs. In general, most pathogens can obtain ideal colonies after 16-20 hours of culture, and a small number of pathogens require several months of culture to form a certain number of colonies. However, in actual clinical work, this situation is alleviated because the patient's condition requires the clinician to quickly and accurately know the pathogenic microorganism species to avoid delaying the optimal treatment timing, and the wide application of various automatic microorganisms and drug susceptibility systems has been alleviated.

2.2 Direct Smear Microscopy

Pathogenic microorganisms are very small in volume and are generally translucent and colorless. After staining, the arrangement, morphology and size of pathogenic microorganisms can be observed by a microscope. This method is relatively simple, the results are obtained quickly, and it is suitable for pathogenic microorganism infection with special morphology. Including spirochete infection, mycobacterium tuberculosis, gonococcal infection, etc., can be used for early preliminary diagnosis of such pathogenic microorganisms. Because this method does not need special equipment and instruments, it is still widely used in basic laboratories.It is an important method for detecting pathogenic microorganisms and is the gold standard for pathogenic microorganism detection. In recent years, the detection of pathogenic microorganisms has gradually developed into high-throughput technology, and such techniques do not require more sample quantities when tested. The operation is simple, the time is obviously shortened, no pollution occurs, the accuracy of the test result is guaranteed, and a high degree of automation can be obtained. At the same time, however, we should also be aware that no matter what kind of detection method has its limitations, different inspection methods should be used in combination in actual work.

3. Application of Modern Molecular Biology Technology in Rapid Diagnosis of Pathogenic Microorganisms

3.1 Development and Application of Probe Hybridization Technology

With the continuous development of molecular biology and the widespread application of genetic engineering technology, it is recognized that nucleotide sequences (DNA) are the material basis for building life. Using the principle of nucleotide base sequence complementation, a specific gene probe is used to detect and identify the species, genus, type and various pathogenic virulence genotypes of various pathogenic microorganisms by nucleic acid hybridization technology. The so-called DNA probe is a labeled single-stranded DNA/RNA fragment containing a specific nucleotide sequence, and the fragment can be used to detect a target DNA sequence complementary thereto in a clinical specimen. It is widely used in the identification of various pathogenic microorganisms and is sensitive, specific, simple and rapid.Gene probes are divided into radioisotope labeled probes and non-radioisotope labeled probes according to their labeling types. Application in rapid diagnosis of pathogenic microorganisms, such as detection of virulence islands and virulence genes of intestinal pathogenic bacteria. Diarrhea-causing Escherichia coli is a group of important pathogenic bacteria

causing diarrhea in humans and animals. Its pathogenicity is closely related to virulence islands or virulence genes. Molecular biological methods, probe hybridization or PCR techniques are required for etiological diagnosis.

In fact, we can treat each pathogen as a unique nucleic acid fragment. The above-described fragments can be combined to prepare a probe under separation and labeling. The label, the nucleic acid probe and the test sample are combined to hybridize, so that the label can be detected, which is detected on the premise that the specific pathogen is present in the article. In this process, the probe can be organically combined with the nucleic acid sequence, thereby detecting whether the sample contains a specific pathogen. In China's current social development, gene probe testing technology is widely used and favored by food safety management departments, which plays an important role in promoting the development of China's food industry. For example, biotin is used to detect gene fragments of Salmonella, which is used as a probe to detect food safety. Practice has proved that it has remarkable effect. Another example is the establishment of a non-radioactive DNA probe using automated enzyme ligation, which has a significant effect on the detection of Listeria monocytogenes.

3.2 Pcr Technology and Its Application in Rapid Diagnosis of Pathogenic Microorganism

PCR (Polymerase Chain Reaction) is an in vitro enzymatic amplification technology of specific genes or cloned sequences mediated by specific oligonucleotides (primers). Invented by dr. Mullis of the united states in 1985, he won the nobel prize in chemistry in 1993. Because PCR technology itself is very simple, it can multiply a small number of original template DNA molecules by geometric series, which can meet the requirements of scientists to operate DNA to the greatest extent. Therefore, PCR technology has developed rapidly in the past ten years. RT-PCR (replication-PCR) was developed on the basis of classical PCR. RNA was used as a template, which was first reverse transcribed into cDNA, and then subjected to normal PCR cycle amplification, in situ-PCR, nest-PCR, and recombinant-PCR. , quantitative-PCR, etc. PCR is a revolution in the field of infectious disease diagnosis, which is sensitive, specific, and easy to automate. PCR technology combined with probe hybridization and DNA sequence analysis can not only be used as a routine diagnosis of Legionella pneumophila, but more importantly, it can be used for Legionella virulence gene detection, Legionella new varieties identification, and pathogenesis of Legionella Research and so on have significant practical value.

With the continuous development of molecular biology technology, multiple PCR, labeled PCR, asymmetric PCR and other different PCR methods have been applied to the detection of food pathogenic microorganisms. Their application makes PCR technology have higher sensitivity and shorter cycle. However, the common PCR technology will be affected by factors such as template preparation, primer quality and specificity, enzyme quality and PCR cycle number. Real-time fluorescence quantitative PCR technology will be affected by the same heterologous DNA background, oligonucleotide hybridization specificity, TaqMan probe ratio, high SYBR Green Ⅰ concentration and other factors in the detection process, which can cause quantitative results to deviate or lead to false positive and false negative results. Therefore, in practical applications, the stability of the above factors should be ensured as much as possible, and a positive control should be set. The results of food microbial testing have a certain lag, and it is impossible to guide practical production quickly and correctly. The emergence of PCR technology has brought a new dawn to food hygiene inspection. Using this technology, pathogenic microorganisms in food can be detected in a timely and accurate manner.

3.3 Gene Chip Technology

Gene chip technology is an organic combination of life science and information science. Gene chip technology uses in situ synthesis or microfabrication to cure tens of thousands of nucleic acid probes onto the surface of the support. Hybridization with the labeled sample enables rapid detection of the sample by detecting the hybridization signal. Gene chip technology is based on the specific nucleic acid hybridization that occurs between the probe on the chip and the target gene

fragment in the sample. With the continuous development of molecular biology technology, multiple PCR, labeled PCR, asymmetric PCR and other different PCR methods have been applied to the detection of food pathogenic microorganisms. Their application makes PCR technology have higher sensitivity and shorter cycle. However, the common PCR technology will be affected by factors such as template preparation, primer quality and specificity, enzyme quality and PCR cycle number. Real-time fluorescence quantitative PCR technology will be affected by the same heterologous DNA background, oligonucleotide hybridization specificity, TaqMan probe ratio, high SYBR Green Ⅰ concentration and other factors in the detection process, which can cause quantitative results to deviate or lead to false positive and false negative results. The main steps are as follows: various gene oligonucleotides are spotted on the surface of the chip, and the microbial sample is treated for nucleic acid extraction and nucleic acid amplification. Then it is labeled with fluorescein, hybridized with oligonucleotide dots on the chip, and finally the fluorescence distribution pattern is quantified and analyzed by a scanner to determine whether certain specific microorganisms exist in the detected sample. Gene chip has the characteristics of high throughput, multi-parameter synchronous analysis, fast full-automatic analysis, high accuracy, high precision and high sensitivity analysis. It is one of the most effective methods to identify harmful microorganisms at present.

4. Prospect of Molecular Biology Technology

Molecular biology is a burgeoning discipline that is developing vigorously, although in recent years articles on molecular biology have taken up most of the space in bio-medical journals at home and abroad, and new technologies and applications are emerging continuously. It is in the ascendant, but not many are really suitable for routine clinical examination. The main reason is that some new methods are not very mature, and the methods are relatively complex. Commercialized medicine boxes and special equipment are too expensive for patients to bear. Taking gene chips as an example, it is still in the stage of development and research, and there is still a distance from clinical practice. In addition, in some clinical trials, the existing methods are quite complete. For example, there are many good methods for daily detection of HBV, and their sensitivity and specificity are not bad. Therefore, except for special cases, it is generally unnecessary to use molecular biological methods for detection. With the increase of PCR inhalation, the carrying contamination rate and the flushing error are all downward parabola. When a certain amount is inhaled, the curve tends to be flat. The specific data is shown in Figure 1 and Figure 2 below.

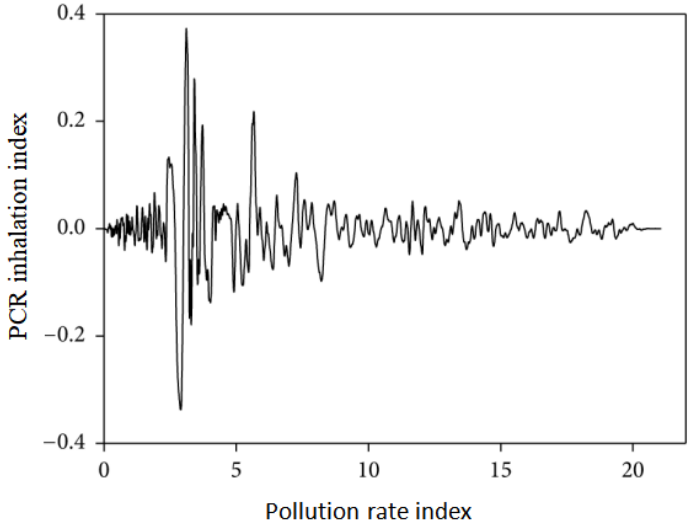

Fig.1 Comparison of Pcr Inhalation and Carrying Pollution Rate

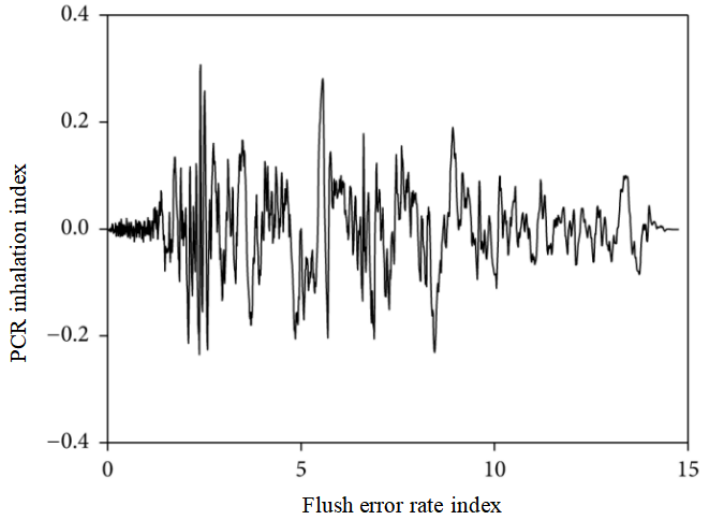

Fig.2 Comparison of Pcr Inhalation Volume and Flushing Error Rate

The flow cell type semi-automatic biochemical analyzer generally has a flow cuvette capacity of several tens of microliters. In order to reduce the cross-contamination of adjacent samples, the cuvette is washed by using excess detection liquid of the latter sample. The greater the difference in concentration between the front and back specimens, the greater the carry-over pollution. Therefore, the abnormal value of the pathological serum seriously interferes with the detection result of the next sample. At present, the detection liquid of most automatic biochemical analyzers controls the minimum inhalation volume. Although a smaller amount of inhalation saves reagents, it also increases the carry-on contamination of the instrument, and even with reliable reagents, carry-over contamination cannot be ruled out. It has been reported in the literature that after testing high-value serum samples, the instrument is washed with distilled water or reagent blank and then the next sample is detected to avoid contamination of the next sample by the high-value sample. However, it can be seen from this experiment that when the amount of inhalation is small, the test result of the next sample is low after the distilled water is rinsed. A low flushing error can be considered a special form of carrying contamination.

5. Conclusion

With the development of the current society, people's material living standards continue to improve and the use of antibacterial drugs is not standardized. Infectious diseases, common pathogen resistance, foodborne infectious diseases, and prevention and control of infectious diseases must be ensured in the population to ensure rapid and accurate detection of pathogens. The emergence of various high-throughput detection technologies has led to the continuous entry of inspection techniques into new fields, just as the detection of pathogens has progressed from histomorphology to molecular and genetic studies. In recent years, with the in-depth research on the detection of pathogenic microorganisms, molecular biotechnology will certainly play a more important role in the detection of pathogenic microorganisms. In the rapid detection of clinical common pathogenic microorganisms, polymerase chain reaction (PCR), gene (DNA) chip technology and biosensor technology play a very important role. It is of great significance to apply these molecular biology techniques reasonably and effectively.

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