CREATION OF DIAGNOSTICS FOR BACTERIAL INFECTIONS BASED ON THE PCR METHOD

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СОЗДАНИЕ ДИАГНОСТИКУМОВ БАКТЕРИАЛЬНЫХ ИНФЕКЦИЙ НА ОСНОВЕ ПЦР МЕТОДА

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Annotation. The most acute task of developing highly sensitive test systems is in the development of diagnostics for especially dangerous infections, such as plague, cholera, anthrax, tularemia and especially dangerous intestinal infections. Despite the fact that, thanks to the development of healthcare, mass epidemics of these diseases are a thing of the past, small local outbreaks of these infections occur almost every year. Early identification of such pathogens and organization of monitoring of their spread

contributes to the timely initiation of anti-epidemic measures and the effective selection of antibacterial therapy [1, 15].

Key words: diagnosticum, especially dangerous infections, intestinal infections, PCR method.

Аннотация. Наиболее остро задача разработки высокочувствительных тест-систем стоит в сфере разработки диагностикумов особо-опасных инфекций, таких как чума, холера, сибирская язва, туляремия и особо-опасные кишечные инфекции. Несмотря на то, что благодаря развитию здравоохранения, массовые эпидемии данных заболеваний давно ушли в прошлое, небольшие локальные вспышки данных инфекций случаются практически ежегодно. Ранняя идентификация подобных возбудителей и организация мониторинга их распространения способствует своевременному началу противоэпидемических мероприятий и эффективному выбору средств антибактериальной терапии [1, 15].

Ключевые слова: диагностикум, особо-опасные инфекции, кишечные инфекции, ПЦР-метод.

Introduction. For a long time, insufficient attention in the world was paid to the control of particularly dangerous intestinal infections, such as, for example, the enterohemorrhagic strain Escherichia coli O157:H7. Early identification of such pathogens and organization of monitoring of their spread contributes to the timely initiation of anti-epidemic measures and the effective selection of antibacterial therapy.

Of particular concern is the possibility of the spread of modified and evolved forms of pathogens of particularly dangerous infections, especially those with multi-resistance to typical first-choice antibiotics. Antibiotic resistance, caused by the presence of episomal genetic determinants, can be transmitted within a wide species spectrum of bacterial pathogens of infectious diseases by horizontal transfer [2, 10, 11].

At the moment, most of the test systems used in clinical practice for the etiological deciphering of especially dangerous infections are based on classical microbiological methods, enzyme-linked immunosorbent assay methods or PCR diagnostics. Taken together, these approaches are quite effective; however, each of these pathogen detection methods has certain disadvantages. In particular, the microbiological determination of a microorganism requires a lot of time to grow it and work under conditions of an increased level of biosafety. Traditional enzyme immunoassay is not sensitive enough for rapid diagnosis in the early stages of infection and rapid etiological deciphering of the pathogen, since it detects at least 1-10 ng of pathogen protein, which significantly exceeds the lethal dose of many bacterial toxins. PCR diagnostics is a very sensitive and specific method of analysis, however, clinical

samples or samples that include food particles, soil and other impurities also contain PCR inhibitors, related microorganisms, and nonspecific DNA that can give a false positive signal. In addition, PCR is not applicable for the detection of pathogenic microorganisms in complex complex environments, for example, when analyzing food products, since in this case it is necessary to enrich the detected targets (analytes) in the sample using affinity labels or selective media, which significantly increases detection time. Finally, PCR can only detect the presence of pathogen DNA, but not the presence of, for example, anthrax toxin. Differentiation into toxigenic and non-life-threatening strains using the PCR method is in some cases difficult or even impossible.

The developed technology of highly effective diagnostics is based on the immuno-PCR method, which combines the advantages of selectivity of immunological identification of the target due to its binding to a specific antibody and high sensitivity of PCR detection [1, 7, 12]. In the field of diagnosing bacterial infections, immuno-PCR strategies have been used to obtain detection systems for salmonella [2, 8], staphylococcal enterotoxin [5], Shiga toxins [6], etc.

The advantages of the immuno-PCR method for diagnosing pathogens of dangerous infections are: high sensitivity, allowing the identification of the pathogen and the pathogens secreted by it in the early stages of infection; high level of compatibility with standard ELISA and PCR technologies used in clinical practice; high specificity of detection, ensured by the applied principle of immunodetection; low probability of receiving false positive signals compared to standard PCR diagnostic methods; the ability to quickly analyze a large number of samples; the low cost of most materials used in the analysis and the possibility of quick extrapolation of the developed method for the detection of any pathogenic and non-pathogenic molecules in the presence of an effective pair of detecting antibodies.

The developed diagnostic method includes the stages of immobilization of an antibody on paramagnetic particles, blocking the surface of the particles to prevent nonspecific binding of the antigen and the DNA matrix used in detection, application of samples containing the antigen, recognition of the antigen by the detecting biotinylated antibody, detection of the antigen-detecting antibody complex with a DNA conjugate with neutravidin and setting up a real-time PCR reaction, the efficiency of which depends on the amount of DNA bound in the resulting complex.

At the first stage of technology development, the format for creating test systems was determined. Immuno-PCR technology involves immobilization of the first (binding) antibody on the solid phase. We compared the effectiveness of using immunological tablets and paramagnetic particles coated with protein G of bacteria of the genus Streptococcus as a solid phase, which make it possible to immobilize an antibody to a specific antigen on the surface of the particles, bind it from liquid samples, and get rid of contaminants contained in the samples at an early stage. stages

of the experiment and separate the DNA template used for the final PCR amplification from other components of the detection complex [1, 6, 13].

Experimental results showed that the use of paramagnetic particles helps to reduce the experimental time and reduce the false positive signal in the PCR amplification reaction. A further reduction in the background signal level was achieved by blocking the surface of the paramagnetic particles with Denhardt's solution containing foreign DNA. The effectiveness of blocking the surface of particles using this solution was higher than when blocking the surface with a solution of bovine serum albumin, casein and other blocking agents used in immunodetection.

DNA binding in this system was carried out through the interaction of a biotinylated detection antibody with biotinylated DNA through molecular bridges formed by the tetravalent protein neutravidin. The detectable template for real-time PCR was a DNA sequence encoding a translation elongation factor found in the genome of the organism Fusarium avenaceum, a fungus that is a parasite of agricultural crops. The probability of finding such DNA in clinical samples is extremely low [3, 9, 16]. The DNA sequence was optimized for PCR amplification with fluorescent signal detection in real time, which further reduced the likelihood of a false positive signal during detection and significantly increased the sensitivity of the method. Real-time monitoring of the PCR reaction was carried out using the TaqMan method using sequence-specific primers and a fluorescent probe.

The detection efficiency using the developed technology was significantly increased by using a non-covalent conjugate of DNA with neutravidin, which is a spatial molecular “network” formed due to the interaction of biotin located at the 5 ends of a double-stranded DNA fragment with tetravalent neutravidin molecule. The most effective is the formation of such complexes in an equimolar ratio of biotinylated DNA to neutravidin protein [1, 13, 14]. Neutravidin is a derivative of the avidin protein, characterized by high specificity and efficiency of interaction with biotin. The use of a molecular “mesh” instead of unbound fragments of biotiated DNA increased the amount of DNA template retained by a single antibody molecule and increased the sensitivity of the method by more than 10 times. The sensitivity parameters for detecting bacterial antigens using immuno-PCR technology reached 1 pg of protein per 1 ml of liquid sample.

The developed technology can be translated into a test system format either using monoclonal antibodies to various epitopes of the detected antigen, or using specific polyclonal antibodies.

Conclusions. Thus, the developed strategy for creating diagnostics based on immuno-PCR can be used to obtain diagnostic test systems for the needs of practical healthcare and environmental and food control.

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