

# Assessment of the significance of immunoenzyme test-system for determining antiendotoxic immunity against the endotoxin of gram negative bacteria

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**Abstract.** The purpose of the study was to determine the specificity and sensitivity of the immunoenzymatic test system that detects antibodies against the antigens of these bacteria in order to evaluate antiendotoxic immunity against GShPB endotoxins. It was found that flat bottom polystyrene solid phase carrier was selected for the proposed experimental test system for the detection of endotoxins of these bacteria by IFA method. The sensitivity of the recommended test system was 75% in colon dysbiosis, 76% in acute tonsillitis, 89% in acute bronchitis, and 95% in urinary tract infections, and the specificity was 5-25%, thus the diagnostic value of this test system it was proved for the first time .

## 1 Introduction

Today, it is difficult to imagine theoretical and practical medicine without the method of immunoenzyme analysis (IFA), because it plays a huge role in the diagnosis of many infectious and non-infectious diseases. It is known that effective, early diagnosis and treatment tactics can be planned using this method. This method is distinguished by its ability to obtain reliable results, allows the diagnosis to be carried out on a pathogenetic scientific basis, does not require special training for specialists, and the equipment used is affordable [6, 7, 9, 12]. In modern conditions, this discovery led to the rapid development of clinical immunology, and increased efficiency of serological reactions.

It is known from the analyzed scientific sources that the basis of IFA is test-systems, with the help of which, if the reaction is carried out correctly, it is possible to obtain true results and make reasonable conclusions. To date, test systems for the detection of endotoxins of gram-negative bacteria have not been developed among known pathogens, so it is not possible to detect them using the IFA method [1, 8, 14].

Gram-negative bacteria are capable of causing not only purulent-inflammatory diseases, but also various other diseases, and their endotoxins are involved in their pathogenesis. Endotoxins are not only the causes of the above-mentioned diseases, but also endogenous intoxication, and it is very difficult to diagnose them in time, because most of these gram-negative bacteria are found in the biotopes of the body and do not normally cause disease, but create the phenomenon of "transient bacteremia" and provide antigenic stimulation, thereby suppressing the immune system. controls its continuous functioning, ensures the effectiveness of the immune response [2, 3, 4, 11, 13].

Based on the above, it is important to create a test system for the detection of gram-negative conditionally pathogenic bacteria (GShPB) using IFA, and to introduce it into clinical practice.

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The purpose of the conducted research In order to assess the antiendotoxic immunity against GShPB endotoxins, it is necessary to determine the specificity and sensitivity of the immunoenzyme test system, which detects antibodies against the antigens of these bacteria.

## 2 Materials and Methods

It should be noted that most of the test-systems manufactured by most companies and recommended for health care use 96-well tablets with a flat bottom or U-shaped bottom as a solid phase carrier. In a series of studies conducted for the purpose of creating an experimental test system, the advantages of flat-bottomed polystyrene solid-phase carrier were determined.

As a result of studies, to isolate GShPB endotoxins as antigens, *Klebsiellae pneumoniae*, *Escherichia coli*, *Citrobacter freindii*, *Proteus vulgaris*, Collection strains of gram-negative bacteria such as *Pseudomonas aeruginosa* were used. The reason that hospital or museum strains are not used is because they have not been fully identified, some of the biological characteristics are variable, and atypical strains can be identified. The selected bacterial cultures were brought to a concentration of  $1 \times 10^9$  microbial bodies/ml and used as such. They were heated to  $80^\circ\text{C}$  for 0.5 hours and inactivated, and immunoglobulin G isolated from rabbit blood serum (IgG) was used as antibodies.

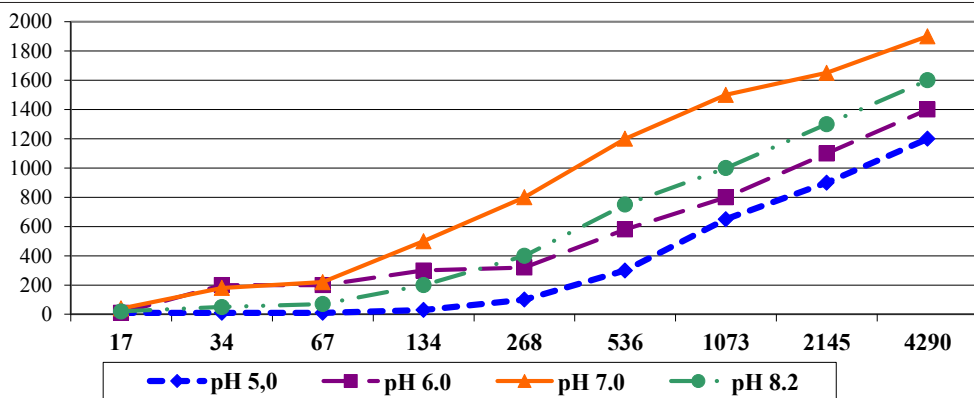
Antigens were isolated by extraction of the day-old culture of the collection strains presented according to Buaven - complex microbial antigen, using trichloroacetic acid . The concentration of complex bacterial antigen was adjusted to  $40\ \mu\text{g/ml}$  and used.

## 3 Results and Discussion.

It was shown that the fixation of GShPB endotoxin to the selected solid phase carrier depends on many factors, including the pH environment. This environment is the main source for the growing reaction in the tablet. It is necessary to take into account the pH environment of the used liquid mixture, which directly affects the degree of adsorption of antigens for the selection of a solid phase carrier and attachment of antigen to it. To create an experimental test system,  $1.0\ \mu\text{g}$  in  $0.1\ \text{M}$  Na-bicarbonate buffer, with a concentration of  $40\ \mu\text{g/ml}$ , was added to the wells of a polystyrene 96-well flat-bottomed tablet, and 4 It was left at a temperature of  $^\circ\text{C}$  for 16 hours. Researches were carried out with the help of the IFA analyzer "Stat Fax-300" (USA), this instrument was standardized and allowed to obtain a true result during the examination.

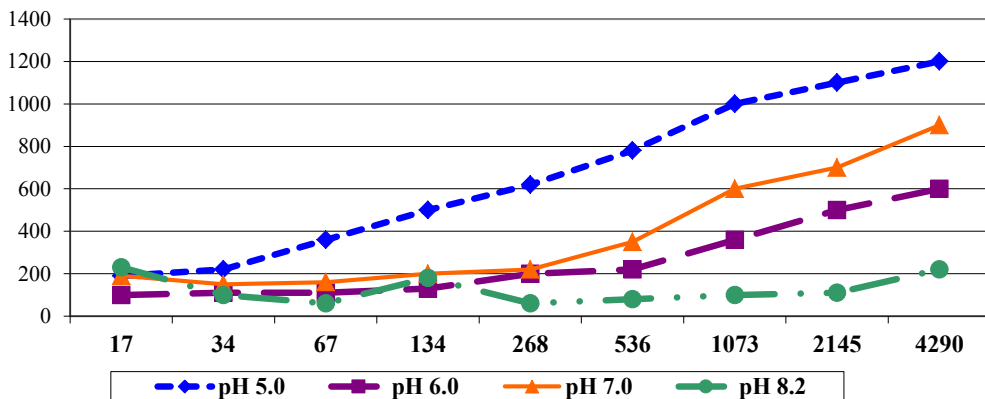
The data did not differ from the results reported in the literature [5, 6, 10]. Solid phase carriers meeting these requirements - 96-well flat-bottomed polystyrene tablets - were selected and used for the experiment in the above-mentioned case.

All three types of carriers were tested in the experiments. Due to the fact that the values of X and Y types of carriers were the same, the data of X and Z types of carriers were obtained. It was proved that the sorption property of polystyrene X with a hydrophobic surface has a strong relationship with the pH and ionic strength of the solution (Fig. 1).



**Fig 1.** Effect of rN in buffer on the binding of serum antibodies to type X solid-phase polystyrene 96-well flat-bottom tablets, R aM, ng/ml.

were obtained when a buffer solution with a low pH corresponding to the isoelectric point of the protein and a Z-type carrier were used (Fig. 2).



**Fig 2.** Effect of pH in buffer on binding of serum antibodies to Z-type solid-phase polystyrene 96-well flat-bottom tablet, R aM, ng/ml.

Blocking agents and immune reagents used by us in the process of developing the test system were dissolved in the same pH medium as the adsorbed antigen.

Research has developed ways to use IFA for mass verification. It was recommended to use blood itself instead of using blood serum for IFA when working in expeditionary mode for mass investigations, because it is difficult to collect blood and separate serum from it in field conditions, although it requires a large amount of blood, it is difficult to maintain sterility, and it is difficult to carry a centrifuge for serum collection. Difficulty, time-consuming causes difficulties. For this, 50  $\mu$ l of blood was taken from the finger using a micropipette and placed in a microtube containing 1 ml of physiological solution containing 0.9% NaCl. The tube with blood was mixed and left in a freezer at 4°C for 18 hours. At the same time, a 1:40-1:50 dilution of the test blood serum was taken, and the supernatant was used for IFA. IFA can be placed in 2 wells of a microplate to perform in screening-test mode. If the results were positive, the tests were repeated.

At the next stage of the work, it was necessary to determine the sensitivity and specificity of the method in order to determine and formalize the practical value of the proposed test system. It is known that these two concepts can be used in any method of disease detection, but they are widely used only in laboratory diagnostics. According to researchers, sensitivity is the ratio of persons recognized as patients as a result of using a diagnostic method to the total number of patients examined using this diagnostic method; specificity is the ratio of people recognized as healthy as a result of using a diagnostic method to the total number of healthy people examined using this diagnostic method.

Absolute and relative (%) numbers of seropositive and seronegative samples were used to study the diagnostic sensitivity and specificity of the obtained results. Sensitivity indicators differed from each other in diseases of different microbial etiology (MEK). The highest sensitivity rate was observed in urinary tract infections (UTI) at 95%, followed by acute bronchitis (89%) and acute tonsillitis (76%). The lowest sensitivity of the test system was observed in children with intestinal dysbiosis (YID) and was 75%.

At the next stage, the diagnostic sensitivity of MEK was determined by the strains whose antigens were prepared, the highest sensitivity was in *Escherichia coli* (88%), followed by *Citrobacter freindii* and *Pseudomonas aeruginosa* - 79% and 70%, respectively. The lowest sensitivity was found in *Proteus vulgaris* and *Klebsiellae pneumoniae* - 56% and 51%, respectively. It was recommended that this condition be taken into account in the serological diagnosis of diseases called GShPB.

The obtained results show that the specificity of this studied test-system was low in MEK (SYI, acute bronchitis, acute tonsillitis), as well as in IID. In healthy children, it showed a high rate - 90%. According to the rule of specificity assessment, this condition is mainly carried out in healthy individuals, including healthy children. If we take into account that the parameter obtained in healthy children was 90%, it was confirmed that the specificity of this diagnostic method is high.

Discussion. Selection of 96-well, flat-bottomed polystyrene tablet for IFA and fixation of antigen to it was shown to be the basis of the experimental test system. Also, it should not be forgotten that the value of the pH medium, which directly affects the level of adsorption of antigens (endotoxin) fixed on the surface of the solid-phase tablet, is the guarantee of obtaining the correct result. In the experiment, the optimal results for the fixation of GShPB endotoxins on a solid-phase carrier were obtained using a negatively charged polar hydrophilic tablet of the Z type, using a medium with a pH of 5.0.

It was found that the recommended test system for studying the level of detection of antibodies against GShPB antigens in blood serum has a high diagnostic sensitivity. The sensitivity was 95% in SYI, 89% in acute bronchitis, 76% in acute tonsillitis, and 75% in IID. The high diagnostic sensitivity of the test system in all cases showed its high efficiency and high role in the assessment of antitoxic immunity. *Escherichia coli* had the highest sensitivity for GShPB (88%), followed by *Citrobacter freindii* and *Pseudomonas aeruginosa* (79% and 70%), the lowest sensitivity was found in *Proteus vulgaris* and *Klebsiellae pneumoniae* (56% and 51%). The specificity of the test system was high in healthy children (90%).

#### **4. Conclusions**

The recommended experimental test system can be used not only in laboratory conditions, but

also in field conditions for mass testing. Not only blood serum, but also a 1:40 or 1:50 solution of blood in physiological solution can be used in it.

The sensitivity of the test system for IFA showing the degree of detection of antibodies against GShPB endotoxins (antiendotoxic immunity) is 75% in IID, 76% in acute tonsillitis, 89% in acute bronchitis, 95% in SYI, the specificity of the test system is 5-25, respectively. %, these parameters proved for the first time the high diagnostic value of this test system.

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