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Assessment of the Importance of Active Reaction for Antigen Attachment to Solid Phase Carrier for Immunoferment Analysis

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Relevance. Antiendotoxic immunity, which is formed in inflammatory diseases of various bacterial (microbial) etiologies in children, is associated with the peculiarities of endotoxin - pyrogenic effect, activation of blood flow and intravascular thrombi formation, impaired hemodynamics, tension of the humoral joint of the immune system, impaired kidney function, weak immunogenic effect, pathogenesis of these nosological units. The identification and evaluation of antiendotoxic immunity in inflammatory diseases of bacterial etiology is of critical importance for determining the early diagnosis of these diseases, the prospect of the end of the disease. These obtained results suggest that the creation of clinical-immunological criteria to prevent the transition of diseases of bacterial etiology among children to chronic manifestations increases the educational value of antiendotoxic immunity.

On a global scale, the endotoxins of Gram-negative bacteria today, their induction of the synthesis of various cytokines and other mediators upon landing in the human body, the absence of organotropy of endotoxins from protein toxins, the property of suppressing the phagocytosis process, the difference in the presence of a non-special effect, received various pathologies and pathogenetic mechanisms of causing pathological conditions were revealed, , the problems of their use for diagnostic purposes remain relevant today, attracting attention in scientific sources with the fact that research work on this problem is rare [1.3.5.7.9.11].

The purpose of the study was to assess the educational value of the comparative determination of the clinical-immunological properties of antiendotoxic immunity in children's blood serum in inflammatory diseases of bacterial etiology.

Research tasks include:

comparative study and assessment of the degree of detection of antibodies against endotoxins of Gram-negative bacteria in children's serum in bacterial (germ) etiological inflammatory diseases (acute tonsillitis, acute bronchitis, urinary tract infection) and colon dysbiosis;

age-related comparative determination of the level of antithelolar Detection against endotoxins of Gram-negative bacteria in diseases of bacterial etiology in children and assessment of the value of antiendotoxic immunity diagnosis;

determination of antibodies against endotoxins of Gram-negative bacteria seropositive I seronegative samples to determine the inter-age clinical-immunological characteristics of the incidence rate and develop immunological criteria on this basis;

development of criteria for determining the specificity and sensitivity of the immunoferment tist-system, which detects antibodies against antigens of Gram-negative bacteria, in order to assess antiendotoxic immunity against endotoxins of Gram-negative bacteria.

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As the object of the study, 251 3-12-year-old sick children with the diagnosis of inflammatory diseases of bacterial etiology (acute tonsillitis, acute bronchitis, urinary tract infection), 61 colon dysbiosis of the same age were identified for comparison purposes, and 25 healthy children were taken.

It is known that IFA is a laboratory immunological method of qualitative detection and quantitative measurement of antigens. The IFA was founded in the 70s of the last 20th century in Switzerland by Engvall and Perlmann, in the United States by da van Weemen and Schuur et al. invited from the parties.

The essence of IFA consists in the specific interaction of antitelo and excitatory antigen in the blood serum, followed by the addition of a conjugate to the resulting antigen-antitelo complex (human IgM, IgG, Ida immunoglobulins against the type in which the enzyme is celebrated). The enzyme causes degradation of the chromogenic substrate and produces a color product that can be visually or photometrically determined. Recording reaction results is carried out in special photometers with vertical light at a given wavelength, while the result is expressed in units of optical density[2.4.6.8.10].

Today there are many options for detection using IFA, but the most optimal option detection has been shown to increase the specificity and sensitivity of the reaction. Based on this, the placement of IFA (indirect ELISA) in a heterogeneous solid-phase carrier of them became of the highest scientific and practical importance. It should be noted that test systems manufactured by most companies and recommended for health practices, used for dental purposes, use flat-bottomed or U-shaped-bottomed 96-Honeycomb tablets as solid phase carriers.

In many of our series studies with the aim of creating an experimental test-system, it was the flat-bottomed polystyrene solid-phase carrier that allowed us to determine the advantages.

Studies from personal research have shown that the fixation of the Gram-negative bacterial antigen on 96-honeycomb flat-bottom tablets is due to many factors, one of the main of which is considered to be an active reaction of the buffer environment (rn). It is found that in order to select a solid-phase carrier and attach an antigen to it, it is necessary to take into account the RN environment in the used liquid mixture, which directly affects the level of antigen adsorption. In this regard, the purpose of this chapter in the experiment was to study and evaluate the effect of RN-the active reaction of the environment to the degree of fixation of gshpb antigens on 96 - honeycomb flat-bottom polystyrene tablets [13.15.17.19.21.23].

In the experiment, collector strains of Klebsiellae pneumoniae, Escherichia coli, Citrobacter freindii, Proteus vulgaris, Pseudomonas aeruginosa were applied for the purpose of obtaining antigens. Strains 1x109 microbial body / ml (m.t./ ml)was used in the form of an inactivated bacterial mass in the concentration, heated to 800s for 30 minutes. This temperature regime allows you to preserve the structure of proteins, neutralize bacterial suspension. In the experiment, Iggs of rabbits were used as antibodies. All information about collictional strains is fully contained in Chapter II, so we did not saturate them.

Antigens are separated by Buaven - a complex microbial antigen, by extraction of the Daily crop of collector strains brought in using uchchloricric acid. The complex bacterial antigen concentration was increased to 40 mcg/ml, and mainly this concentration was used. During the research, polysaccharide antigen was also isolated and studies were carried out, but we did not include the results obtained with this antigen in our dissertation work due to its very low detection effect, low effective diagnostic value. The extraction of the complex microbial antigen, on which the modification introduced on our side is presented in detail in Chapter II, so we did not find it necessary to dwell on this information.

It is known that sodium-carbonate (0.05 mol/l, rn 9.0), borate (0.05 ml/l, rn 8.0) and tris-HCl (0.05 mol/l, rn 8.0) buffered physiological solution (rn 7.2-7.5) are used for the purpose of loading antigens, their rn values and ionic strength can change during loading.

Experimental test-to create a system, a polystyrene 96-honeycomb flat-bottom tablet with a pH of 9.7±0.1 was added to the honeycomb, 1.0 mcg in a 0.1 m Na-bicarbonate buffer, with a concentration of 40 mcg/ml, a complex bacterial antigen was added and left at a temperature of 40s (in a thermostat) for 16 hours (one night). After the end of microbial antigen fixation, the buffer was purified using a 0.01 m Na-phosphate buffer (pH 7.3±0.1) via a 0.15 M NaCl and 0.1% tvin-20 washout. The results were calculated spectrophotometrically using the" Stat Fax-300 " (US) IFA analyzer at a wavelength of 492 nm.

Prepared protein antigens were intensively adsorbed into the solid phase carrier at high values of the transferred active reaction (rn), when their charge was negative and at maximum magnitude. At the same time, the interaction of immune reagents in the implementation of the later stages of IFA was optimal at RN physiological values. When incubating Antigen-loaded test tablets with immune reagents, it was found that a significant decrease in the RN value of the buffer medium would lead to its desorption. When this case was proven, it was achieved that this was not allowed in the later stages of test-system preparation.

In order to create an experimental test-system, it was determined and indicated that the solid-phase carrier used in the experiment (96-hole flat-bottomed polystyrene tablet) should have the following characteristics:

it must have a high binding capacity compared to the immobilizing reagent;

the property of desorption of the reagent used in small quantities;

low level of nospesific binding;

must have a reversible property.

The results obtained Taranov A.G. [2000] did not differ from the results. In the case of the origin in the above, solid phase carriers – 96-honeycomb flat-bottom polystyrene tablets-were selected by us to meet these requirements for the experiment.

As you know, according to the method of processing the surface of the tablet, the solid-phase polystyrene carriers currently used for passive adsorption are divided into 3 types:

Type X-Charge index 1, Surface characteristic non-polar hydrophobic, IgG bond average;

Type Y-charge index 12, surface characteristic polar hydrophobic, IgG bond effective;

Type Z, Charge index 200, surface characteristic polar hydrophilic, IgG binding is inefficient.

In experiments conducted by us, all three types of carriers were examined. Due to the fact that the indicators of the X and Y types of carriers are the same, we included only the data of carriers of the X and Z types in our dissertation work. The effect of the RN environment used on binding proteins to X and Z polystyrene is shown in Figures 1 and 2, respectively.

The sorbtsional nature of X polystyrene with a hydrophobic surface has been shown to have a strong correlation with the RN and ionic strength of the solution (Figure 1).

In the experiment, optimal results were obtained when a low RNase buffer solution and a Z-type carrier were used to match the isoelectric point of the protein (fig. 2).

The protein antigens of the strains of microorganisms used in the experiment were adsorbed for 14 hours in 40s harort. After The Binding of immune complexes in the Test-tablet, the blocking of vacancies in the solid-phase carrier was carried out in the later stages of the analysis in order to prevent the nospesific Binding of other immune reagents to the Fixated antigen. Various proteins and noionic detergents were used – a 1% solution of bull whey albumin, a 0.5% gelatin solution, a 5% normal serum solution, solutions such as a 0.05-0.5% Triton X-100 and a twine-20.

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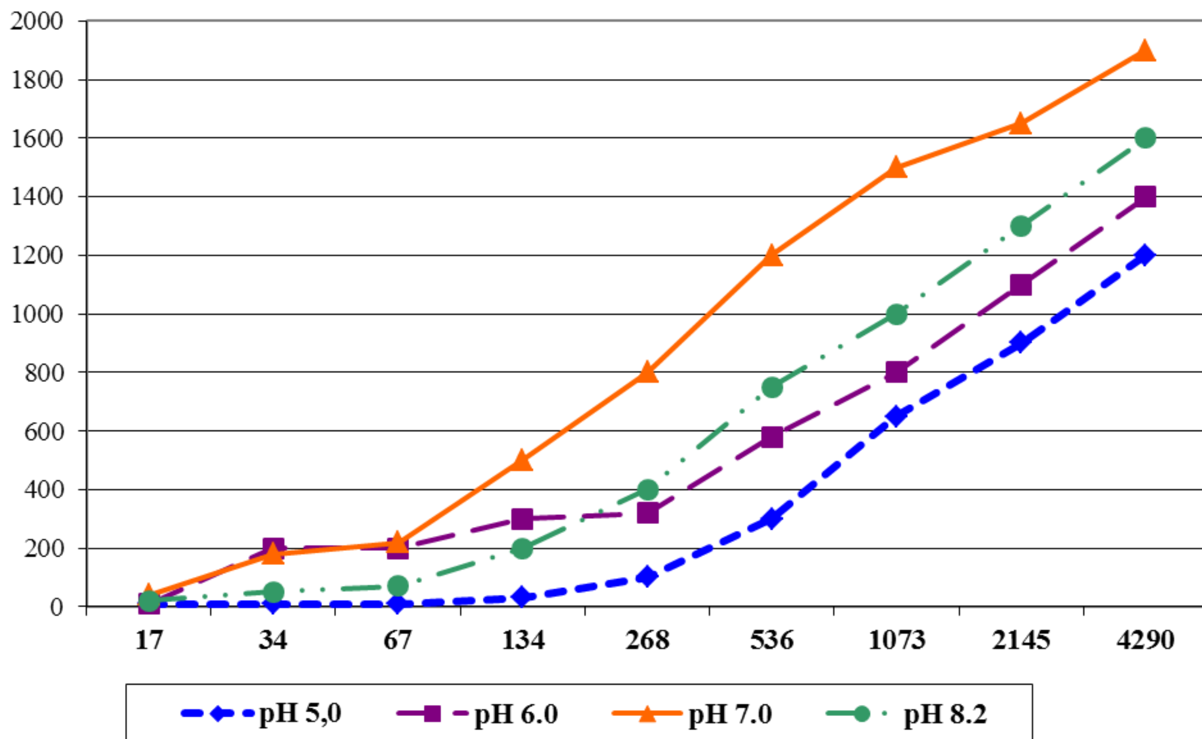

Figure 1. The effect of RN in the buffer on The Binding of serum antibodies toa type x solid-phase polystyrene 96-honeycomb flat-bottom tablet, R amng/ml.

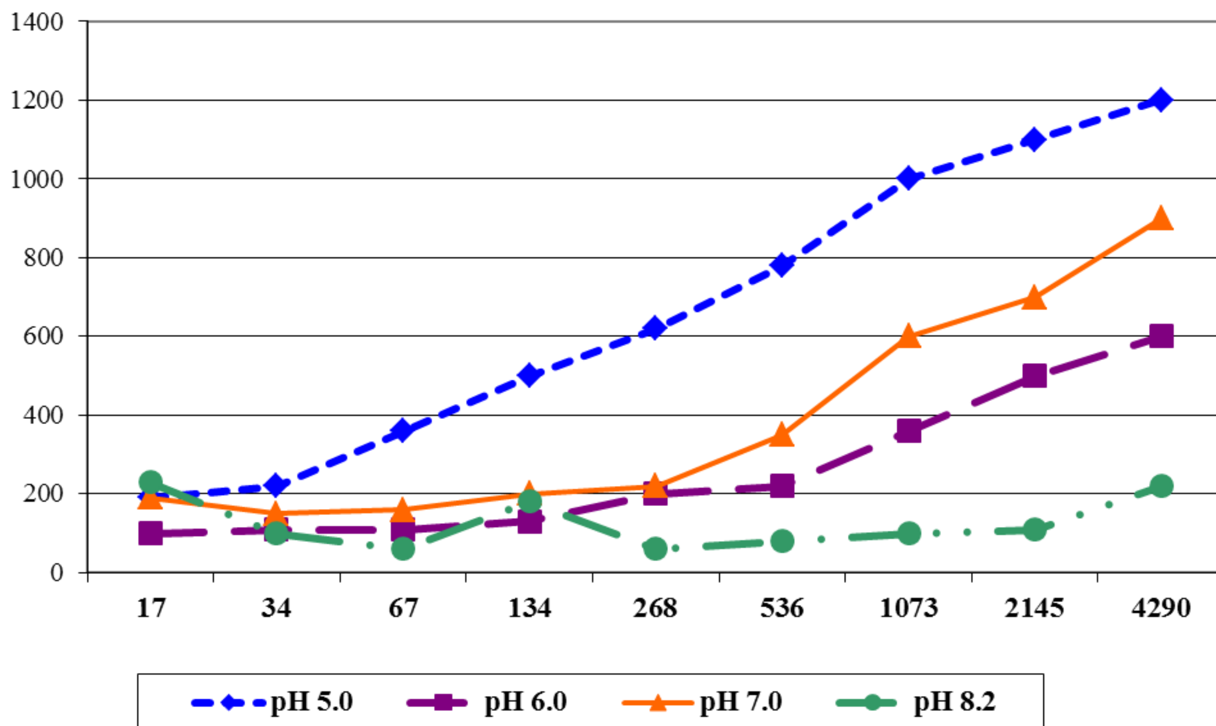

Figure 2. The effect of RN in the buffer on The Binding of serum antibodies to a type Z solid-phase polystyrene 96-honeycomb flat-bottom tablet, R am, ng/ml.

The blockers and immune reagents used were dissolved in the same buffer solution as the adsorbed antigen. The obtained immune adsorbents were stored for a long time (for 8 months) at a temperature of 40s without losing the specific activity of immune reagents. In an attempt to prevent their desorption, a 0.02% sodium azide-retaining blocking solution was added. Since sodium azide is an inhibitor of peroxidase of erkalampir (horseradish), an experimental test-tablets were washed with a buffer solution to stop the action of 0.02% sodium azide before incubating with an immune reagent that is conjugated with this enzyme.

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The trial substrates of adsorbed immune reagent and the incubation period and conditions in the experiment with reagents at different stages of IFA were empirically selected [12.14.16.18.20.22].

Thus, the results obtained to assess the importance of an active reaction to attach an antigen to a solid-phase carrier for IFA showed that when choosing a solid-phase carrier for IFA (96-honeycomb flat-bottomed polystyrene tablet) and fixing an antigen to it, the RN value of the buffer medium directly affecting the adsorption level of loaded antigens should be taken into account, as well, the activity was obtained using a buffer solution with a low reaction (rn 5,0).

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