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ANALYSIS OF AN ACTIVE REACTION FOR ANTIGEN ATTACHMENT TO SOLID PHASE CARRIER IN IMMUNOENZYME ANALYSIS.

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Abstract:

The purpose of the study. The purpose of the experiment is to evaluate the effect of environmental pH on the degree of attachment of antigens of microorganisms to 96-well polystyrene tablets. It was found that for the selection of a solid phase carrier and attachment of an antigen to it, it is necessary to take into account the pH of the environment, which directly affects the level of antigen adsorption

Keywords: Immunoenzymatic analysis, solid phase carrier, buffer medium, protein antigens.

INTRODUCTION. Enzyme immunoassay (IFT) is a laboratory immunological method of qualitative determination and quantitative measurement of antigens [1, 2]. The IFT method was proposed in the 70s of the last century in Sweden by Engvall and Perlmann, van Weemen and Schuur et al. in the USA [3, 4].

The essence of the enzyme immunoassay is the specific interaction of the antibody and the stimulus antigen, followed by the addition of the conjugate to the resulting complex (human IgM, IgG, IgA immunoglobulins against the type targeted by the enzyme). The enzyme breaks down the chromogenic substrate and produces a colored product that can be detected visually or photometrically. Registration of reaction results is carried out in special photometers with a vertical beam at a certain wavelength. The result is expressed in optical density units. [1, 5].

There are many setup options, of which heterogeneous solid phase IFT (indirect ELISA) has become the most practical. Most commercial diagnostic kits use 96-well tablets as the solid phase [5, 6, 7].

Fixation of the stimulus antigen on 96-well tablets depends on many factors, one of the main of which is the active reaction of the buffer medium (rN).

In this regard, **the aim of the study** was to evaluate the effect of the active response (rN) of the medium on the level of antigen fixation of microorganisms on 96-well polystyrene tablets in an experiment.

MATERIAL AND METHODS. Gram-positive cocci, gram-negative bacteria, Candida sp. For comparison, antigens were obtained from Pseudomonas aeruginosa, Klebsiella pneumoniae, and Candida albicans (Table 1). Cultures of microorganisms were obtained from UzR SSV EMYuK "microorganisms of human infections".

Table 1
In the experiment, a complex microbial antigen was obtained, cultures of microorganisms.

Strains	Registration number no	Source of acquisition
Pseudomonas aeruginosa	003778/155	C opr o culture
Enterococcus faecalis	003460/ "SV"	Urine culture
Staphylococcus aureus	003994 / Wood - 46	Hemoculture
Staphylococcus aureus	003702 /14-B	B iliculture
Staphylococcus epidermidis	003063 \306	Pus in bones
Staphylococcus epidermidis	004145 / MZ-87	Nasal mucosa
Klebsiella pneumoniae	000691/691	Coproculture
Escherichia coli	002673/477	Coproculture
Candida albicans	/ 7	Palate mucosa

Inactivated bacterial strains in the form of substances germ body 10^{9} was used in the amount of body/ ml concentration, heated to 80^{0} C for 30 minutes. This mode allows you to preserve the structure of proteins and neutralize the bacterial suspension. Rabbit IgGs were used as antibodies in the experiment

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RESULTS AND DISCUSSION. Antigens according to Buaven are complex microbial antigens, which were isolated by extracting the micro-organisms from the daily inoculum with the help of trichloroacetic acid.

It is known that in order to load antigens 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) is used. Their rN values and ionic strength can change [6].

To create experimental test systems, 1.0 μg of antigen in 0.1 M Na-bicarbonate buffer with pH 9.7±0.1 was added to the wells of a polystyrene 96-well tablet and left for 16 hours at ^{40} C. After microbial antigen fixation, the buffer was purified by washing with 0.01M Na-phosphate buffer (pH 7.3±0.1) with 0.15M NaCl and 0.1% Twin-20. The results were calculated spectrophotometrically at a wavelength of 492 nm using an immunoenzymatic tablet analyzer "Stat Fax-300" (USA).

Are intensively adsorbed to the solid phase carrier at high values of the active reaction (rN), when their charge is negative and maximal. At the same time , the interaction of immune reagents during the subsequent stages of IFT was optimal at physiological values of rN. During the incubation of antigen-loaded test-lancets with immune reagents, it was found that a significant decrease in the rN value of the buffer medium leads to its desorption.

In order to create an experimental test-system, the solid-phase carrier used in the experiment (96-well polystyrene tablet): should have a high binding capacity compared to the immobilized reactant; the ability to desorb a small amount of reagent; having a low level of non-specific binding; should be renewable [5]. We have selected test tablets that meet these requirements for the experiment.

According to the method of surface cleaning, polystyrene carriers currently used for passive adsorption are divided into 3 types: Type X - charge index i 1, surface characteristics , non-polar hydrophobic, IgG binding is moderate ; Type Y - charge index i 12, surface characteristic, polar hydrophobic, IgG the link is effective ; Z type , charge index i 200 , surface characteristic polar hydrophilic , IgG connection is ineffective .

All three types of carriers were tested in the experiment. K ' indicators of X and Y types of carriers due to being the same we fa q at We present the data of carriers X and Z. The effect of rN environment on the binding of proteins to polystyrene X and Z is shown in Tables 1 and 2, respectively.

The sorption property of X polystyrene with a hydrophobic surface has a strong relationship with the pH and ionic strength of the solution (Fig. 1).

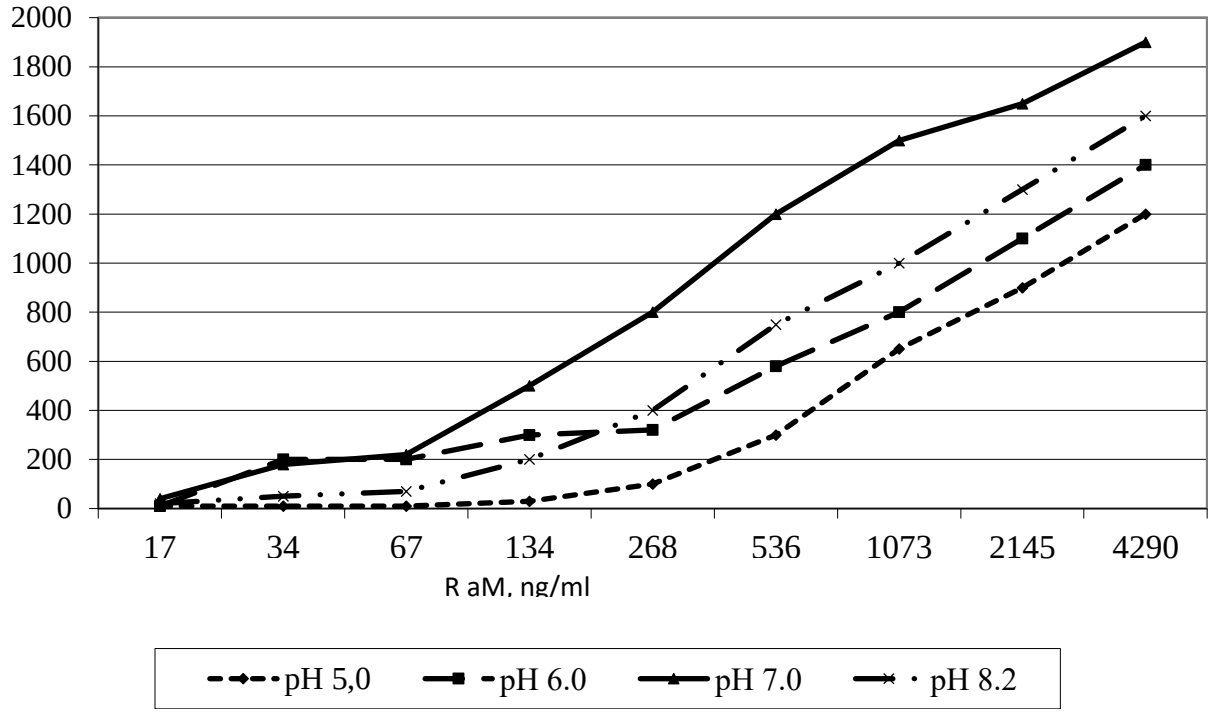

Figure 1 . Effect of rN on the binding of antibodies to X-type polystyrene .

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

Protein antigens of strains of microorganisms used in the experiment were adsorbed at a temperature of 40 C for 14 hours. After binding of immune complexes on the test plate, in order to prevent non-specific binding of other immune reagents to the fixed antigen, the blank spaces in the solid phase carrier were blocked at the next stages of the analysis. Various proteins and nonionic detergents were used - 1% solution of bovine serum albumin, 0.5% gelatin solution, 5% normal serum solution, 0.05-0.5% Triton X -100 and Tween-20 solutions.

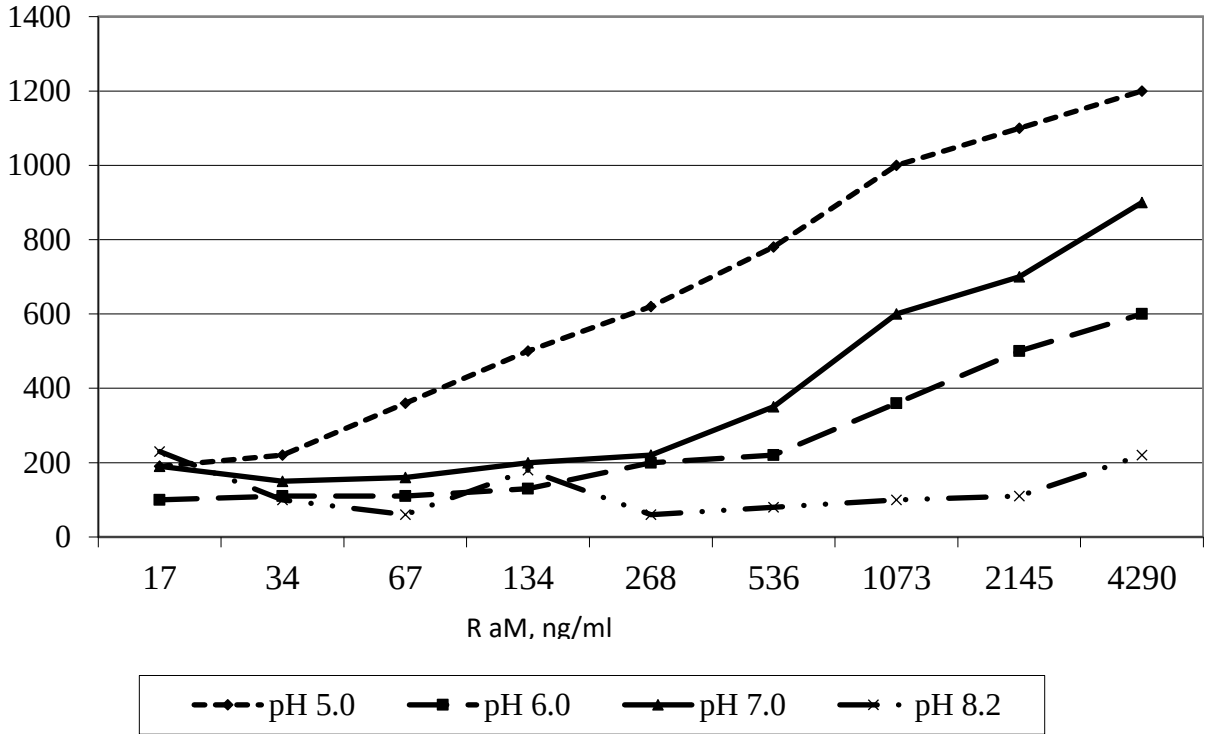

Figure 2 . Effect of rN on binding of antibodies to Z- type polystyrene .

The blocking agents and immune reagents used were dissolved in the same buffer solution as the adsorbed antigen. The resulting immune adsorbents were stored for a long time (for 8 months) without losing the specific activity of immune reagents at a temperature of 4 ^{0} C. In order to prevent their desorption, a blocking solution containing 0.02% sodium azidine was added. Because sodium azidine is an inhibitor of horseradish peroxidase, the experimental test plates were washed with a buffer solution to block the effect of sodium azidine before incubation with this enzyme-conjugated immunoreagent.

The duration and conditions of incubation of the adsorbed immunoreagent with test substrates and reagents at different stages of IFT were chosen empirically.

CONCLUSIONS

1. When choosing a solid phase carrier (polystyrene tablet with 96 wells) and fixing the antigen to it, it is necessary to take into account the rN value of the buffer medium, which directly affects the adsorption level of loaded antigens .

2. In the experiment, optimal results for the fixation of microbial antigens were obtained on negatively charged polar hydrophilic polystyrene of the Z type, using a buffer solution with a low reaction (pN)

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