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Poliovirus-binding inhibition ELISA based on specific chicken egg yolk antibodies as an alternative to the neutralization test

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ABSTRACT

The first application results of enzyme immunoassay system (ELISA) – binding inhibition ELISA (BI ELISA) for the detection of antibodies to polioviruses of three types based on the use of specific antibodies from chicken yolk (IgY) are presented. This variant of ELISA is a "surrogate" neutralization test (NT). When comparing the results of the detection of antibodies in 90 sera of children who were vaccinated with oral and inactivated poliovirus vaccines, good correlation (r = 0.67, 0.61, 0.76 for types 1, 2, 3 respectively) between the BI ELISA and the NT results was shown. The presented variant of ELISA is the further stage of the development of "IgY-technology" for laboratory diagnostics of viral infections, namely poliovirus infection, which can be used for seroepidemiological studies as an analogue of the NT which requires the use of life poliovirus with the required standard of biosafety containment facilities.

The neutralization test (NT) remains the "gold standard" in serological diagnostic and seroepidemiological studies of viral infections, in particular, infections associated with poliovirus (*Enterovirus* genus of the *Picornaviridae* family). This is primarily due to the functional nature of the NT, in which protective antibodies – which may have different avidity levels – are necessary for this assay. However, the NT has a number of known shortcomings: relatively technical complexity, low productivity and long duration (usually more than several days) in terms of obtaining result, which may limit the use for large seroepidemiological studies. In addition, the NT utilizes cell cultures and live viruses that require compliance with approved biosafety requirements. In accordance with the plans of the WHO Global Polio Eradication Initiative, work with polioviruses (wild, Sabin strains) in the near future will be sharply limited by the requirements of the biosafety containment and will be possible only in the small number of specially accredited institutions (World Health Organization (WHO, 2013). Already, such restrictions are applied to the work with poliovirus type 2 regardless of its origin (wild, vaccine-derived or Sabin strains). The inability to use classical NT with live polioviruses for diagnostic and seroepidemiological studies by most laboratories will create considerable difficulties for an adequate assessment of the epidemiological situation with poliomyelitis. Thus, the development of an alternative method for determination of the functional neutralizing antibodies to poliovirus without using live virus is very relevant. Importantly, attempts to replace the NT with known non-functional serological techniques (ELISA) to determine specific antibodies of a certain class (IgM, IgG, etc.) showed a low correlation with the NT results (Ivanov, Dragunsky, 2005). The first "functional" variant of ELISA, as a surrogate of the NT for the determination of neutralizing antibodies to poliovirus (Binding Inhibition ELISA, or blocking ELISA), was proposed by several groups since 1983 (Hagenaars et al., 1983; Edevag et al., 1995; Herremans et al., 1997; Hashido et al., 1999; Rezapkin et al., 2005; Ivanov et al., 2005; Schepp et al., 2017). All of these variants were based on the use of polyclonal and monoclonal antibodies (solid phase sensitization, detector antibodies) from laboratory animals. In this study, we present the results of the development of an ELISA as a surrogate variant of the NT for the detection of antibodies to poliovirus based on the use of specific antibodies of class Y (IgY) isolated from egg yolks of chickens immunized with poliovirus.

Leghorn laying hens (4–6 months old) were immunized 3 times with an interval of 2 weeks (Ivanov et al., 2016) with purified wild-type poliovirus strains of three types: Mahoney (type 1), MEF -1 (type 2) and Saukett (type 3), (Plotkin et al., 2018). Each virus type (approximately 9.0 $\log_{10}$ $TCID_{50}$/ml) was injected in a volume of 1 ml at four points of pectoral muscles without adjuvant (Ivanov et al., 2016). IgY preparations (from yolks taken 2 weeks after the 3rd immunization) were obtained by precipitation with sodium sulfate: two precipitation steps with 19.1% and 14.0% sodium sulfate at room temperature, two

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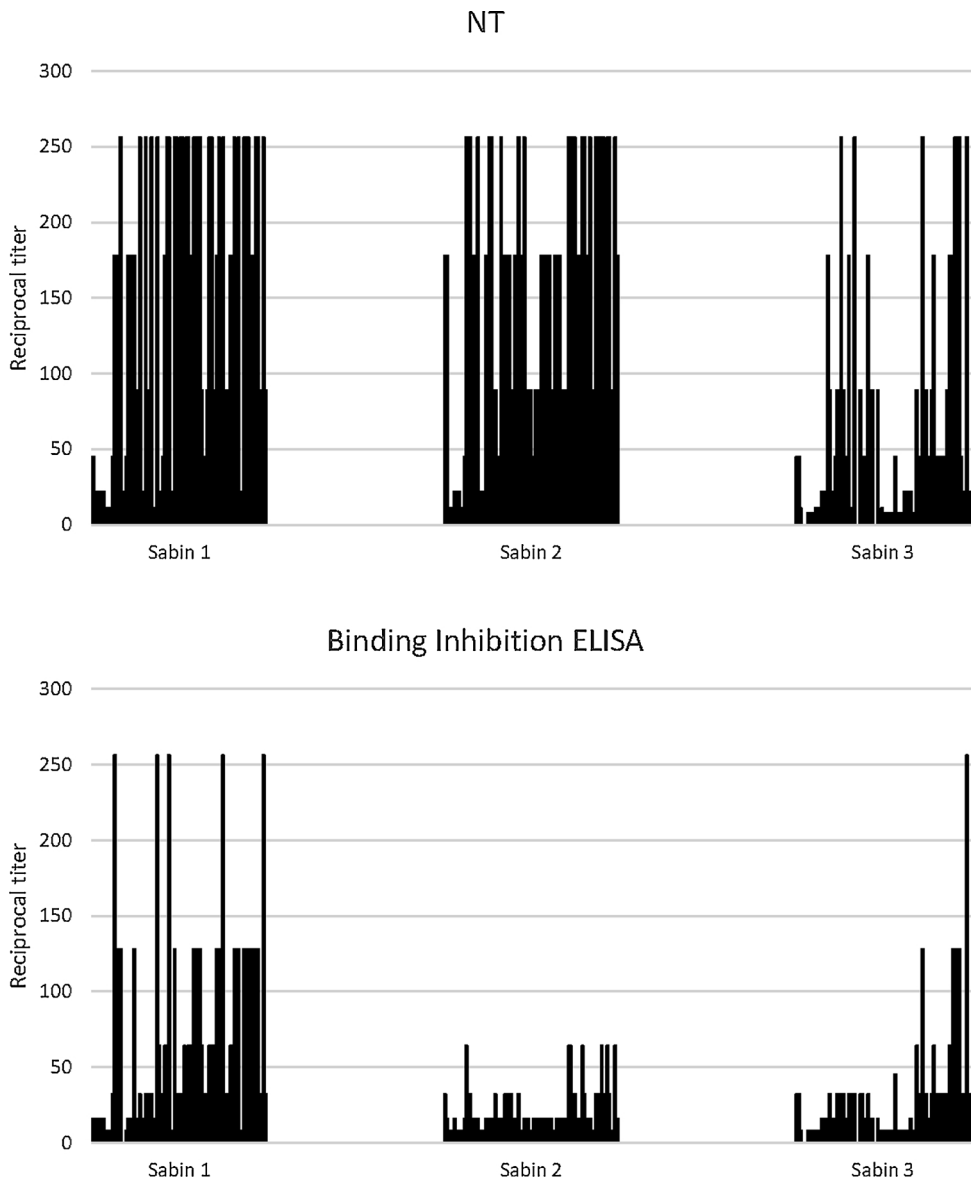

Fig. 1. Comparative assessment of the NT and BI ELISA: profiles of poliovirus specific antibodies (90 serum samples) measured against Sabin 1–3 strains.

centrifugations (10,000 and 12,000 x *g*) and dialysis against 0.01 M PBS pH 7.4 (Akita and Nakai, 1993), followed by affinity purification on a HiTrap column (Amersham) according to the manufacturer's instructions. The IgY preparations were sterilized by filtration (Millipore filters with 0.45 µm pore diameter), stored at + 4 °C and used in ELISA as immunosorbents for each type of poliovirus.

Sera of rabbits against each wild poliovirus strain were prepared according to the procedure (Ivanov et al., 2016) and used for ELISA as a detection antibody. Specific activity of purified IgY and rabbit sera was determined in the NT against wild-type poliovirus types 1, 2, 3 on the HEp-2 cell culture according to the standard WHO protocol (World Health Organization (WHO, 1997). For ELISA, IgY preparations with NT titers > 1: 2048 and sera of rabbits > 1:1024 were used. As standard antigens, laboratory series of monovalent inactivated poliovirus vaccines (types 1, 2, 3) based on Sabin strains (s-IPV) were used (Ivanov et al., 2016).

Sera were obtained from a bank of sera collected in the frame of an annual national study to determine the level of collective immunity to poliomyelitis, performed on the order of The Russian Federal Service for Surveillance on Consumer Rights Protection and Human Wellbeing (Rospotrebnadzor). Written informed consent was obtained from all subjects (their legal representatives) by the primary clinical sites. A total of 90 sera from children (51 boys, 39 girls) aged 3–4 years were investigated. All children were immunized against poliomyelitis in accordance with the National Immunisation Schedule of the Russian Federation and received the following polio vaccination course: 2 IPV (Inactivated Poliovirus Vaccine) vaccinations at age 3 and 4,5 months and 3 OPV (trivalent Oral Poliovirus Vaccine) revaccinations at age 6, 18, 20 months. Prior to the study by the NT and BI ELISA, the sera were subjected to a single freeze/thaw (−20 °C) cycle.

Binding Inhibition ELISA (BI ELISA). This variant was developed on the basis of previously described methods (Ivanov et al., 2005, 2014). The Costar immunoplates (Corning, 9018) were coated 18 h at + 4 °C in the humid atmosphere with IgY preparations against wild poliovirus types at the concentration of 10 µg/ml (100 µl per well) in carbonate-bicarbonate buffer pH 9.6 (Sigma, C3041-100 CAP). In parallel, in Costar nonbinding microplates (Corning, 3798), mixtures of the test sera with standard antigens (laboratory series of monovalent inactivated poliovirus vaccines (types 1, 2, 3) based on Sabin strains (s-IPV) (Ivanov et al., 2016) were prepared: 60 µl of serum in 2-fold dilutions starting from 1:8 to 1:1024 in ELISA buffer (phosphate buffered saline, PBS, pH 7, 2, 1% calf serum (Gibco) and 0.05% Tween-20), and

60 µl of s-IPV (types 1, 2, 3) in ELISA buffer at dose of 4–8 antigenic units (according to titration data of D-antigen). As controls (control of 100% binding of antigen), mixtures of standard antigens were used in the indicated doses with ELISA buffer. The mixtures of test sera and standard antigens were kept at room temperature for 18 h (+ 22–25 °C). The immunoplates with adsorbed specific IgY were washed 2 times with washing solution (physiological saline with 0.05% Tween-20), then PBS solution with 1% calf serum (Gibco) was added (200 µl/well) to block unbound sites and incubated in humid atmosphere (CO_2 incubator) for 1 h at +37 °C. After next washing (2 times), the mixture of test sera and standard antigens were added (100 µl /well) and incubated in the humid atmosphere for 2 h at + 37 °C. After washing (5 times), detector antibodies - rabbit antisera to types 1, 2, 3 of wild-type polioviruses diluted 1:500 in ELISA buffer were added (100 µl per well) and incubated in the humid atmosphere for 1 h at + 37 °C. The wells were washed 5 times, peroxidase conjugate against rabbit IgG (monoclonal anti-rabbit IgG, γ-chain specific, Sigma, A1949-1VL) was added (100 µ/well), incubated in the humid atmosphere for 1 h at + 37 °C. The wells were washed 5 times, TMB substrate (Sigma, T0440-100 ml) was added (100 µl/well), incubated for 30 min in the dark chamber at room temperature, the reaction was stopped with 2 M sulfuric acid (50 µl/well). The optical density (OD) was measured at 450 nm. The result was considered positive if the OD of wells with poliovirus antigen plus test serum was decreased by ≥50% compared to control (antigen plus ELISA buffer), and the result was expressed as end-point titer (Ivanov et al., 2005). Example: mean OD value of standard antigen + ELISA buffer = 0.560, so positive result (end-point titer) is ≤0.280 (test serum dilution giving OD reduction by ≥50%).

Neutralization test. The sera were tested against Sabin strains (type 1 - LSc 2 ab, type 2 - P712, Ch, 2 ab, type 3 – Leon, 12a_1b) on HEp-2 cell culture according to the WHO protocol (World Health Organization (WHO, 1997). The internal standard (human serum sample with antibodies against Sabin strains titrated in parallel with The Second International Standard for anti-poliovirus sera – SIS 66/202) was used as the reference serum.

Statistical analysis. To determine the correlation coefficient (r), a regression analysis was used when comparing the results of the NT and BI ELISA.

Fig. 1 shows the results of two tests comparing the NT and BI ELISA with the titration of 90 sera. Arithmetic mean values of the NT/BI ELISA titers were: for type 1–128/60; for type 2–131/20 and for type 3–54/25. In this case, the sensitivity of BI ELISA relative to the NT for type 1 was 98% (88/90), for type 2–100% (100/100), for type 3–98% (88/90). Correlation coefficient r for type 1–0.67, for type 2–0.61, for type 3–0.76, which corresponds to the results obtained by other authors, for example, Hashido et al (Hashido et al., 1999). Similar results were also obtained earlier when specific antibodies of mammals (rabbits) were used as an immunosorbent (Ivanov et al., 2005).

A possible problem with the use of inactivated polioviruses (laboratory series of IPV based on Sabin strains) as standard antigens for the detection of antibodies is the change in antigenic determinants due to formalin exposure. However, good correlation between the NT and BI ELISA results (r = 0.67, 0.61, 0.76 for types 1, 2, 3 respectively) and sensitivity (98–100%) of BI ELISA-relative to the NT obtained in this study indicate the absence of possible changes in antigenic determinants. Of course, the NT, being a biological (functional) test, remains out of competition, but considering the requirements of the present containment issues, surrogate version of the NT (BI ELISA) using specific yolk IgY as an immunosorbent, can act as reliable alternative to the NT. One of the main positive aspects of BI ELISA is the possibility to use it for large-scale seroepidemiological studies, serological monitoring of the effectiveness of poliovirus vaccines, etc. The BI ELISA, like the NT, does not have a linear response, therefore the comparison of these methods is largely based on the coincidence of the positive-negative results (the BI ELISA sensitivity relative to the NT).

The use of immunoglobulins of class Y ("IgY-technology") for various immunoassays (ELISA, RIA, fluorescent antibody technique, immunochistochemistry) in recent years has demonstrated the effectiveness of this approach (Schade et al., 2005). Despite the fundamental similarity of the two analogs (mammalian IgG and avian IgY), whose biochemical characteristics are well-studied (Shimizu et al., 1992), IgY has several advantages, especially valuable for its use in diagnostic purposes (as immune reagents): 1) birds high immunoreactivity against foreign proteins of infectious origin (viral, bacterial, parasitic), as well as to proteins of toxins and poisons; 2) low cross-reactivity with mammalian proteins due to large phylogenetic distance between birds and mammals: the inability of IgY to activate the mammalian complement system, the inability to bind to the rheumatoid factor and the Fc receptors, which provides low level of nonspecific reactions (Schade et al., 2005).

Thus, the presented results demonstrate the further development of "IgY-technology" in combination with a surrogate version of the NT (BI ELISA) for use in the large-scale seroepidemiological studies of poliomyelitis. The simplicity of obtaining preparative quantities of highly active specific yolk immunoglobulins as an immunosorbent, their low cross-reactivity with mammalian proteins (human sera being tested) makes their use in various serological techniques extremely promising.

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Conflict of interest

None declared.

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References

Akita, E.M., Nakai, S., 1993. Comparison of the four purification methods for the production of immunoglobulins from eggs laid by hens immunized with an enterotoxigenic E. coli strain. J. Immunol. Meth. 160, 207–214.

Edevag, G., Wahren, B., Osterhaus, A.D.M.E., Sundquist, V.-A., Grandström, M., 1995. Enzyme-linked immunosorbent assay-based inhibition test for neutralizing antibodies. J. Clin. Microbiol. 33, 2927–2930.

Hagenaars, A.M., Van Delft, R.W., Nagel, J., Van Steenis, G., Van Wezel, A., 1983. A modified ELISA technique for the titration of antibodies to polio virus as an alternative to a virus neutralization test. J. Virol. Methods 6, 233–239.

Hashido, M., Horie, H., Abe, S., Doi, Y., Hashizume, S., Agboatwalla, M., Isomura, S., Nishio, O., Hagiwara, A., Inouye, S., 1999. Evaluation of enzyme-linked immunosorbent assay based on binding inhibition for the type-specific quantification of poliovirus neutralization-relevant antibodies, 1999. Microbiol. Immunol. 43 (1), 73–77. https://doi.org/10.1111/j.1348-0421.1999.tb02375.x.

Herremans, M.M.P.T., Reimerink, J.H.J., Ras, A., van der Avoort, H.G., Kimman, T.G., van Loon, A.M., Conyn-Van, Spaendonck M.A., Koopmans, M.P., 1997. Evaluation of a poliovirus binding-inhibition assay as an alternative to the virus neutralization test. Clin. Diagn. Lab. Immunol. 4, 659–664.

Ivanov, A.P., Dragunsky, E., 2005. ELISA as possible alternative to the neutralization test for evaluating the immune response to poliovirus vaccines. Expert Rev. Vaccines 4 (2), 167–172. https://doi.org/10.1586/14760584.4.2.167.

Ivanov, A.P., Dragunsky, E.M., Ivanova, O.E., Rezapkin, G.V., Potapova, S.G., Chumakov, K.M., 2005. Poliovirus-binding inhibition ELISA for evaluation of immune response to oral poliovirus vaccine. A possible alternative to the neutralization test. Hum. Vaccine 1 (3), 102–105.

Ivanov, A.P., Kozlov, V.G., Klebleeva, T.D., Ivanova, O.E., Kiktenko, A.V., 2014. An enzyme-linked immunosorbent assay based on specific class Y (IgY) antibodies from egg yolks to quantify D-antigen in inactivated poliovirus vaccines. Probl. Virol. 6, 39–42 (In Russian).

Ivanov, A.P., Klebleeva, T.D., Ivanova, O.E., Ipatova, E.G., Gmyl, L.V., Ishmukhametov, A.A., 2016. Experimental approaches to the development of inactivated poliovirus vaccine based on Sabin strains. Epidemiol. Vaccine Prev. 15 (4), 59–64. https://doi.org/10.31631/2073-3046-2016-15-4-59-64. (In Russian).

Plotkin, S.A., Orenstein, W.A., Offit, P.A., Edvards, K.M., 2018. Vaccines, seventh edition. Elsevier, pp. 841–865.

Rezapkin, G., Martin, J., Chumakov, K., 2005. Analysis of antigenic profiles of inactivated poliovirus vaccine and vaccine-derived polioviruses by block-ELISA method. Biologicals 33 (1), 29–39. https://doi.org/10.1016/j.biologicals.2004.11.001.

Schade, R., Calzado, E.G., Sarmiento, R., Chacana, P.A., Porankievich-Asplund, J., Terzolo, H.R., 2005. Chicken egg yolk antibodies (IgY-technology): a review of progress in production and use in research and human and veterinary medicine. ATLA 33, 129–154.

Schepp, R.M., Berbers, G.A., Ferreira, J.A., Reimerink, J.H., van der Klis, F.R., 2017. A novel multiplex poliovirus binding inhibition assay applicable for large serosurveillance and vaccine studies, without the use of live poliovirus. J. Virol. Methods 241, 15–23. https://doi.org/10.1016/j.jviromet.2016.12.006.

Shimizu, M., Nagashima, H., Sano, K., Hashimoto, K., Ozeki, M., Tsuda, K., Hatta, H., 1992. Molecular stability of chicken and rabbit immunoglobulin G. Biotechnol. Biochem. 56, 270–274.

World Health Organization (WHO), 1997. Manual for Virological Investigation of Poliomyelitis. WHO/EPI/GEN/97.1. WHO, Geneva. http://whqlibdoc.who.int/hq/1997/WHO_EPI_GEN_97.01.pdf.

World Health Organization (WHO) Polio Eradication and Endgame Strategic Plan 2013-2018. http://www.polioeradication.org/Portals/0/Document/Resources/StrategyWork/PEESP_EN_A4.pdf46.