Recombinant Human Parvovirus B19 VP2 VLP

for Parvovirus B19 Antibody Detection

Introduction

For many years, enzyme-linked immunosorbent assay (ELISA) was routinely used for in-vitro diagnostics of infectious diseases. As advanced automation entered routine laboratories, and new needs for flexibility in testing arose, chemiluminescent immunoassays (CLIA) became more and more popular. CLIA technology is a very versatile and reliable method allowing fast and economic testing of patient samples. However, with the increasing complexity of CLIA, developers also face new technical challenges, e.g. demands for higher quality of raw materials or optimization of bead coupling.

Virion\Serion's Parvovirus B19 VP1 Antigen (BA122VSVP1) will now be complemented with our newly developed **Human Parvovirus B19 VP2 VLP (BA122R01)**. Our former Parvovirus B19 VP2 VLP antigen (BA122VSVP2) will be discontinued and replaced by **Human Parvovirus B19 VP2 VLP (BA122R01)** due to improved production processes resulting in a higher quality and availability.

The protein production in an ISO 13485 certified environment guarantees the expected high quality, traceability and lot-to-lot consistency paired with readily available bulk quantities.

Background

Parvovirus B19 is the most important member of the parvovirus family with respect to clinical human disease. It is found worldwide and replicates in the actively dividing erythroid cells.

Transmission of virus from host to host is primarily via oral and nasal secretions, and in many cases infection is inapparent. Approximately 10 % to 15 % of those infected develop, after an incubation time

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of 13 days to 17 days, Erythema infectiosum which manifests as a mild rash spreading over the cheeks and progressing to the trunk and limbs which can last up to three weeks. Complications include arthralgia and lymphadenopathy. In developed countries, 2 % to 10 % of children are infected by the age of five, while half of people over the age of 20 and 85 % of people over the age of 70 have serological evidence of exposure to the virus. Infection during pregnancy of a non-immune woman leads in 30 % of cases to transmission of the virus to the fetus and in 7 % of such cases results in Hydrops fetalis.

Serological testing of Parvovirus B19 antibodies is indicated for patients, from the groups mentioned above, with uncharacteristic clinical pictures, with suspected acute or recent infection. This applies especially to pregnant women in institutions such as schools, hospitals and child-care facilities.

The human Parvovirus B19 capsid consists of two capsomer proteins, VP1 and VP2. Accounting for 96 % of all capsid proteins, VP2 is the major structural protein [1].The use of high-quality VP2 antigens is essential, especially in IgM diagnostics [2].

Virion\Serion's **Human Parvovirus B19 VP2 VLP (BA122R01)** consists of multiple VP2 capsid proteins (60 kDa) which are expressed in insect cells and self-assembled into an icosahedral capsid (virus-like particle (VLP)). Furthermore it's highly purified by His-tag affinity chromatography ensuring high quality and availability. Read on to learn more about efficient IgG and IgM detection with the new Human Parvovirus B19 VP2 VLP (BA122R01).

Order Information and Related Products

Code	Description	Packaging
BA122R01 ***NEW!***	**Human Parvovirus B19 VP2 VLP** Human Parvovirus B19, recombinant VP2 VLPs (VP2 virus like particles, conformational epitopes, VP1 aa 228-781). Multiple VP2 capsid proteins (60 kDa) self-assembled into an icosahedral capsid (VLP) Source: recombinant protein, expressed in SF9 insect cells Affinity tag: His-Tag	1 mg
BA122VSVP1	**Parvovirus VP1** Parvovirus B19, recombinant VP1 (full length, linear epitopes, VP1 aa 1-781) Source: recombinant protein, expressed in E. Coli Molecular weight: 87 kDa	1 mg

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Material and Methods

Antigen

Recombinant Human Parvovirus B19 VP2 VLP (BA122R01) is produced in insect cells and highly purified by His-tag affinity chromatography.

Immunoassay

Human Parvovirus B19 VP2 VLP was immobilized on magnetic beads with 5 µm in diameter, similar to those which are commonly used for CLIA. The coupling of the antigens as well as the subsequent immunoassays were performed in an automated workflow on a KingFisher™ Flex System (ThermoFisher Scientific™). For IgG or IgM detection, antigen coupled magnetic particles were incubated with diluted human sera for 15 minutes at 37 °C and then washed. Bound Parvovirus IgG or IgM antibodies were detected after 15 minutes incubation at 37 °C with fluorescently labeled secondary antibody by using a Cytoflex S flow cytometer (Beckman Coulter). Reference ELISA were performed according to manufacturer's instructions.

Dynamic light scattering and electron microscopy

Dynamic light scattering (DLS) on a Zetasizer Ultra (Malvern Panalytical GmbH) was used for the measurement of size distribution and the detection of aggregates of the different Parvovirus B19 VP2 VLP lots in solution. For evaluation, the volume distribution was used, which shows the total volume of particles in the different size bins.

For electron microscopy, the sample was dripped onto copper mesh, stained with uranyl acetate (negative staining procedure) and then examined under a Transmission Electron Microscope JEM-2100 (JEOL) at 200 kV.

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Results

Biochemical Analysis

Three independent lots of recombinant Parvovirus B19 VP2 VLP were analyzed using dynamic light scattering (DLS) to check the homogeneity. Figure 1 shows the comparison of the of three independent Parvovirus VP2 VLP lots in direct comparison with the discontinued BA122VSVP2 highlighting a strong consistency and homogeneity with less than 1 % aggregates detected by DLS in all lots. In addition, the discontinued BA122VSVP2 was also analyzed to demonstrate the high level of comparability. The hydrodynamic diameter of 35.9 d.nm ± 2.52 d.nm is very close to the diameter of actual Parvoviruses[3].

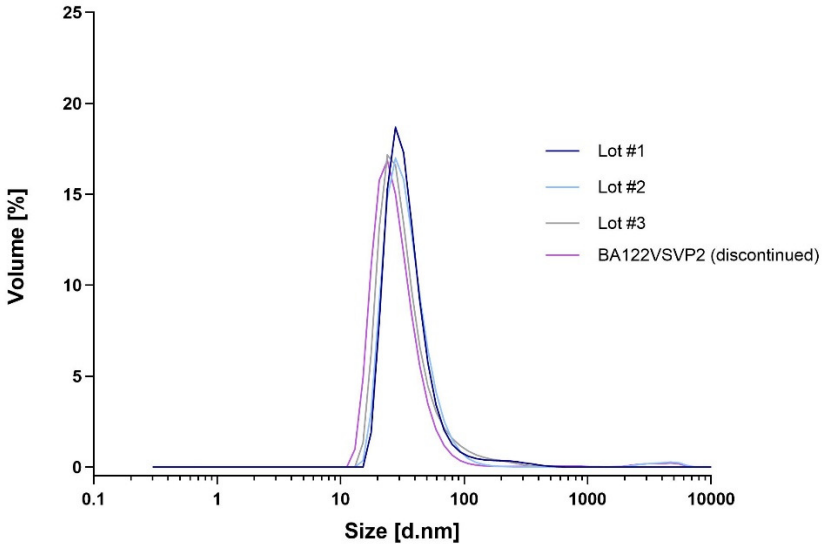

Figure 1: DLS analysis (volume distribution) of three independent lots of recombinant Parvovirus VP2 VLP (BA122R01) and discontinued BA122VSVP2. Peak overlay illustrates a high conformity.

In addition three independent lots of BA122R01 and the discontinued BA122VSVP2 were compared by SDS-PAGE. The excellent lot to lot consistency is shown in Figure 2. In summary, these analytical methods demonstrate a high level of purity and homogeneity within the independent lots.

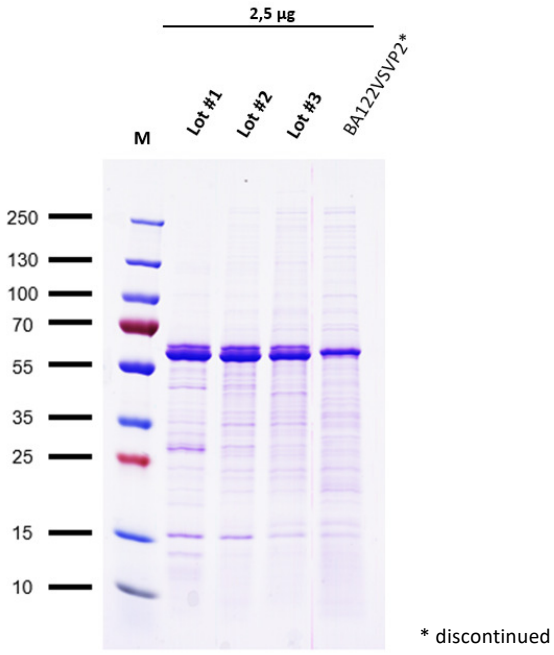

Figure 2: Comparison of three independent lots of BA122R01 and discontinued BA122VSVP2 by SDS-Page, stained with Coomassie solution.

Electron microscopy

In order to prove the formation of virus-like particles, electron micrographs were taken. Figure 3 clearly shows that BA122R01 is perfectly present as a virus-like particle with a diameter of approximately 30 nm. This is in perfect agreement with the DLS results shown in Figure 1.

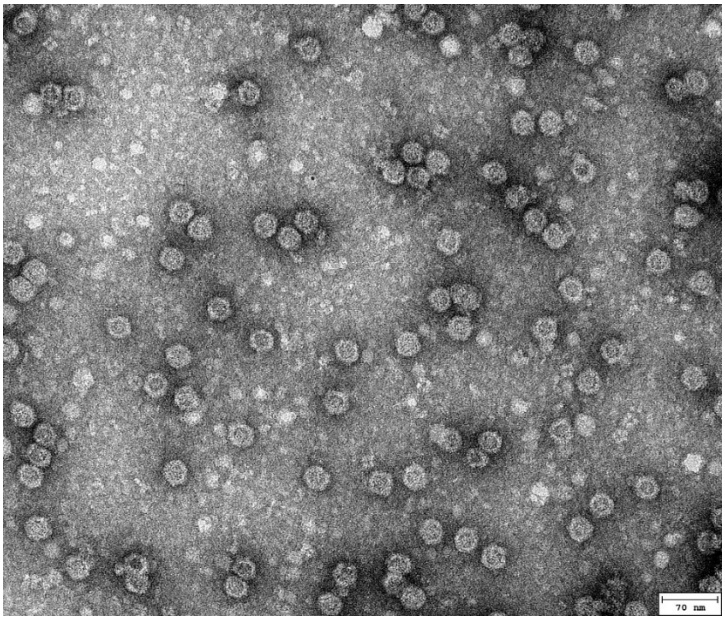

Figure 3: Electron microscopy was performed to prove VLP-formation. A sample of BA122R01 was dripped onto copper mesh, stained with uranyl acetate and examined under an electron microscope at 200 kV.

Lot to Lot Consistency

Binding of IgG and IgM antibodies by two independent lots of Human Parvovirus B19 VP2 VLP was assessed by bead-based immunoassay with a fluorescence readout using a flow cytometer. Therefore 13 human serum samples and dilution series of two high positive human serum samples for IgG and 14 human serum samples and dilution series of two high positive human serum samples for IgM, covering a wide range from Parvovirus IgG or IgM negative to highly positive, were evaluated.

Using Passing-Bablok regression analysis, very high correlation coefficients of $r \geq 0.98$ could be achieved (Figure 4), underlining a high lot to lot correlation. The observed slopes for IgG of 0.89 and IgM of 0.92 indicate good reactivity for both antigen lots (Figure 4). In summary, the high reproducibility proves excellent lot to lot consistency.

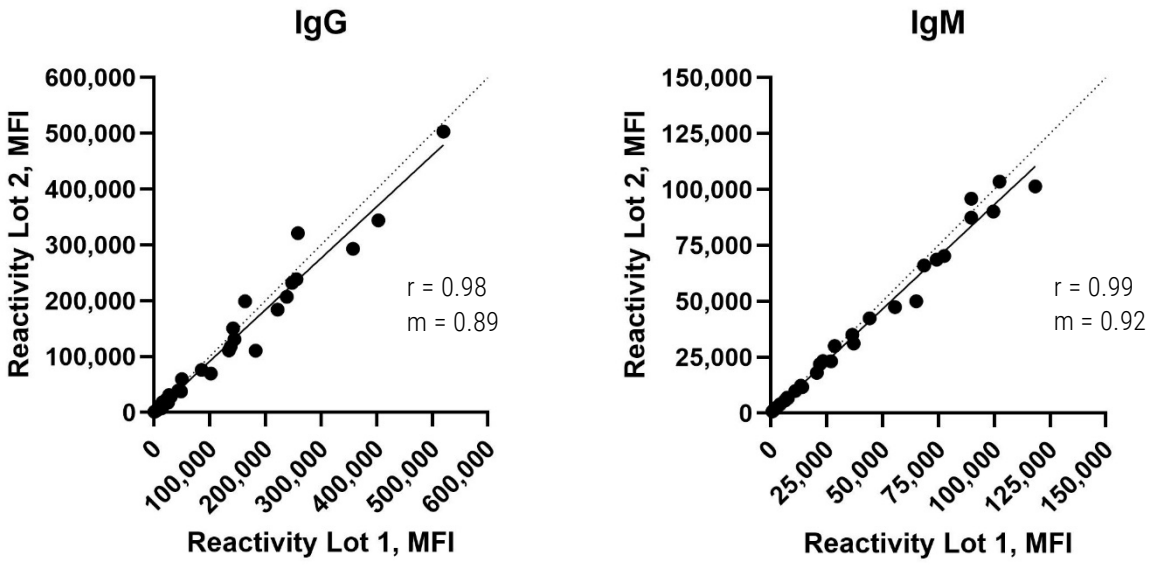

Figure 4: A comparison of two independent lots of Human Parvovirus B19 VP2 VLP was done using an IgG and IgM bead-based immunoassay. As a fluorescent read-out was performed, the assay signals are displayed as median fluorescence intensity (MFI). The statistical analysis was performed using Passing-Bablok regression. Pearson correlation coefficient r = 0.98 (IgG) and r = 0.99 (IgM) as well as slope m = 0.89 (IgG) and m = 0.92 (IgM).

Reactivity and Measurement Range

In order to evaluate the reactivity of IgG and IgM antibodies towards the recombinant Human Parvovirus B19 VP2 VLP antigen, human serum samples were tested using a bead-based immunoassay. The positive and negative status of the samples was confirmed by two commercially available parvovirus IgG and IgM ELISAs, respectively.

For this purpose, 87 human serum samples, comprising 44 positive and 43 negative samples, were subjected to IgG analysis. The mean reactivity observed for positive samples was 268,720 MFI, in comparison to 6,410 MFI for negative samples (Figure 5). The ratio between the mean value of the

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positive samples and the mean value of the negative samples (p/n ratio) was 42, indicating a high potential for distinguishing between positive and negative samples.

A total of 79 human samples were subjected to IgM analysis, of which 33 exhibited positive results and 46 displayed negative outcomes. The median reactivity observed for positive samples was 60,918 MFI, in comparison to 999 MFI for negative samples (Figure 5). The p/n ratio was 61, indicating that the recombinant Human Parvovirus B19 VP2 VLP antigen demonstrates excellent IgM reactivity.

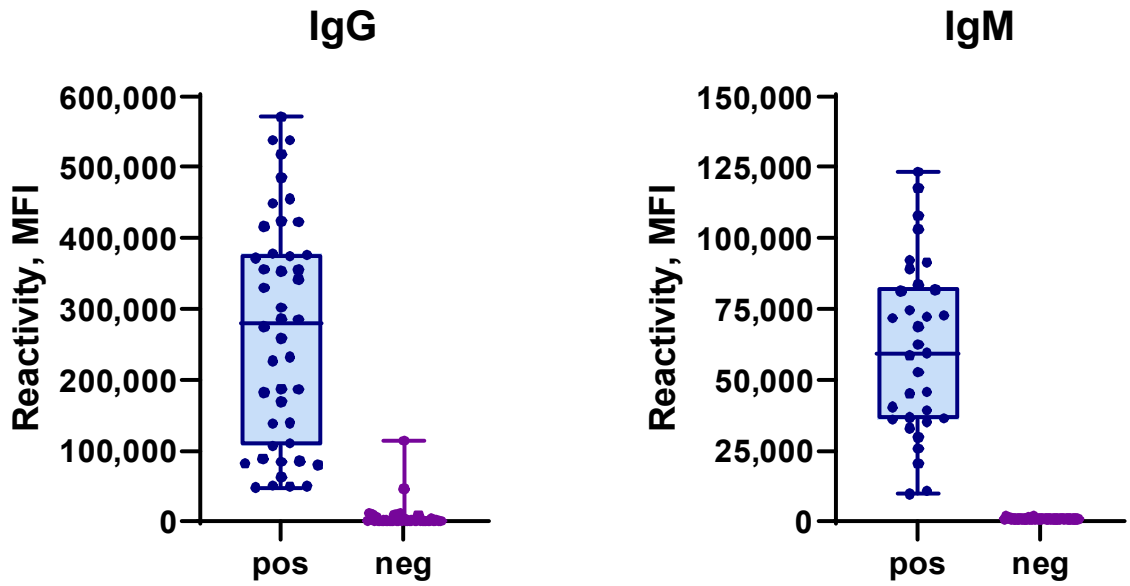

Figure 5: To demonstrate the reactivity of human parvovirus B19 VP2 VLP, an IgG bead-based immunoassay was performed on 87 human specimens, of which 44 were positive and 43 were negative, and an IgM bead-based immunoassay was performed on 79 human samples, 33 positive and 46 negative. The ratio of the mean of positive samples to the mean of negative samples was 42 for IgG and 61 for IgM analysis.

The WHO International Standard (2nd International Standard for Anti-Parvovirus B 19 plasma, human, NIBSC code: 01/602) with an assigned unitage of 77 IU/mL was tested with a bead-based IgG immunoassay. The standard was diluted 100-fold to a concentration of over 1,000,000-fold and analyzed in duplicates (Figure 6).

The quantitative measurement of antibodies ranged from 770 x 10^{-3} IU/mL to 23 x 10^{-6} IU/mL, demonstrating a remarkably broad measurement range of four orders of magnitude. The limit of detection was found to be less than 0.1 x 10^{-3} IU/mL.

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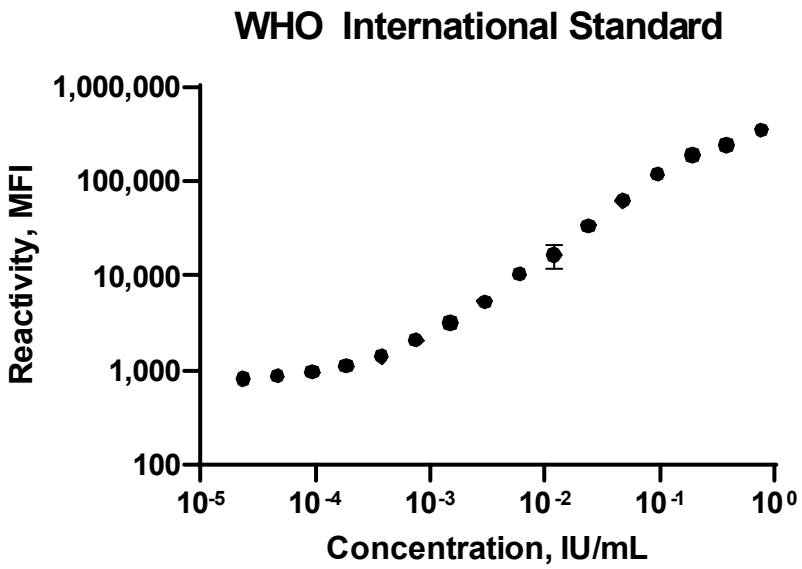

Figure 6: WHO International Standard for Anti-Parvovirus B 19 was diluted over a wide range and tested with bead-based IgG immunoassay. The achieved measurement range is as wide as four orders of magnitude, and the limit of detection is less than 0.1 x 10^{-3} IU/mL.

Sensitivity and Specificity

A method comparison was performed to assess the diagnostic efficacy of recombinant human Parvovirus B19 VP2 VLP antigen for the detection of IgG and IgM antibodies. An IgG bead-based immunoassay was performed on 87 human specimens, of which 44 were positive and 43 were negative. The positive and negative status of the samples was confirmed by two commercially available parvovirus IgG ELISAs. Receiver operating characteristic (ROC) analysis showed an area under the curve (AUC) of 0.99, indicating excellent diagnostic performance (Figure 7).

An IgM bead-based immunoassay was performed on 79 human samples, including 33 positive and 46 negative samples, which had been tested with two commercial parvovirus IgM ELISAs. The observed AUC was 1.00 (Figure 7), indicating that the recombinant human parvovirus B19 VP2 VLP antigen is highly suitable for IgM detection.

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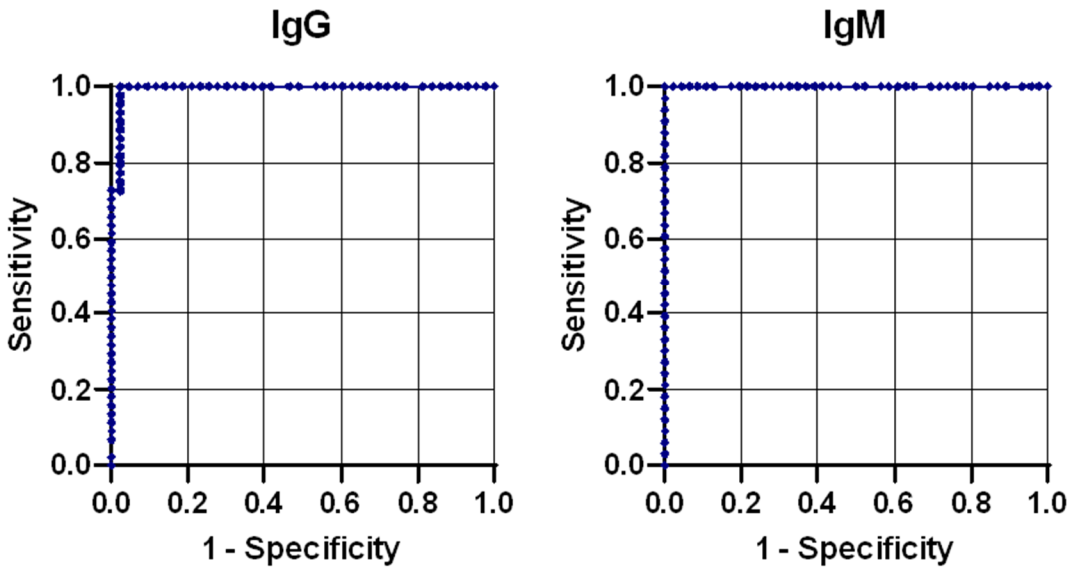

Figure 7: To evaluate the diagnostic performance of human parvovirus B19 VP2 VLP, an IgG bead-based immunoassay was performed on 87 human specimens, of which 44 were positive and 43 were negative. ROC analysis showed an AUC of 0.99. In addition, an IgM bead-based immunoassay was performed on 79 human samples, 33 positive and 46 negative. The observed AUC was 1.00.

To determine the sensitivity and specificity of the recombinant human Parvovirus B19 VP2 VLP antigen, an ideal cut-off value was defined to minimize the number of false positive and false negative results. For IgG, this optimized cut-off value was 47,000 MFI, giving a high sensitivity of 100 % and specificity of 97.7 % (Table 1). The results of the IgM immunoassay showed an optimal threshold of 5,000 MFI, with a sensitivity of 100 % and a specificity of 100 % (Table 1).

Table 1: Recombinant human Parvovirus B19 VP2 VLP antigen: sensitivity and specificity were determined for IgG (n = 87) and IgM (n = 79).

	Sensitivity	Specificity
IgG	100 %	97.7 %
IgM	100 %	100 %

Summary

The new recombinant human Parvovirus B19 VP2 VLP is a highly purified protein, which ensures the highest quality and availability. Our experience in the production of human Parvovirus VP2 VLPs has also enabled us to make production even more efficient, allowing us to produce higher volumes with improved quality. An excellent lot-to-lot consistency, an optimal broad measurement range and a very

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low limit of detection have been demonstrated by a bead-based immunoassay. Finally, the excellent specificity and sensitivity achieved make it an ideal candidate for the reliable detection of IgG and IgM in serological IVD assays.

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