Instructions for Use BIOSAVE® ANTIGEN Assay CE

Detection of HUMAN PATHOGENS in human serum, liquor or feces by LATEX AGGLUTINATION

Total assay time: about 7 min. to max. 25 min. (treatment with pronase)

INTENDED USE
The Biosave® Antigen assay is a rapid assay for the qualitative determination of antigens in human serum, liquor and feces. The Biosave® Ag assay is a latex agglutination assay and is determined for in vitro diagnostic use only.

PRINCIPLE OF THE ASSAY
The test is based on the method of latex agglutination (indirect agglutination). The latex particles, which have been coated with antibodies from rabbits against the antigen in question, are provided as a stabilized suspension. Prepared patient specimen and latex detection reagent are placed within the application sites on the agglutination plate. Both liquids are mixed gently. Any autologous antigen present in patient sample will bind to specific antibodies on the latex particles and cause visible agglutination reaction. If patient specimen does not contain the antigen under investigation, no agglutination is observed and the reaction mixture remains milky white and turbid.
Unspecific reactions (e.g. caused by rheuma factors) can be find out by a parallel test with latex control reagent. The latex particles of the control reagent are coated with normal rabbit immunoglobuline. In most cases proteins are responsible for unspecific reactions. This proteins can be destroyed by the protease pronase. If the antigen is no protein, a treatment with pronase can increase the specifity of the test. Rheumatism-factors may also be removed by applying anti-human IgG sorbent (Biosorb®).

LIMITATIONS OF THE PROCEDURE
The Biosave® Antigen Latex Agglutination Assay is a test for the qualitative detection of the antigen. As in any antigen tests a negative result does not exclude an infection. Clinical interpretations should not only be based on the results of one assay. In any case, results should always be interpreted in the context of the general clinical picture, the timing of specimen collection and other laboratory findings. To confirm negative and equivocal test results or to detect seroconversion, it may be recommended to repeat the assay within 10-14 days using a fresh serum specimen.

BIOSAVE® REAGENTS
Latex detection reagent: stabilized suspension of latex particles coated with antibodies from rabbits against the specific antigen, ready to use, in a dropper vial.
Latex control reagent: stabilized suspension of latex particles coated with normal rabbit globulin, ready to use, in a dropper vial.
Antibody control: goat anti-rabbit serum, lyophilized.
Positive control: stabilized antigen extract, ready to use, in a dropper vial.
Negative control: stabilized human serum, without agglutinating antibodies, lyophilized.
Pronase: for treatment of serum samples, lyophilized.
Diluent buffer: buffer for sample dilution, ready to use.
Disposable test cards
Instruction for use

MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED
Pipettes to deliver serum samples and controls
Applicator sticks
Mechanical plate shaker (optional)
Timer
High intensity incandescent lamp (optional)

STORAGE AND STABILITY
Store Biosave® latex reagent and Biosave® controls at the temperature specified on the label. The **unopened** reagents are stable up to the expiry date indicated on the label if the recommendations are strictly followed. Do not use any of these reagents after they have expired. After first use the reagents have to be well closed and stored at the temperature specified on the label. These reagents must be consumed as soon as possible. Stability on reuse does not necessarily correlate with expiry date.
Caution: Do not freeze the latex reagents, otherwise irreversible clotting of the latex particles will occur!
The disposable test cards may be stored indefinitely at room temperature. These items are nevertheless issued with an expiry date that appears on the product label. It serves no other purpose but to allow easy stock control.

SAFETY PRECAUTIONS
1. The positive control contains antigen extract and must be considered potentially infectious and handled with appropriate care.
2. All human sera used in the manufacture of the preparations from human sera (controls) must be considered potentially infectious and handled with appropriate care.
3. All liquid reagents (latex detction reagent, buffers etc.) contain sodium azide or thimerosal as preservative, as indicated on the label. Thimerosal and sodium azide are toxic. Avoid contact with skin and eyes. Do not inhale or ingest. Azide containing reagents must not be brought into contact with any copper or lead containing containers, for example certain drain pipes, as this could lead to formation of explosive metal azides.
4. The safety regulations of trade associations and of the respective institute (laboratory) are strictly be followed (see notices, laboratory guidlines, safety instructions etc.).
5. Actual Good Laboratory Practice rules (GLP guide lines) should always be followed.
6. Materials and reagents used in the test must be disposed off in compliance with the applicable legal regulations and the workplace has to be disinfected.

MATERIAL FOR TESTING
Serum or liquor are both suitable for testing. Serum/liquor are stable for about 1 week if held at 5-10°C. If storage or repeated testing of samples is required for longer periods, samples should be stored at or below -20°C.
Serum:
For a better specificity of the assay, it is recommended that all serum specimens be treated with pronase as described below.
1. Add 200 µl of serum specimen to 200 µl of the pronase solution. The vial have to be closed well.
2. Incubate serum/pronase solution at 56°C for 15 minutes.
3. Immediately boil the serum/pronase solution for a full 5 minutes to terminate enzymatic digestion.
4. Allow solution to cool to room temperature before performing the assay. For titration note that patient samples has been diluted 1:2 with the pronase solution.
Liquor:
Liquor should be inactivated by placing in a boiling water bath for 5 minutes prior to each test. This manipulation limits nonspecific interference.

Bios® Labordiagnostik GmbH, Hofmannstr. 7, D-81379 München,
Fon/Fax: +49 89/898895-41/40, e-mail: bios@bios-world.com

PREPARATION OF REAGENTS

Reconstitute lyophilized reagents with the indicated volume of deionized or destilled water. For reconstitution mix gently, don't heat or shake intensive.
Heat inactivate the **negative control** at 56°C for 30 min each day of use.

QUALITY CONTROL AND TROUBLE SHOOTING

Positive and negative control are both testet with the latex detection reagent. By parallel test the positive control, the negative control and the antibody control are testet with the latex control reagent. With the positive and the negative control no agglutination occurs with the latex control reagent, the antibody control shows a positive reaction.
If the obtained control values do not fall within the expected range, the test is invalid and has to be repeated.
Do not use clumpy latex reagent (expired, liquid dried out, frozen etc.).

ASSAY PROCEDURE

Before starting the assay, allow all components to equilibrate to room temperature. This takes about 5 minutes. Please protect all reagents from sunlight and keep away from heaters. The reagents are formulated ready to use and thus do not require any further dilution for the assay. Agitate all reagents gently before use.
Controls have to be run once for the day. Note: controls do not need to be run on each card with each patient sample. Label an appropriate number of application sites on the agglutination plate with a pencil to identify patient and control reactions. For the control run 2 wells for the positive control, 2 wells for the negative control and 1 well for the antibody control are needed. The positive control and the negative control are both testet with latex detection reagent and latex control reagent, the antibody control is only tested with latex control reagent. For each patient sample 2 wells are needed because each sample must be tested with the latex detection reagent and the latex control reagent.
1. Holding the positive control vial in a vertical position, squeeze one free-falling drop of reagent into each of the two designated rings.
2. Place 25 µl of the antibody control and negative control to the appropriate rings.
3. Place 25 µl of patient specimen in each of the two designated rings.
4. Holding the detection latex in a vertical position, squeeze one free-falling drop of reagent into one of the positive control, one of the negative control and to the patient specimen.
5. In a similar fashion, add one drop of the latex control reagent to the second positive control, the second negative control, the antibody control and to the second patient specimen ring.
6. Thoroughly mix the contents in each well, using a separate applicator stick for each application site.
7. Agitate the test card gently for about 5 minutes (either by hand or using a mechanical plate shaker with 125 ± 25 rpm).
8. Evaluate the test immediately. Otherwise incorrect results may occur due to drying. It is recommended to observe the test wells for agglutination using a high intensity incandescent lamp. Rate the results on a scale ranging from negative to 4 +.
Restore the latex reagent and the positive and negative control at 5-10° C immediately after use.

EVALUATION AND INTERPRETATION OF RESULTS

Positive:
If agglutination of the latex particles can be observed within the appropriate time, this indicates the presence of specific antigens against the antibodies on the latex particles. The specimen (control or patient sample) is considered positive.
The positive control gives an agglutination within the indicated time.
Negative:
A homogeneous and milky suspension with no visible clumping after the appropriate incubation time is considered as negative result (control or patient sample).

REFERENCES

Singer J.M., Plotz C.M.: The Latex Fixation Test. I. Application to the Serologic Diagnosis of Rheumatoid Arthritis. Am. J. Med. 21, 1956, 888-892

Bios® Labordiagnostik GmbH, Hofmannstr. 7, D-81379 München,
Fon/Fax: +49 89/898895-41/40, e-mail: bios@bios-world.com