Instructions for Use BIOGNOST® ANTIBODY Assay CE

Detection of IgG, IgM and IgA ANTIBODIES in human serum by INDIRECT IMMUNOFLUORESCENCE

Total assay time: 90-120 minutes

INTENDED USE
The Biognost® IFA is an indirect fluorescent antibody assay for the qualitative and/or semi-quantitative determination of antibodies in human serum. The Biognost® IFA is intended for use as an aid in the in vitro diagnostic.

PRINCIPLES OF THE ASSAY
The test is based on the classical method of the indirect immunofluorescence. The substrate slides have been coated with antigen. First, a sample of patient serum is placed on the test slide and incubated. If the serum sample contains any antibody to the respective antigen it will bind to its target antigen on the slide. Unbound immunoglobulins and other sample components are subsequently removed by rinsing the slide with the provided wash buffer. Don't stirr during the washing-procedure! Otherwise the immobilized substrate may be damaged or washed away. During a second incubation step, bound antibodies are optically labeled with FITC-conjugated anti-human immunoglobulin (the conjugate). Excess conjugate is removed by washing the slides once again. The antigen/human antibody/conjugate complexes formed can be visualized by fluorescence microscopy at a 400 to 500fold magnification.
If the specific detection of IgM or IgA antibodies is desired, samples are first treated with a specific absorbent (Biosorb®) that removes IgG and rheumatoid factor before applying these samples to the antigen-coated slide (refer to sections COMPONENTS and MATERIAL FOR TESTING)
If the investigated patient specimen does not contain any antibodies to the respective antigen, no specific antigen-antibody complexes will form and consequently no specific fluorescence will be seen under the microscope.

LIMITATIONS OF THE PROCEDURE
The indirect immunofluorescense technique is used for the detection of diverse antibodies in patient specimens. Because the affinity of antibodies for the test antigen(s) can greatly vary between individual patients, test results are difficult to standardise in absolute terms and individual antibody titers do not necessarily reflect the severity of the investigated disease. On the other hand, because this technique is based on substrate slides that present the (theoretically) optimal range of relevant antigens, indirect immunofluorescence is ideally suited for antibody screening.
Where positive controls with known antibody titres are available, test results can be evaluated semiquantitatively. However, users are reminded that assessment of the clinical picture should not be based on these results alone. Results always should be interpreted in the general context of related information (e.g. clinical symptoms and signs, timing of specimen collection, other laboratory findings, manufacturer-specific characteristics of the assay components, in-house reference range for the respective test) and in conjunction with other available patient data.

BIOGNOST® REAGENTS
Biognost® assays are available as test kit or individual components:
Substrate slide: coated with the respective antigen.
Positive control: stabilized human serum containing antibodies to the respective antigen, ready to use.
Negative control: stabilized human serum containing no antibodies (IgG, IgM, IgA) as determined by immunofluorescence assay, ready to use.
Conjugate: mono- or polyspecific anti-human immunoglobulin, with or without Evans blue counterstain, ready to use or concentrate; working dilution as indicated on the label.
PBS buffer: easily soluble dry powder blend; prepares 500 ml or 1000 ml buffer solution, pH 7.5, containing 10 mM sodium phosphate + 150 mM sodium chloride.
Mounting medium, blotting templates, coverslips, instructions for use.

MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED
Appropriate containers to prepare serial dilutions of specimens
Precision pipettes and pipette tips to deliver 1-1000 µl
Vortex mixer
A 500 ml or 1000 ml graduated cylinder to prepare phosphate buffered saline solution
Distilled or deionized water
Humidified incubation chamber
Incubator (37°C)
Staining troughs
Wash bottle for the buffer
Timer
Darkfield fluorescence microscope with filters allowing excitation at 450-490 nm and emission at 560-590 nm (for best sensitivity, incident excitation should be used in preference over transmission excitation). Do not use immersion oil.

STORAGE AND STABILITY
Store all slides, controls, conjugates and Biosorb® at the temperature specified on the label. To prevent the antigen-coated slides from drying up and denaturing they must be kept in the properly sealed laminated aluminium pouch supplied. The **unopened** reagents are stable until 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.
The air-sealed dry powder blend of phosphate-buffered saline may be stored indefinitely at room temperature or below if **unopened**.
The mounting medium, the blotting templates and the coverslips may be stored indefinitely at room temperature or below. 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. The PBS wash buffer (pH 7.5) should be freshly prepared on the day of use as it contains no preservatives. In case there is any buffer solution left over that one still wants to use the following day, it should be kept properly covered at 5-10°C. Discard PBS buffer solution if any turbidity, colouration or flocculent precipitate appears or if the pH has changed.

SAFETY PRECAUTIONS
1. All kits and assay components listed in the Instructions for use (see section COMPONENTS) are for in vitro diagnostic use only.
2. All human sera used in the manufacture of the preparations from human sera (controls) listed in the section COMPONENTS must be considered potentially infectious and handled with appropriate care.
3. All liquid reagents, such as controls, conjugate etc. contain 0.09% sodium azide. Sodium azide is poisonous. Don't swallow and avoid any contact with skin and mucous membranes. Azide containing reagents must not be brought into contact with any copper or lead containing objects, for example certain drain pipes, as this could lead to formation of explosive metal azides.
4. As specified on the product label, some of the conjugates contain the dye Evans blue for counterstaining. Evans blue is a possible carcinogen (a class 1* poison according to the Swiss poisons schedule). Although the dye concentration is very low (maximally 0.2 mg/ml), users are advised to take care not to swallow these conjugates and to avoid any contact with the skin.

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

5. The safety regulations of trade associations and of the respective institute (laboratory) should be strictly followed (see notices, laboratory guidlines, safety instructions etc.).
6. Actual Good Laboratory Practice rules (GLP guide lines) should always be followed.
7. 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 and plasma are both suitable for testing. Serum and plasma samples 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 subdivided into small portions (50 µl), snap-frozen in liquid nitrogen and stored at or below -20°C. Larger volumes of serum or plasma should not be exposed to repeated freeze-thaw cycles because this can cause aggregation of proteins and degradation of some serum and plasma components. Because azide does not interfere with the assay, serum and plasma samples also may be stabilized with 0.09% azide. Such samples can be stored for prolonged periods (up to 1 year) at 5-10°C without loss of analyte.
However, if serum samples are to be assayed for IgA or IgM antibodies, they first should be suitably pretreated to avoid any false positive reactions due to rheumatoid factors in conjunction with IgG antibodies and prevent any potential competition for antigen binding sites from IgG which could lead to false negative results. A ready-to-use separation system available from Bios® can be used for this purpose (Biosorb®, refer to section Separation systems). Although specifically optimized for IgG/IgM separation, this system is also suitable for the pretreatment of samples in IgA assays. It removes excess IgG from the sample while both IgM and most of the IgA remain. During this procedure samples will become diluted. The resulting dilution factor for IgM and IgA is 1:5 and must be taken into account when preparing final sample dilutions for testing.

QUALITY CONTROL AND TROUBLE SHOOTING

Both a positive control for each parameter to be interpreted and a negative control should be included with each run. Each control must demonstrate expected reactions as indicated on the label in order to validate the test. If the obtained control values do not fall within the expected range, the test is invalid and has to be repeated.
The titer obtained for the positive controls should match the control value stated on the label within ± 1 to 2 dilution steps. If this is not the case check the following: Have any components been used in the assay that were not originally supplied by Bios® (e.g. coverslips, mounting medium etc. from another supplier); was the Biognost® buffer freshly prepared; are there any functional deficiencies concerning the fluorescence microscope, such as oil-smeared objective lenses, poor optical adjustment, weak light source; have Biognost® slides and Biognost® reagents been stored correctly or have any components been used which are already expired; was the incubation chamber sufficiently humidified (during slide processing application sites must not be allowed to dry up at any stage), have slides been labeled with a felt-tipped marker pen etc. Never force the warming up process of the reagents by applying heat.
Any non-specific binding of the conjugate may be identified by including a buffer blank in the test run; i.e. phosphate-buffered saline is applied in lieu of a patient sample to one of the slide wells and processed according to the usual test procedure.
Assay sensitivity and specificity are continually monitored in the Bios® control laboratory to assure consistent test performance. Bios® applies all serum and control standards available from WHO or other official institutions for assay standardization. Biognost® positive and negative controls provided are calibrated according to standards or sera from clinically characterized patients or blood donors available.
Warranty by Bios is extended only if directions of use are strictly followed, if solely Bios products are verifably applied in the test, and if the test is accomplished by qualified personnel.

TEST PREPARATIONS

If the conjugate is provided as concentrate, prior to testing, it has to be diluted with PBS buffer as indicated on the label. Note that the conjugate is not stable once diluted. It must be prepared freshly for each test run.

ASSAY PROCEDURE

Before starting the assay, allow the Biognost® slides, controls and conjugates to equilibrate to room temperature. This takes about 5 min. The controls and conjugates are formulated ready for use and thus do not require any further dilution for the assay.
For directions regarding the use of Biosorb® as IgG/IgM (IgG/IgA) separation system and ensuing the final sample dilution please refer to section COMPONENTS and to Instructions for use Cat.No. S0001tie.
The Biognost® controls have already been pretreated if necessary and thus should not be subjected to any further immunoglobulin class separation or absorptions.
The antigen on all Biognost® slides has already been fixed. Substrates may be destroyed by a second fixation step in customer's lab.
Prior to testing, serum samples have to be appropriately (screening tests or titrations) diluted with phosphate-buffered saline, either with or without 1% bovine serum albumin. Common dilution series: 1:2; 1:4; 1:8 etc. or 1 :5 ; 1 :10 ; 1 :20 etc.
Before commencing the assay, the distribution and identification plan for all specimens and controls to be tested should be carefully established on a form. This form is the basis for interpretation and documentation of results.

1. Tear open the laminated aluminium foil at the prepunched notch and carefully remove slide. Take care not to touch any application sites. If slides are to be labeled, this is best done with a pencil. Never use any of the felt-tipped marker pens for writing on your slides.
2. Apply a sufficient volume of the controls or appropriately diluted patient serum to completely cover the application site (between 15 and 50 µl, depending on the well size of the chosen slides).
3. Incubate slides 30 min at room temperature in a humidified incubation chamber.
Exception: viruses and intracellular germs:
IgG detection: incubate slides 30 min at room temperature in a humidified incubation chamber,
IgM/IgA detection: incubate slides 30 min at 37°C or 60 min at room temperature in a humidified incubation chamber.
Protect slides from direct sunlight; keep away from heaters.
4. Remove slides from chamber, drain off excess liquid and carefully rinse slides with phosphate buffered saline. (Do not aim the buffer stream directly onto application sites!)
5. Immerse slides 2x 5 min in a bath of phosphate-buffered saline; use large staining troughs and change buffer between cycles. Do not agitate slides in the buffer bath.
6. Briefly dry slides with the blotting templates. The substrate must not be allowed to dry at this stage. Therefore, proceed immediately with step 7.
7. Apply the appropriate conjugate to each application site such that the conjugate completely covers the well (between 15 and 50 µl, depending on the well size of the chosen slides). One drop dispensed from Biognost® conjugate vials corresponds to about 25 µl.
8. Incubate slides 30 min at room temperature in a humidified incubation chamber. Protect slides from light and keep away from heaters.
9. Repeat steps 4-6 above and immediately proceed with next step.
10. Mount coverslips by placing 2 or 3 drops of mounting medium on each slide and, to avoid entrapping air bubbles, carefully lower the cover-slip from one end of the slide to the other. Evaluate slides by fluorescence microscopy. For best results this should be done immediately.
Any overflowing mounting medium should be wiped off with buffer moistened paper toweling to prevent slides from sticking to the slide platform of the microscope or to the base of the slide storage box.
For long-term preservation, the mounted coverslips may be sealed around the edges with a few drops of clear nail polish; if stored at or below -20°C such slide preparations will keep for several years.

This assay can also be processed by automation.

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

EVALUATION OF SLIDES AND INTERPRETATION OF RESULTS

Slides are viewed at 400 to 500fold magnification under a darkfield fluorescence microscope (overview at 100fold magnification). To avoid loss of sensitivity by photobleaching, do not dwell on the same viewing field any longer than really necessary for evaluation. For best results, as many viewing fields as practicable should 1be examined in rapid succession.

A test run can only be interpreted if the controls included in the run demonstrate the expected results.

Consult the full-length instructions for use for detailed information about evaluation and interpretation of the different assays.

Please note, IgM fluorescence is less brilliant than IgG fluorescence. Also IgM fluorescence patterns are different from IgG patterns and may vary in addition from serum to serum. Titers given on our IgM controls may not be reproduced on each lot of concerning substrate slide. But undiluted positive control should always give the positive result. The possible deviation of ± 2 titer steps cannot be avoided in IFA methodology (refer to “Quality control and trouble shooting”). But this possible variation in assay activity has to be taken into consideration when assay results are interpreted.

Fluorescence patterns:

To evaluate the assay, fluorescence patterns of the tissue structures or the respective pathogens have to be determined.

Positive:

Specific fluorescence shows a typical, light apple green colour and its intensity is commonly rated on a scale from 1+ (weak), through 2+ (intermediate), 3+ (bright) to 4+ (very bright).

Negative:

A fluorescence rating of less than 1+ is considered a negative result. Any yellowish or dark green fluorescence is unspecific and to be disregarded.

Titer:

Titers are reported as the reciprocal of the highest specimen dilution that yields a fluorescence intensity rated at least 1+. For example, if the 1:80 dilution was rated 1+ while the 1:160 dilution gave a negative result then the titer of this specimen would be reported as 80.

REFERENCES

1. Weller T.H., Coons A.H.: Fluorescent Antibody Studies with Agents of Varizella and Herpes Zoster Propagated in vitro. Proc. Soc. Exp. Biol. Med. 86, 1954, 789-794.

2. Riggs J.L., Siewald R.J., Burckhalter J.H., Downs C.M., Metcalf T.G.: Isothiocyanate Compounds as Fluorescent Labeling Agents for Immune Serum. Am. J. Pathol. 34, 1958, 1081-1097.

Bios® Labordiagnostik GmbH, Hofmannstr. 7, D-81379 München,

Fon/Fax: +49 89/898895-41/40, e-mail: bios@bios-world.com