Instructions for Use BIOGNOST® ANTIGEN Assay CE

DFA: Detection of ANTIGENS by DIRECT IMMUNOFLUORESCENCE TECHNIQUE: a one-step technique based on FITC-labeled mono- or polyclonal antibodies
IFA: Detection of ANTIGENS by INDIRECT IMMUNOFLUORESCENCE TECHNIQUE: a two-step technique based on mono- or polyclonal primary antibodies and FITC-labeled second antibodies

INTENDED USE
The Biognost® Ag DFA is a direct, the Biognost® Ag IFA an indirect immunofluorescence assay for the qualitative determination of pathogens directly from patient material or after isolation. The Biognost® Ag assay is intended for use in the in vitro diagnostic.
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PRINCIPLES OF THE ASSAY
The test is based on the classical immunofluorescence method. The patient specimen is first placed on a glass slide and subjected to a suitable fixation procedure (cf. section "Specimen Collection and Preparation"). It is then covered with an antibody reagent of the appropriate antigen specificity, either a fluorescein conjugated antibody (DFA) or an unlabeled antiserum/primary antibody (IFA), and incubated. In case of a positive specimen, the mono- or polyclonal antibodies will bind to the specific microbial target antigens. Excess antibody is subsequently removed by rinsing the test slide with the provided wash buffer.
For DFA a coverslip is mounted. For IFA during a second incubation step, any bound primary antibodies are labeled with FITC-conjugated second antibody (the conjugate). Excess conjugate is removed by washing the slide once again. Then a coverslip is mounted.
The antigen/conjugate (DFA) or antigen/primary antibody/conjugate (IFA) complexes formed are visualized by fluorescence microscopy at a 400 to 500fold magnification. Sensitivity and specificity of the conjugate/primary antibody can be doublechecked using corresponding antiserum/conjugate control slides.
Don't stirr during the washing procedure! Otherwise the immobilized antigen may be damaged or washed away.

LIMITATIONS OF THE PROCEDURE
The Biognost® Immunofluorescence assays for microbial antigens allow the specific, qualitative detection of the offending pathogen either directly in the patient specimen or in the microbial isolate. A positive test result establishes the diagnosis of infection with the pathogen under consideration, provided that not more than a single patient specimen has been examined per slide. If more than one positive test result is obtained with different patient specimens examined on one and the same slide (or on different slides that have been treated in the same staining trough/s), positive test results should be reexamined. Cross-contamination between different samples applied to different application sites may happen during the washing procedure. In such cases, all positive test results should be confirmed individually (application of only a single specimen sample per slide and per staining trough).
However, in common with other antigen detection methods, a negative test result can never exclude with certainty infection of the patient with the pathogen in question. There is always a chance that the specific infectious agent is not represented in the particular specimen collected for analysis, or in that portion of the specimen actually used in the test. Therefore, if negative results are obtained in cases where specific infection is strongly suspected, the test should be repeated, preferably on a fresh specimen. Or the result should be confirmed with a serological assay or by culture.

BIOGNOST® REAGENTS FOR ANTIGEN ASSAYS
DFA: Pathogen specific FITC labelled conjugate (poly- or monoclonal, ready to use or concentrated), control slides, PBS, mounting medium, coverslips.
IFA: Pathogen specific antibody (poly- or monoclonal, ready to use or concentrated), FITC labeled second antibody, controls (antigen coated control slides or empty slides with positive and negative suspensions), PBS, mounting medium, coverslips.

ADDITIONAL MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED
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) (optional)
Large 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 trans-mission excitation). Do not use immersion oil.

STORAGE AND STABILITY
Store all antibody solutions (primary antibodies and conjugates) as well as the control slides 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 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.
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 the patient specimens used in antigen detection assays are potentially infectious and must be handled with appropriate care.
2. The antibody solutions (primary antibodies and conjugates) and the mounting medium contain 0.09% sodium azide or other preservative. All preservatives are poisonous. 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.
3. 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). Users are advised to take care not to swallow these conjugates and to avoid any contact with the skin.
4. The safety regulations of trade associations and of the respective institute (laboratory) should be strictly 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.

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SPECIMEN COLLECTION AND PREPARATION

Specimen collection, stabilization of specimen, transportation and preparation of the sample for the assay contribute considerably to the relevance of test result. So prior to running Biognost® antigen assays laboratories should install antigen collection and handling procedure (together with clinicians and pathologists).
In the case of gastrointestinal infections, the microbial antigen in question is best determined in stool specimens which may be preserved in 10% formalin. (Preservation with polyvinyl alcohol or thimerosal-iodine-formaldehyde is not suitable).
In the case of respiratory infections, specific antigen detection is possible in any specimen obtained by one of the following methods: bronchoalveolar lavage, bronchoscopic biopsy, transtracheal aspiration, (induced) sputum, throat or nasopharyngeal swab, pleural puncture, autopsy and culture (culture confirmation test).
When using sputum specimens for testing, pretreatment of samples with dithio-threitol or N-acetyl cysteine reduces samples' viscosity.
HSV is readily detectable in vesicular fluid or vesicle scrapings).
Reliable detection of intracellular microbial antigens is only possible if the collected specimen contains a sufficient number of infected cells. To preserve microbial infectivity in cases where parallel analysis or confirmation of test results by cell culture is desired, specimens should be immediately transferred into a suitable transport medium after being collected from the patient.

Fixation/Inactivation:

A separate test slide is used for each patient. Apply the appropriately prepared specimen to the test slide and allow the slide to air-dry completely at room temperature (15-30 min, depending on the thickness of the substrate). Please note that samples which have not been properly dried onto the slide tend to become easily detached during the washing steps. Next, the test slide is subjected to a heat- and/or acetone- and/or formalin-fixation step (single-, double- or triple fixation). When all residual solvent has evaporated from the slide, the sample is ready for incubation with the antigen-specific antibody, that is either unlabeled primary antibody for the indirect immunofluorescence technique (IFA) or fluorescein conjugated antibody for the direct technique (DFA).
For specimen collection and preparation for eucaryotes please refer to actual literature.

QUALITY CONTROL AND TROUBLE SHOOTING

A control slide for positive and negative control of the antiserum and/or the conjugate should be included with each run. If the control slide does not demonstrate the expected reactions, the test is invalid and has to be repeated. In case control slides do not show perfect results 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 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 slides and reagents been stored correctly or have any components been used which have 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.
To avoid unbonding or destroying of the fixed antigen, do not stir during washing procedures.
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.

ASSAY PROCEDURE

Biognost® conjugates for the direct immunofluorescence technique are regularily available in a convenient ready-to-use form, if no mode of dilution is given on the label. Once thawed, the ready-to-use conjugates can be directly used in the test run. Some conjugates are available as concentrated stock solution and dilution is indicated on the label. If no mode of dilution is given on the label, user has to determine the optimal working dilution of the concentrated conjugate (on the label conjugate "conjugate concentrate"). However, the user-diluted conjugate is not stable for prolonged periods and thus a fresh working dilution has to be prepared for each test run. For the indirect immunofluorescence technique, Biognost® primary antibodies as well as the secondary antibodies (conjugates) are regularily supplied as ready-to-use reagents. Otherwise dilution is specified on the label.

I. Direct Immunofluorescence Assay (DFA):

1. Apply to the test slide a sufficient volume (one or several drops) of either the ready-to-use or the appropriately diluted conjugate such that the fixed patient specimen is completely covered.
2. Incubate slides 30 minutes at room temperature or at 37°C (intracellular organisms) in a humidified incubation chamber. Protect slides from direct sunlight; keep away from heaters.
3. Remove slides from chamber, drain off excess liquid and carefully rinse slides with phosphate buffered saline. (Do not aim the buffer stream directly onto the application sites!)
4. 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.
5. Briefly dry slides around the application site with absorbent paper. The substrate must not be allowed to dry at this stage. Therefore, proceed immediately with step 6.
6. Mount coverslips by placing 2 or 3 drops of mounting medium on each slide and, to avoid entrapping air bubbles, carefully lower the coverslip from one end of the slide to the other. 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. Evaluate slides by fluorescence microscopy. For best results this should be done immediately. Alternatively, slides may be stored for up to two hours in a dark and cool place and have to be protected from desiccation.

II. Indirect Immunofluorescence Assay (IFA):

1. Apply to the test slide a sufficient volume (one or several drops) of the primary antibody such that the fixed patient specimen is completely covered.
2. Incubate slides 30 minutes at room temperature or at 37°C (intracellular organisms) in a humidified incubation chamber. Protect slides from direct sunlight; keep away from heaters.
3. Remove slides from chamber, drain off excess liquid and carefully rinse slides with phosphate buffered saline. (Do not aim the buffer stream directly onto the application sites!)
4. 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.
5. Briefly dry slides around the application site with absorbent paper. The substrate must not be allowed to dry at this stage. Therefore, proceed immediately with step 6.
6. Apply the appropriate conjugate (FITC-labeled secondary antibody) to the slide as described in step 1.
7. Incubate 30 min at room temperature in a humidified incubation chamber. Protect slides from direct sunlight and keep away from heaters.
8. Repeat steps 3-5 and refer to I., 6.

EVALUATION

Slides are viewed at 400 to 500fold magnification (general view at 100fold magnification) under a darkfield fluorescence microscope (filter range: 450-490 nm). 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 be examined in rapid succession.
Slide preparations should be promptly evaluated under the microscope following the last processing step. If this is impossible, prepared slides must be stored in a dark cool place and well protected against drying up. For long-term preservation - for instance, if the preparations are to be kept for teaching purposes - they have to be sealed with clear nail polish along the edges of the coverslip and stored at or below -20°C.

Immunofluorescence staining patterns:

When evaluating the slides, only the staining of characteristic microbial structures must be taken into account.

Positive:

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

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A specimen is considered positive for the investigated pathogen if any fluorescence of pathogen-typical structures which is rated at least 1+ can be seen.

Negative:

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

Target antigens and target structures:

The Biognost® antibody reagents specifically recognize antigen structures that are characteristic for the investigated pathogen (bacteria, CPE, etc.). If fluorescence microscopy reveals a clearly visible, light apple green fluorescence of any such pathogen structures, the examined specimen is considered positive; already the presence of a single fluorescent antigen structure is sufficient proof that the patient is infected with the investigated pathogen.

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.

REFERENCES

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