Microsporidia IFAT

Indirect immunofluorescence assay for the specific diagnostic of human intestinal microsporidiosis

2 x 50 tests for in vitro diagnostic use and for professional laboratory use

Instructions for use for article N° **8100**
N° CE : H-CH/CA01/IVD/12173 - UDI-DI: 07640158218105

Intended use:

The Bordier Microsporidia IFAT kit is intended for the direct qualitative detection of both species spores in human stool. This test thus allows a species diagnosis.

Background:

Microsporidiosis is a worldwide opportunistic infection caused by *Microsporidia*, a group of obligate intracellular parasitic fungi. *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis* are the two species responsible for gastrointestinal disease in humans. Infections caused by *E. intestinalis* are treated with albendazole, while fumagillin has been shown to be effective for eradicating *E. bieneusi*. Thus, species identification is important for defining the appropriate treatment. Humans can be infected by ingesting *Microsporidia* spores. Infective sporoplasm invades host cells, multiplicates and matures into spores. The host cell membrane is disrupted and releases the spores to the surroundings. These free mature spores can infect new cells, thus continuing the cycle. Infection occurs on severely immunocompromised persons. The most important symptom is diarrhea. Diagnosis is based on microscopic examination of spores in fecal samples, an immunofluorenscent assay or detection of DNA by PCR.

Principle and presentation:

The kit contains monoclonal antibodies and the fluorescent conjugate allowing 2 x 50 immunofluorescence assays on microscope slides. Monoclonal antibodies will bind specifically to samples spores attached to the slide wells. Unfixed antibodies will be washed away. The presence of spore-specific antibodies is detected with a fluorescent anti-mouse IgG conjugate. A second washing step will remove the unbound conjugate. After mounting between slide and coverslip, reading is performed with a fluorescence microscope.

Material contained in the kit (2 x 50 assays):

MAB1	8100-01	Monoclonal antibody anti- ***E. bieneusi*** ready to use (red caps)	**2 x 0.5 ml**
MAB2	8100-02	Monoclonal antibody anti- ***E. intestinalis*** ready to use (green caps)	**2 x 0.5 ml**
CONJ	8100-03	Ready to use fluorescent (488nm) anti-mouse IgG conjuguate containing Evans blue	**1 x 2 ml**

Shelf life and storage:

Store the kit at 2° to 8°C (transport at ambient temperature), avoid long term exposure of the components to direct light. The expiry date and the lot number of the kit are printed on the back of the box. After initial opening, all reagents are stable until the expiry date when stored at 2-8°C.
Note: once slides are mounted and sealed, they are stable for 6 months if stored at 2-8°C and protected from direct light.

Equipment needed but not provided with the kit:

Pipettes (ml and µl). Flasks. Dilution tubes. Distilled water. Manual or automatic equipment for rinsing slides wells. Centrifuge. Vortex mixer. Timer. Methanol. PBS (Phosphate-Buffered Saline). Filters (best = 50 µm or 100 µm). Epoxy coated glass slides (75 mm x 25 mm) with 8 compartments 5 mm in diameter or equivalent. Coverslip (60 mm x 24 mm). Anti-fading fluorescence mounting medium. Immersion oil. Fluorescence microscope (x1000).

Preparation of reagents before use:

All reagents in the kit are ready to use.

Specimen collection and preparation:

Use human stools. Fresh and untreated samples can be stored for 48 hours at 2-8°C, otherwise store at -20°C or below. Avoid repeated freezing and thawing. Samples treated with 10% formaldehyde can be stored for 2 months at room temperature.

Warnings and precautions:

Toxic compounds are found in following concentration:

Component	Reference	Sodium azide (N_aN_3)	Merthiolate	Evans Blue
Monoclonal antibodies	8100-01 and -02	0.02 %	0.02 %	/
Fluorescent conjugate	8100-03	0.01 %	0.002 %	0.0002 %

At the used concentrations, sodium azide and merthiolate do not have any toxicological risk on contact with skin and mucous membranes.

- Monoclonal antibodies (8100-01 and -02) are from mouse.
- Conjugate is made with antibodies from goat.
- Treat all reagents and samples as potentially infectious material.
- Do not interchange reagents of different lots.
- Do not use reagents from other manufacturers with reagents of this kit.
- Do not use reagents after their expiry date.
- Close reagent vials tightly immediately after use and do not interchange screw caps to avoid contamination.
- Use separate and clean pipettes tips for each sample.
- Do not re-use slides and coverslips.
- The description of symbols used on the labels can be found on the website www.bordier.ch.

Disposal consideration:

All materials used for this test are generally considered as hazardous waste. Refer to national and regional laws and regulations for the disposal of hazardous waste.

Method:

- Filter through 50 µm (best) or 100 µm filter.
- Centrifuge at 700g for 15 minutes, discard the supernatant and resuspend the pellet (dilution 3 times) with PBS.
- Put 2 µl of the fecal sample suspension to be tested on well slides and dry for one hour. Count 2 wells per sample: 1 for each monoclonal antibody.
- Fix the slides with methanol, let dry 5 minutes.
- Add 20 µl of either of the monoclonal antibodies on the wells and incubate for 30 min at room temperature in a humid atmosphere.
- Wash 3 times with a drop of PBS, aspirating the PBS on the side of the wells without touching the sample.
- Add 20 µl of conjugate to each well. Incubate for 30 min at room temperature in a humid atmosphere, in the dark.
- Aspirate the conjugate, wash with a drop of PBS as specified above. Dip the slides in 3 changes of PBS.
- Drain the buffer from the slides. Dry the slides underneath and carefully around the samples, without touching the sample.
- Add 2 drops of anti-fading fluorescence mounting medium, place a coverslip on the wells, avoiding the formation of air bubbles.

Reading is made with a fluorescence microscope, equiped with the appropriate fluoresceine filter (488 nm) and an immersion objective (x 1000).
Note: mounted slides can be sealed with varnish for long term storage (possible rereading) and safer handling.

Interpretation:

To assess the specificity of the labeling, a positive and negative sample (not included) could be processed in parallel with the stool samples.
The monoclonal antibodies react exclusively with the spore walls of Microsporidia. ***E. bienusi*** spores (1.3 x 0.7 µm) and ***E. intestinalis*** spores (1.7 x 1.0 - 1.1 µm) are surface labeled with a marqued peripheral fluorescence.
Quality controls of current lots are published on our website: www.bordier.ch as positive samples pictures.
In case of positive or doubtful result, we recommend performing a DNA detection test by PCR if such a test is available or required by national regulations.

Analytical performances:

Analytical specificity:
Among 67 positive samples with the device for *E. bieneusi*, none were found positive *for E. intestinalis*. Among 6 positive samples with the device for *E. intestinalis*, none were found positive for *E. bieneusi.*
Fluorescence could sometimes be observed with unidentified "chain-linked" bacteria with the anti-*E. intestinalis* monoclonal antibody. However, the size and shape of microsporidia spores allow to differentiate them from these bacteria.
There is no known interference in stool samples.

Precision:
Repeatability was assessed by testing 2 human samples containing spores of each species separated into 8 wells of a slide in a single assay. Reproducibility was assessed by testing the 2 samples on 3 different assays. In all cases, the results obtained were consistent with the expected results.

The following performances cannot be evaluated because this test is qualitative : analytical sensitivity (limits of detection and quantitation), accuracy, trueness, measuring range, linearity, expected values in normal and affected populations.

Clinical performances:

Diagnostic sensitivity and specificity:
Among 72 positive samples for *E. bieneusi* by PCR and/or Microsporidia IFAT, 52 are positive with both techniques, 3 only by PCR and 15 only by IFAT. Control by transmission electron microscope (TEM) shows that these 15 samples are positive for *E. bieneusi*, suggesting that IFAT is more sensitive than PCR.
Among 12 positive samples for *E. intestinalis* by PCR and/or Microsporidia IFAT, 6 are positive with both techniques and 6 only by PCR. A control by TEM shows that these 6 samples are positive for *E. bieneusi*, suggesting that IFAT is more specific than PCR, especially since these 6 samples were detected positive for *E. bieneusi* by IFAT.
In a prospective study on 1237 stool samples, 11 samples were found positive by Microsporidia IFAT and by PCR. 7 samples were found doubtful in Trichrome and 98 in Uvitex 2B. None were found positive by PCR and IFAT.

Incidents :

Any serious incident occurring in connection with the device shall be notified to the manufacturer and to the competent authority of the Member State in which the user and/or the patient is established.

References:

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