**ENGLISH**

**INTENDED USE**

The Detect IF *Pneumocystis carinii* (*P. carinii*) test is an indirect qualitative immunofluorescence kit for the detection of *P. carinii* oocysts in human bronchoalveolar lavage fluid and induced sputum. It is intended to aid in the diagnosis of suspected *P. carinii* infection. Results should be interpreted in light of all clinical and diagnostic information.

**INTRODUCTION**

*Pneumocystis carinii* is a eukaryotic microorganism of uncertain taxonomy. Recent ribosomal RNA homology studies have shown identical nucleic acid sequences with some fungi, but classification of the organism is still the subject of discussion. ¹

*P. carinii* is ubiquitous, infecting man and other mammals; the route of infection is presumed to be airborne. ¹ It is a major pathogen in the immunocompromised, especially patients with AIDS²,³ where it is an established cause of pulmonary infection. Starvation, haematological malignancies, collagen vascular diseases, primary cellular immune deficiency and immunosuppressive therapy, for example in transplant patients and leukaemic patients on cytotoxic drugs are factors that increase the likelihood of infection with *P. carinii* pneumonia.

Onset of *P. carinii* pneumonia may be apparently rapid or occur insidiously. When clinically evident, features are increased respiration rate and spiking fever. Chest films show a diffuse infiltrate; pulmonary function tests show alveolar-capillary block resulting from impaired gas exchange in alveoli, causing hypoxaemia and hypercapnia.

Currently, *P. carinii* pneumonia may be diagnosed by the observation of *P. carinii* in either open lung or transbronchial lung biopsy material, bronchoalveolar lavage⁴,⁵ or induced sputum. It can be visualised with a variety of non-specific stains including Gomori methenamine silver, toluidine blue-O, Gram-Weigert, Giemsa and Wright-Giemsa. Because all these stains react with yeasts and other structures, *P. carinii* must be distinguished on the basis of morphology. Staining techniques are time consuming and often require a high level of technical expertise in the interpretation of results. Monoclonal antibodies specific for *P. carinii* oocysts have become available, allowing the development of immunofluorescent techniques to rapidly and unambiguously identify *P. carinii* oocysts in bronchoalveolar material⁶,⁷ and induced sputum.⁸,⁹ The Detect IF *P. carinii* test uses a murine monoclonal antibody reactive with both human and rodent *P. carinii* in a simple and rapid test for the detection and identification of *P. carinii* in human bronchoalveolar lavage fluid (BAL) and induced sputum (IS).

**PRINCIPLE OF THE ASSAY**

Bronchoalveolar lavage fluid or pre-treated induced sputum specimens are centrifuged and washed. The pellets are resuspended, placed on slides and fixed. The specimens are Enzyme-digested. Murine anti- *P. carinii* antibody and fluorescently labelled anti-mouse antibody are added in turn after incubation, rinsing, wicking, and air-drying steps. On viewing with a fluorescence microscope, oocysts show as medium bright to bright apple green and may be evenly or unevenly labelled. The presence of *P. carinii* oocysts in bronchoalveolar lavage fluid or induced sputum indicates *P. carinii* infection.

**K I T C O M P O N E N T S**

| REAG A | Anti-*P. carinii* Monoclonal Antibody | 1 × 1 mL | Murine anti-*P. carinii* monoclonal antibody, bovine serum albumin, 0.1% (w/v) sodium azide. **Ready-to-use.** |
| REAG B | FITC-Conjugated Anti-Mouse Antibody | 1 × 1 mL | Fluorescein-isothiocyanate (FITC) conjugated anti-mouse antibody, Evans Blue counterstain. **Ready-to-use.** |
| REAG C | Enzyme (Lyophilised) | 1 vial | Pre-treatment enzyme for clinical specimens. **Reconstitute with 200 µL 0.001M HCl (supplied) and dilute before use. N.B. DANGER** |
| REAG D | Dilute Hydrochloric Acid (0.001M HCl) | 1 × 0.5 mL | For Enzyme reconstitution. **Ready-to-use.** |
| REAG E | Enzyme Diluent | 1 × 3 mL | Tris buffer with enzyme activator. **Ready-to-use.** |
| SPCM SLDS | Patient Specimen Slides | 25 slides | PTFE-coated (yellow) slides with four square specimen wells. |
| REAG F | Mounting Medium | 2 × 3 mL | Phosphate-buffered glycerol, Citifluor photobleaching retardant. **Ready-to-use.** |
STORAGE OF REAGENTS

Handling and Procedural Notes

1. Store kit components at 2-8°C and use until the expiry date on the labels. Do not use expired reagents.
2. Do not mix different kits.
3. Do not freeze kits.
4. The lyophilised Enzyme must be reconstituted before use, see Preparation for the Assay section. All other reagents are ready-to-use.
5. After reconstitution with 200 µL 0.001M HCl, the lyophilised Enzyme is stable for up to 3 months from the date of reconstitution if stored at 2-8°C.
6. Do not expose Mounting Medium to direct light during storage. Store at 2-8°C or at 18-25°C.
7. Patient Specimen Slides can be stored at 18-25°C.
8. A precipitate may form in the Enzyme Diluent. Should this occur do not try to redissolve it, there is no detrimental effect on the efficacy of the test.
9. Avoid contamination of reagents. Use a new disposable pipette tip for each reagent or sample manipulation.

Specimen Collection, Storage and Pre-treatment

The assay is for use with human bronchoalveolar lavage and induced sputum specimens. Ideally, up to 30 mL bronchoalveolar lavage and 2-4 mL induced sputum should be collected into sterile vessels by appropriate procedures, and tested as soon as possible after collection. To inactivate any human immunodeficiency virus that may be present, it is strongly advised that the suspension of clinical material is diluted with an equal volume of absolute ethanol and incubated for ten minutes at room temperature (18-25°C) before processing. Dispose of waste materials in accordance with local regulations.

Sputum specimens should be pre-treated (homogenisation or incubation) by the addition of “Sputasol”, “Sputolysin” or similar mucolytic agent for ten minutes at room temperature (18-25°C) before assay.

WARNINGS AND PRECAUTIONS

For in vitro diagnostic use only.

Safety Precautions

1. Adhere strictly to the instructions in this booklet, particularly for handling and storage conditions for kit reagents and clinical samples.
2. All patient samples should be considered potentially infectious and handled with the same precautions as any other potentially biohazardous material. The CDC/NIH Health Manual "Biosafety in Microbiological and Biomedical Laboratories", 5th Edition, 2007, describes how these materials should be handled in accordance with Good Laboratory Practice.14
3. Do not pipette by mouth.
4. Do not smoke, eat, drink or apply cosmetics in areas where kits and samples are handled.
5. Any skin complaints, cuts, abrasions and other skin lesions should be suitably protected.
6. The Anti-P. carinii Antibody and FITC-Conjugate contain sodium azide which can react with lead and copper plumbing to form highly explosive metal azides. On disposal, drain with large quantities of water to prevent azide build-up.
7. Material safety data sheets for all hazardous components contained in this kit are available on request from Axis-Shield Diagnostics.

<table>
<thead>
<tr>
<th>Reagent A</th>
<th>EUH032 -</th>
<th>Contact with acids liberates very toxic gases.</th>
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<tbody>
<tr>
<td>DANGER</td>
<td>WARNING</td>
<td>May cause allergy or asthma symptoms or breathing difficulties if inhaled.</td>
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<tr>
<td>Reagent C</td>
<td>H334 -</td>
<td>Avoid breathing dust/fumes/gas/mist/vapours/spray.</td>
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<td></td>
<td>PREVENTION</td>
<td>In case of inadequate ventilation wear respiratory protection.</td>
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<td></td>
<td>P261 -</td>
<td>IF INHALED: If breathing is difficult, remove victim to fresh air and keep at rest in a position comfortable for breathing.</td>
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<td></td>
<td>P285 -</td>
<td>If experiencing respiratory symptoms: Call a POISON CENTER or doctor/Physician.</td>
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<td>P304+341 -</td>
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<td>P342+311 -</td>
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**PREPARATION**

**Materials/Equipment Required but not Provided**

1. Precision pipettes to dispense 5 µL, 15 µL, 20 µL and 200 µL.
2. Centrifuge for volumes up to approximately 30 mL at 3,000 x g.
3. Distilled/ultrapure water.
4. Wash bottle containing distilled/ultrapure water.
5. Analar or equivalent grade acetone.
6. Incubator at 37°C.
7. Humidified slide incubation chamber at 37°C.
8. Microscope coverslips, 18 x 18 mm and 50 x 20 mm.
9. Ultraviolet microscope equipped for viewing fluorescein and Evans Blue fluorescence.
10. Cytospin (e.g. Cytospin 2), optional.
11. Timer for 5 to 30 minutes.
12. Wicking/tissue material.
13. Appropriate mucolytic agent for induced sputum specimens, e.g. Sputolysin (Behring Diagnostic) or Sputasol (Oxoid). Use as recommended by the manufacturer; alternatively, use 0.1% Dithiothreitol (w/v) solution in a 1:1 ratio with specimen volume and incubate at 37°C for as long as required.

N.B. Dithiothreitol can irritate eyes and skin. If contact with skin or eyes occurs irrigate with water for at least 10 minutes. If discomfort exists, seek medical attention.

**Preparation for the Assay**

Allow all reagents to equilibrate to room temperature.

Reconstitute lyophilised Enzyme with 200 µL 0.001M HCl; this results in a 10X concentrate. Record the reconstitution date on the label and allow to stand at room temperature (18-25°C) for ten minutes. Mix gently by inversion, ensuring all particulate material is in solution. Reconstituted enzyme is stable for 3 months at 2-8°C.

**ASSAY PROTOCOL**

**Pre-treatment of Specimens**

Patient specimens should be tested as soon as possible after collection. When performing an assay be aware of the potential HIV status of specimens and take all the recommended precautions for dealing with such specimens. Induced sputum specimens should be pre-treated with a mucolytic agent, e.g. Sputasol. Non-mucoid specimens such as BAL will normally not require the mucolytic procedure.

**Protocol**

1. Centrifuge specimens for 15 minutes at 3,000 x g, and wash the particulate/pelletable material in distilled/ultrapure water. Repeat once or twice, ensuring the pellet is fully resuspended between washes.
2. Resuspend the final pellet in a small amount of distilled/ultrapure water, such that the density of the material is not excessive, and vortex.
3. Spread 10-20 µL over the entire area of one or more Patient Specimen Slide wells. Evaporate to dryness at 37°C. If a Cytospin (e.g. Cytospin 2) is available, spin 0.4 to 0.5 mL BAL or IS at 900 rpm, using one white and one tan filter.
4. Fix specimens by overlaying 1-2 drops Analar (or equivalent quality) acetone. Allow to evaporate at room temperature.
5. Rinse Cytospin preparations with a stream of distilled/ultrapure water to remove salts from the specimen, as they reduce the efficacy of enzyme digestion.
6. Air-dry slides.
7. Dilute the reconstituted Enzyme 1 in 10 (1+9) with Enzyme Diluent. Dilute only enough reconstituted Enzyme for immediate requirements.
8. Overlay dried and fixed specimens with 20 µL diluted Enzyme. Ensure the entire well area is covered by reagent.
9. Incubate slides for EXACTLY 30 minutes in a humidified chamber set at 37°C. Over-digestion of oocysts will result if incubation is continued for more than 30 minutes. Oocysts may become less characteristic and less readily identifiable.
10. Rinse slides with distilled/ultrapure water by running a stream of water over the surface of the wells. Do not direct the jet directly at the specimen.
11. Wick and air-dry the slides.
12. Add **15 µL** anti-*P. carinii* Antibody to specimens. Ensure that the entire well area is covered by reagent. Incubate in a humidified chamber for 15 minutes at 37°C.

13. Rinse wells as described in step 10, wick and air-dry.

14. Add **15 µL** FITC-Conjugated Anti-Mouse Antibody to specimens. Ensure that the entire well area is covered by reagent. Incubate in a humidified chamber for 15 minutes at 37°C.

15. Rinse wells, wick and air-dry.

16. Place a drop of Mounting Medium onto every well in use and apply a coverslip of appropriate size. Invert the slide on an absorbent tissue and gently press to exclude excess Mounting Medium and air bubbles.

17. Examine specimens for bright to medium bright apple-green oocysts, which may be evenly or unevenly labelled. Cellular debris and other material may be counterstained with Evans Blue, which will fluoresce red. Examine the entire specimen area.

### INTERPRETATION OF RESULTS

**POSITIVE RESULT** - Five or more fluorescent oocysts over the whole slide.

**EQUIVOCAL RESULT** - One to five fluorescent oocysts.

**NEGATIVE RESULT** - No fluorescent oocysts. If *P. carinii* infection is still suspected, repeat the assay with a heavier inoculum.

### PERFORMANCE DATA

**CENTRE 1**<sup>10</sup>

223 BAL and IS specimens from HIV patients with respiratory tract symptoms were evaluated and compared with modified Grocott stain. Overall agreement = 90.6%.

Of 21 (9.4%) discrepant results, six subsequent specimens were obtained, and five out of six results were positive by both tests.

**CENTRE 2**<sup>8</sup>

135 IS specimens were evaluated and compared with Grocott stain. Overall agreement = 88.9%.

Fifteen results (11.1%) were Detect IF *P. carinii* positive/equivocal and Grocott negative. The authors concluded that this indicates an increased sensitivity for *P. carinii* in cytological preparations of IS with the immunofluorescence technique compared to conventional stains.

**CENTRE 3**<sup>9</sup>

254 BAL and IS specimens from 75 patients with AIDS, other immunocompromised patients, including transplant patients, and patients diagnosed as ‘atypical pneumonia’ were evaluated and compared with Grocott stain. Overall agreement = 94.1%.

Fifteen results (5.9%) were Detect IF *P. carinii* positive/equivocal and Grocott negative. The authors concluded that the Detect IF test was more reliable and sensitive than the Grocott technique.

**CENTRE 4**<sup>6</sup>

50 BAL and 50 IS specimens were tested for *P. carinii* infection using indirect immunofluorescence, direct immunofluorescence, modified Wright-Giemsa stain and modified silver stain. A positive specimen was defined as any smear which was positive by two or more methods.

Using this definition, the sensitivity and specificity of DETECT IF *P. carinii* were as follows.

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<th>BAL</th>
<th>IS</th>
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<tr>
<td>Sensitivity</td>
<td>86%</td>
<td>97%</td>
</tr>
<tr>
<td>Specificity</td>
<td>100%</td>
<td>100%</td>
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**CENTRE 5**

152 BAL specimens from patients with clinical evidence of *P. carinii* pneumonia were evaluated and compared with Grocott stain. Results for each method were compared to the clinical evidence of *P. carinii* pneumonia (PCP). In five cases, the clinical evidence result was equivocal; four were Detect IF positive and Grocott negative, one was Grocott positive and Detect IF negative.

Overall agreement of DETECT IF with clinical evidence of PCP = \( \frac{146}{147} = 99.3\% \) (one result was equivocal with IF)

Overall agreement of Grocott stain with clinical evidence of PCP = \( \frac{140}{147} = 95.2\% \)

Overall agreement of DETECT IF with Grocott = 94.5%
LIMITATIONS OF USE

1. A negative result does not exclude the possibility of *P. carinii* infection. Results should be interpreted in light of all clinical and diagnostic information. If necessary, obtain a further specimen.

2. Excess mucous in specimens may prevent adequate staining.

3. The FITC-conjugated anti-mouse antibody has the potential to cross-react with *Candida albicans* when present in patient samples which can be misinterpreted as false positive results.\(^\text{15}\)
REFERENCES

For in vitro diagnostic use

Catalogue number

Lot

Reagent A – F

Specimen Slide

60 tests

Protect from light

See instructions for use

Use by

Store at 2-8°C

Manufactured by

Global Trade Item Number

Axis-Shield Diagnostics Limited
The Technology Park, Dundee DD2 1XA, United Kingdom.
Tel: +44 (0) 1382 422000, Fax: +44 (0) 1382 422088.
E-mail: shield@axis-shield.com
Website: www.axis-shield.com

Ver: 2014/11