An Indirect Immunofluorescence Test for the Detection of P. jirovecii in Human Clinical Specimens / Test d'immunofluorescence indirecte pour la détection de P. jirovecii dans les échantillons cliniques humains / Prueba indirecta de inmunofluorescencia para la detección de P. jirovecii en muestras clínicas humanas / Indirekter Immunofluoreszenztest (IFFT) zur Bestimmung von P. jirovecii in Humanproben / Test di immunofluorescenza indiretta per la rivelazione di P. jirovecii in campioni clinici umani / Teste de imunofluorescência Indirecta para Detecção de P. jirovecii em Amostras Clínicas de Origem Humana / En indirekte immunofluorescens test for opdagelse af P. jirovecii i kliniske humanprøver / En indirekt immunofluorescenstest för detektion av P. jirovecii i humana kliniska prov / Μια εξέταση εμμέσου ανοσοφθορισμού για την ανίχνευση της P. jirovecii σε ανθρώπινα κλινικά δείγματα

FOR PROFESSIONAL USE ONLY

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ΓΙΑ ΕΠΑΓΓΕΛΜΑΤΙΚΗ ΧΡΗΣΗ ΜΟΝΟ

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ENGLISH INTENDED USE

The MONOFLUO™ KIT P. jirovecii test is an indirect qualitative immunofluorescence kit for the detection of P. jirovecii oocysts in human bronchoalveolar lavage fluid and induced sputum. It is intended to aid in the diagnosis of suspected P. jirovecii infection. Results should be interpreted in light of all clinical and diagnostic information.

INTRODUCTION

Pneumocystis jirovecii (P. carinii) is a unicellular, eukaryotic organism that is present in the lungs of many mammalian species, including man. The Pneumocystis organisms found in humans were originally referred to as P. carinii f. sp. hominis, the subspecies name used to distinguish the Pneumocystis organisms found in humans from Pneumocystis organisms found in other mammals. Recently the Pneumocystis organism found in humans was recognized as a distinct species and renamed Pneumocystis jirovecii.

The organism is spread by airborne routes, usually causing asymptomatic infection. It is a major pathogen in the immunocompromised, especially patients with AIDS, 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. jirovecii pneumonia.

Onset of P. jirovecii 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. jirovecii pneumonia may be diagnosed by the observation of P. jirovecii in open lung or transbronchial lung biopsy material, bronchoalveolar lavage 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. jirovecii 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. jirovecii oocysts have become available, allowing the development of immunofluorescent techniques to rapidly and unambiguously identify P. jirovecii oocysts in bronchoalveolar material and induced sputum. The MONOFLUO™ KIT P. jirovecii test uses a murine monoclonal antibody reactive with both human and rodent P. jirovecii in a simple and rapid test for the detection and identification of P. jirovecii 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. jirovecii 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. jirovecii oocysts in bronchoalveolar lavage fluid or induced sputum indicates P. jirovecii infection.

KIT COMPONENTS

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<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Anti-P. jirovecii Monoclonal Antibody</td>
<td>1 x 1ml</td>
<td>Murine anti-P. jirovecii monoclonal antibody, bovine serum albumin, 0.1% (w/v) sodium azide. Ready-to-use.</td>
</tr>
<tr>
<td>B</td>
<td>FITC-Conjugated Anti-Mouse Antibody</td>
<td>1 x 1ml</td>
<td>Fluorescein-isothiocyanate (FITC) conjugated anti-mouse antibody, Evans Blue counterstain. Ready-to-use.</td>
</tr>
<tr>
<td>C</td>
<td>Enzyme (Lyophilised)</td>
<td>1 vial</td>
<td>Pre-treatment enzyme for clinical specimens. Reconstitute with 200µl 0.001M HCl (supplied) and dilute before use.</td>
</tr>
<tr>
<td>D</td>
<td>Dilute Hydrochloric Acid (0.001M HCl)</td>
<td>1 x 0.5ml</td>
<td>For Enzyme reconstitution. Ready-to-use.</td>
</tr>
<tr>
<td>E</td>
<td>Enzyme Diluent</td>
<td>1 x 3ml</td>
<td>Tris buffer with enzyme activator. Ready-to-use.</td>
</tr>
<tr>
<td></td>
<td>Patient Specimen Slides</td>
<td>25 slides</td>
<td>PTFE-coated (yellow) slides with four square specimen wells.</td>
</tr>
<tr>
<td>F</td>
<td>Mounting Medium</td>
<td>2 x 3ml</td>
<td>Phosphate-buffered glycerol, Citifluor photobleaching retardant. Ready-to-use.</td>
</tr>
<tr>
<td></td>
<td>Pack Leaflet</td>
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</table>
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 200µl 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 30ml bronchoalveolar lavage and 2-4ml 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.15
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. jirovecii 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 Bio-Rad.

A ANTIBODY

Harmful
R22: Harmful if swallowed.
R32: Contact with acids liberates very toxic gas.
S23: Do not breathe fumes.
S36: Wear suitable protective clothing.
S60: This material and its container must be disposed of as hazardous waste.

C ENZYME

Harmful
R42: May cause sensitisation by inhalation.
S22: Do not breathe dust.
S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S36/37: Wear suitable protective clothing and gloves.
S45: In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible)
S60: This material and its container must be disposed of as hazardous waste.
PREPARATION

Materials/Equipment Required but not Provided

1. Precision pipettes to dispense 5µl, 15µl, 20µl and 200µl.
2. Centrifuge for volumes up to approximately 30ml 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 18mm and 50 x 20mm.
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 200µl 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 10-20µl 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 0.5ml 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 20µl 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 15µl anti- P. jirovecii 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 **15µl** 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. jirovecii* infection is still suspected, repeat the assay with a heavier inoculum.

**PERFORMANCE DATA**

**CENTRE 1**

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**

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

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

**CENTRE 3**

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 MONOFLUO™ KIT *P. jirovecii* positive/equivocal and Grocott negative. The authors concluded that the MONOFLUO™ KIT *P. jirovecii* test was more reliable and sensitive than the Grocott technique.

**CENTRE 4**

50 BAL and 50 IS specimens were tested for *P. jirovecii* 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 MONOFLUO™ KIT *P. jirovecii* were as follows.

<table>
<thead>
<tr>
<th>Specimen Type</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAL</td>
<td>86%</td>
<td>100%</td>
</tr>
<tr>
<td>IS</td>
<td>97%</td>
<td>100%</td>
</tr>
</tbody>
</table>

**CENTRE 5**

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

Overall agreement of MONOFLUO™ KIT *P. jirovecii* with clinical evidence of PCP = 146/147 = 99.3% (one result was equivocal with IF)

Overall agreement of Grocott stain with clinical evidence of PCP = 140/147 = 95.2%

Overall agreement of MONOFLUO™ KIT *P. jirovecii* with Grocott = 94.5%

**LIMITATIONS OF USE**

1. A negative result does not exclude the possibility of *P. jirovecii* 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. 

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For in vitro diagnostic use / Pour diagnostic in vitro / Para uso diagnóstico in vitro
In Vitro Diagnosticum / Per uso diagnostico in vitro
Para utilizar no diagnóstico / Kun for in vitro diagnostik.
För diagnostisk användning in vitro / Για διαγνωστική χρήση in vitro

Catalogue number / Numero catalogue / Numero de catalogo / Bestellnummer
Numero di catalogo / Número de catálogo Bestellungsnummer / Katalognummer

Lot / Lot / Lote / Ch.-B. / Lotto
Lote / Parti / Lot / Παρτίδα

60 tests / 60 determinations / 60 pruebas / 60 Bestimmungen
60 tests / 60 testes / 60 tests / 60 tester / 60 προσδιορισμοί

Caution / Avertez / Adverta / Verwarnen / Cautela
Cuidado! / Advarsel / lakttag försiktighet / Προσοχή

See instructions for use / Voir les consignes d’utilisation
Ver las instrucciones de uso / Gebrauchsinformation beachten
Vedere le istruzioni per l’uso / Ver as instruções de utilização / Se brugsvejledningen / Se instruktionaler för användning / Δείτε τις Οδηγίες χρήσης

Use by / Utiliser avant / Utilizar antes de / Verwendbar bis / Scadenza
Utilizar antes de / Brug før / Använd före / Ημερομ. Λήξης

Store at 2-8°C / Conserver a 2-8°C / Conservar a 2-8°C / Lagerung bei 2-8°C
Conservare a 2-8°C / Armazenar entre 2 e 8°C / Opbevar ved 2-8°C
Förvara vid 2-8°C / Φυλάσσετε στους 2-8°C

Manufactured by / Fabrique par / Fabricado por / Hergestellt von / Prodotto da
Fabricado por / Fremstillet af / Tillverkad av / Κατασκευάζεται από την

Keep away from light / Conserver à l’abri de la lumière / Mantener alejado de la luz /
Vor Licht schützen / Tenere al riparo dalla luce / Manter ao abrigo da luz / Må ikke udsættes for lys / Förvara iväg från ljus / Φυλάξτε το μακριά από το φως

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