

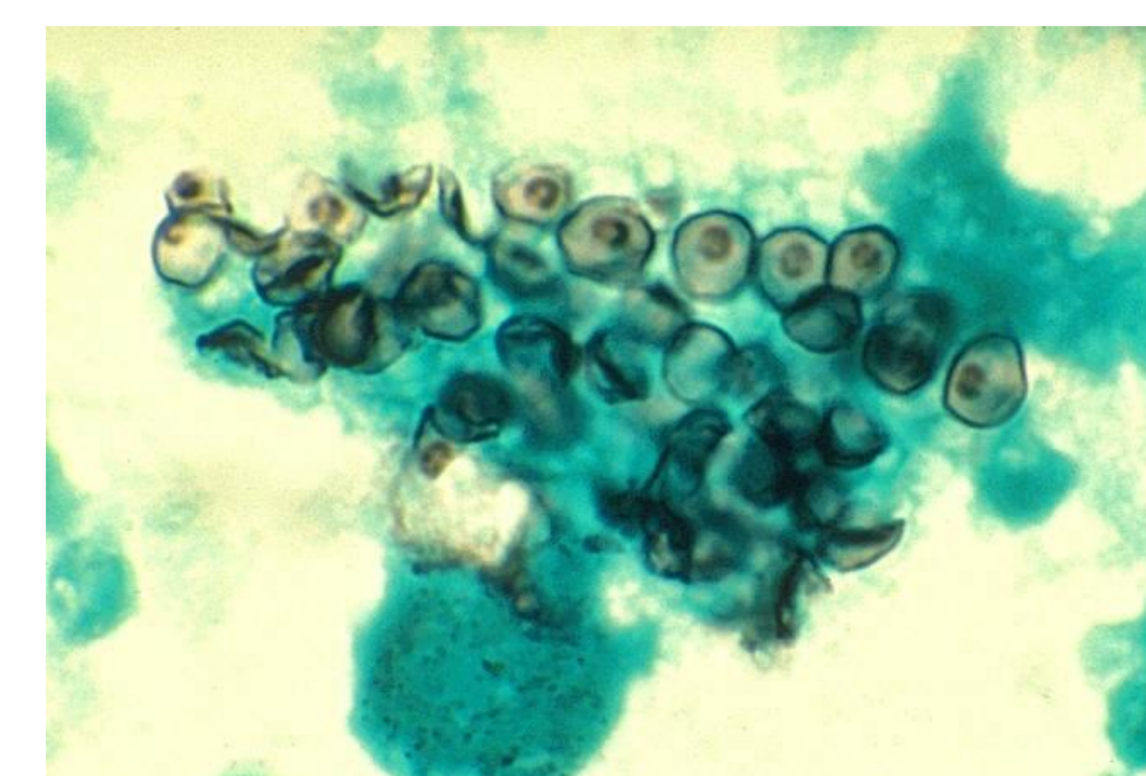
# Diagnosis of *Pneumocystis jirovecii* pneumonia by detection of DNA in blood and oropharyngeal wash, compared with sputum

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## Background

- *Pneumocystis jirovecii* pneumonia (PCP) remains one of the commonest opportunistic infections in the UK
- Diagnosis is generally by immunofluorescence or polymerase chain reaction (PCR) on respiratory specimens
- However, many patients do not expectorate and laboratory confirmation can be difficult
- This study was designed to compare sensitivity and specificity of PCR on alternative samples to evaluate less invasive, simpler methods of diagnosing PCP



## Methods

- A prospective study of individuals being investigated for PCP as part of clinical care at the infectious diseases unit, North Manchester General Hospital
- Consenting individuals provided specimens as follows, on the same day where possible:
  - Sputum, induced if necessary
  - Blood (3.5ml in EDTA)
  - Oropharyngeal wash (OPW: gargle with 10ml normal saline)
- Laboratory methods
  - All specimens were analysed by in-house real-time TaqMan<sup>®</sup> PCR assay, targeting a 250 base-pair regions of the mitochondrial large subunit rRNA gene
  - Clinical and laboratory data were collected using a standardised case report form
  - Results from PCR on OPW and blood were compared with PCR on sputum for sensitivity and specificity

## Results

### Description of patients

- 45 participants provided 45 sputa, 31 OPW and 41 blood samples
- 41 (91%) were male; 38 (84%) Caucasian; median age 39 years (interquartile range [IQR] 34, 47)
- One HIV-negative renal transplant recipient; 44 HIV-positive with median CD4 count 64 (IQR 15, 160)
- 9/44 (20%) of HIV+ on antiretroviral therapy (ART) at the time of recruitment; 4 had undetectable plasma HIV RNA.
- Of 35/44 not on ART, median HIV RNA 164550 copies/ml

### PCP prophylaxis and treatment

- 10/45 had prior episodes of PCP
- 9/45 taking PCP prophylaxis (6 dapsone; 3 cotrimoxazole)
- 39/45 started empirical treatment for PCP a median 2 days before samples taken (range 11 days before – 2 days after samples)

### PCR on OPW compared with sputum

- Sensitivity 53% (95% confidence interval [CI] 29, 77%)
- Negative predictive value 55% (95% CI 32, 77%)
- Specificity 100%, Positive predictive value 100% (i.e. no false negatives)
- If samples taken <=2 days after treatment start, sensitivity 80% (8/10; 95% CI 51, 100%)
- In 22/31 participants, OPW and sputum were taken on the same day (the remainder taken from -1 to +3 days from sputum specimen)



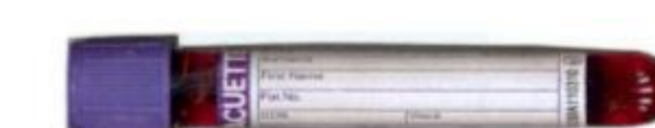
### Laboratory results

- PCR for PCP was positive in 27/45 (60%) sputum specimens
- Results from paired OPW/sputum and blood/sputum specimens are shown in the table

PCR results from OPW and blood, vs. sputum	Sputum PCR positive (+)	Sputum PCR negative (-)	Total
OPW PCR+	9	0	9
OPW PCR-	10	12	22
Total	19	12	31
Blood PCR+	12	0	12
Blood PCR-	12	17	29
Total	24	17	41

### PCR on blood compared with sputum

- Sensitivity 50% (95% confidence interval [CI] 29, 71%)
- Negative predictive value 59% (95% CI 40, 77%)
- Specificity 100%, Positive predictive value 100% (i.e. no false negatives)
- If samples taken <=2 days after treatment start, sensitivity 57% (8/14; 95% CI 29, 86%)
- In 28/41 participants, blood and sputum were taken on the same day (the remainder taken from -3 to +3 days from sputum specimen)



## Limitations

- Small, proof-of-concept study with not all specimens taken on the same day or before treatment commenced

## Conclusions

- In this small demonstration study, PCP PCR using oropharyngeal wash or blood is relatively insensitive, particularly if taken during treatment
- However, results from OPW early in or prior to treatment are promising and OPW could be used if obtained promptly
- In this study, PCP PCR on either OPW or blood had 100% specificity (no false positives compared with sputum). Therefore, obtaining invasive respiratory specimens may not be required if PCP DNA is detected in these specimens
- Larger studies on early OPW may provide more confidence in its use and avoid invasive investigations