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Citation for final published version:

White, P. Lewis, Backx, Matthijs and Barnes, Rosemary 2017. Diagnosis and management of *Pneumocystis jirovecii* infection. *Expert Review of Anti-infective Therapy* 15 (5) , pp. 435-447. 10.1080/14787210.2017.1305887 file

Publishers page: <http://dx.doi.org/10.1080/14787210.2017.1305887>  
<<http://dx.doi.org/10.1080/14787210.2017.1305887>>

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1 **Title:** Diagnosis and Management of *Pneumocystis jirovecii* infection

2

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DRAFT CONFIDENTIAL

16 **ABSTRACT**

17 *Pneumocystis jirovecii* is a ubiquitous fungus causing pneumonia in humans. Diagnosis  
18 was hampered by the inability to culture the organism, with diagnosis based on  
19 microscopic examination of respiratory samples. Performance of microscopy was  
20 improved by immunofluorescent (IF) testing using monoclonal antibodies targeting  
21 both cyst and trophic forms. Although microscopy is specific, with positivity used to  
22 define disease, poor sensitivity meant negativity could not exclude it.  
23 New assays can assist in the diagnosis. PCR has permitted testing of respiratory samples  
24 other than bronchoalveolar lavage (BAL), easing sampling pressures. PCR has greater  
25 sensitivity than IF but questions remain as to the significance of low level positivity, in  
26 respect to colonisation versus disease associated with low fungal burdens. Conversely,  
27 PCR negativity in BAL samples can exclude disease, provided sampling is adequate. The  
28 presence of 1-3- $\beta$ -D-Glucan in serum is also a useful biomarker, providing high  
29 sensitivity. However, 1-3- $\beta$ -D-Glucan is not specific to *Pneumocystis jirovecii* pneumonia  
30 (PCP), and definitive thresholds for PCP are not available. Combination testing has the  
31 potential to both diagnose and exclude PCP. Recommendations on prophylactic and  
32 therapeutic management will be discussed with reference to new guidelines for PCP.

## 33 INTRODUCTION

34 *Pneumocystis jirovecii* pneumonia (PCP) was an early indicator of the HIV epidemic and  
35 occurred in 70-80% of AIDS patients.<sup>1-3</sup> The incidence of PCP associated with HIV has  
36 fallen, a result of earlier HIV diagnoses, better anti-retroviral therapy and the use of  
37 prophylaxis. Most HIV associated cases of PCP now occur in patients with undiagnosed  
38 HIV.<sup>4,5</sup> There is an increasing population of susceptible non-HIV patients, including  
39 those with solid malignancies, solid organ transplant and haematopoietic stem cell  
40 transplant recipients, patients receiving immuno-suppressive therapies for auto-  
41 immune and inflammatory conditions and those with genetic primary immune  
42 deficiency disorders.<sup>6</sup> A national study over the decade 2000-2010 showed an annual  
43 average increase in incidence of 9%, and the largest cohort associated with PCP were  
44 those suffering from underlying haematological malignancy.<sup>5</sup> Cases of PCP have also  
45 been diagnosed in less typical scenarios, such as in non-HIV individuals suffering from  
46 Dengue fever and those with pre-existing lung disease.<sup>5,7</sup>

47 Children are exposed to *Pneumocystis* at early age, between the ages of 2-4 years old  
48 over 80% of children will have generated antibodies. Reactivation of latent infection  
49 was a presumed source of infection in susceptible hosts later in life.<sup>8,9</sup> However, several  
50 documented PCP outbreaks confirm anthropophilic transmission, likely by airborne  
51 dispersal. Furthermore, typing revealed that infection was associated with place of  
52 diagnosis rather than place of birth.<sup>10</sup> Increased risk for developing PCP is associated  
53 with immuno-suppression, primarily a reduction in the CD4 lymphocyte count or  
54 lymphocyte dysfunction. A summary of risk factors is listed in Table 1.

55 The primary manifestations are associated with the respiratory tract, with extra-  
56 pulmonary disease, potentially associated with any organ, a rare manifestation.

57 Symptoms are generally non-specific, including fever, non-productive cough, worsening

58 chest pain, shortness of breath (especially on exertion), with the severity of symptoms  
59 often greater in non-HIV patients.<sup>11</sup> In mild cases initial examination may appear  
60 normal, although under exertion heart rate and oxygenation levels may become  
61 abnormal.<sup>11</sup> In HIV+ patients the onset of symptoms can be indolent often delaying  
62 diagnosis by weeks, whereas in non-HIV patients PCP presentation is acute, often  
63 fulminant, particularly after corticosteroid administration.<sup>12</sup> The mortality rate in HIV+  
64 patients ranges from 17-30%, whereas in non-HIV patients are higher ranging from 28-  
65 53%.<sup>12</sup>

66 Given the non-specific nature of the clinical findings further investigations specific to  
67 *Pneumocystis* are necessary to confirm a diagnosis of PCP even in symptomatic high-risk  
68 patients and diagnosis should not be based on clinical presentation and radiology.<sup>13</sup>

69 Microscopic examination and molecular testing of respiratory samples are available but  
70 both have different performance limitations. Alternatively, serum/plasma samples can  
71 be tested for the presence of (1-3)- $\beta$ -D-Glucan (BDG), although this assay cannot  
72 differentiate between the broad range of fungal pathogens it is capable of detecting.

73 Clinical investigations (e.g. radiology) can provide insight in likelihood of PCP by  
74 showing evidence of the disease process or potential host response to infection, but  
75 again lack aetiological specificity. Recent guidelines for the diagnosis and management  
76 of PCP are available but the evidence is lacking or weak in many areas.<sup>11-17</sup>

## 77 **DIAGNOSIS**

### 78 **Understanding test formats**

79 The incidence of a disease influences utility of diagnostic tests, and can determine the  
80 optimal testing strategy in different clinical settings. Before ordering any test, clinicians  
81 should decide how the test result (positive or negative) would affect the management of  
82 their patient. If both outcomes are the same then the test has no clinical value. Clinicians  
83 often focus on a purely diagnostic approach, but many tests are better suited to exclude  
84 a diagnosis, avoiding the need for unnecessary therapy. Testing can also be used  
85 prognostically to monitor disease and assess the duration and response to therapy.  
86 For most cohorts the incidence of PCP is relatively low and the pre-test probability of  
87 disease is small compared to the pre-test probability of not having disease.  
88 Consequently, negative results are better suited to excluding disease through a high  
89 sensitivity and negative predictive value (NPV). With high sensitivity comes potential  
90 false positivity but specificity can be improved by intensifying the diagnostic work-up  
91 through repeat and combination testing, and multi-disciplinary interpretation of results.  
92 Different sample types, for example upper and lower respiratory tract specimens and  
93 even blood samples, may shift the emphasis of the result from sensitivity/NPV to high  
94 specificity/positive predictive value (PPV).

### 96 **Radiological investigations**

97 Chest radiography (CXR) may be normal during the early stages of disease, but can  
98 worsen rapidly, particularly in the non-HIV population.<sup>11, 12, 17</sup> Computerised  
99 tomography (CT) scans are more sensitive than conventional radiographic techniques,  
100 providing evidence of infection even during the early stages of disease in non-HIV  
101 patients, and there is a role for CT despite CXR negativity.<sup>18, 19</sup> CXR typically presents

102 with bilateral, diffuse interstitial infiltrates that progress to bilateral consolidations.<sup>11,</sup>  
103 18,20  
104 CT generally demonstrates bilateral, symmetric patchy ground-glass attenuation.  
105 Consolidations may be present in mid or late stages of disease.<sup>12,18</sup> Other findings  
106 include nodules, cysts, pneumothoraces, upper lobe localization, linear opacities and  
107 septal thickening.<sup>11,12,20,21</sup> Cavitation, intra-thoracic adenopathy and pleural effusions  
108 are less likely.<sup>11,16,21</sup> The radiological presentation of PCP is not specific and can imitate  
109 other pathogens (e.g. bacterial pneumonia).<sup>22</sup> Radiology cannot provide an aetiological  
110 diagnosis, but may be used to initiate empirical therapy in high risk patients. This  
111 should trigger efforts to achieve a mycological diagnosis of the organism from the  
112 respiratory tract.  
113 Recent developments for the imaging of PCP include the successful application of ultra  
114 low dose chest CT, fluorodeoxyglucose positron emission tomography (FDG-PET) and  
115 bronchoscopic probe-based confocal laser endomicroscopy.<sup>23-25</sup> CT has also been used  
116 to determine the severity and prognosis of PCP infection.<sup>18,21</sup>

### 118 **Non-Microbiological Laboratory Investigations**

119 Overall lymphocyte count should be determined, as values <10% of the norm has been  
120 associated with a poor prognosis in PCP infection.<sup>26</sup> Lymphocyte function in addition to  
121 absolute numbers may also be significant and the role of recent immunosuppressant  
122 drugs and other biological response modifying agents should be considered.  
123 Hypoxaemia will vary depending on the severity of disease and HIV status, and  
124 regularly presents as a mild and severe reduction arterial oxygen in HIV+ and non-HIV  
125 patients, respectively.<sup>11,12</sup> Serum lactate dehydrogenase (LDH) elevation is a suggestive  
126 marker, with levels >500ml/dL associated with PCP.<sup>11</sup> Extracellular LDH indicates cell

127 damage or cell death, with elevated levels correlating with lung tissue damage, but it is  
128 not specific for PCP and is of little use outside the HIV+ population. In a study of LDH in  
129 performance in HIV+ and non-HIV cohorts the sensitivity and specificity were  
130 100%/47% and 63%/43%, respectively, showing that within HIV+ cohort a negative  
131 result could be used to confidently exclude disease, but positivity required confirmatory  
132 testing.<sup>27</sup> The use of procalcitonin serum concentration to differentiate PCP from other  
133 respiratory infections and/or colonisation is not clear.<sup>28-30</sup>  
134 Clinical factors have also been used to predict mortality. In large observational cohort  
135 study of 451 HIV+ patients five significant predictors (Age, recent intravenous drug use,  
136 total bilirubin, serum albumin and alveolar-arterial oxygen gradient) were determined  
137 through multivariate analysis and incorporated into model to predict PCP mortality.<sup>31</sup>

138

### 139 **Conventional Techniques – Culture**

140 The difficulty in culturing *Pneumocystis* has hindered both diagnosis and research and  
141 development. Several methods using various co-culture cell lines were described but  
142 failed to attain widespread use.<sup>32</sup> Most attempts have used rat-models and  
143 subsequently *P. carinii* not *P. jirovecii*. In 1999, *P. carinii* initially isolated from rat lung  
144 was cultured using continuous axenic cultivation.<sup>33</sup> This complex technique has been  
145 successfully applied to the recovery of *P. carinii* from lungs and BAL fluid of rats and  
146 used to investigate life-cycle, but has limited use in routine diagnostics.<sup>34-36</sup>  
147 In 2014, the first successful cultivation and propagation of *P. jirovecii* direct from BAL  
148 was achieved using a three-dimensional air-liquid interface culture system formed by  
149 CuFi-8 respiratory epithelial cell line.<sup>32</sup> While this represents a major breakthrough and  
150 provides the potential to perform antifungal susceptibility testing, it still requires cell



151 culture, limiting its use in routine diagnostics laboratories, being replaced by direct  
152 molecular methods.

153

#### 154 **Conventional Techniques – Microscopy**

155 The gold standard for the diagnosis of PCP remains the histological and microscopic  
156 identification of ascus (cysts containing ascospores) and trophic forms using Wright's-  
157 Giemsa, toluidine blue O, calcofluor white or Grocott-Gomori stains, in tissue, BAL and  
158 induced sputum. While Gomori stains the cell wall of the ascus form, Giemsa will stain  
159 both ascus and trophic forms but do not stain the cell wall. Toluidine blue is a generic  
160 stain for nucleic acids and polysaccharides, while calcofluor white stains chitin and  
161 cellulose, neither is specific for *Pneumocystis*. The performance of conventional stains  
162 has been superseded by IF microscopy using anti-*P. jirovecii* monoclonal antibodies.<sup>12</sup>  
163 However, in the majority of studies only the ascus form was targeted and a combination  
164 of stain and/or IF kit to detect both ascus and trophic forms is recommended. IF kits  
165 that detect both forms are available (e.g. Monofluo™ *Pneumocystis jirovecii* IFA or  
166 Merifluor *Pneumocystis* kits).<sup>12</sup>  
167 In a comparison of four staining methods sensitivities were 73.8%, 76.9%, 48.4% and  
168 90.8%, for calcofluor white, Grocott-Gomori, Diff-Quik (modified Wright's-Giemsa) and  
169 Merifluor *Pneumocystis* respectively.<sup>37</sup> The sensitivity of the Diff-Quik method was  
170 significantly lower than the other methods. For conventional stains the corresponding  
171 specificity was >99%, whereas for the IF antibody assay (Merifluor *Pneumocystis* kit) it  
172 was 94.7%, significantly lower than the other methods.<sup>37</sup> The authors concluded that  
173 the Merifluor *Pneumocystis* kit was a useful screen to exclude PCP but the  
174 specificity/PPV was insufficient to confirm disease. However, the positive likelihood  
175 ratio (less affected by prevalence) for the Merifluor *Pneumocystis* kit was 17.1, and

176 subsequent positive results are associated with PCP. Conversely, none of the non-IF  
177 methods generated a negative likelihood ratio  $\leq 0.1$ , and cannot be used to exclude  
178 disease confidently.

179 For microscopic approaches a primary screen with a highly sensitive IF method  
180 confirmed by a secondary specific method is recommended.<sup>12</sup> A summary of the  
181 comparative performance of various microscopic staining and fluorescent techniques  
182 for the diagnosis of PCP is shown in table 2.<sup>38-40</sup>

183 It is important to consider the influence of specimen type and quality on assay  
184 performance. There is no standardised approach to sampling the respiratory tract and  
185 protocols will vary across centres affecting the quality of BAL and sputa. When  
186 comparing both IF and conventional staining on sputum and BAL, the sensitivity was  
187 lower when testing sputum across all assays.<sup>40</sup> In a meta-analysis involving seven  
188 studies with 160 cases and 162 controls the sensitivity and specificity of staining and IF  
189 of induced sputum was determined using BAL testing as a reference.<sup>41</sup> Overall  
190 sensitivity and specificity when testing induced sputum was 55.5% and 98.6%,  
191 respectively, although the sensitivity when IF testing (67.1%) was significantly greater  
192 than conventional staining (43.1%).<sup>41</sup>

193

### 194 **(1-3)-B-D-Glucan**

195 The use of assays to detect (1-3)- $\beta$ -D-Glucan (BDG) is now widely accepted and permits  
196 the testing of easily obtainable serum/plasma specimens. Clinical trials of BDG  
197 performance for the diagnosis of PCP are lacking but various meta-analyses of clinical  
198 evaluations exist (Table 3).<sup>42-44</sup> Overall, sensitivity is high and BDG negativity can be  
199 used to exclude PCP, although false negatives have been noted.<sup>45</sup> Specificity is  
200 suboptimal ( $<90\%$ ).<sup>42-44</sup> A BDG positive result alone cannot be considered diagnostic of

201 PCP, due to the assays broad detection range coupled with a patient cohort that may  
202 susceptible to other fungal pathogens. The result should be interpreted along with  
203 radiological findings together with a PCP specific assay. Specificity will also be affected  
204 by non-infective factors such as potential sources of false positivity.<sup>46</sup> For the diagnosis  
205 of PCP there was no difference in the overall accuracy of BDG assays developed by  
206 different manufacturers.<sup>43</sup> In one meta-analysis, BDG performance when testing  
207 samples from HIV+ versus HIV- patients was comparable, although in a more recent  
208 study sensitivity was deemed to be significantly lower in the non-HIV population (HIV+:  
209 92% versus HIV-: 85%), potentially a result of the greater burden of organism seen in  
210 HIV+ PCP.<sup>43, 44</sup>

211 BDG assays utilise a single positivity threshold for the detection of invasive fungal  
212 disease and it is not possible to confidently determine organism specific fungal  
213 aetiology based on the strength of positivity. However, for cases of PCP it is not unusual  
214 to see positivity greater than the upper limit of the assay (e.g. Fungitell >500pg/ml),  
215 even in the absence of IF staining of respiratory samples.<sup>47, 48</sup> In the study of Damiani *et*  
216 *al.* the median Fungitell BDG concentration across 17 cases of PCP was 1945pg/ml  
217 (range: 122-8000pg/ml), with 10 of the cases generating concentrations >500pg/ml,  
218 and 14 cases with concentrations >300pg/ml.<sup>49</sup> Both control and *Pneumocystis*  
219 colonised patients had BDG concentrations below 90pg/ml. Differentiation of  
220 colonisation from infection was also possible using the Beta-Glucan test Wako™  
221 (colonisation: 49pg/ml versus infection: 173pg/ml).<sup>50</sup> Compared to the Fungitell assay  
222 the overall BDG concentrations generated by the Wako assay were lower for all  
223 categories of infection, potentially reflecting the differences in reaction kinetics and  
224 subsequent positivity thresholds and highlighting the necessity to independently  
225 validate different kits. When testing serum by the Fungitell assay using a positivity

226 threshold of 300pg/ml the sensitivity, specificity, LR+ve and LR-ve were 91%, 92%,  
227 11.4 and 0.1, respectively indicating that the assay could be used to both confirm and  
228 exclude disease.<sup>30</sup>

229 With *Pneumocystis* primarily infecting the respiratory tract a limitation of BDG is poor  
230 clinical utility when testing respiratory samples. *Candida* species are common  
231 commensals of the mucosal membranes and airway colonisation by other fungi is  
232 possible the presence of elevated BDG concentrations are not indicative of disease, and  
233 could be misleading in symptomatic patients. In one study the specificity of BDG testing  
234 of BAL samples was only 68%, compared to 92% when testing serum and  
235 reproducibility was poor with only 5.9% of retested BAL samples confirming the earlier  
236 result.<sup>48</sup> Even when using higher positivity thresholds BDG specificity when testing BAL  
237 fluid remained compromised (241pg/ml: 39%; 783pg/ml 79%).<sup>30</sup> While there has been  
238 a successful attempt to differentiate PCP infected from colonised/uninfected patients  
239 based on BDG concentration. Others have found receiver operator characteristic curve  
240 analysis to be of limited use in defining BDG BAL threshold.<sup>30, 48, 51</sup>

241

## 242 **Molecular Investigations**

243 The use of molecular based tests for the diagnosis of PCP continues to be described with  
244 too many studies to be discussed individually.<sup>13</sup> While the focus on development of  
245 local assays provides technological diversity, it prevents methodological  
246 standardisation, which remains limited, and can affect the outcomes of meta-analyses.  
247 Nevertheless, meta-analyses determining the performance of PCP PCR show excellent  
248 performance for diagnosis (LR+ve:  $\geq 10$ ), but more so the exclusion of PCP (NPV:  $\geq 99\%$ ,  
249 LR-ve:  $\leq 0.03$ ) (Table 4).<sup>52-54</sup>

250 Sub-group analysis using microscopy as the reference standard showed performance  
251 (Se: 97%, Sp: 93%) comparable to the combined population, whereas specificity was  
252 increased to 96% when using other reference standards.<sup>52</sup> Comparison of performance  
253 in HIV+ and HIV- cohorts was similar. <sup>54</sup> When testing BAL the sensitivity and specificity  
254 were 100% and 87%, respectively, but when induced sputa were incorporated  
255 sensitivity was 97% and specificity was 93%. <sup>54</sup> Comparison of performance when PCR  
256 testing BAL fluid with oropharyngeal wash fluid (OW) showed OW to have significantly  
257 lower sensitivity (76%) but higher specificity (93%), indicating that the PCR detection  
258 of *Pneumocystis* in the upper airways is a good indicator PCP (LR+ve 10.4, compared to  
259 8.0 in BAL).<sup>54</sup> While PCP PCR negativity when testing BAL fluid appears to provide the  
260 ability to confidently exclude PCP, false negatives associated with a mutation in the  
261 large sub-unit mitochondrial rRNA has been noted and as with all molecular based  
262 assay surveillance for genetic drift is required, but complicated by the lack of  
263 surveillance cultures. <sup>55</sup> The use of nasopharyngeal aspirates cannot be used to exclude  
264 PCP, but may provide a useful adjunct diagnostic test in combination with other  
265 markers (e.g. BDG). <sup>56</sup>

266 From a technical perspective, the use of commercial kits for cell wall disruption and  
267 nucleic acid extraction affected specificity, while targeting the ITS region for PCR  
268 amplification improved sensitivity, but, along with targeting the large sub-unit  
269 mitochondrial rRNA, decreased specificity. <sup>54</sup> The use of nested-PCR provided  
270 significantly lower specificity which could be attributed to its potential to detect sub-  
271 clinical levels of *Pneumocystis*, although could also be an effect of the contamination  
272 prone process. <sup>53</sup> Nowadays the use of conventional PCR amplification systems has been  
273 superseded by real-time (quantitative) PCR platforms that are associated with  
274 improved specificity but also have been used to differentiate *Pneumocystis* infection

275 from colonisation.<sup>54, 57-59</sup> When interpreting the significance of the burden the  
276 underlying condition of the patient and quality of sample must be considered. For  
277 example, in one study using a real-time PCR cycle threshold (Ct) of 27 was associated  
278 with 100% specificity for the diagnosis of PCP in HIV+ patients, yet the optimal Ct in  
279 HIV- patients was 31 cycles and associated specificity was 80%.<sup>57</sup> Conversely, an upper  
280 Ct of 35 cycles generated a sensitivity of 80% and 1/5 PCP HIV- cases would be missed.  
281 When setting thresholds to confirm or exclude disease it is critical that specificity and  
282 sensitivity are  $\geq 95\%$ , respectively. Otherwise the utility of the assay is compromised  
283 and results of limited clinical value.

284 When interpreting low level PCP PCR positives (Ct >35) it is important to determine  
285 both the quality of sampling and also understand the presentation of clinical disease in  
286 HIV-patients with a low fungal burden but significant immune response. Theoretically,  
287 human DNA can be used as a surrogate for sample assessment. Low levels of human  
288 DNA could represent poor sample quality, whereas if a large quantity is present it could  
289 represent a strong immune response. For reference it is essential to know the typical  
290 burden of human DNA in respiratory samples and it is also requires that sampling is  
291 standardised, which for BAL remains highly variable. The sampling of the upper  
292 respiratory tract is less variable and has been associated with greater specificity.<sup>54</sup>

293 Given the broad range of available PCP PCR assays it may be wise for centres to  
294 incorporate commercially manufactured and standardised tests that have developed an  
295 understanding of how to interpret, in particular low level positives. In a comparative  
296 study of three commercial assays (*Pneumocystis jirovecii* (carinii) – FRT PCR Kit  
297 (AmpliSens), MycAssay *Pneumocystis* (Myconostica) and real-time PCR *Pneumocystis*  
298 *jirovecii* (Bio-Evolution)) the sensitivity and specificity when testing proven/probable  
299 PCP was 100%, 100%, 95% and 83%, 93% and 100%, respectively, and sample

300 concordance between the Amplisens and MycAssay were excellent (Kappa: 0.85).<sup>60</sup> One  
301 interesting concept is the development of a commercial real-time PCR for both the  
302 detection of organism and dihydropteroate synthase (DHPS) point mutations associated  
303 with resistance to sulfa-based drugs such as sulfamethoxazole and dapson, used for  
304 both prophylaxis and treatment of PCP.<sup>61</sup> Using a positivity threshold of 32 cycles the  
305 sensitivity and specificity of the PneumoGenius® assay were 70% and 82%,  
306 respectively. Performance may have been affected by the classification of disease based  
307 on clinical findings in high risk hosts responding to PCP therapy but missing a  
308 mycological criterion. Nevertheless, the assay was able to screen for sulfa-resistance  
309 direct from 89 samples and showed a 4.5% resistance rate.<sup>61</sup>  
310 With more than 60 types of *P. Jirovecii* identified and approximately 30% of PCP cases  
311 infected with multiple types, the ability to investigate transmission and clusters has  
312 been hampered by the difficulty in cultivating *Pneumocystis*.<sup>62,63</sup> Molecular based  
313 methods can also be used to determine the epidemiology and transmission of infection  
314 and to investigate potential outbreak scenarios and multi-locus sequence typing and  
315 multi-locus real-time mutation frequencies have been used.<sup>10,64,65</sup>

316

### 317 **Combination testing**

318 While the reference standard for the diagnosis of PCP remains microscopic evidence,  
319 usually IF, within a respiratory specimen its limited sensitivity cannot be used to  
320 exclude disease.<sup>12,13,17</sup> The question remains whether by combining more sensitive  
321 tests specificity of diagnosis can be improved while maintaining confidence in exclusion.  
322 In the adult haematology population, current guidelines suggest a diagnostic algorithm  
323 involving real-time PCR and IF testing of BAL in patients with a clinical suspicion of  
324 disease. If both are positive, a diagnosis of PCP is confirmed and vice versa.<sup>13</sup> If PCR is

325 positive, but IF negative, diagnosis is made if high burdens are detected, For low  
326 burdens, additional BDG testing is recommended. If PCR is negative but IF positive then  
327 this is considered technically inconsistent and the quality of either result is questioned.  
328 <sup>13</sup> This begs the question why IF is still being performed, rather than being replaced  
329 with PCR in combination with BDG testing. In a study comparing circulating biomarkers  
330 with PCP lung burden 96% of (25/26) patients that were BAL PCP PCR positive but IF  
331 negative were also positive by BDG, as were all (10/10) patients that were BAL PCP PCR  
332 and IF positive. <sup>66</sup> Conversely, 29% (10/34) of PCP PCR and IF negative were BDG  
333 positive, although 15/34 were diagnosed with proven/probable invasive aspergillosis.  
334 Given the panfungal nature of BDG, it makes sense to perform a primary investigation  
335 using PCP PCR, and if positive confirm, dependent on pulmonary burden, with BDG  
336 testing. <sup>49</sup> When BAL samples are not available BDG testing of serum is recommended  
337 where negativity can be used to exclude PCP, but positivity should be confirmed by PCR  
338 (or IF) testing of less invasive respiratory samples. <sup>13</sup>  
339 The combination of BDG testing in association with LDH levels permits a fully non-  
340 invasive sampling regime and has been successfully evaluated for the diagnosis of PCP.  
341 When using optimal thresholds (BDG: 400pg/ml; LDH: 350U/l) specificity was 84%. <sup>67</sup>  
342 A further serological biomarker multi-centre study evaluated BDG, LDH, Krebs von den  
343 Lungen-6 antigen (KL-6, a potential marker of interstitial pneumonitis) and S-adenosyl  
344 methionine (SAM, a metabolic intermediate possibly exogenously required by  
345 *Pneumocystis*) to aid in the diagnosis of PCP. <sup>68</sup> The best overall performance was by  
346 combining BDG with KL-6 (Se: 94% Sp: 90%). Although sensitivity was slightly higher  
347 when combining BDG with LDH, specificity was compromised (Sensitivity: 97%  
348 Specificity: 72%). For all these approaches it could be argued that the absence of  
349 organism specific assay compromises confidence in diagnosis, and incorporating a



350 *Pneumocystis* specific PCR is required. If this is the case then the combination of  
351 PCR/BDG is preferable to using another non-specific serological biomarker.

352

### 353 **MANAGEMENT**

354 PCP can run a fulminant course, particularly in HIV negative individuals and early  
355 treatment improves prognosis. Disease can be stratified according to mild, moderate or  
356 severe depending on presenting symptoms, oxygen saturation and chest radiographic  
357 changes. Requirement for mechanical ventilation and vasopressors is a poor prognostic  
358 feature.

359 Clinicians should commence antimicrobials on the basis of clinical suspicion and before  
360 diagnostic investigations have been performed. Increasingly, sensitive molecular and  
361 biomarker detection is picking up patients who have only minimal symptoms or who  
362 are asymptomatic and this can present some diagnostic dilemma. Prophylaxis of at risk  
363 patients is also considered a mainstay of management. Guidelines for the prophylaxis  
364 and treatment have been developed for different groups and are summarized in Table 5.  
365 Although included within the fungal kingdom on the basis of cell wall composition and  
366 structure combined with nucleotide sequence similarity, *Pneumocystis jirovecii* is not  
367 susceptible to polyene and azole antifungal drugs, due to the absence of ergosterol from  
368 its cell wall. The different morphological forms also show varying susceptibility to  
369 other drugs with *in vitro* inhibition of ascospores (cyst) but not trophic forms by  
370 echinocandins. Trimethoprim, sulfa drugs and pentamidine form the main stays of  
371 treatment. Corticosteroids are of proven benefit in HIV positive individuals with disease  
372 but a beneficial role has not been established for other patient groups. The most  
373 effective way of preventing PCP in people living with HIV is by immune-reconstitution

374 through the administration of effective anti-retroviral therapy. Prophylaxis should be  
375 administered until immune reconstitution has been achieved.

376

### 377 **Prophylaxis**

378 Recommendations for prophylaxis are comprehensively reviewed in the ECIL guideline  
379 although this focuses on patients with haematological malignancies and undergoing  
380 SCT.<sup>16</sup>

381 Prophylaxis is recommended in risk groups that include HIV positive patients with CD4  
382 counts less than 200 cells/mm<sup>3</sup>, transplant patients, and patients with high-risk  
383 haematological malignancies as well as a growing number of patients receiving disease  
384 modifying drugs and aggressive chemotherapeutic regimens for an array of  
385 inflammatory and malignant diseases.<sup>69</sup> This last group is increasing rapidly and  
386 includes patients receiving TNF blockade (infliximab, adalimumab, etanercept), anti-  
387 IL1 therapies (alemtuzamab). B-cell blockade (Rituximab) and selective T cell blockade  
388 in addition to anti-purine drugs, bendamustine, nucleoside analogues and high-dose  
389 steroids for prolonged periods.<sup>70</sup>

390 Cotrimoxazole remains the drug of choice for both prophylaxis and treatment.

391 Systematic review and meta-analysis have shown significant benefit in preventing PCP  
392 and reducing PCP related mortality although the trials analysed focused on  
393 haematological malignancy and solid organ transplant patients and tended to be small  
394 and of poor quality.<sup>71</sup> The benefit in HIV populations is well documented and the effect  
395 on survival is compelling but there are few data in other groups of patients particularly  
396 those receiving disease modifying drugs. Prophylaxis is still not universally used in  
397 haematological patients receiving rituximab despite recommendations for prophylaxis  
398 in rheumatoid arthritis.<sup>69</sup>

399 A variety of different prophylactic regimens of cotrimaxazole have been used, Daily,  
400 alternative day, and thrice weekly have all been used and the optimum regimen in  
401 different patient groups has not been determined. ECIL guidelines recommend either  
402 one single strength tablet (480mg) daily or one double strength (960mg) table three  
403 times a week. <sup>16</sup>

404 Intolerance of cotrimaxazole and adverse events (including, rashes and marrow  
405 suppression) are relatively frequent and may necessitate use of second-line agents.  
406 Inhaled pentamidine, dapsone and atovaquone have all be used effectively but are  
407 considered inferior to cotrimaxazole on the basis of largely retrospective comparisons  
408 and should only be used after careful consideration.

409 It may be possible to reintroduce cotrimaxazole when adverse events resolve.

410 Inhaled pentamidine has the advantage that it is administered monthly but requires a  
411 jet nebulizer and side-room facilities for effective and safe administration. Dapsone can  
412 trigger methaemoglobinaemia in susceptible individuals and patients should be  
413 screened for glucose-6- phosphate dehydrogenase deficiency before use. Other serious  
414 side-effects include a potentially fatal idiosyncratic dapsone-hypersensitivity syndrome  
415 causing fever, skin rash, eosinophilia, and major organ dysfunction. Atovaquone is  
416 generally better tolerated and probably as effective as the other second line agents. Use  
417 tends to be limited by higher drug acquisition costs.

## 418 **Treatment**

419 Recommendations are comprehensively reviewed in the ECIL guideline although this  
420 focuses on patients without HIV disease.<sup>15</sup>

421 High dose cotrimoxazole is the treatment of choice given intravenously at  
422 20mg/kg/day in 2-4 divided doses. For severe disease, primaquine plus clindamycin is  
423 used for intolerant and refractory cases. Intravenous pentamidine has also been used  
424 but experience is confined to case reports. For mild to moderate disease, atovaquone  
425 may be used second-line. The use of echinocandins is not recommended.

426 Adjunctive corticosteroids (50-80mg daily) have established benefit in severe disease in  
427 patients with HIV but use in other patients should be considered on a case to case basis.

428 Treatment durations of 14-21 days are recommended depending on response and  
429 severity of disease. Patients can be slow to respond and may actually deteriorate  
430 clinically in the first few days of treatment. Assessment of failure to respond cannot be  
431 made confidently during the first week of treatment.

432

## 433 **CONCLUSIONS**

434 With the incidence of PcP increasing through infection in high-risk non-HIV-infected  
435 patients, it is essential that every effort is made to optimize the diagnosis of PcP. While the  
436 development of culture-based methods is a breakthrough in the field, they come at a time  
437 when reliance on culture to attain a microbiological diagnosis is less and the role of PcP  
438 culture more suited to the academic scenario. Non-mycological laboratory markers and  
439 clinical presentation although satisfactory to initiate therapy in high-risk individuals do not  
440 provide a definitive diagnosis. Diagnosis by IF remains the reference standard, but the  
441 development of non-culturebased strategies has aided the diagnosis of other fungal  
442 diseases (e.g. invasive aspergillosis) and the combination of PcP  
443 PCR along with BDG testing may be suitable alternative, especially given the low incidence  
444 of disease. With both prophylaxis and treatment based on the primary use of

445 cotrimoxazole, the emergence of resistance to sulfa-based drugs is of concern, and in the  
446 absence of culture, molecular techniques are the only route available to identifying  
447 resistance in Pneumocystis.

448 Expert commentary

449 A weakness in the diagnosis of PcP remains the resistance to move away from microscopic  
450 based diagnosis. It is accepted that sensitivity is far from optimal and false negatives will  
451 occur, but this conflicts with the low incidence of disease that dictates testing be used to  
452 exclude disease, with subsequent sensitivity paramount. For a disease where recent  
453 incidences across HIV, hematology and solid organ transplantation were approximately 1%  
454 or less, the pretest probability of not having PcP is approximately 99% and it is  
455 far easier to use a highly sensitive ( $\geq 95\%$ ) test to confidently exclude disease than a highly  
456 specific ( $\geq 95\%$ ) assay to confirm it [19–21]. For example, for a disease with a prevalence of  
457 1% and an assay with a good sensitivity and specificity of 90% the posttest probability of  
458 disease associated with a positive result is 8.3%, whereas the posttest probability of no  
459 disease associated with a negative result is 99.9%. Increasing specificity to 95% and 99%,  
460 respectively, increases the posttest probability of disease, when the assay is positive to  
461 15.4% and 47.6%, respectively. So even with an excellent specificity of 99% it is  
462 more likely that the patient does not have disease. If this applied specifically to PcP and  
463 typical performance of PCR and IF microscopy then it is clear that even though IF provides  
464 a greater degree of diagnostic confidence it is still not infallible, and its lower sensitivity  
465 limits its application to exclude disease. For PCR to take over as the reference method for  
466 PcP diagnosis standardization is required and commercially produced kits, international  
467 collaborative efforts of the Fungal PCR initiative and external quality control exercises  
468 (Quality Control for Molecular diagnosis (QCMD)) will assist this process. With a reliance on  
469 testing lower respiratory tract specimens (e.g. BAL), the testing for PcP will always be  
470 balanced against the risk of obtaining the sample (e.g. during thrombocytopenia).  
471 Consequently, clinical diagnosis, based on risk factors, symptoms and response to therapy,  
472 will occur, but in cases not responding to therapy, this could reflect a pneumonia caused by  
473 a different etiology or possibly a case of treatment-resistant PcP. Moving away from  
474 testing BAL specimens to less-invasive specimens, such as upper respiratory samples or  
475 even blood, alleviates the clinical pressure and also removes the need to standardize  
476 bronchoscopy, which varies considerably between centers and impacts on test

477 performance and interpretation. It is unlikely that a single noninvasive test will be able to  
478 provide both a diagnosis and the ability to exclude disease, but combining PCR of upper  
479 respiratory tract specimens with BDG testing of serum may do so. Currently, large scale  
480 performance data is limited but the ability to offer this noninvasive approach will surely  
481 appeal to clinicians and it is hoped there will be sufficient evidence in the near future to  
482 confirm the applicability of this strategy.

#### 483 Five-year view

484 Within the next five years, diagnosis of PcP will become less reliant on IF, with the potential  
485 for IF to become obsolete. The standardization of PCR through the efforts of the Fungal PCR  
486 initiative and through commercial development coupled with increasing prospective  
487 information on the performance of real-time PCR will provide greater understanding of  
488 interpretation of low-level PCR positives, across a range of patient populations. Combining  
489 PCR with BDG will further reduce the requirement for IF diagnosis. The development of  
490 syndromic testing using multiplex molecular methods may allow PcP to be detected  
491 alongside a range of other respiratory pathogens (e.g. Abbott IRIDICA) in a single assay.  
492 Whether BDG could be combined with Pneumocystis-specific immunology (antibodies or  
493 antigen) and provide a totally serological approach is yet to be proved. Although antibody  
494 ELISA tests targeting the major surface glycoproteins (Msg A, Msg B and Msg C) in  
495 Pneumocystis have shown promise, there is very little in the way of standardization and  
496 commercialization [84].

497 There is also the problem of positivity in healthy individuals who have been exposed to  
498 Pneumocystis, and as antibody levels peak almost a month post recovery, whether  
499 significant antibody positivity will occur too late to be clinically useful  
500 [84–86].

501 The application of next-generation sequencing (NGS) in relation to Pneumocystis is limited  
502 by the lack of culture. The ability to perform cell culture may alleviate the problem and  
503 should be focused on strains of Pneumocystis which are resistant to treatment to identify  
504 new molecular mechanisms of resistance. NGS may also provide further insights into  
505 transmission and sources of infection, allowing improved infection control measures to be  
506 applied. By combining direct PCR testing of nucleic acid extracted from respiratory  
507 specimens, NGS can provide enhanced broad-range diagnosis in symptomatic

508 patients, but also an understanding of the respiratory microbiome and the prevalence of  
509 Pneumocystis colonization in asymptomatic individuals.

510 From a clinical perspective, it is likely that the population at risk of PcP will expand with  
511 cases diagnosed in novel cohorts and the application of resistance monitoring is likely to  
512 become a standard procedure and has already been trialled in Europe where anthropophilic  
513 transmission and suboptimal prophylaxis were identified as risk factors [87].

514 Key issues

515 The population of patients at risk from PCP is growing and changing. While the  
516 incidence of disease in the HIV cohort may be reducing due to successful anti-  
517 retroviral therapy the incidence in other populations (Haematology, particularly  
518 conditions affecting lymphocyte count and function; solid organ transplant  
519 recipients, including renal transplants; solid malignancy; rheumatoid conditions;  
520 pre-existing chronic lung conditions; patients with connective tissue disorders  
521 and those receiving immuno-modulatory therapies) is increasing.

- 522 • In high risk patients clinical presentation and radiology is sufficient for initiating  
523 empirical therapy but should not be used as definitive diagnosis, and on  
524 commencing therapy every effort should be made to achieve an organism  
525 specific mycological diagnosis.
- 526 • The reference method for the diagnosis of PCP is the microscopic examination of  
527 respiratory samples, preferably BAL fluid, with immuno-fluorescent staining  
528 using anti-*Pneumocystis* antibodies targeting both ascus and trophic forms.
- 529 • Negative microscopy cannot be used to exclude PCP, but given the incidence of  
530 disease exclusion of disease is a sensible use of mycological testing. Both PCP  
531 PCR of BAL and BDG of serum/plasma can be used to exclude PCP when  
532 negative.

- 533 • BDG testing of serum and plasma is very sensitive (>90%) but not sufficiently  
534 specific and given the broad detection range coupled with the susceptibility of  
535 the at-risk patient population it should be combined with an organism specific  
536 test. The BDG testing of respiratory samples is not recommended and adds very  
537 little to testing serum/plasma.
- 538 • Standardisation of PCP PCR methodology would be beneficial although meta-  
539 analyses of current methodology provide high ( $\geq 90\%$ ) sensitivity and specificity  
540 when testing BAL fluid. PCP PCR sensitivity is reduced when testing upper  
541 respiratory samples, although specificity is increased. Commercial PCP PCR tests  
542 will assist in methodological standardisation and have the ability to identify  
543 genetic markers associated with resistance to sulfa-based therapy direct from  
544 the specimen. Molecular based methods can be used to identify origin of  
545 infection, transmission routes, outbreaks situations as well as epidemiology and  
546 evolution of the organism.
- 547 • Combination testing, involving IF microscopy and PCR on BAL, or in the absence  
548 of BAL, BDG on serum/plasma and PCR/IF on an upper respiratory sample is  
549 recommended. Albeit there is a strong argument for combining PCR and BDG  
550 alone.
- 551 • Guidelines for the prophylaxis and treatment of PCP in HIV, solid organ  
552 transplantation, haematology and rheumatoid conditions are available.

553

#### 554 Funding

555 This paper was not funded.

#### 556 Declaration of interest

557 PL White has received project funding from Myconostica, Luminex, and Renishaw diagnostics, was sponsored by  
558 Myconostica, MSD and Gilead Sciences to attend international meetings, was on a speaker's bureau for Gilead Sciences, and  
559 provided consultancy for Renishaw Diagnostics Limited. RA Barnes has received an educational grant and scientific fellowship



560 award from Gilead Sciences and Pfizer, is a member of the advisory board and speaker bureau for Gilead Sciences, MSD,  
561 Astellas, and Pfizer, and was sponsored by Gilead Sciences and Pfizer to attend international meetings. The authors have no  
562 other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict  
563 with the subject matter or materials discussed in the manuscript apart from those disclosed.  
564

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824

825

DRY

826 **Table 1.** A summary of risk factors for *Pneumocystis pneumonia*.

Underlying condition	Risk Factor
HIV/AIDS <sup>a</sup>	<p>CD4 count &lt;200cells/<math>\mu</math>l,</p> <p>CD4 cell percentage &lt;14%,</p> <p>Previous PCP,</p> <p>Oral Candidiasis,</p> <p>Higher HIV burden,</p> <p>Ongoing bacterial pneumonia.</p>
Haematological malignancy <sup>b</sup>	<p>CD4 count &lt;200cells/<math>\mu</math>l,</p> <p>Lymphocytopenia,</p> <p>Immuno-suppression to prevent rejection of allogeneic haematopoietic SCT.</p> <p>For autologous SCT patients receiving purine analogues or high dose corticosteroids.</p> <p>GVHD,</p> <p>ALL patients or those with lymphoproliferative disorders (CML, NHL, and multiple myeloma) as a result of chemotherapy including R-CHOP14, FCR, AVBD, gemcitabine or high-dose methotrexate.</p> <p>Monoclonal antibodies (e.g. rituximab).</p>

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Solid-organ transplantation<sup>c</sup>

CD4 count <200cells/ $\mu$ l,  
Corticosteroids,  
Anti-lymphocyte therapy,  
Mycophenolate mofetil,  
Calcineurin inhibitors,  
CMV disease,  
Graft rejection,  
Prolonged neutropenia,  
Exposure to cases of PCP.

Inflammatory Disease

Administration of multiple ( $\geq 3$ )  
immunomodulatory medications,  
including: Calcineurin inhibitors and/or  
anti-TNF therapy.  
Corticosteroids.

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827 <sup>a</sup> Information collated from CDC, NIH, HIVMA/IDSA guidelines (11)

828 <sup>b</sup> Information collated from ECIL guidelines (16)

829 <sup>c</sup> Information collated from American Society of Transplantation guidelines (17)

830

831 **Table 2.** Studies comparing the performance of various microscopic staining and  
 832 fluorescent kits for the detection of PCP. When interpreting results the influence of  
 833 incorporation bias on performance parameters should be considered, as in many  
 834 studies the results, particularly in combination with the other tests have been used to  
 835 define cases and controls.

Reference	Assay	Performance Parameter						
		Sensitivity	Specificity	PPV	NPV	LR+ve	LR-ve	DOR
37	CW	73.8%	99.6%	98.0%	93.4%	184.5	0.26	709.6
	MF	90.8%	94.7%	81.9%	97.5%	17.1	0.097	176.3
	DQ	48.4%	99.6%	96.9%	88.0%	121.0	0.51	237.3
	GMS	76.9%	99.2%	96.2%	94.2%	96.2	0.23	418.3
38	CB	74.3%	99.6%	92.9%	98.0%	165.6	0.3	552.0
	MoF	60.0%	99.3%	87.5%	96.9%	89.2	0.4	223.0
	Giemsa	34.6%	100%	100%	95.1%	>346 <sup>a</sup>	0.7	>494.3 <sup>a</sup>
39	GMS	50%	100%	100%	96.5%	>500 <sup>a</sup>	0.5	>1000 <sup>a</sup>
	Giemsa	50%	100%	100%	96.5%	>500 <sup>a</sup>	0.5	>1000 <sup>a</sup>
40 <sup>b</sup>	MoF	93.1%	100%	100%	95.5%	>931 <sup>a</sup>	0.07	>13300 <sup>a</sup>
	DQ	87.9%	97.6%	98.1%	85.4%	36.6	0.12	305.0
	GMS	89.7%	95.2%	96.3%	87.0%	18.7	0.11	170.0
	PCIF	94.8%	88.1%	91.7%	92.5%	8.0	0.06	133.3

836 <sup>a</sup> Values have been generated using a specificity of 99.9% to overcome  $\infty$

837 <sup>b</sup> Results represent combined induced sputum and bronchoalveolar lavage fluid testing

838 **Key:** CW: Calcofluor white                      GMS: Grocott-Gomori methenamine silver  
 839            MF: Merifluor *Pneumocystis*            CB: Calcofluor blue  
 840            DQ: Diff-Quik                                    MoF: Monofluo™ *Pneumocystis jirovecii*  
 841            PCIF: *P. carinii* IF kit

842 **Table 3.** The performance of (1-3)- $\beta$ -D-Glucan Testing for the diagnosis of *Pneumocystis*  
 843 pneumonia as determined by meta-analyses. The table contains data determined for  
 844 range of susceptible patients testing with various BDG assays.

845

Parameter	Study		
	Karageorgopoulos (40)	Onishi (41)	Li (42)
Cases/Total (n/N)	357/2080	286/2331	433/2195
Sensitivity (%)	94.8	95.5	90.8
Specificity (%)	86.3	84.3	78.1
PPV (%)	54.3	46.0	50.5
NPV (%)	99.0	99.3	97.2
LR +ve	6.9	6.1	4.1
LR -ve	0.06	0.05	0.12
DOR	115	122	34.2

846

847 **Key:**

848 **PPV:** Positive predictive value

849 **NPV:** Negative predictive value

850 **LR +ve:** Positive likelihood ratio

851 **LR -ve:** Negative likelihood ratio

852 **DOR:** Diagnostics Odds ratio

853 **Table 4** The performance of PCR for the diagnosis of *Pneumocystis* pneumonia as  
 854 determined by meta-analyses. The table provides the performance for PCP PCR when  
 855 testing both HIV+ and HIV- patients, when testing upper and lower respiratory tract  
 856 specimens, and is irrespective of differing technical details.  
 857

Parameter	Study		
	Summah (50) <sup>a</sup>	Fan (51)	Lu (52)
Cases/Total (n/N)	506/2330	606/1793	416/2505
Sensitivity (%)	97	98	99
Specificity (%)	94	91	90
PPV (%)	82	85	66
NPV (%)	99	99	>99
LR +ve	16.2	10.9	9.9
LR -ve	0.03	0.02	0.01
DOR	540	545	990

858  
 859 <sup>a</sup> Due to incomplete information the case and total population were calculated using  
 860 sample numbers.

861  
 862 **Key:**  
 863 **PPV:** Positive predictive value  
 864 **NPV:** Negative predictive value  
 865 **LR +ve:** Positive likelihood ratio  
 866 **LR -ve:** Negative likelihood ratio  
 867 **DOR:** Diagnostics Odds ratio



**Table 5.** Therapeutic Recommendations for the management of *Pneumocystis pneumonia* in adults

Recommendation	Guidelines (population)			
	CDC, NIH, HIVMA/IDSA (HIV) <sup>11</sup>	ECIL (Haematology) <sup>14,16</sup>	American Society of Transplantation (SOT) <sup>(17)</sup>	
Prophylaxis	Population	1) CD4 count <200cells/ $\mu$ l 2) CD4 cell <14% 3) CD4 count 200-250 cells/ $\mu$ l in the absence of regular 3 month CD4 monitoring 4) Not patients receiving pyrimethamine/sulfadiazine for toxoplasmosis	1) ALL 2) allogeneic HSCT, steroids (>20mg/day/4 weeks) 3) Alemtuzumab 4) Fludarabine/cyclophosphamide /rituximab Optional: Lymphoma with R-CHOP14 or escalated BEACOPP, nucleoside analogues, radiotherapy for brain tumours/metastasis with steroids	1) All SOT, especially lung transplant 2) Increasing immuno-suppression to prevent graft rejection 3) Recurrent or chronic CMV infection 4) Prolonged course of corticosteroids (>20mg for $\geq$ 2weeks) 5) Prolonged neutropenia 6) Episodes of autoimmune disease
	Duration	Until CD4 count $\geq$ 200 cells/ $\mu$ l for > 3 months	1) Induction to end of maintenance 2) Engraftment for at least 6 months until immuno-competent	A minimum 6-12 months post-transplant for all SOT recipients. Patients with lung or small bowel

		3) More than 6 months post completion	grafts or those prior PCP or chronic CMV disease may require lifelong prophylaxis
		4) Minimum of 6 months post completion	
Therapy <sup>a</sup>	<p>Front line:</p> <p>Trimethoprim/sulfamethoxazole one single-strength (80mg TMP/400mg SMX) daily or one double strength tablet (160mg TMP/800mg SMX)/daily.</p> <p>Second line:</p> <p>Trimethoprim/sulfamethoxazole one double strength tablet (160mg TMP/800mg SMX) three times per week</p> <p>Dapsone (50mg twice daily)</p> <p>Dapsone (200mg) + pyrimethamine (75mg) + leucovorin (25mg) weekly</p> <p>Dapsone (50mg daily) +</p>	<p>Front line:</p> <p>Trimethoprim/sulfamethoxazole one single-strength (80mg TMP/400mg SMX)/day or double strength tablet (160mg TMP/800mg SMX)/day or three per week.</p> <p>Second line:</p> <p>Dapsone (50mg twice daily)</p> <p>Pentamidine aerosols (300mg per month)</p>	<p>Front line:</p> <p>Trimethoprim/sulfamethoxazole one single-strength (80mg TMP/400mg SMX)/day or double strength tablet (160mg TMP/800mg SMX)/day or three per week.</p> <p>Second line:</p> <p>Dapsone (50-100mg once a day)</p>

pyrimethamine (50mg weekly) +  
 leucovorin (25mg weekly)  
 Pentamidine aerosols (300mg per  
 month)  
 Atovaquone 1500mg daily

Targeted Treatment	Population	HIV/AIDS patients with suspected/diagnosed PCP	Haematological malignancy, solid cancer, solid organ transplant, autoimmune/inflammatory conditions with suspected/diagnosed PCP	All SOT with suspected/diagnosed PCP
	Duration	3 weeks	A minimum of 14 days	At least 14 days, extended to 21 days for severe cases
	Therapy <sup>a</sup>	Frontline: Trimethoprim/sulfamethoxazole (15-20mg/kg TMP; 75-100mg/kg SMX per day)  For moderate to severe disease (i.e. hypoxemia) adjunctive corticosteroids should be used	Frontline: Trimethoprim/sulfamethoxazole (15-20mg/kg TMP; 75-100mg/kg SMX per day)	Frontline: Trimethoprim/sulfamethoxazole (15-20mg/kg TMP; 75-100mg/kg SMX per day) with TMP administered by IV every 6-8h. For hypoxemic patients potentially in combination with 40-60mg of prednisolone (twice daily)

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Second line for severe disease:

Primaquine and clindamycin  
(30mg/(600mgx3)) per day

Pentamidine IV (4mg/kg/day)

Second line for mild/moderate  
disease:

Dapsone (100mg daily) +  
trimethoprim (15mg daily)

Atovaquone (750mg BID)

Second line:

Primaquine and clindamycin  
(30mg/(600mgx3)) per day

Pentamidine IV (4mg/kg/day)

Second line:

IV Pentamidine (Initially 4mg/kg/day  
over 1-2h) Recipients of  
pancreas/islet transplants should  
receive an alternative second line  
therapy.

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<sup>a</sup> Where possible only the recommendation receiving an "A" grading or the preferred drug of choice have been listed.