**Supplementary appendix**

**Title:** PCR on respiratory tract specimens of immunocompromised patients to diagnose Pneumocystosis (PcP): A systematic review and meta-analysis

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**Key Words:** *Pneumocystis* PCR, Pneumocystosis, PcP, meta-analysis, systematic review

**Supplementary table 1: Summary of results of previous meta-analyses on PCR diagnosis of PcP and their limitations**

|  |  |  |  |
| --- | --- | --- | --- |
| **Author, year** | **Sensitivity (95% CI)** | **Specificity (95% CI)** | **Limitations** |
| Lu et al, 201112 | Overall: 99% (96-100%)BAL: 99% (99-100%)IS: 97% (92-100%) | Overall: 90% (87-93%)BAL: 87% (83-91%)IS: 93% (90-95%) | * Only 13 included studies.
* No analysis of URT samples.
* No risk of bias assessment.
 |
| Fan et al, 201313 | BAL: 98% (91–100%)  | 91% (83–96%) | * Only 16 included studies.
* BAL samples only.
 |
| Summah et al 201314 | Overall: 97% (93-99%)BAL: 98% (94-99%) | Overall: 94% (90-96%)BAL: 93% (89-96%) | * Only 10 included studies – 2330 samples.
* Subgroup analysis for BAL only.
* PCR compared to both proven and probable PcP.
 |
| Senécal et al 202215 | NPA: 89% (80-96%)OW: 77% (66-85%)IS: 99% (51-100%) | NPA: 98% (93-100%)OW: 94% (90-96%)IS: 96% (88-99%) | * Non-invasive samples only i.e. no BAL
* Only 7 studies on IS PCR and, 13 URT PCR
* All types of PCR pooled together
* Inclusion of case-control studies.
 |

95% CI; 95% confidence interval, BAL; bronchoalveolar lavage, IS; induced sputum, URT; upper respiratory tract, NPA; nasopharyngeal aspirate, OW; oral wash, PCR; polymerase chain reaction; PcP; *Pneumocystis* pneumonia

**Supplementary 2: Search strategy**

Search Strategy: 1 exp Pneumocystosis/ 2 exp Pneumocystis pneumonias/ 3 exp Pneumocystis carinii Pneumonia/ Pneumocystis jirovecii pneumonia/ / PCP/ PJP 4 (Pneumocystis or Pneumocystosis or "P. jirovecii" or "P. carinii" ).ti,ab.5 or/1-4 6 exp Polymerase Chain Reaction/ 7 pcr.ti,ab. 8 "polymerase chain reaction\*".ti,ab. 9 or/6-8 10 5 and 911 exp Animals/ not Humans/12 10 not 11.

1-54

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**Supplementary 4: Study characteristics**

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| **Study (n=55)** | **Country** | **Centres** | **Patients (n)** | **Patient population** |
|  |  |  |  |  |
| Alanio 2011 | France | 1 | 238 | Adults, HIV (69) and non HIV (169) including haemotological malignancy, solid organ transplant, solid malignancy and autoimmune on immunosuppressants |
| Almeida-Silva 2023 | Brazil | 1 | 40 | Adult patients (40), all with HIV and CD4<200 |
| Alshahrani 2020 | Saudi Arabia | "Multi" | 100 | Adults, form of immunocompromise not specified  |
| Azoulay 2009 | France | 1 | 448 | Age range not specified, all non-HIV including haematological malignancy, solid malignancy, solid organ and stem cell transplant, autoimmune on immunosuppressants  |
| Barbin 2022 | Iran | 7 | 200 | Age range not specified. All 200 hospitalized patients receiving prolonged immunosuppressive therapies (kidney transplant recipients, patients with autoimmune disorders and malignant disorders under chemotherapy) |
| Caliendo 1998 | USA | 1 | 168 | Age range not specified, HIV and non-HIV including solid organ transplant recipients |
| Cartwright 1994 | USA | 1 | 205 | Age range not specified, HIV (112), non-HIV including malignancies (73) and other causes of immunocompromise not specified  |
| Chawla 2011 | India | 1 | 50 | Adults with HIV |
| Chotiprasitsakul 2020 | Thailand | 1 | 222 | Adults with HIV (37) and non-HIV (185), including haemotological malignancy, solid organ transplant, solid malignancy and autoimmune on immunosuppressants |
| Chouaid 1995 | France | 1 | 49 | Adults with HIV  |
| Chumpitazi 2011 | France | 1 | 54 | Adults and children, with HIV (5) and non-HIV (49) including haemotological malignancy, solid organ transplant, solid malignancy and autoimmune on immunosuppressants |
| Church 2015a | Canada | 1 | 127 | Age range not specified, HIV (9) and non-HIV (78) including malignancy, solid organ and stem cell transplant recipients  |
| Desoubeaux 2017 | France | 1 | 481 | Adults with HIV (6) and non-HIV (42) including haemotological malignancy, solid organ transplant, solid malignancy and inherited immunodeficiencies |
| Eisen 1994 | Australlia | 1 | 20 | Age range not specified, all with HIV  |
| Evans 1995a | UK | 1 | NS | Age range or immunocompromise not specified  |
| Evans 1996b | UK | 3 | 53 | Age range not specified, HIV (11) and non-HIV (42) with other forms of immunocompromise non-specified |
| Fillaux 2008 | France | 1 | 101 | Adults and children, HIV (22) and non-HIV, malignancy (36) and other non-specified |
| Fischer 2001 | USA | 1 | 175 | Age range or immunocompromise not specified  |
| Franconi 2022 | Italy | 1 | 18 | Age range not specified. Both HIV and non-HIV patients included all on immunosuppressive therapies. |
| Goterris 2019 | Spain | 1 | 36 | Age range not specified, HIV (5) and non-HIV (31) including haemotological malignancy, solid organ transplant, solid malignancy and autoimmune on immunosuppressants |
| Graves 1997 | USA | 1 | 74 | Age range not specified, HIV (52) and non-HIV (22) not specified  |
| Hauser 2011 | UK, Austria, Switzerland, USA | 6 | 110 | Adults with HIV (9) and non-HIV (101) including solid organ transplant and malignancy |
| Helweg 1998 | Denmark | 1 | 76 | Age range not specified, all with HIV |
| Hoarau 2017 | Reunion | 1 | 133 | Age range not specified, HIV (13) and non-HIV (120) including haemotological malignancy, solid organ transplant, solid malignancy |
| Huang 1999 | USA | 1 | NS | Age range and immunocompromise not specified  |
| Huggett 2009 | UK | NS | 61 | Age range not specified, all with HIV |
| Juliano 2015 | USA | 1 | 43 | Adults with HIV |
| Kaouech 2008 | Tunisia | NS | 54 | Children and adults, HIV (20) and non-HIV (34) including haemotological malignancy, cirrhosis, autoimmune, immunodeficiency |
| Kilic 2020 | USA | 1 | 125 | Age range not specified, HIV (35) and non-HIV (90) including malignancies, stem cell or bone marrow transplant, solid organ transplant and other immunosuppressed condtions |
| Larsen 2004 | USA | 1 | 108 | Age range not specified, all with HIV  |
| Lipschik 1992  | USA | NS | 133 | Age range not specified, HIV (65) and non-HIV (68) including maligancy, autoimmune and other not specified |
| Mathis 1997 | Switzerland | 1 | 507 | Age range not specified, HIV (295) and non-HIV (164) including organ transplant, haemotological malignancy, immunosuppressants |
| Matsumura 2012 | Japan | 1 | 147 | Age range not specified, HIV (13) and non-HIV (134) including solid and haemotological malignancy, autoimmune, organ transplant |
| Mejia Bautista 2020 | USA | 1 | NS | Age range and immunocompromise not specified  |
| Moodley 2017 | South Africa | 1 | 266 | Adults with HIV |
| Moonens 1995 | Belgium | 1 | 43 | Age range not specified, HIV (14) and non-HIV (29) including solid organ transplant recipients and immunosuppressants |
| Rhabodonirina 1997 | France | 1 | 105 | Age range not specified, all with HIV  |
| Robberts 2007 | South Africa | 1 | NS | Age range and immunocompromise not specified  |
| Robert-Gangneux 2014 | France  | 1 | 659 | Age range not specified, HIV and non-HIV including haemotological malignancy, solid malignancy, transplant recipients and immunosuppressants |
| Roux 1994 | France | 1 | 165 | Age not specified, HIV (120) and non-HIV (45) not specified  |
| Salsé 2021 | France | 1 | 219 | Adult patients, HIV (12) and non-HIV (207) including haematological malignancy, solid tumours and autoimmune or inflammatory disease  |
| Samuel 2011 | South Africa | 1 | 202 | Children with HIV (129) and non-HIV (71) including malnutrition, receiving immunosuppressive therapy or immunodeficiency disease other than HIV |
| Savoia 1997 | Italy | 1 | 123 | Age range not specified, all with HIV  |
| Sasso 2016 | France | 1 | 148 | Adults and children with HIV (40) and non-HIV (108) including solid malignancy, haemotological malignancy, solid organ and stem cell transplant, autoimmune and underlying respiratory disease |
| Sing 2000 | Germany | 1 | 375 | Adults and children with HIV (89) and non-HIV (245) including transplant recipients, malignancy and other non-specified |
| Singh 2015 | India | 1 | 185 | Adults with HIV (64) and non-HIV (121) including renal transplant recipients, malignancy, and other non-specified  |
| Takahashi 2002 | Japan | 1 | 81 | Adults and children, HIV (26) and non-HIV (55) including haemotological malignancy, connective tissue diseases and renal transplantation |
| Tamburrini 1993 | Italy | 1 | 52 | Adults with HIV (48) and non-HIV (4) |
| Tia 2012 | Thailand | 1 | 102 | Age range not specified, HIV (66) and non-HIV (36) including underlying solid organ malignancies, haematological malignancies, systemic lupus erythematosus and organ transplantation  |
| To 2013 | Hong Kong | "Multi" | 117 | Age range not specified, HIV (10) and non-HIV (107) including lung disease, malignancy, stem cell or solid organ transplant recipient, autoimmune on immunosuppressants |
| Torres 2000 | USA | 2 | 47 | Adults with HIV |
| Veintimilla 2023 | Spain | 1 | 299 | Adults, both HIV (53) and. Non-HIV (246) including patients with haematological malignancy, solid tumours, transplant recipients, on immunosuppressants |
| Wakefield 1991 | UK | 1 | 47 | Age range not specified, all with HIV  |
| Wang 2017 | China | 1 | 71 | Age range not specified, non-HIV including solid organ transplant, malignancy, autoimmune condition on immunosuppressants |
| Zingale 2003 | Italy | 1 | 173 | Adults with HIV |

NS; not specified

**Supplementary 5: PCR techniques**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Study (n=55)** | **Type of microscopy in reference standard** | **PCR**  | **DNA extraction** | **Sample volume for DNA extraction (μL)** | **Target gene**  | **Internal control** |
| Alanio 2011 | High probability': Giemsa stain + indirect IF +/- direct IF (if prev were neg). Decided by 2 blinded clinicians. | In-house qPCR | QIAamp DNA mini kit | 200 | mtLSU rRNA  | Yes |
| Almieda 2023 | IFA | In-house nested cPCR/Commerical qPCR (Viasure) | QIAamp DNA Mini kit | NS | mtLSU rRNA |  |
| Alsharani 2020 | Clinically suspected': Direct IF  | Commerical qPCR (MycAssay)  | QIAamp DNA mini kit | NS | mtLSU rRNA  | Yes |
| Azoulay 2009 | Definitive': Giemsa + indirect IFA (2x slides if prev neg and PCR pos). 'Probable': negative microbiology, response to treatment | In-house cPCR | QIAamp DNA mini kit | NS | LSU rRNA  | Yes |
| Barbin 2022 | GMS and giemsa | In-house cPCR | phenol chloroform | NS | mtLSU rRNA |  |
| Caliendo 1998 | Direct IFA | Commercial colorimetric cPCR (Roche) | Organic | 100 | 18s rRNA  | Yes |
| Cartwright 1994 | Direct IFA | In-house PCR-EIA (ELISA) | Organic | 500-2000 | mtLSU rRNA  | No |
| Chawla 2011 | GMS and giemsa. | In-house cPCR | QIAamp DNA mini kit | NS | mtLSU rRNA | Yes |
| Chotiprasitsaku 2020 | Direct IFA | Commerical qPCR (Biopharm AG) | magLEAD | NS | mtLSU rRNA | Yes |
| Chouaid 1995 | Giemsa, Musto, GMS. IS also by direct IFA. | In-house cPCR | Phenolchloroform | NS | mtLSU rRNA | Yes |
| Chumpitazi 2011 | Confirmed': Giemsa, GMS  | In-house qPCR touch-down | Qiagen EZ1 DNA tissue kit | 400 | MSG | Yes |
| Church 2015 | Modified gold standard': TDO, GMS OR direct IFA OR if neg by IFA + pos x2 by PCR, considered positive. Also made comparison to IFA only.  | In-house qPCR | Quickgene 810 | 180 | mtLSU rRNA | Yes |
| Desoubeaux 2017 | Direct IFA | In-house qPCR | QIAamp DNA mini kit | 200 | mtLSU rRNA | Yes |
| Eisen 1994 | Direct IFA | In-house cPCR | Organic | 500 | mtLSU rRNA | Yes |
| Evans 1995a | Direct IFA | In-house nested cPCR | Phenolchloroform | NS | mtLSU rRNA | Yes |
| Evans 1996b | Direct IFA | In-house cPCR | Phenolchloroform | 20 | mtLSU rRNA | Yes |
| Fillaux 2008 | Direct IFA | In-house qPCR | Roche, isopropanol-based | 1500 | MSG | Yes |
| Fischer 2001 | Direct IFA or GMS (depending on centre) | In-house immunofluorescent cPCR  | InstaGene Matrix (Bio-Rad) | 5000-50,000 | MSG/mtLSU | Yes |
| Franconi 2022 | GMS | Commercial qPCR (Unyvero/Saccace) | Commerical Unyvero kit | 180 | 26s rRNA |  |
| Goterris 2019 | Direct IFA | qPCR | QIAamp DNA minikit | NS | DHPS/ mtSSU rRNA | Yes |
| Graves 1997 | Direct IFA | cPCR | Organic | 5000 | Dihydrofolate reductase gene | Yes |
| Hauser 2011 | Direct IFA | Commercial qPCR (MycAssay)  | MycXtra fungal DNA extraction kit | <1000-60,000 | mtLSU rRNA | Yes |
| Helweg 1998 | Giemsa and direct IFA on BAL | In-house cPCR | Phenolchloroform | 2000 | mtLSU rRNA | Yes |
| Hoarav 2017 | Musto stain on BAL | Commercial qPCR (Fast Track Diagnostics) | NS | 600 | mtLSUrRNA | Yes |
| Huang 1999 | Direct IFA | In-house cPCR | DNA Extraction Reagent (Perkin Elmer) | IS (1000-3000), OW (10,000-15,000), BAL (5000-10,000) | MSG/mtLSU rRNA | Yes |
| Huggett 2009 | GMS  | In-house qPCR (HSP70) and cPCR (MtLSU rRNA) | DNeasy tissue kit and subsequently the QIAamp UltraSens Virus Kit | 200 (DNeasy), 750 (Qiamp) | HSP70 DNA/ mtLSU rRNA | Yes |
| Juliano 2015 | Microscopy' non-specified on BAL/IS | In-house qPCR  | QIAamp DNA mini kit | 15,000 | DHPS/MSG | Yes |
| Kaouech 2008 | Giemsa, GMS, TDO | In-house cPCR | QIAamp DNA Mini kit | NS | MSG | Yes |
| Kilic 2020 | Direct IFA | Commercial qPCR (DiaSorin Molecular) | Diasorin Molecular | NS | mtLSU rRNA | Yes |
| Larsen 2004 | Diff-quik on BAL/IS | In-house touch-down qPCR TD  | NucliSens kit | NS | MSG | Yes |
| Lipschik 1992  | Indirect IFA, TDO | In-house cPCR (nested and simple) | Phenol chloroform | 5000-10,000 (BAL), 1000-3000 (IS) | mt rRNA  | Yes |
| Mathis 1997 | Direct IFA | In-house cPCR  | Biorad mesh | 1500 (BAL), 50-500 for other specimens | mtLSU rRNA | Yes |
| Matsumura 2012 | GMS | In-house qPCR | QIAamp DNA mini kit | NS | DHPS | Yes |
| Mejia 2020 | Direct IFA | In-house qPCR | Diasorin Molecular | NS | NS | NS |
| Moodley 2017 | Direct IFA | In-house qPCR | Roche MagNA Pure Compact  | NS | mtLSU rRNA | Yes |
| Moonens 1995 | Direct IFA | In-house cPCR (nested and simple) | Phenolchloroform | 10,000 | mtLSU rRNA | Yes |
| Rhabodonirina 1997 | GMS and giemsa. | In-house cPCR (simple and nested) | GeneReleaser  | NS | mtLSU rRNA | Yes |
| Robberts 2007 | Direct IFA | In-house nested cPCR | DNA purification kit, Wizard SV Genomic | 200-1000 | mtLSU rRNA | Yes |
| Robert-Gagneux 2014 | Giemsa and direct IFA on BAL | In-house qPCR | QIAamp DNA mini kit | 1000 | mtLSU rRNA | Yes |
| Roux 1994 | Giemsa, TDO or direct IFA | In-house cPCR | Phenolchloroform | 10,000 | mtLSU rRNA | NS |
| Salse 2021 | GMS and giemsa on BALF | Commercial qPCR (RealStar)/In-house qPCR/ | EZ1 DNA Tissue Kit (Qiagen) | 2000 | mtLSU rRNA/MSG |  |
| Samuel 2011 | Direct IFA | In-house qPCR | Nuclisens EasyMAG | NS | MSG | Yes |
| Saovia 1997 | Direct wet mount, Giemsa, TDO | In-house nested cPCR | Organic | NS | mtLSU rRNA | Yes |
| Sasso 2016 | Direct Giemsa, GG  | In-house qPCR, commercial qPCRs (Amplisens, McAssay, Bioevolution) | EZ1 DNA Tissue kit (Qiagen®) | 2000 | MSG/ mtLSU rRNA | NS |
| Sing 2000 | Giemsa + GMS | In-house nested cPCR | Qiagen DNeasy tissue kit | NS | mtLSU rRNA | Yes |
| Singh 2015 | GMS | In-house cPCR (LAMP/nested) | Qiagen DNeasy tissue kit | 200 | mtLSU rRNA/18s rRNA | Yes |
| Takahashi 2002 | Diff-quik on BAL | In-house cPCR | Phenolchloroform | NS | 5S rDNA | Yes |
| Tamburrini 1993 | Direct IFA | In-house cPCR | Phenolchloroform | 20 | mtLSU rRNA | Yes |
| Tia 2012 | Giemsa and IFA on BAL | In-house cPCR (single round/nested) | QIAamp DNA mini kit | 1000 | mtLSU rRNA | Yes |
| To 2013 | GMS  | In-house qPCR | QIAamp DNA kit and DNeasy plant minikit  | 200 | mtSSU rRNA | Yes |
| Torres 2000 | GMS or Giemsa.  | In-house cPCR (semi-quantitative +1 to +3) | NS | 5000-25,000 | ITS | NS |
| Veintimilla 2023 | Direct IFA (matched sample) | Commercial qPCR (Progenie Molecular) | MagnaPure Compact instrument using the Nucleic Acid Isolation kit I (Roche Diagnostics GmbH®), | 1500 | LSU rRNA |  |
| Wakefield 1991 | GMS and others. | In-house cPCR | Phenolchloroform | 1000 | LSU rRNA  | NS |
| Wang 2017 | GMS | In-house qPCR | QIAamp DNA Mini kit | NS | HSP70 gene | NS |
| Zingale 2003 | Giemsa and Grocott | In-house qPCR | Qiagen tissue kit | NS | LSU rRNA/ DHPS | NS |

IFA; immunofluorescent assay, qPCR; quantitative PCR, GMS; Grocott methenamine silver, TBO; toluidine blue 0, NS; not specified,**Supplementary 6: Risk of bias and applicability concerns graph: review authors' judgements about each domain presented as percentages across included studies**



**Supplementary 7: Overall risk of bias and applicability concerns for 55 selected studies**

**Supplementary 8: Coefficients of the binomial regression**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| testpos | Coefficient | Std. Err | z | P  | 95% Conf. Interval |
|   |  |  |  |  |  |   |
| 1.disease | 6.3783 | 0.4202 | 15.18 | 0.000 | 5.5546 | 7.2020 |
|   |  |  |  |  |  |  |
| sample |  |  |  |  |  |  |
| BALF and IS | 0.8159 | 0.4684 | 1.74 | 0.082 | -0.1021 | 1.7340 |
| IS | 0.6293 | 0.2253 | 2.79 | 0.005 | 0.1877 | 1.0708 |
| upper and lower | 0.3061 | 0.4224 | 0.72 | 0.469 | -0.5218 | 1.1340 |
| URT | -0.3457 | 0.4846 | -0.71 | 0.476 | -1.2955 | 0.6041 |
|   |  |  |  |  |  |  |
| disease#sample |  |  |  |  |  |  |
| 1#BALF and IS | -1.2122 | 0.7732 | -1.57 | 0.117 | -2.7278 | 0.3033 |
| 1#IS | -1.1679 | 0.4393 | -2.66 | 0.008 | -2.0290 | -0.3068 |
| 1#upper and lower | -0.5417 | 0.7111 | -0.76 | 0.446 | -1.9355 | 0.8522 |
| 1#URT | -2.1379 | 0.6340 | -3.37 | 0.001 | -3.3806 | -0.8952 |
|   |  |  |  |  |  |  |
| pcr |  |  |  |  |  |  |
| conventional | -0.8802 | 0.3017 | -2.92 | 0.004 | -1.4715 | -0.2888 |
|   |  |  |  |  |  |  |
| disease#pcr |  |  |  |  |  |  |
| 1#conventional | 0.1903 | 0.4801 | 0.4 | 0.692 | -0.7507 | 1.1313 |
|   |  |  |  |  |  |  |
| \_cons | -2.1415 | 0.2386 | -8.97 | 0.000 | -2.6092 | -1.6738 |
|   |  |  |  |  |  |  |
| study |   |  |  |  |  |  |
| var(disease)  | 1.0850 | 0.5367 |  |  | 0.4115 | 2.8608 |
| var(\_cons)  | 0.8637 | 0.2237 |  |  | 0.5199 | 1.4350 |
|   |  |  |  |  |  |  |
| study  |  |  |  |  |  |  |
| cov(disease,\_cons) | -0.0744 | 0.2442 | -0.3 | 0.761 | -0.5531 | 0.4043 |

Mixed-effects logistic regression. Dependent variable: “testpos”. Predictors: “disease”, “sample”, and “pcr”. Two interactions were included: “disease\*sample” and “disease\*pcr”. The group variable was “study”. The random effect intercept was “study”, the random coefficient was “disease”. Note that the baseline level for “sample” was “BALF”, for “pcr” was “qPCR”. Number of observations = 9,564. Number of groups = 49. Log likelihood = -2893.602. Wald chi2(11) = 457.44. Prob from chi2 = 0.0000. BALF; bronchoalveolar lavage fluid, IS; induced sputum, URT; upper respiratory tract. Std. Error; standard error, 95% CI; 95% confidence interval.

**Supplementary 9: Sensitivity, specificity, DOR, LR+ and LR- calculated from the coefficients of the binomial regression model.**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **sens** | ll | ul | **spec** | ll | ul | **DOR** | ll | ul | **LR+** | ll | ul | **LR-** | ll | ul |
| qPCR, BALF | 0.987 | 0.968 | 0.995 | 0.893 | 0.844 | 0.927 | 635 | 269 | 1498 | 9.194 | 5.727 | 12.661 | 0.014 | 0.001 | 0.027 |
| cPCR, BALF | 0.972 | 0.932 | 0.988 | 0.954 | 0.930 | 0.970 | 710 | 305 | 1652 | 21.178 | 12.438 | 29.918 | 0.030 | 0.004 | 0.056 |
| qPCR, BALF and IS | 0.982 | 0.903 | 0.997 | 0.792 | 0.623 | 0.898 | 210 | 45 | 986 | 4.720 | 1.667 | 7.773 | 0.022 | NE | 0.060 |
| cPCR, BALF and IS | 0.961 | 0.803 | 0.993 | 0.905 | 0.801 | 0.957 | 234 | 49 | 1112 | 10.110 | 2.564 | 17.657 | 0.043 | NE | 0.116 |
| qPCR, IS | 0.980 | 0.944 | 0.993 | 0.815 | 0.721 | 0.883 | 217 | 78 | 601 | 5.303 | 3.024 | 7.583 | 0.024 | 0.000 | 0.049 |
| cPCR, IS | 0.956 | 0.887 | 0.984 | 0.917 | 0.866 | 0.950 | 243 | 90 | 656 | 11.511 | 5.985 | 17.036 | 0.047 | 0.001 | 0.094 |
| qPCR, upper and lower | 0.990 | 0.949 | 0.998 | 0.848 | 0.747 | 0.914 | 536 | 113 | 2541 | 6.529 | 3.033 | 10.025 | 0.012 | NE | 0.032 |
| cPCR, upper and lower | 0.977 | 0.912 | 0.994 | 0.933 | 0.882 | 0.963 | 599 | 156 | 2306 | 14.659 | 6.158 | 23.159 | 0.024 | NE | 0.058 |
| qPCR, URT | 0.892 | 0.710 | 0.965 | 0.905 | 0.809 | 0.955 | 78 | 26 | 238 | 9.340 | 2.997 | 15.682 | 0.120 | NE | 0.245 |
| cPCR, URT | 0.787 | 0.502 | 0.931 | 0.960 | 0.911 | 0.982 | 87 | 27 | 284 | 19.424 | 5.358 | 33.490 | 0.222 | NE | 0.446 |

Sensitivity, specificity, DOR, LR+ and LR- calculated from the coefficients of the binomial regression model. “ll”: lower limit, and “ul”: upper limit of the 95% confidence interval. In the lower limit of LR-, the expression “NE” (not evaluable) appears on some instances. Indeed, the corresponding results obtained by the calculating algorithm had a negative sign, which is impossible as the LRs are ratios between two positive numbers (proportions). This fact is due to the use of an approximate method to calculate the 95% confidence intervals of the LRs, the “delta method”. qPCR; quantitative PCR, cPCR; conventional PCR, BALF; bronchoalveolar lavage fluid, IS; induced sputum, URT; upper respiratory tract, LR; likelihood ratio, sens; sensitivity, spec; specificity, ll; lower limit, ul; upper limit.

**Supplementary 10: Summary Receiver Operating Characteristic (ROC) Plots.**

Bivariate analysis of the sensitivity and specificity of the PCR as a diagnostic tool for PCP. PCP qPCR plots on bronchoalveolar lavage fluid (BALF) samples (A), and induced sputum (IS) samples (B), and upper respiratory tract (URT) specimens (C).

A: Bivariate analysis of the diagnostic accuracy on the 32 studies where the sample was obtained from BALF only.



B: Bivariate analysis of the diagnostic accuracy on the 11 studies where the sample was from IS only.



C: Bivariate analysis of the diagnostic accuracy on the 8 studies where the sample was from URT origin only.

