Diagnostic accuracy of PCR alone and compared to galactomannan in bronchoalveolar lavage for the diagnosis of invasive aspergillosis: Systematic review

Supplemental material:

Supplementary Table 1: QUADAS-2 tool adapted for our review

Patient selection – Risk of bias	1. Was a consecutive or random sample of patients enrolled? 2. Was a case-control design avoided? 3. Did the study avoid inappropriate exclusions? If case control = high risk, if all yes = low risk. Otherwise unclear
Patient selection – Concerns regarding applicability	Is there concern that the included patients do not match the review question? Considered high = not strictly adherent with EORTC/MSG host criteria, low = host + clinical factor by EORTC/MSG. Otherwise unclear.
Index test – Risk of bias	Were the index test results interpreted without knowledge of the results of the reference standard? If no = high risk, if yes = low risk. Otherwise unclear
Reference standard – Risk of bias	Were the reference standard results interpreted without knowledge of the results of the index test? If no = high risk, if yes = low risk. Otherwise unclear
Reference standard – Concerns regarding applicability	Is there concern that the target condition as defined by the reference standard does not match the review question? Considered high = reference standard not strictly

	adherent with EORTC criteria 2002 or 2008, low = reference standard EORTC criteria 2002 or 2008 or these could be reapplied using individual patient description. Otherwise unclear.
Flow and timing - Risk of bias	1. Was there an appropriate interval between index test(s) and reference standard? 2. Did all patients receive a reference standard? 3. Did patients receive the same reference standard? 4. Were all patients included in the analysis? If any no = high risk, if all yes = low risk. Otherwise unclear Q1 considered appropriate interval if BAL performed within 2 weeks of infection onset.

The following signaling questionswere considered irrelevant for our review:

Domain	**Signalling question**	**Reason for exclusion**
Index test – Risk of bias	If a threshold was used, was it pre-specified?	Results of PCR are read as positive or negative. Real-time PCR might have a threshold of copies, however again, result is read as positive or negative
Index test – Concerns regarding applicability	Is there concern that the index test, its conduct, or interpretation differ from the	Per inclusion criteria only PCR tests were included and all PCR tests were

	review question?	considered in our review
Reference standard – Risk of bias	Is the reference standard likely to correctly classify the target condition?	EORTC/ MSG clinical criteria considered as adequate reference standard, even though it has limitations
Flow and timing - Risk of bias		The answer in all studies was yes, therefore this question was excluded

Supplementary Table 2: Excluded trials and reason for exclusion

5 - No reference standard or EORTC/MSG definitions inappropriately applied and data in publication did not permit re-application of consensus definitions

2 - Used previously known Aspergillus-positive samples

4 - No BAL performed or no cases of IA

5 - Population assessed not at risk for IA or data for patients at risk could not be separated from patients not at risk

2 - Bronchoscopy used for screening

2 - Case series / case reports

1 - Non-relevant

16 - Incomplete data reported to construct 2X2 tables for sensitivity and specificity

3 - Inclusion of same patients as Buchheidt 2001 included in the review and Reinwald 2012. The publications with more patients or full subgroup analyses were selected for inclusion.

Supplementary Table 3: Study characteristics

Study ID	City, Country	Year start	Year end	Study design	N patients	N (%) Haematological malignancy/ N (%) HSCT	Study population	Criteria used for IPA definition (adherence to the reference standard Y / N)	N patients with proven / probable
Bretagne 1995(6)	Cre`teil, France	1992	1993	Cohort, prospective	28	11 (39.3%) / 9 (32.1%)	Immunocompromised patients with respiratory signs and unexplained fever	NIAID-MSG 1989 (N)	3 (10.7%)
Buchheidt 2001 (7)	Mannheim, Germany	1995	1998	Cohort, prospective	67	61 (89.5%) / 4 (6.0%)	Patients with haematological malignancies, neutropenia , fever unresponsiveness to the first line antibacterial treatment, and/or newly arisen nonspecific pulmonary infiltrates proven by conventional chest radiography	NIAID-MSG 1989 (Y)	9 (13.4%)
Frealle 2009 (15)	Lille, France	2000	2004	Cohort, retrospective	55	55 (100.0%) / 3 (5.5%)	Haematological cancer patients, HSCT at risk for IA	EORTC\MSG 2002 (Y)	23 (41.8%)
Hadrich 2011 (19)	Sfax, Tunisia	2004	2007	Case-Control, prospective	42	42 (100%) / 0 (0.0%)	Haematological cancer patients with febrile neutropenia and persistent fever for more	EORTC\MSG 2008 modified (N)	14 (33.3%)

							than 96 h refractory to broad-spectrum antibacterial treatments on whom BALs were performed		
Hayette 2001 (20)	Lie`ge, Belgium	1997	1998	Cohort, prospective	74	16 (21.6%) / 0 (0.0%)	Haematological malignancies, HSCT, SOT, high dose steroids. All patients undergoing bronchoscopy	NIAID-MSG 1989 modified (N)	10 (13.5%)
Jones 1998 (21)	Manchester, UK	NS	NS	Cohort, prospective	69	69 (100.0%) / NS	Haematological cancer patients with febrile episodes unresponsive to antibiotics	Authors defined. Based on NIAID-MSG 1989 (N)	5 (7.2%)
Khot 2008 (22)	Seattle, WA, US	2002	2003	Cohort, retrospective	94	94 (100.0%) / 8 (61.5%)	Patients with haematological malignancies or undergoing HSCT with pneumonia or pulmonary nodules	EORTC\MSG 2002 (Y)	13 (13.8%)
Luong 2011 (24)	Pittsburgh, PA, US	2000	2010	Cohort, retrospective	150	0 (0.0%) / 0 (0.0%)	Lung transplant recipients who underwent bronchoscopy for surveillance or diagnostic evaluation	EORTC\MSG 2008 modified (N)	16 (10.6%)
Musher 2004 (27)	Seattle, WA, US	1993	2002	Case-Control, retrospective	93	93 (100.0%) / 44 (88.0%)	Haematological malignancies, bronchoscopy to evaluate pulmonary nodules or infiltrates	EORTC\MSG 2002 modified (N)	46 (49.4%)

							that were detected after or while being evaluated for HSCT		
Orsi 2012 (28)	Modena, Italy	NS	NS	Case-Control, retrospective	19	8 (42.1%) / NS	Critically ill immunocompromised patients undergoing BAL for evaluation of pulmonary infiltrates	EORTC\MSG 2008 (Y)	6 (31.6%)
Raad 2002 (29)	Houston, TX, US	1996	1997	Cohort, prospective	249	165 (66.3%) / NS	Haematological malignancies, other cancer with chest radiographic findings suggestive of pneumonia	EORTC\MSG 2002 modified (Y)	32 (12.8%)
Rantakokko -Jalava 2003 (30)	Turku, Finland	NS	NS	Cohort, prospective	66	NS / NS	Haematological malignancies, HSCT, SOT, high dose steroids, bronchiectasis at risk for IA	EORTC\MSG 2002 (Y)	11 (16.6%)
Reinwald 2012 (31)	Mannheim, Germany	2000	2011	Cohort, retrospective	226	214 (94.7%) / 53 (23.5%)	haematological patients at high risk for fungal infections with new lung infiltrates detected by high-resolution CT	EORTC\MSG2002 (Y)	48 (21.2%)
Roselló 2011 (32)	Barcelona, Spain	NS	NS	Cohort, prospective	42	9 (21.4%) / NS	Haematological malignancies, SOT immunodeficiency with risk factors for IA	EORTC\MSG 2008 (Y)	7 (16.6%)
Sanguinetti	Rome, Italy	2001	2002	Cohort,	44	44 (100.0%) /	Patients with haematological malignancies	EORTC\MSG 2002	20 (45.4%)

2003 (33)				prospective		NS	and lung infiltrates	(Y)	
Shahid 2008 (34)	Aligarh, India	2004	2006	Cohort, prospective	69	0 (0.0%) / 0 (0.0%)	Patients with lung carcinoma, receiving chemotherapy or chronic steroid therapy	EORTC\MSG 2002 modified (N)	23 (33.3%)
Tang 1993 (39)	London, UK	NS	NS	Cohort, prospective	23	14 (60.9%) / 14 (60.9%)	Haematological malignancy, HSCT, SOT and clinical or radiological evidence of respiratory disease	Authors defined. Based on NIAID-MSG 1989 (N)	5 (21.7%)
Torelli 2011 (41)	Rome, Italy	2010	2011	Cohort, prospective	158	52 (42.6%) / NS	Haematological malignancies, COPD, cirrhosis, cancer receiving chemotherapy, solid organ transplant recipient, HIV, steroid use, or recipient of T-cell immunosuppressant with pneumonia, fever, suspected invasive fungal infection	EORTC\MSG 2008 modified (N)	17 (10.7%)
Verweij 1995 (43)	Nijmegen, The Netherlands	NS	NS	Cohort, prospective	19	17 (89.5%) / 5 (26.3%)	Neutropenic patients with fever persisting despite treatment with broad-spectrum antibacterial agents and pulmonary infiltrates	Authors defined. Based on NIAID-MSG 1989 (Y)	9 (47.3%)

NR – not required, NS – not stated, HSCT – hematologic stem cell transplant, SOT – solid organ transplant, COPD- chronic obstructive pulmonary disease, HIV – human immunodeficiency virus, ICU – intensive care unit, NIAID-MSG – National institute of allergy and infectious diseases – mycoses study group. EORTC\MSG - European Organisation for Research and Treatment of Cancer – Mycoses Study Group.

Supplementary Table 4: QUADAS-2 risk of bias assessment

Study ID	**Patient selection – Risk of bias**	**Patient selection – Concerns regarding applicability**	**Index test – Risk of bias**	**Reference standard – Risk of bias**	**Reference standard – Concerns regarding applicability**	**Flow and timing - Risk of bias**
Bretagne 1995 (6)	H	L	L	U	U	U
Buchheidt 2001 (7)	L	L	L	L	L	L
Frealle 2009 (15)	L	L	L	L	L	L
Hadrich 2011 (19)	H	U	L	U	H	U
Hayette 2001 (20)	H	L	H	L	H	U
Jones 1998 (21)	H	U	L	H	U	U
Khot 2008 (22)	L	U	L	U	L	U
Luong 2011 (24)	H	L	L	U	H	U
Musher 2004 (27)	H	L	L	U	H	H
Orsi 2012 (28)	H	L	U	U	U	L
Raad 2002 (29)	L	L	L	U	L	U

Rantakokko-Jalava 2003 (30)	L	U	H	U	L	U
Reinwald 2012 (31)	L	U	U	L	L	L
Roselló 2011 (32)	L	U	H	U	U	U
Sanguinetti 2003 (33)	L	U	L	L	L	L
Shahid 2008 (34)	H	U	L	L	H	L
Tang 1993 (39)	L	L	L	U	U	U
Torelli 2011 (41)	L	H	L	L	H	L
Verweij 1995 (43)	H	U	L	L	L	U

H – high risk, U – unclear, L – low risk

Supplementary Table 5: PCR methods

Study ID	PCR method	Volume used for PCR	Cell wall disruption method	DNA extraction method	Cycle number	Primer gene	Primer sequence	Internal / inhibition control	Contamination control	Aspergillus spp. detected by primer
Bretagne 1995 (6)	Standard	1·5 cc	Beads beating	Phenol-chloroform	40	Mitochondrial DNA fragment	5' GAA AGG TCA GGT GTT CGA GTC AC 3' (804 to 826)/ 5' CTT TGG TTG CGG GTT TAG GGA TT 3' (914 to 938)	Y	Y	A. fumigatus, A. flavus, A. terreus, A. niger
Buchheidt 2001 (7)	Nested	1·5 cc	Lyticase	Phenol-chloroform	23+35	18S rRNA	5' CGG CCC TTA AAT AGC CCG 3' (1296–1313) / 5' GA CCG GGT TTG ACC AAC TTT 3' (1681–1700)	Y	Y	Various A. spp.
Frealle 2009 (15)	Real-time, LightCycler	NS	Lyticase	QIAgen	55	18S rRNA	5'-CTGTTAGTGCGGGAGTTCAAAXTCT-3' / 5'-CTGAGCTAATTTCTTTCAACCCA	Y	Y	A. fumigatus , A. flavus , A. niger, A. terreus

							AGGGA-3’			
Hadrich 2011 (19)	Real-time, TaqMan, PCR-ELISA	NS	NS	QIAgen	45	18S rRNA	5 ′ -AAG CTC GTA GTT GAA CCT TG-3 ′ / 5′-ATG GTC CTA GAA ACC AAC AA-3 ′ (45-294)	Y	NS	A. fumigatus , A. flavus, A. terreus , A. niger , A. nidulans , A. ustus , A. glaucus , A. versicolor
Hayette 2001 (20)	Nested	1-5 cc	NS	Phenol-chloroform	30+30	Alkaline protease gene	5' AGCACCGACTACATCTAC 3' / 5' GAGAT GGTGTTGGTGGC 3'	Y	NS	A. fumigatus , A. flavus, A. terreus , A. niger , A. nidulans , A. glaucus
Jones 1998 (21)	PCR-ELISA	0·2 cc	Lyticase	Phenol-chloroform	40	Mitochondrial DNA	5' GAAAGGTCAGGTGTTCGAGTCA	Y	Y	A. fumigatus , A. flavus , A.

						fragment	3' (804-826) / 5' CTTGGTTGCGGGTTTAGGGATT 3' (916-938)			niger
Khot 2008 (22)	Real-time, TaqMan	2-5 cc	Beads beating	Master Pure Yeast kit	45	18S rRNA	5' GAT AAC GAA CGA GAC CTC GG 3' / 5' AGA CCT GTT ATT GCC GCG C 3'	Y	Y	Various A. spp.
Luong 2011 (24)	Real-time	0·5 cc	NS	QIAgen	NS	18S rRNA	pan A. 5' GTGGAGTGATTTGTCTGCTTAAT TG 3' (1215–1239) / 5' TCTAAGGGCATCACAGACCTGTT 3' (1345–1367) A. fumigatus 5' GCCCGCCGTTTCGAC 3' (86-100) / 5' CCGTTGTTGAAAGTTTTAACTGA TTAC 3' (195-221) A. terreus 5' CATTACCGAGTGCGGGTCTTTA 3' (12-33) / 5'	Y	Y	pan-Aspergillus, A. fumigatus, A. terreus

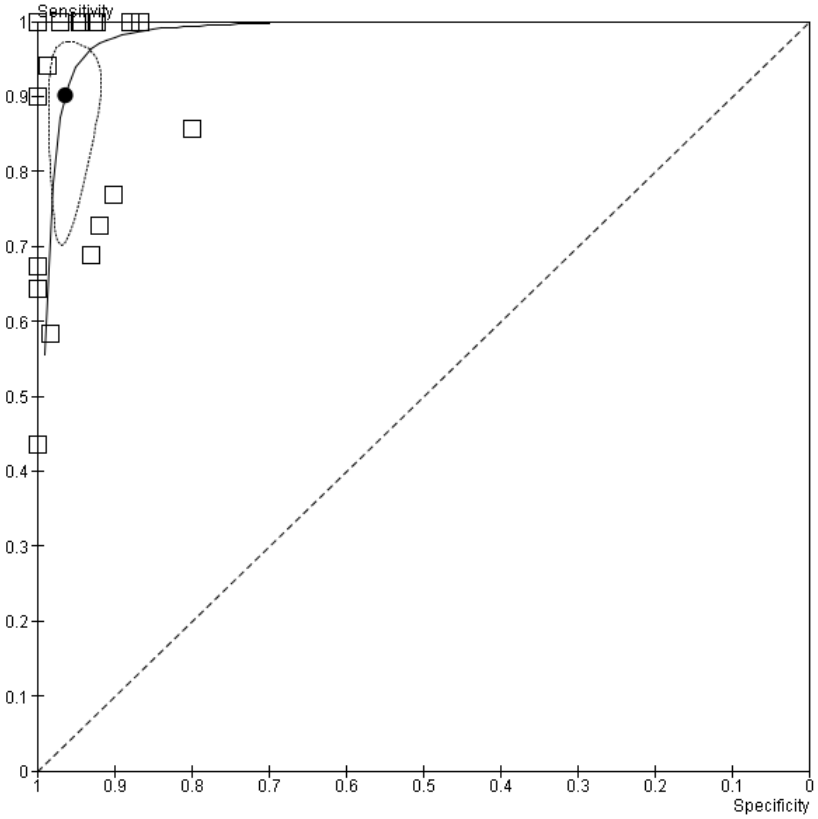
							CCCGCCGAAGCAACAAG 3' (65-81)			
Musher 2004 (27)	Real-time	1·5 cc	NS	Master Pure Yeast kit	45	18S rRNA	5' GATACCGTYGTAGTCTTA 3' / 5' TG TCTGGACCTGGTGAGT 3'	Y	Y	Various A. spp.
Orsi 2012 (28)	Real-time	1-2 cc	Beads beating	MycXtra™	36	18S rRNA	5' GAT AAC GAA CGA GAC CTC GG 3' / 5' AGA CCT GTT ATT GCC GCG C 3'	Y	Y	Various A. spp.
Raad 2002 (29)	Standard	1 cc	NS	Phenol-chloroform	40	Mitochondrial DNA fragment, Alkaline protease gene	ALP 5' AGCACCGACTACATCTAC 3' / 5' GAGATGGTGTTGGTGGC 3' mitochondrial 5' GAAAGGTCAGGTGTTCGAGTCAC 3' / 5' CTTTGGTTGCGGGTTTAGGGATT 3'	Y	Y	A. fumigatus, A flavus, A. terreus, A. niger
Rantakokko-Jalava 2003 (30)	Real-time, LightCycler	5-10 cc	Lyticase	QIAgen , DNA-Pure yeast	45	Mitochondrial DNA fragment	5' GAA AGG TCA GGT GTT CGA GTC A 3' / 5' CTT GGT TGC GGG TTT AGG GAT T 3'	Y	Y	A. fumigatus, A. flavus, A. terreus, A.

				genomic kit, High Pure PCR template preparation kit, Master Pure Yeast kit						niger
Reinwald 2012 (31)	Nested	1.5 cc	Lyticase	Phenol-chloroform	58	18S rRNA	AFU7S CGG CCC TTA AAT AGC CCG AFU7AS GA CCG GGT TTG ACC AAC TTT	Y	Y	Various A. spp
Roselló 2011 (32)	Real-time, SmartCycler	0·4 cc	NS	QIAgen	NS	18S rRNA	NS	NS	NS	Various A. spp
Sanguinetti 2003 (33)	Real-time, iCyclerIq, Nested (used in analysis)	1·5 cc	NS	QIAgen	40+30	18S rRNA	5' CCG ATT ACG TCC CTG CCC TT 3' / 5' TTG ACC AAC TTT CCG GCT CTG 3'	NS	Y	A. fumigatus, A. flavus, A. glaucus, A. niger, A.

										terreus
Shahid 2008 (34)	Standard	0·1 cc	NS	Phenol-chloroform	35	Mitochondrial DNA fragment	5' GAA AGG TCA GGT GTT CGA GTC AC 3' / 5' CTT TGG TTG CGG GTT TAG GGA TT 3'	Y	Y	A. fumigatus, A. flavus, A. niger
Tang 1993 (39)	Standard	0·25 cc	Beads beating	Phenol-chloroform	42	Alkaline protease gene	5'AGCACCGACTACATCTAC3' / 5' GAGAT GGTGTTGGTGGC 3'	NS	Y	A. fumigatus, A.flavus
Torelli 2011 (41)	Real-time, SmartCycler, Real-time in-house	5 cc	Beads beating	MycXtra	36	18S rRNA	5' GAT AAC GAA CGA GAC CTC GG 3' / 5' AGA CCT GTT ATT GCC GCG C 3'	Y	Y	Various A. spp. And specially A. fumigatus, A. flavus, A. terreus, A. niger
Verweij 1995 (43)	Standard	5-10 cc	Beads beating	Phenol-chloroform	30	18S rRNA	5' CCTGGT TGATCCTGCCAGTA 3' / 5' GCTTGATCCTTCTGCA GGTT 3'	NS	NS	Various A. spp

PCR – polymerase chain reaction, ELISA - Enzyme-Linked Immunosorbent Assay, Y – yes, NS – not stated

Supplementary Figure 1: HSROC for PCR in the diagnosis of proven or probable IPA.



Studies points are scaled by the inverse of their standard errors. The summary point with 95% confidence region is shown: sensitivity 90.2% (95% CI 77.2-96.1%), specificity 96.4% (95% CI 93.3-98.1%).