Supporting information:

Table 1: Limitations of current diagnostic tests for histoplasmosis and coccidioidomycosis

diagnostic tests	limitations	
	histoplasmosis	**coccidioidomycosis**
culture	long turn-around time, may take up to 8 weeks [8]	time intensive
	dangerous, as BSL-3 agents	dangerous, as BSL-3 agents
	sensitivity may range from 0 to 74% depending on the clinical manifestations [8]	sensitivity may range from 25 to 64% depending on the clinical manifestation [16,84]
	microscopy of hyphae may be confounded with *Chrysosporium* spec. and *Sepedonium* spec. [85]	may be confounded with other arthroconidial species
microscopy	several protozoa can show intracellular organisms of similar size: *Leishmania* spec., *Trypanosoma cruzi, Toxoplasma gondii* [10]	spherules in tissue may be confounded with sporangia of *Rhinosporidium seeberi* and adiaspores of *Emmonsia* spec. [10]
	several fungi may be confounded with *Histoplasma* in tissue sections: *Pneumocystis jirovecii*, *Coccidioides* spec. endospores, *Candida glabrata,* capsule-deficient *Cryptococcus* spec., small variants of *Blastomyces dermatitidis, T. marneffei, Paracoccidioides* spec., *Emergomyces* spec. [8,10-12]	mycelial forms may be encountered in *Coccidioides* infections if the transport is delayed, or from boundaries of old cavitary lesions in the lung, in skin lesions and in ventricular fluid during CNS infection [9]
		presence of *Coccidioides* endospores, without typical spherules may be difficult to differentiate from some yeast cells including *Blastomyces*, *Histoplasma* and *Candida* [10]
specific antigen detection	cross-reactions are possible with *Es. africanus, Blastomyces* spec*., Paracoccidioides brasiliensis, Talaromyces marneffei, Coccidioides immitis* and *posadasii, Sporothrix schenkii* [13,14,19]	no commercially available kits
		cross-reactions are possible with *Histoplasma, Blastomyces, Paracoccidioides* [15,16]
	limited sensitivity in immunocompetent patients and/or localized disease [13,17,18,20]	limited sensitivity in immunocompetent patients
antibody detection tests	sensitivity limited in patients with decreased cellular immunity [8]	sensitivity limited in patients with decreased cellular immunity [24]
	may be false negative at the beginning of the acute infection [8]	may be false negative at the beginning of the acute infection [24]
	cross-reactions are possible with other fungal diseases (*Blastomyces*, *Cryptococcus*, *Coccidioides*, *Aspergillus*,…) and other granulomatous diseases [25]	cross-reactions are possible with other fungal diseases (*Histoplasma*, *Cryptococcus*,…) [23]

Es.: *Emergomyces*; *T*.: *Talaromyces*;

Table 2: *Histoplasma* panel content and raw Cq results of the centres

Sample number	DNA content	Centre 1	Centre 2	Centre 3	Centre 4: assay 1	Centre 4: assay 2	Centre 4: assay 3	Centre 5: assay 1	Centre 5: assay 2
1	Triton 0.1	negative	negative	negative	negative	negative	negative	negative	negative
2	*H. capsulatum* (RKI09-0599) 10 pg/5µl	27.31/27.43	31.2/31.55	23.72/23.77	25.50	26.00	23.39	positive	*H. capsulatum*
3	*C. albicans* (RKI11-0116) 100 pg/5µl	negative	negative	negative	negative	negative	negative	negative	*C. albicans*
4	*H. capsulatum* (RKI09-0599) 1 pg/5µl	30.99/31.35	34.66/34.87	27.65/27.27	28.96	30.10	27.20	positive	negative
5	*Es. europaeus* (RKI17-1077) 100 pg/5µl	negative	negative	35.59/35.6	negative	negative	negative	negative	negative
6	*H. capsulatum* (RKI09-0599) 100 pg/5µl	23.79/23.77	27.17/27.56	19.93/19.67	21.30	21.91	19.80	positive	*H. capsulatum*
7	*H. capsulatum* (RKI09-0599) 0.01 pg/5µl	37.77/38.07	37.28/38.42	34.52/33.71	35.10	35.51	33.81	negative	negative
8	*Es. africanus* (RKI17-1221) 100 pg/5µl	negative	negative	35.53/35.81	negative	negative	negative	negative	negative
9	*H. capsulatum* (RKI09-0599) 0.001 pg/5µl	39.93/40.66	negative	37.21/37.29	37.00	38.02	36.61	negative	negative
10	*H. capsulatum* (RKI09-0599) 0.1 pg/5µl	34.77/34.78	36.65/36.4	31.53/31.16	32.34	33.89	30.71	positive	negative
11	*H. capsulatum* (RKI09-0599) 1000 pg/5µl	20.34/20.34	23.82/23.82	15.97/15.91	17.76	18.20	15.99	positive	*H. capsulatum*
12	*A. fumigatus* (RKI13-0959) 100 pg/5µl	negative	negative	negative	negative	negative	negative	positive	*A. fumigatus*
13	*B. dermatitidis* (RKI16-1033) 100pg/5µl	negative	negative	25.74/25.85	32.61	negative	negative	negative	*B. dermatitidis*
14	*P. brasiliensis* (RKI16-1032) 100pg/5µl	negative	negative	negative	negative	negative	negative	negative	*P.brasiliensis* or *lutzii*

RKI: Robert Koch Institute; ATCC: American Type Culture Collection; *H.*: *Histoplasma*; *C.*: *Candida*; *Es.*: *Emergomyces*; *A.*: *Aspergillus*; *B.*: *Blastomyces*; *P.*: *Paracoccidioides*

19 **Table 3:** *Coccidioides* panel content and raw Cq results of the centres

Sample number	DNA content	Centre 1	Centre 2	Centre 3	Centre 4	Centre 5
1	*Coccidioides posadasii* (RKI06-0090) 10 pg/5µl	26.28/26.63	34.16/34.06	28.28/28.64	25.48	*Coccidioides posadasii*
2	Triton 0,1	negative	negative	negative	negative	negative
3	*Coccidioides posadasii* (RKI06-0090) 0.01 pg/5µl	37.31/38.03	43.71/ negative	40.48/42.40	35.53	negative
4	*Coccidioides posadasii* (RKI06-0090) 1 pg/5µl	30.38/30.47	37.02/36.2	32.79/30.02	28.94	negative
5	*Trichophyton violaceum* (RKI16-0839) 100 pg/5µl	negative	negative	negative	negative	*Trichophyton violaceum*
6	*Coccidioides posadasii* (RKI06-0090) 0.001 pg/5µl	42.82/ negative	negative	negative	35.73	negative
7	*Candida albicans* (RKI11-0116) 100 pg/5µl	negative	negative	negative	negative	*Candida albicans*

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8	*Uncinocarpus reesii* (RKI19-0061) 100 pg/5µl	negative	negative	41.96/negative	negative	negative
9	*Coccidioides posadasii* (RKI06-0090) 0.1 pg/5µl	33.66/33.53	41.10/39.67	37.81/35.7	33.81	negative
10	*Coccidioides posadasii* (RKI06-0090) 100 pg/5µl	22.7/22.77	29.40/29.65	25.89/25.87	21.61	*Coccidioides posadasii*

RKI: Robert Koch Institute; ATCC: American Type Culture Collection

Table 4: Sensitivity and specificity of protocols for the detection of *Histoplasma capsulatum* DNA

Centre	type of PCR (target)	*Histoplasma capsulatum* panel	
		sensitivity (%)	specificity (%)
1	*Histoplasma* qPCR (ITS1)[10,31]	100	100
2	*Histoplasma* qPCR (ITS1)[25]	85,7	100
3	*Histoplasma* RT-qPCR (mtSSU)[9]	100	57,1
4	test 1: *Histoplasma* qPCR (ITS2)	100	71,4
	test 2: *Histoplasma* qPCR (ITS1)[18]	100	100
	test 3: *Histoplasma* qPCR (*COX2*), Hagen	100	100
5	test 1: *Histoplasma-specific* cPCR (ITS1)	71,4	100
	test 2: panfungal cPCR (ITS)[60,61]	42,8	100

qPCR : real-time PCR; ITS: internal transcribed spacer; RT-qPCR: reverse transcriptase real-time PCR; mtSSU: mitochondrial ribosomal small subunit RNA; *COX2*: cytochrome C oxidase 2; cPCR: conventional PCR

Table 5: Lowest detected number of genome equivalents and DNA quantity of *Histoplasma capsulatum* and *Coccidioides* in the two corresponding panels

	***Histoplasma capsulatum* panel**			***Coccidioides* spec. panel**		
	concentration (pg/5µl)	quantity (fg)	GE	concentration (pg/5µl)	quantity (fg)	GE
Centre 1	0,001	1	0,0213	0,001†	1†	0.0332 †
Centre 2	0,01	4	0,0851	0,01†	4†	0.1330 †
Centre 3	0,001	1,6	0,034	0,01	16	0.5320
Centre 4: assay 1	0,001	1,6	0,034	0,001	1,6	0.0532
Centre 4: assay 2	0,001	1,6	0,034	0,001	not applicable	not applicable
Centre 4: assay 3	0,001	1,6	0,034	0,001	not applicable	not applicable
Centre 5: assay 1	0,1	100	2,13	0,1	not applicable	not applicable
Centre 5: assay 2	10	104	213	10	10^4	332

GE: genome equivalents; † positive in one replicate out of 2.

Centre	type of PCR (target)	*Coccidioides* spec. panel	
		sensitivity (%)	specificity (%)
1	*Coccidioides* qPCR (ITS2)[15,34]	91,7	100
2	*Coccidioides* qPCR (ITS1)[27]	75	87,5
3	*Coccidioides* RTqPCR (mtSSU)	83.3	100
4	*Coccidioides* duplex qPCR (*PRA2*)[50]	100	100
5	panfungal cPCR (ITS)[60,61]	33,3	100

Table 6: Sensitivity and specificity of protocols for detecting *Coccidioides* spec. DNA

qPCR: real-time PCR; ITS: internal transcribed spacer; RT-qPCR: reverse transcriptase real-time PCR; mtSSU: mitochondrial ribosomal small subunit RNA; *PRA2*: proline-rich antigen 2; cPCR: conventional PCR

References

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Kaufman L, Brandt B. Fluorescent-antibody studies of the mycelial form of Histoplasma capsulatum and morphologically similar fungi. J Bacteriol. 1964;87(1):120-126. doi:10.1128/jb.87.1.120-126.1964