Clin. Lab. 2025;71:XXX-XXX

ORIGINAL ARTICLE

Evaluation of a Manual DNA Extraction Method Combined with In-House *Aspergillus* Real-Time PCR

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SUMMARY

Background: **To improve the molecular diagnostic yield for *Aspergillus* spp. from respiratory samples, we developed and evaluated a new DNA extraction method directly from respiratory samples combined with in-house *Aspergillus* real-time PCR.**

Methods: **We developed a method using beads and resin, where a sample is centrifuged to separate the supernatant and pellet. The pellet undergoes bead beating, is mixed with the supernatant, washed with AL buffer and distilled water, followed by the addition of Chelex-100 resin and boiling to extract the DNA template from the supernatant. As a comparator method, the Qiagen kit was used. To evaluate the efficiency of DNA extraction, nucleic acids extracted by the two methods were tested using in-house *Aspergillus* real-time PCR, and the cycle threshold (Ct) values were compared. The evaluation was conducted using contrived sputum samples with reference strains and 100 clinical respiratory specimens.**

Results: **Using contrived sputum samples at various concentrations, for *A. niger* and *A. flavus*, the Ct values were significantly lower when nucleic acids were extracted using the beads plus resin method compared to the Qiagen kit. For clinical samples, the beads plus resin method demonstrated a higher sensitivity of 88% (44/50) for the pan-*Aspergillus* primer/probe set compared to 76% (38/50) with the Qiagen kit, and significantly lower Ct values were obtained with our method ($p < 0.001$).**

Conclusions: **We developed a DNA extraction method that outperforms a commercial kit, enabling effective molecular detection of *Aspergillus* spp. directly from respiratory specimens to aid in diagnosing invasive pulmonary aspergillosis.**

(Clin. Lab. 2025;71:xx-xx. DOI: 10.7754/Clin.Lab.2025.250209)

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Manuscript accepted February 15, 2025

Supplementary Data

Table S1. Comparison results of in-house *Aspergillus* real-time PCR using two extraction methods from 50 clinical specimens with *Aspergillus*-positive cultures.

No.	Culture result		In-house *Aspergillus* real-time PCR (Ct value)			
	Organisms	Colony count	Pan-*Aspergillus* primer/probe		Species-specific primer/probe	
			Beads plus resin method	Qiagen	Beads plus resin method	Qiagen
1	*A. fumigatus*, *A. niger*	3	35.78	36.56	*A. fumigatus* (32.11)	*A. fumigatus* (34.88)
					A. niger (29.13)	*A. niger* (29.78)
2	*A. niger*	3	26.51	31.57	*A. niger* (28.43)	*A. niger* (32.54)
3	*A. niger*	3	22.45	28.08	*A. niger* (23.01)	*A. niger* (27.4)
4	*A. niger*	4	32.33	32.57	*A. niger* (31.11)	*A. niger* (31.78)
5	*A. fumigatus*	60	20.53	27.2	*A. fumigatus* (26.03)	*A. fumigatus* (26.15)
6	*A. niger*	10	29.25	37.01	*A. niger* (27.21)	*A. niger* (29.53)
7	*A. fumigatus*	50	21.86	27.62	*A. fumigatus* (27.56)	*A. fumigatus* (28.41)
8	*A. fumigatus*, *A. niger*	17	25.77	26.96	*A. fumigatus* (30.48)	*A. fumigatus* (31.41)
					A. niger (31.4)	*A. niger* (33.21)
9	*A. fumigatus*	10	21.1	27.63	*A. fumigatus* (25.41)	*A. fumigatus* (28.76)
10	*A. fumigatus*	3	33.27	33.56	*A. fumigatus* (33.74)	*A. fumigatus* (34.12)
11	*A. fumigatus*	1	negative	negative	negative	negative
12	*A. niger*, *A. terreus*	9	29.66	29.46	*A. niger* (28.43)	*A. niger* (29.11)
					A. terreus (25.05)	*A. terreus* (31.02)
13	*A. niger*	5	31.81	33.26	*A. niger* (31.22)	*A. niger* (32.98)
14	*A. fumigatus*	7	29.15	30.22	*A. fumigatus* (26.9)	*A. fumigatus* (29.45)
15	*A. niger*	13	30.66	32.64	*A. niger* (29.89)	*A. niger* (32.46)
16	*A. fumigatus*	17	22.21	27.22	*A. fumigatus* (17.41)	*A. fumigatus* (25.41)
17	*A. niger*	10	29.32	29.42	*A. niger* (26.55)	*A. niger* (29.1)
18	*A. fumigatus*	5	30.42	negative	*A. fumigatus* (35.4)	negative
19	*A. fumigatus*	1	27.16	negative	*A. fumigatus* (31.74)	negative
20	*A. terreus*	5	28.57	negative	*A. terreus* (26.7)	negative
21	*A. niger*	3	negative	negative	negative	negative
22	*A. flavus*, *A. fumigatus*	10	22.68	28.34	*A. fumigatus* (29.11)	*A. fumigatus* (33.08)
23	*A. niger*, *A. fumigatus*	2	negative	negative	negative	negative
24	*A. fumigatus*	70	29.22	36.52	*A. fumigatus* (16.75)	*A. fumigatus* (29.24)
25	*A. awamori*	4	31.69	32.54	negative	negative
26	*A. niger*	1	negative	negative	negative	negative
27	*A. fumigatus*	20	25.77	30.04	*A. fumigatus* (29.39)	*A. fumigatus* (31.73)
28	*A. fumigatus*	4	19.02	22.46	*A. fumigatus* (21.75)	*A. fumigatus* (22.1)
29	*A. fumigatus*	10	25.05	25.16	*A. fumigatus* (27.46)	*A. fumigatus* (30.57)
30	*A. fumigatus*	10	23.93	24.17	*A. fumigatus* (17.07)	*A. fumigatus* (28.35)
31	*A. fumigatus*	6	29.13	negative	*A. fumigatus* (30.29)	negative
32	*A. fumigatus*	4	negative	negative	negative	negative
33	*A. fumigatus*	1	23.01	30.29	*A. fumigatus* (25.91)	*A. fumigatus* (25.73)
34	*A. fumigatus*	2	28.28	negative	*A. fumigatus* (32.69)	Negative
35	*A. fumigatus*	20	29.62	39.14	*A. fumigatus* (28.49)	*A. fumigatus* (37.15)

Table S1. Comparison results of in-house *Aspergillus* real-time PCR using two extraction methods from 50 clinical specimens with *Aspergillus*-positive cultures (continues).

No.	Culture result		In-house *Aspergillus* real-time PCR (Ct value)			
	Organisms	Colony count	Pan-*Aspergillus* primer/probe		Species-specific primer/probe	
			Beads plus resin method	Qiagen	Beads plus resin method	Qiagen
36	*A. fumigatus*	4	31.4	negative	*A. fumigatus* (24.17)	Negative
37	*A. fumigatus*	7	32.24	37.27	*A. fumigatus* (33.33)	*A. fumigatus* (31.71)
38	*A. niger*	2	33.65	34.49	*A. niger* (33.36)	*A. niger* (31.88)
39	*A. fumigatus, A. niger*	5	negative	negative	negative	negative
40	*A. terreus*	5	34.32	38.24	*A. terreus* (36.3)	*A. terreus* (37.31)
41	*A. niger*	6	36.32	35.47	*A. niger* (34.23)	*A. niger* (37.23)
42	*A. fumigatus*	5	28.43	34.15	*A. fumigatus* (31.44)	*A. fumigatus* (37.41)
43	*A. niger, A. fumigatus*	10	30.26	36.09	*A. fumigatus* (29.41)	*A. fumigatus* (35.78)
44	*A. terreus*	2	29.89	34.78	*A. terreus* (28.07)	*A. terreus* (28.68)
45	*A. fumigatus*	16	26.55	32.21	*A. fumigatus* (22.68)	*A. fumigatus* (29.41)
46	*A. fumigatus*	9	26.82	39.48	*A. fumigatus* (31.12)	negative
47	*A. fumigatus*	10	28.41	30.44	*A. fumigatus* (30.51)	*A. fumigatus* (29.41)
48	*A. fumigatus*	10	23.05	24.04	*A. fumigatus* (27.89)	*A. fumigatus* (27.1)
49	*A. fumigatus*	8	26.7	30.1	*A. fumigatus* (27.77)	*A. fumigatus* (29.14)
50	*A. fumigatus*	5	24.16	30.34	*A. fumigatus* (28.4)	*A. fumigatus* (30.47)