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Reply to "Insufficient Demonstration of Long-Term Stability of *Aspergillus* Galactomannan"

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We thank Dr. Johnson and coauthors for reemphasizing in their letter (1) some of the limitations of our study of the stability of galactomannan in frozen specimens (2), namely, the small sample size, lack of clinical information, and potential for confounding factors in pooled specimens. We also acknowledge that our findings differ from those of several other studies, including their own.

While clinical information is desirable, the point of our study was the stability of galactomannan in specimens that had been stored frozen. As the assay uses monoclonal antibodies that are specific for galactomannan and as false-positive results are caused by galactomannan present in other fungi or components produced by *Penicillium* or *Aspergillus* or present in contaminated medications or electrolyte solutions, the lack of clinical information is unlikely to influence observations about galactomannan stability.

Greater variability was observed in the retrospective study of frozen specimens that had initially been tested 5 years earlier than in the prospective study, which used pools created at the time of initial testing. Greater variability in archived specimens is understandable given the multiple factors affecting variability: assay-to-assay, kit-to-kit, and operator-to-operator variation. Furthermore, and very importantly, the assay was not developed for quantification and does not use a standard curve for calculation of galactomannan concentration. That said, the agreement in the retrospective study was good. Samples (10 of the 30 tested) that showed a >20% decline in their galactomannan level and galactomannan index (GMI) are listed in Table 1. Only one of the 10 specimens was negative (had an index of less than 0.5) with repeat testing, and 5 of the 10 had relatively low initial GMIs (0.83 to 2.48), in which greater variability is expected. Only 2 specimens showed a major decline, both of which were BAL fluid specimens (GMIs, 2.58 to 0.61 [76%] and 7.10 to 2.78 [62%]).

The prospective study of pooled specimens was conducted to control for the variables affecting the retrospective study. Pooled specimens were used because of the insufficient volume of individual specimens to study four prospective time points. No reduction in GMI was observed over the 11 months for any of the pools. In fact, the GMIs increased: 21.4% of slightly positive serum samples, 2% of highly positive serum samples, 13% of slightly positive BAL fluid samples, and 29% of highly positive BAL fluid samples.

TABLE 1 Comparison of initial and repeat GMIs in specimens exhibiting a >20% reduction after storage for 5 years

Specimen	Initial GMI	Repeat GMI	% reduction
Serum	0.83	0.51	39
BAL fluid	0.97	0.44	55
BAL fluid	1.24	0.93	25
Serum	2.05	1.13	45
BAL fluid	2.58	0.61	76
Serum	3.25	1.78	45
BAL fluid	3.66	2.86	22
BAL fluid	6.62	5.19	22
BAL fluid	7.1	2.78	62
BAL fluid	7.7	5.15	33

In conclusion, our findings support the stability of galactomannan in specimens that have been stored frozen and the use of frozen specimens in evaluating new diagnostic tests based on galactomannan detection. Retrospective studies are unlikely to resolve this controversy. A statistically powered prospective study testing specimens from individual patients at 0 and 11 months, by the same operator using the same kit lot, might settle the controversy.

REFERENCES

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