Instability of *Aspergillus* Galactomannan in Stored Clinical Samples

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We read with interest the recent paper by Wheat et al. (1) reporting on the long-term stability of *Aspergillus* galactomannan in bronchoalveolar lavage fluid (BALF) and serum specimens stored at -20°C. Their findings contrasted with previously published results from Johnson et al. (2), who had observed a significant loss of signal in serum—but not BALF—samples upon long-term storage. We here expand the data on this controversial subject, showing loss of signal in both BALF and serum samples.

Our laboratory is based in a 600-bed hospital hosting a large hematopoietic stem cell transplant program. We introduced the *Aspergillus* galactomannan assay (Platelia *Aspergillus* Ag; Bio-Rad Laboratories Inc.) in 2000 and have performed more than 15,000 tests to date. We do not comply with the manufacturer’s recommendation that sera with optical density indices (ODIs) of ≥0.5 be retested to confirm positivity but rather request a second serum sample, as suggested by Maertens et al. (3). For this study, all serum and BALF samples with ODIs of ≥0.5 tested between January 2009 and April 2013 were stored at -80°C on the day of initial testing. Before initial testing, specimens were kept at 2 to 8°C for not more than 2 days according to the manufacturer’s instructions. Twenty-one specimens were retested in two batches in 2011 and 2012 for preliminary data. All other specimens were retested between May and September 2013, using four different lots. Specimens were thawed on the day of retesting. Initial and frozen samples were tested singly. Equipment and procedures remained unchanged during the study period.

Two hundred ninety-three (196 serum, 97 BALF) specimens were included. Detailed ODI and storage duration values are presented in Table 1. Upon retesting, mean index change was -0.45 for serum and -0.37 for BALF specimens, which resulted in a loss of positivity for 45% and 23% of specimens, respectively. Linear regression analysis of ODIs from initial versus frozen samples is shown in Fig. 1. Signal loss remained significant across the four lots when analyzed separately, and there was no correlation between storage duration and signal loss (data not shown). Absolute signal loss appeared to be independent from initial ODI value, while loss of positivity was inversely proportional to initial ODI value (Table 2).

Decline in galactomannan signal was reported by several investigators after short-term (positive samples kept at 2 to 8°C retested per package insert instructions) (2, 4, 5) and long-term (frozen samples) storage (2, 6, 7). The biological basis of this phenomenon remains unclear. Enzymatic degradation, galactofuranose side chain hydrolysis caused by sample acidification, and freezing-induced damage to nonpolysaccharide galactofuranose-bearing antigens are among the considered hypotheses. Data presented by Wheat et al. (1) strongly suggest that freezing itself is not at play.

Table 1

<table>
<thead>
<tr>
<th>Specimen type (n)</th>
<th>Storage duration in days (mean, median, range)</th>
<th>Initial ODI (mean, median, range)</th>
<th>Frozen ODI (mean, median, range)</th>
<th>Decline in signal* (95% CI, P value)</th>
<th>No. (%) with loss of positivity*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum (196)</td>
<td>796, 812, 72 to 1,624</td>
<td>1.72, 1.08, 0.50 to 7.87</td>
<td>1.27, 0.69, 0.03 to 7.50</td>
<td>-0.45 (-0.25 to -0.65, P = 0.0001)</td>
<td>89 (45)</td>
</tr>
<tr>
<td>BALF (97)</td>
<td>674, 623, 112 to 1,335</td>
<td>2.85, 2.25, 0.51 to 7.43</td>
<td>2.48, 1.65, 0.11 to 6.40</td>
<td>-0.37 (-0.11 to -0.65, P = 0.005)</td>
<td>22 (23)</td>
</tr>
</tbody>
</table>

*Mean difference between frozen and initial ODIs.

**Two-tailed P value, paired t test.

*Frozen ODI of <0.5.

*CI, confidence interval.

FIG 1 Correlation between optical density indices (ODIs) from frozen and initial samples. (A) Serum, n = 196. (B) Bronchoalveolar lavage fluid (BALF), n = 97. Slopes and R² values are indicated. 95% CI, 95% confidence interval.

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This is corroborated by the lack of correlation between storage duration and signal loss in the present study. Signal decline was previously associated with false positivity, suggesting either contamination or the existence of an unstable cross-reacting antigen (4, 6). This could not be ascertained in our study, as clinical correlation was not performed. Discrepancies found across long-term stability studies merit further consideration. One major difference between previous studies and our own is the fact that we do not routinely retest positive samples. Thus, we may have witnessed lately what was in fact the initial loss reported by other investigators (2, 4, 5). Alternatively, previous studies including small numbers of samples may have been underpowered to detect a significant signal loss. Finally, high initial ODI values in the study by Wheat et al. (1) may explain why they failed to observe loss of positivity. Indeed, our data show that the initial ODI value is the main driver of positivity loss, as the magnitude of signal loss is similar across low-, intermediate-, and high-positive samples. More data are needed to unveil the exact mechanisms behind galactomannan signal loss. This could be critical for clinical laboratories to ensure optimal specimen processing and for research projects where specimens are collected and stored for further testing.

ACKNOWLEDGMENT

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REFERENCES


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**TABLE 2 Stability of galactomannan signal according to initial ODI value**

<table>
<thead>
<tr>
<th>Specimen type (n)</th>
<th>Initial ODI category (n)</th>
<th>Initial ODI (mean)</th>
<th>Frozen ODI (mean)</th>
<th>Decline in signal (95% CI, P value)</th>
<th>No. (%) with loss of positivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum (196)</td>
<td>Low (90)</td>
<td>0.72</td>
<td>0.46</td>
<td>−0.26 (−0.15 to −0.37, P &lt; 0.0001)</td>
<td>57 (63)</td>
</tr>
<tr>
<td></td>
<td>Intermediate (34)</td>
<td>1.22</td>
<td>0.92</td>
<td>−0.30 (−0.06 to −0.54, P = 0.017)</td>
<td>14 (41)</td>
</tr>
<tr>
<td></td>
<td>High (72)</td>
<td>3.20</td>
<td>2.44</td>
<td>−0.76 (−0.26 to −1.27, P = 0.0037)</td>
<td>18 (25)</td>
</tr>
<tr>
<td>BALF (97)</td>
<td>Low (27)</td>
<td>0.68</td>
<td>0.516</td>
<td>−0.17 (−0.06 to −0.27, P = 0.0027)</td>
<td>16 (59)</td>
</tr>
<tr>
<td></td>
<td>Intermediate (11)</td>
<td>1.24</td>
<td>0.81</td>
<td>−0.43 (−0.20 to −0.66, P = 0.0022)</td>
<td>3 (27)</td>
</tr>
<tr>
<td></td>
<td>High (59)</td>
<td>4.14</td>
<td>3.68</td>
<td>−0.45 (−0.03 to −0.87, P = 0.035)</td>
<td>3 (5)</td>
</tr>
</tbody>
</table>

*a* Mean difference between frozen and initial ODIs.  
*b* Two-tailed P values, paired t-test.  
*c* Frozen ODI of <0.5.  
*d* Low, ODI of 0.50 to 0.99; intermediate, ODI of 1.00 to 1.49; high, ODI of ≥1.50.  
*e* CI, confidence interval.
Correction for Dufresne et al., Instability of *Aspergillus* Galactomannan in Stored Clinical Samples

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