Sir,

I have read with interest the recently published paper by Liss et al., 1 which implies that most common antimicrobials contain sufficient 1,3-β-D-glucan (BDG) contamination to render patients receiving them positive by the most commonly used BDG assay, Fungitell®. Close reading of the methods and results, however, raises multiple questions concerning the methodology employed, observations described and conclusions drawn.

First, BDG titres were assayed using two independent methods, Fungitell and a BDG-specific ELISA kit (catalogue no. 172516; United States Biological, Salem, MA, USA), using reconstituted or ready-to-use antimicrobial solutions. Using these methods, it was determined that an antimicrobial with a BDG titre as low as 89 pg/mL (Fungitell result for rifampicin (Riemser batch 4124)) would be sufficient to produce patient positivity. However, using the author’s mathematical model for determining patient BDG titre upon infusion (6000 mL x 80 pg/mL of the antimicrobial), one calculates that ~5.4 L of this solution would need to be infused with no loss of BDG to natural clearance as excess fluid was eliminated. 2 Five of 35 antimicrobials tested were found to have >1000 pg/mL. At 1006 pg/mL (colistin, Sobi, batch 7916), 477 mL would need to be infused to produce a BDG titre ≥80 pg/mL under the same circumstances.

Second, the concordance between the Fungitell-assayed BDG titre and BDG titre assayed with the ELISA is very strong (Figure 1; slope = 1.016; \( r^2 = 0.9991 \)). While the Fungitell reportable range is 31–500 pg/mL, the ELISA kit instructions for use state that the reportable range is 50–1000 ng/mL.

Third, while it is certainly possible to have BDG-contaminated parenterals cause patient positivity (BDG-contaminated intravenous immunoglobulins, for example) 3 the authors’ conclusions concerning antimicrobials are undermined by the very consistent findings of the very high negative predictive value for the BDG assay in patient populations that are nearly universally receiving antimicrobials. 4–5 In addition, multiple publications have analysed the impact of antimicrobials upon patient BDG titres without finding systematic false positivity. 6–9

It would be useful to have these observations addressed by Liss et al. 4 in order to evaluate the results and conclusions of their paper.

Transparency declarations

M. A. F. is an employee of Associates of Cape Cod, Inc., East Falmouth, MA, the manufacturer of the Fungitell kit.

References

1,3-β-D-Glucan contamination of common antimicrobials—authors’ response

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Sir,

We thank Dr Finkelman1 for his interest in our work and are grateful for the opportunity to address aspects that have remained unclear.

Dr Finkelman2 states that ‘Using these methods, it was determined that an antimicrobial… would be sufficient to produce patient positivity’. However, we do not make this claim in our article.2 Careful reading reveals that we did not test patient materials or attempt to extrapolate our measurements to in vivo concentrations of 1,3-β-D-glucan (BDG). We determined which reconstituted solutions contain enough BDG to test above the positivity cut-off. We concede that a single dose of most tested antibiotics would likely be insufficient to cause a positive test in patients. However, patients who develop invasive fungal disease are likely to have received multiple doses of antibiotics before the diagnosis of an invasive fungal disease is even suspected.3,4 Consequently, we explicitly encouraged further exploration of the cumulative in vivo impact of infusion solutions contaminated with BDG.

In his second remark, Dr Finkelman2 questions the technical accuracy of our work. We would thus like to point out that to avoid any likely source of error we employed quality control measures beyond those described in the original article. To exclude the non-specific reaction with the Limulus Amoebocyte Lysate (LAL) enzyme-based system, we used an antibody-specific detection by a competitor ELISA (BioAssay; United States Biological, Salem, MA, USA) as a secondary detection method. We validated and used the ELISA kit outside the detection range specified by the manufacturer. Word count limits did not allow for this information: in brief, we saturated capture antibodies with 990 ng/mL BDG. After incubation with the competitor glucans and the tested drugs and washing steps, we incubated with horseradish peroxidase substrate until the standards showed a visible stain. Table 1, column 4 shows the original data. To increase test accuracy, we again washed the plates and incubated them for 15 min at room temperature with an anti-horseradish peroxidase conjugated to R-phycocerythrin (Cayman Chemicals Company, Ann Arbor, MI, USA). After the final washing step, Promega GloMax® was used for detection. Results were presented in the original paper. Finally, we retested frozen aliquots of the samples with a third assay covering the drug BDG level range directly (MyBioSource Inc., San Diego, CA, USA; Table 1, column 8). We summarize correlation data of Passing–Bablok regression for all three methods in Table 2. All analyses showed consistent results, reassuring us that all tests were suitable and that non-specific, i.e. non-BDG-mediated false-positive results were not generated by the LAL enzyme-based system described in our article.

Dr Finkelman’s3 comment that the repeatedly demonstrated high negative predictive value of BDG undermines our conclusion is misleading. Considering the low prevalence of invasive fungal infections and the high sensitivity of BDG testing, a high negative predictive value will result regardless of the test’s limited specificity. Therefore, a low negative predictive value cannot dismiss concerns of infusion-related false-positive results.

Lastly, we point out that other researchers have reported an association between antibiotic exposure and false-positive BDG assay results.5–9 In light of these findings, BDG contamination of anti-infective infusions appears frequent; it probably depends on manufacturing processes and varies widely between lots. As long as the BDG content of each infusion solution remains unknown and the kinetics of infused BDG unexplored, we believe in cautious clinical interpretation of BDG test results in patients exposed to such contaminants.

Transparency declarations

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References
