Development of External Quality Assessment Scheme for β-D-Glucan Testing

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Introduction

β-D-glucan (BDG) is a key pan-fungal biomarker used in the diagnosis of invasive fungal infections (IFIs) (Posteraro B, et al., 2011) which pose a serious risk to immunocompromised patients and are increasing in incidence in the UK. This rise is driven by a growing population undergoing intensive chemotherapy, organ transplantation, and immunosuppressive therapies. Common IFI pathogens include *Candida* spp., *Aspergillus* spp., and other opportunistic fungi, which can be difficult to detect early with conventional methods. BDG is found in the cell walls of many pathogenic fungi, including *Candida*, *Aspergillus*, *Fusarium*, and *Pneumocystis jirovecii*, but is absent in *Cryptococcus* and the Mucorales (Stone M, et al., 2025). Galactomannan (GM), a more specific biomarker for *Aspergillus* spp., is widely used and supported by established external quality assessment (EQA) schemes. However, EQA provision for BDG is currently limited. Given the complexity and variability of BDG assays, robust EQA is essential to ensure test accuracy and consistency. To address this gap, two pilot distributions were performed to evaluate integration of BDG into the fungal biomarker EQA scheme. Since April, BDG has also been included as a reporting option.

Method

Mycology EQA participants were invited via questionnaire to a BDG pilot, leading to two pilot distributions. The existing Fungal Biomarker scheme has since been designed to allow reporting of both GM and BDG. For all distributions, serum specimens are prepared by spiking with varying concentrations of *Aspergillus fumigatus* filtrate.

Pilot 1

A total of two specimens: a BDG positive with high levels of filtrate (5545) and a negative specimen (5546), were dispatched.

- The specimens were distributed on 04/02/2019.
- Results were submitted by participants prior to the closing date of 18/03/2019

Pilot 2

A total of three specimens: a negative (5579), a low positive (5580), and a positive spiked with high levels of filtrate (5581) were dispatched.

- The specimens were distributed on 22/07/2019
- Results were submitted by participants prior to the closing date of 05/08/2019.

Enhanced Distribution

A total of three positive specimens spiked with filtrate were included as additional reporting options within the fungal biomarkers scheme (5842A, 5842B and 5842C).

- The specimens were distributed on 02/06/2025
- Results were submitted by participants prior to the closing date of 16/06/2025.

Results

Pilot 1

- 13.0% (3/23) of laboratories reported specimen 5545 positive as intended (figure 1).
- 82.6% (19/23) of laboratories reported specimen 5546 as intended, whilst 13% (3/23) reported positive and 4.4% (1/23) reported an equivocal result (figure 2).

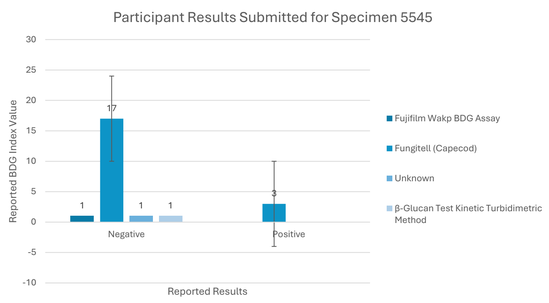

Figure 1: Pilot 1, specimen 5545 participant result concordance.

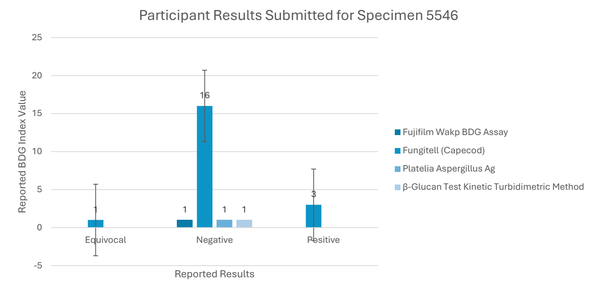

Figure 2: Pilot 1, specimen 5546 participant result concordance.

Pilot 2

- 46.2% (6/13) of laboratories reported specimen 5579 positive as intended, (figure 3). 23.1% (3/13) reported a negative result and 30.8% (4/13) reported an equivocal result.
- 61.5% (8/13) of laboratories reported specimen 5580 negative as intended (figure 4). 7.7% (1/13) reported a positive result and 30.8% (4/13) reported an equivocal result.
- 92.3% (12/13) of laboratories reported specimen 5581 positive as intended (figure 5). 7.7% (1/13) did not submit a result for this specimen.

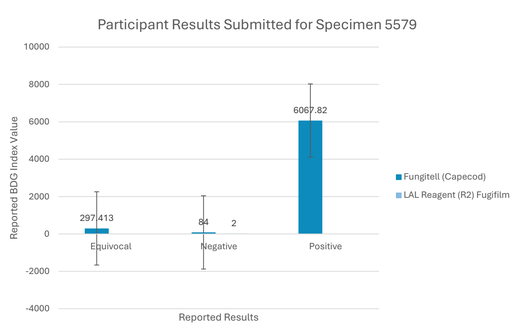

Figure 3: Pilot 2 Specimen 5579 participant result concordance.

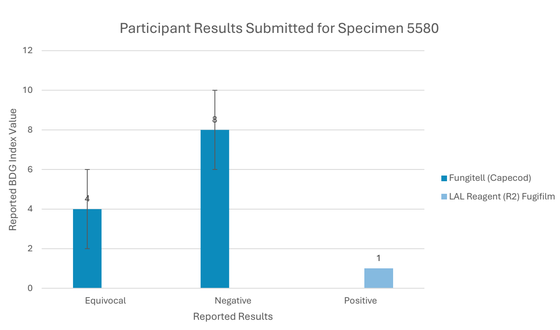

Figure 4: Pilot 2 Specimen 5580 participant result concordance.

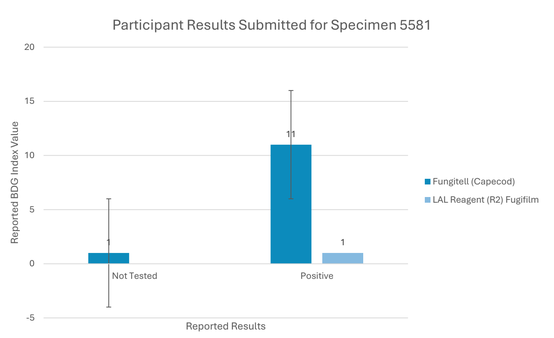

Figure 5: Pilot 2 Specimen 5581 participant result concordance.

Enhanced Distribution

- Specimen 5842A: All participants reported the intended positive result (figure 6).
- Specimen 5842B: All participants reported a positive result (figure 7).
- Specimen 5842C: All participants reported the intended positive result (figure 8).

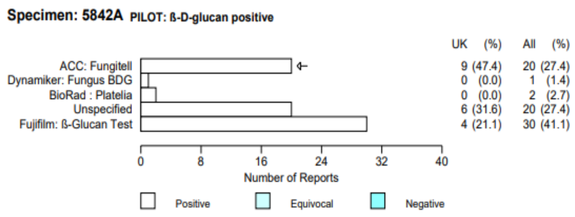

Figure 6: Enhanced distribution, specimen 5842A, participant result concordance.

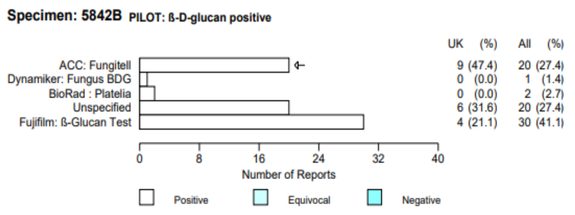

Figure 7: *Enhanced distribution, specimen 5842B, participant result concordance.*

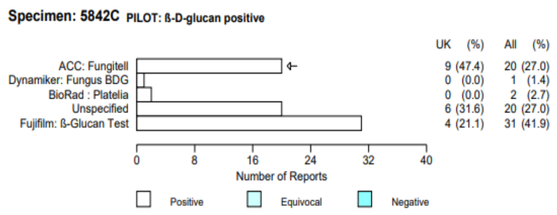

Figure 8: Enhanced distribution, specimen 5842C, participant result concordance.

Discussion

The first pilot results were not as expected, only a small number of participants submitted results that matched the intended results. 3/23 laboratories submitted incorrect results for both specimens, two of which are suspected to have been mismatched specimens. The low levels of detection in the positive specimen is suspected to be attributed to analyte degradation, further investigations will follow to confirm.
The second pilot results were also not as expected, a small increase in number of participants submitted results that matched the intended results. 4/23 laboratories submitted incorrect results for two specimens, again these are suspected to have been mismatched specimens. The low levels of detection in the clinically relevant positive specimen continues to be suspected of being attributed to analyte degradation.
The analysis of the second pilot results showed there was a 92.3% concordance of results submitted by participants with the intended for specimen 5581. The reason for such a high concordance is likely due to, specimen 5581 having a high positive BDG content.

In the enhanced distribution, three specimens were provided: two designated as positive and one as negative (specimen 5842B). Unexpectedly, all three specimens demonstrated very high BDG index values, indicating likely contamination rather than true positivity. Subsequent investigation identified the source of contamination as the gauze used during specimen preparation.
For the preparation of serum specimens in this scheme, serum is filtered to remove clots. A BDG-free alternative was sourced and will now be used to support optimisation of the multi-biomarker analyte EQA scheme. Two further enhanced distributions are scheduled for this year, which will contribute to the development and planned introduction of the elevated scheme.

Conclusion

In conclusion, the first and second pilot results submitted by the participants have a low concordance rate with the intended results, for the detection of BDG. Participants got a higher rate of concordant results when the quantity of BDG in the specimen was high. Participants got a lower concordance rate when the BDG of the specimen was low.
The enhanced distribution demonstrated higher concordance with the intended results overall; however, issues were observed across all specimens. BDG levels were markedly higher than intended in the positive specimens, and notably, the negative specimen was reported as positive by all participants. This highlighted the need for further optimisation of specimen preparation.
Investigations identified the gauze used during serum filtration as the likely source of BDG contamination. To address this, a BDG-free alternative has been sourced, and procedures are being optimised and standardised. These improvements will be implemented prior to future enhanced distributions, supporting the continued development and refinement of the fungal biomarker EQA scheme.
Two enhanced distributions of the FB EQA scheme containing BDG will be then dispatched to the participants who have part-taken in our recent pilots and enhanced distribution, with whom further participant feedback reports will be published and distributed.

Acknowledgements

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References

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