New UK NEQAS EQA Scheme: Fungal Biomarkers

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Introduction and purpose

United Kingdom National External Quality Assessment Service (UK NEQAS) for Microbiology is an external quality assessment (EQA) provider with participating laboratories worldwide. EQA is an invaluable, educational tool for clinical laboratories and a means to monitor, evaluate and improve their own performance, quality and reliability of their service.

Invasive aspergillosis (IA) is a leading cause of infection-related morbidity and mortality in severely immunocompromised individuals[1]. The European Organisation for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group (EORTC) and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (MSG) recommend galactomannan (GM) antigen detection as an element of diagnosing IA[2].

Routinely, laboratories use ELISA based testing for the detection of GM antigen. The variability in storage of specimens, stability of the antigen and interpretation of the results with the assay have been reported[2-4] and therefore warranted the need for an EQA scheme to assess the performance of laboratories providing a service in the detection of GM.

UK NEQAS introduced a new EQA scheme to focus on biomarkers of fungal associated infectious diseases, with GM introduced as the first fungal biomarker. This study analysed the performance of clinical laboratories participating in the Fungal Biomarkers scheme from August 2016 to February 2019.

Methods

Twenty two simulated specimens spiked with various levels of GM antigen were dispatched to between 44 and 88 participants in eight distributions (the first two of which were pilot distributions) from August 2016 to February 2019. Participant laboratories were based in 16 different countries (Table 1).

Table 1: Countries participating laboratories in UK NEQAS for Microbiology Fungal Biomarkers EQA scheme

Countries participating in UK NEQAS for Microbiology EQA for Fungal Biomarkers	
Austria	Italy
Belgium	Malta
Switzerland	Netherlands
Germany	Portugal
Denmark	Sweden
France	Slovenia
Ireland	Turkey
Israel	United Kingdom

Twenty specimens were simulated serum specimens, of which 14 were positive for GM antigen and six were negative. Two specimens were simulated bronchoalveolar lavage (BAL) specimens, both of which were positive for GM antigen.

Participants examined the specimens for the absence or presence of the antigen. Results were then analysed to determine the performance of participants, with each participant issued with a report. Figure 1 illustrates a sample report of a distribution.

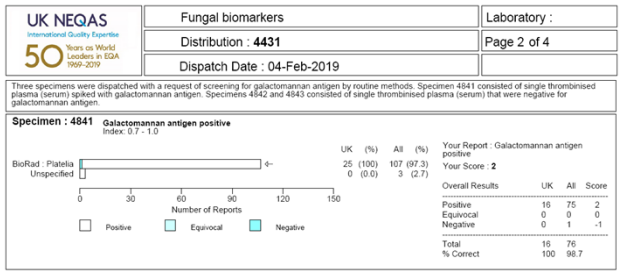

Figure 1: Example distribution report for the Fungal Biomarker scheme

Results

Results were received from between 41 and 76 participants. Results for all specimens are shown in Table 2 for serum specimens and Table 3 for BAL specimens.

- 74 participants reported to use the Biorad Platelia *Aspergillus* galactomannan EIA. Two participants did not specify a method.
- Participants demonstrated very good concordance (>97%) with intended results for the seven specimens containing high levels of GM in serum (≥0.9 index value).
- A decline in participant performance was observed when testing three weak positive specimens designed close to the positive cut off reading of the Platelia Aspergillus ELISA (BioRad) for serum (0.5). These three specimens with median reported index values between 0.63 and 0.66 determined concordances <83%.

Table 2: Participants' overall performance with simulated serum specimens

Specimen	Distribution	Date	GM Intended result	Median reported index value	Participant concordance (%)
3670	4057 (Pilot)	29/08/2016	Negative	0.10	97.6
3692	4057 (Pilot)	29/08/2016	Positive	1.62	97.6
3671	4058 (Pilot)	30/01/2017	Positive	0.85	95.1
3715	4058 (Pilot)	30/01/2017	Positive	0.65	80.5
3859	4111	05/06/2017	Negative	0.30	96.2
3860	4111	05/06/2017	Positive	0.63	82.7
4267	4111	05/06/2017	Positive	0.79	96.1
4026	4166	25/09/2017	Positive	0.68	96.6
4027	4166	25/09/2017	Positive	1.52	100
4216	4226	29/01/2018	Positive	1.00	100
4269	4226	29/01/2018	Positive	0.90	100
4467	4310	04/06/2018	Negative	0.10	90.4
4468	4310	04/06/2018	Negative	0.31	86.1
4469	4310	04/06/2018	Positive	0.68	97.2
4643	4368	24/09/2018	Positive	0.66	82.8
4644	4368	24/09/2018	Positive	1.20	98.3
4645	4368	24/09/2018	Positive	1.00	98.2
4841	4431	04/02/2019	Positive	0.90	100
4842	4431	04/02/2019	Negative	0.20	82.1
4843	4431	04/02/2019	Negative	0.22	89.3

Table 3:Participants' overall performance with simulated BAL specimens

Specimen	Distribution	Date	GM Intended result	Median reported index value	Participant concordance (%)
3716	4058 (Pilot)	30/01/2017	Positive	1.40	100
4268	4166	25/09/2017	Positive	0.94	84.2* (not scored)

- A decline in participant performance was also observed with three specimens spiked with low levels of GM, designed to be well below the cut off reading. These three specimens with median reported index values between 0.1 and 0.22 determined concordances <91%. The reasons for this are unclear.
- Median index values were grouped into index ranges and the mean concordances were plotted on a graph. Figure 2 shows that on average, specimens in the GM index ranges close to the cut off value (0.2 - 0.39 and 0.6 – 0.79) had concordances <89% and GM index ranges >0.8 had concordances >98%.

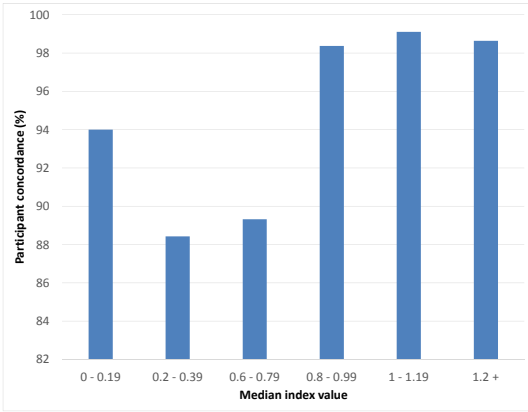

Figure 2: Mean participant concordance of specimens grouped by median reported index values for GM

- One simulated BAL specimen designed with a GM index value of 1.0 - 1.2 , intended to be close to the positive cut off value of the ELISA for BAL (1), proved challenging to participants, and resulted in 84.2% concordance and a median reported index value of 0.94. Manufacturer's instructions recommend that if a GM index value is below the positive cut off and the symptoms of the patient suggestive of IA, the test should be repeated. It is probable some participants did not perform repeat testing to eliminate false negatives.

Discussion / Conclusions

- The Biorad Platelia *Aspergillus* galactomannan EIA[5] kit continues to be the most common ELISA test used for clinical testing. New kits have become available, including kits for multiplex PCR for *Aspergillus* sp., lateral flow assays and competitor ELISA kits. Until recently, many of these alternative assays lacked certification for use in clinical settings, however this is beginning to change with new options becoming available. UK NEQAS will continue to capture data to track trends in methods used for GM antigen testing.
- There are limitations of the GM ELISA. A meta-analysis determined that with a positive cut off of 0.5, the sensitivity of the GM ELISA was 78% and the specificity was 85%. They concluded that in a population of 100 patients with disease prevalent of 11%, two patients with IA would be missed and 13 patients may be treated unnecessarily[6].
- Detection of the cell wall polysaccharide antigen is widely used as a diagnostic marker, and participation in this EQA scheme is an invaluable tool which will help monitor performance in laboratories providing a service in the detection of galactomannan antigen in clinical specimens.
- This review of participant results has shown that the accuracy of the test is greater for specimens with a GM index value >0.9, whilst results with an index value below this should be treated with greater caution and repeat testing of another aliquot of the same specimen is highly recommended (as per manufacturer's instructions).

References

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