

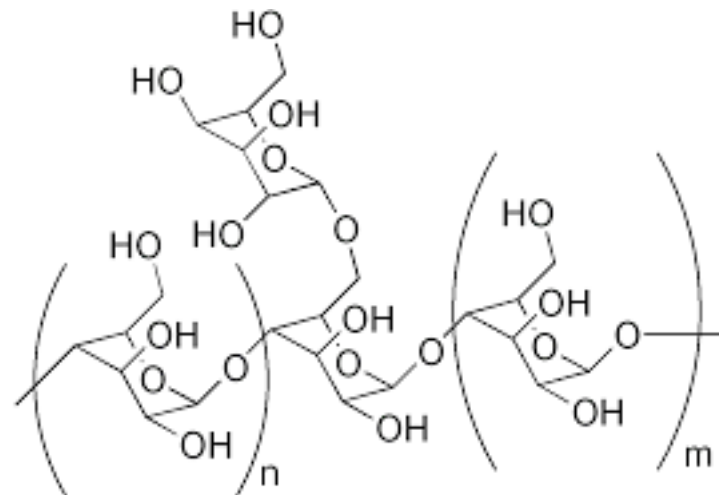
**Invasive Aspergillosis: EQA of
Galactomannan as an Indicator of
Disease**

UK NEQAS Scientific Meeting 2015

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Galactomannan (GM)

- Cell wall polysaccharide of *Aspergillus* species
- Released into the bloodstream of patients symptomatic of invasive aspergillosis (IA)
- Circulating antigen is detected using the Platelia™ *Aspergillus* Ag kit (Bio-Rad)



Development of EQA for fungal biomarkers

- Galactomannan antigen
- β -D glucan antigen
- Cryptococccal antigen
- Aspergillus DNA

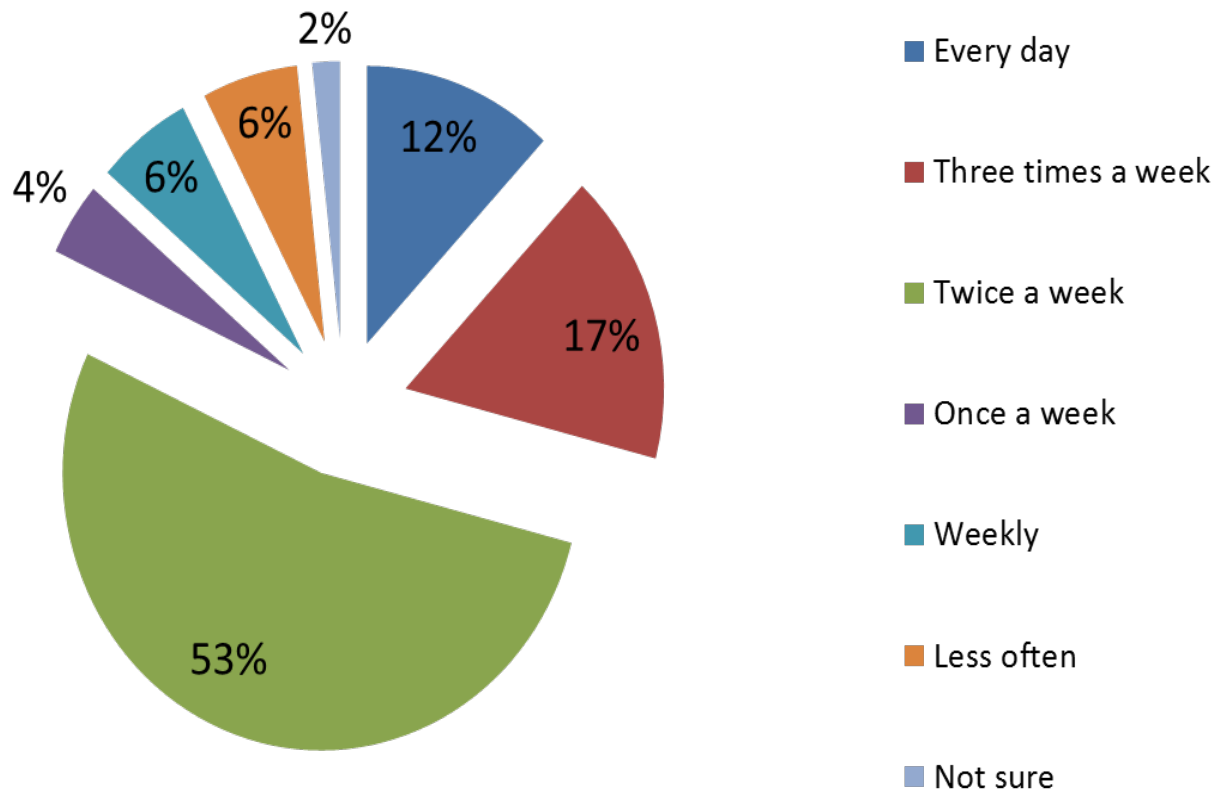
EQA in the detection of the galactomannan antigen in serum samples

- EORTC/MSG guidelines (2008) recommend galactomannan detection is a contributing indicator in the diagnosis of IA
- Many diagnostic laboratories perform the test of GM detection
- There is presently no EQA schemes available for laboratories to participate in the UK

- Conducted in February 2015
- Questionnaire sent to participants of the UK NEQAS mycology (MY) and antifungal susceptibility (MS) schemes
- BSMM, ECMM, UKCMN, EAPCRI, EORTC
- Sent to 400 members
- 168 (42%) responses

How frequently do you run the GM assay in the laboratory?

Frequency of GM detection



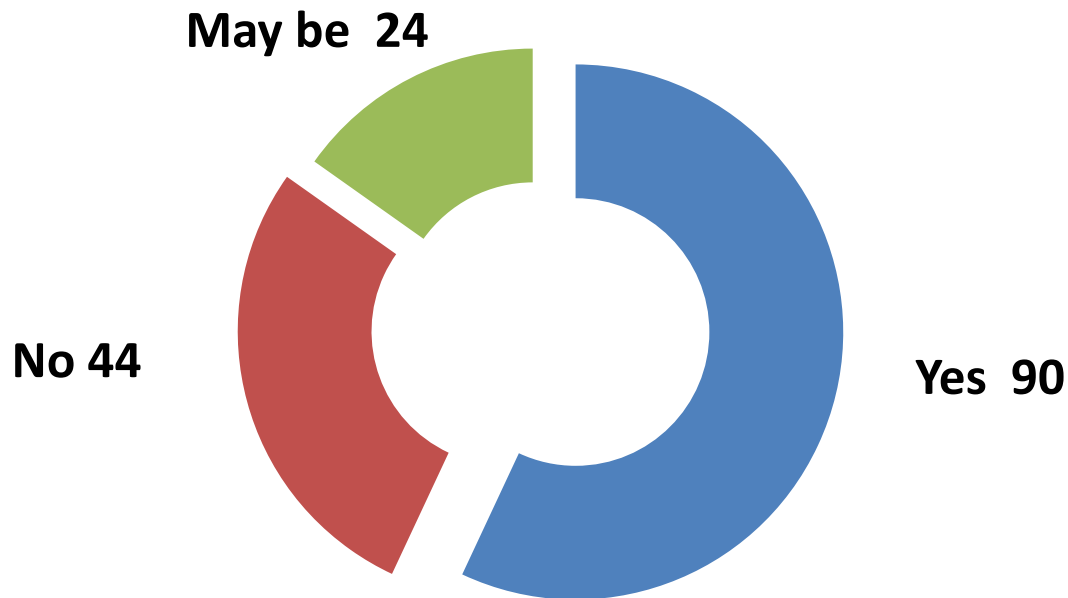
What type of clinical samples do you test for Galactomannan?

Specimen type	Number of laboratories
Serum	124
Broncho-alveolar lavage (BAL) fluid	109
Plasma	24
Cerebral Spinal Fluid (CSF)	14
Pleural fluid	2
Sputum	2
Biopsy (special cases)	1
All respiratory specimens	1
Not sure	2

What temperature do you store samples at during testing?

Temperature °C	Number of laboratories
4 - 8	66
4 to 8 1 day/ - 20 thereafter	1
- 20	23
- 80	8
Ambient	14
Not sure	14

Would you be interested in participating in an EQA scheme for the detection of galactomannan?



Number of Laboratories

Objectives of study

- To investigate various concentrations and stability of galactomannan antigen in spiked serum for the possibility of distribution in an EQA scheme.
- To distribute pilot simulated samples for the detection of GM antigen in serum specimens
- Collate and analyse results received from participating laboratories

Method: Filtration

- **Serum:** Serum samples were spiked with an inoculum of a filtered suspension of *Aspergillus fumigatus* species complex
- The bulk serum was inoculated with an NCPF strain of *A. fumigatus* species complex and incubated overnight at 37°C aerobically in an orbital shaker (to prevent conidial growth).
- The 'fungal balls' were removed via filtration through a 0.4µm and a 0.2µm filter.

Method: Sample preparation

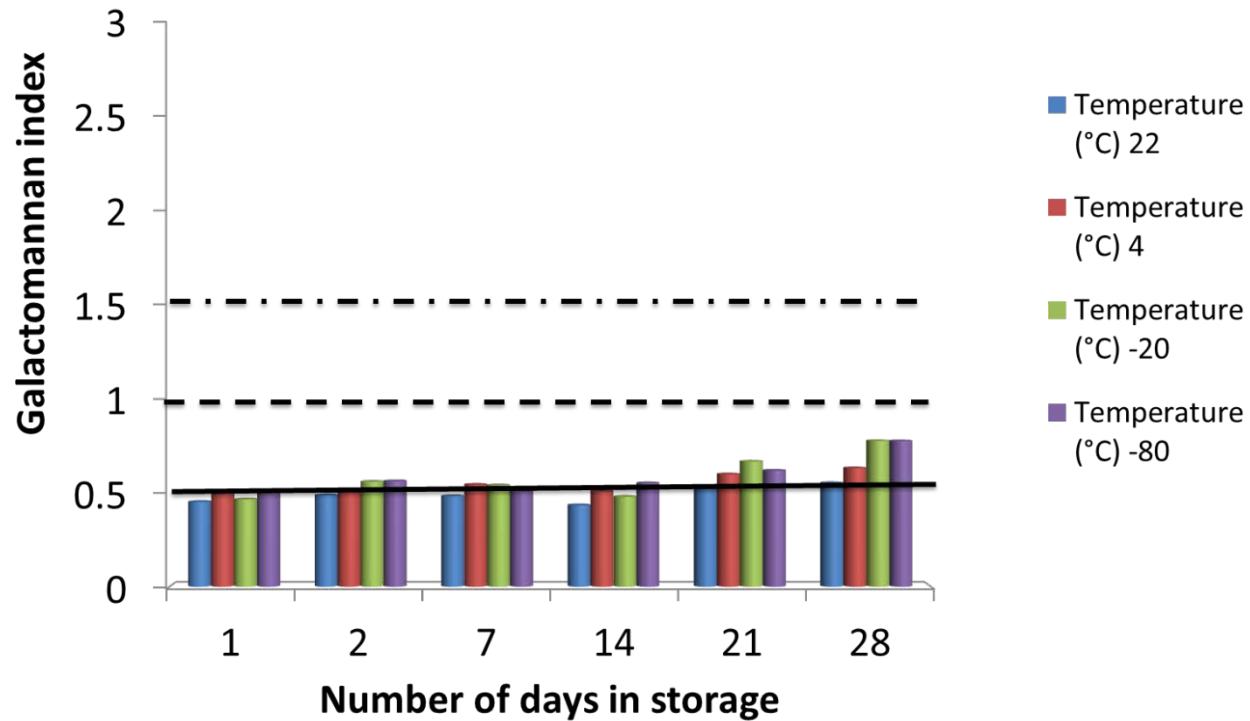
- Serial dilutions were prepared (from 1: 10⁵ to 1: 5 x 10⁶) and tested using the Platelia™ ELISA kit (Biorad), to determine the concentrations of GM in each dilution
- Dilutions were prepared to obtain final galactomannan indexes (GMI) of >2 (high positive), >1 (positive) , ≤ 0.5 (equivocal) and ≤ 0.2 (negative)
- Serum samples associated with each set of concentrations were dispensed in 1mL volumes into sarstedt tubes and stored at 22°C, 4°C, - 20°C and - 80°C

Method: Sample testing

- Each set of concentrations stored at the specified storage conditions were tested for GM at 1, 2, 7, 14, 21 and 28 days
- Each sample was tested in duplicate for the GMI and mean determined to assess the values of GMI over time

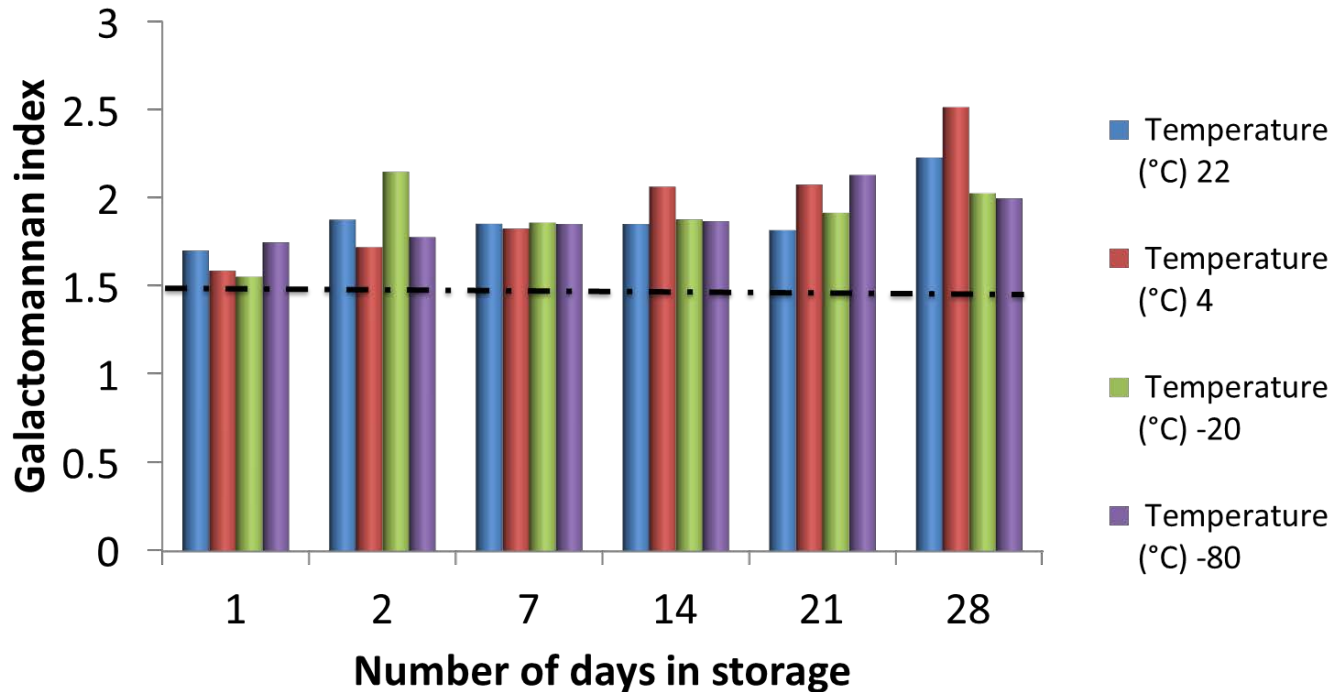
Results: 2.5ng/mL

Stability of GM (~2.5ng/mL) over 28 days



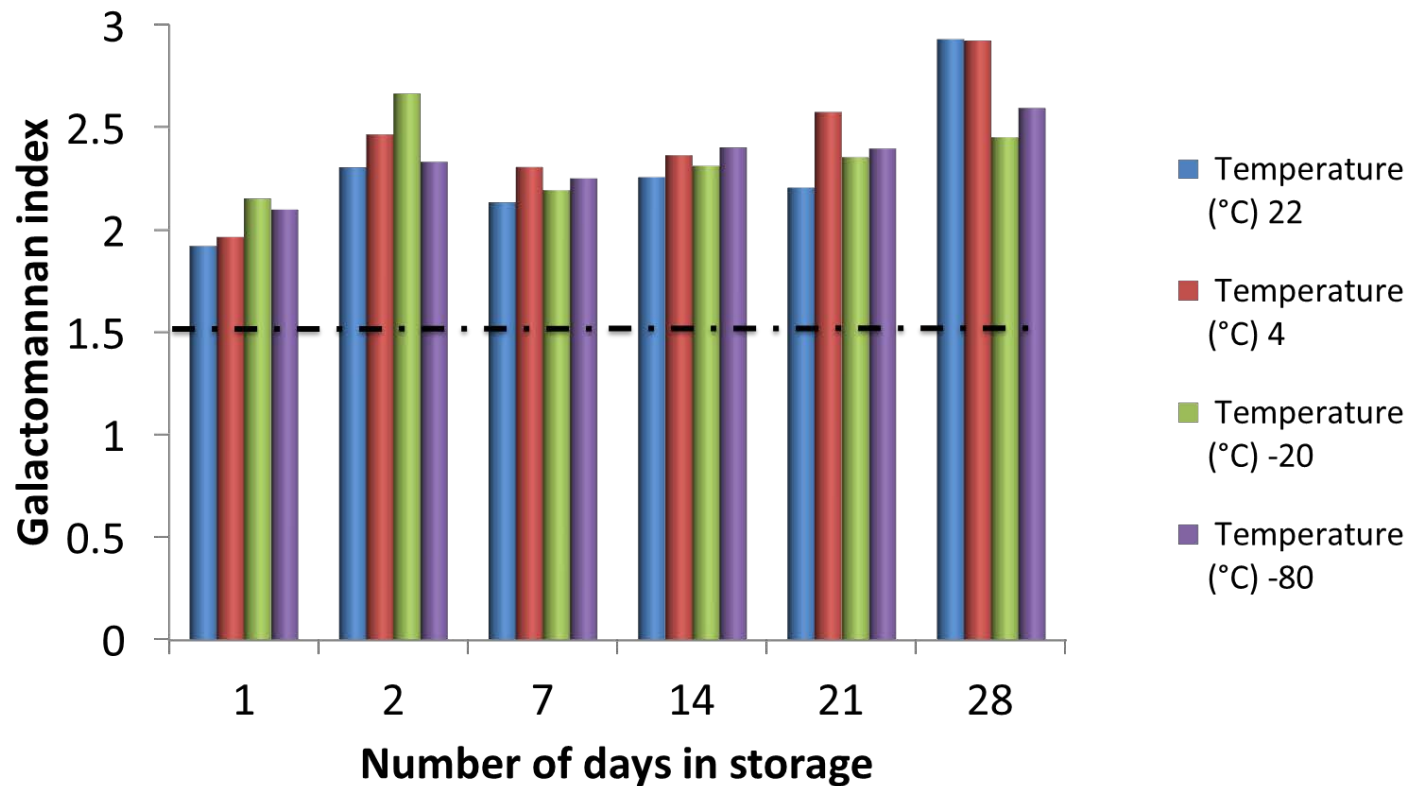
Results: 5ng/mL

Stability of GM (~5ng/mL) over 28 days

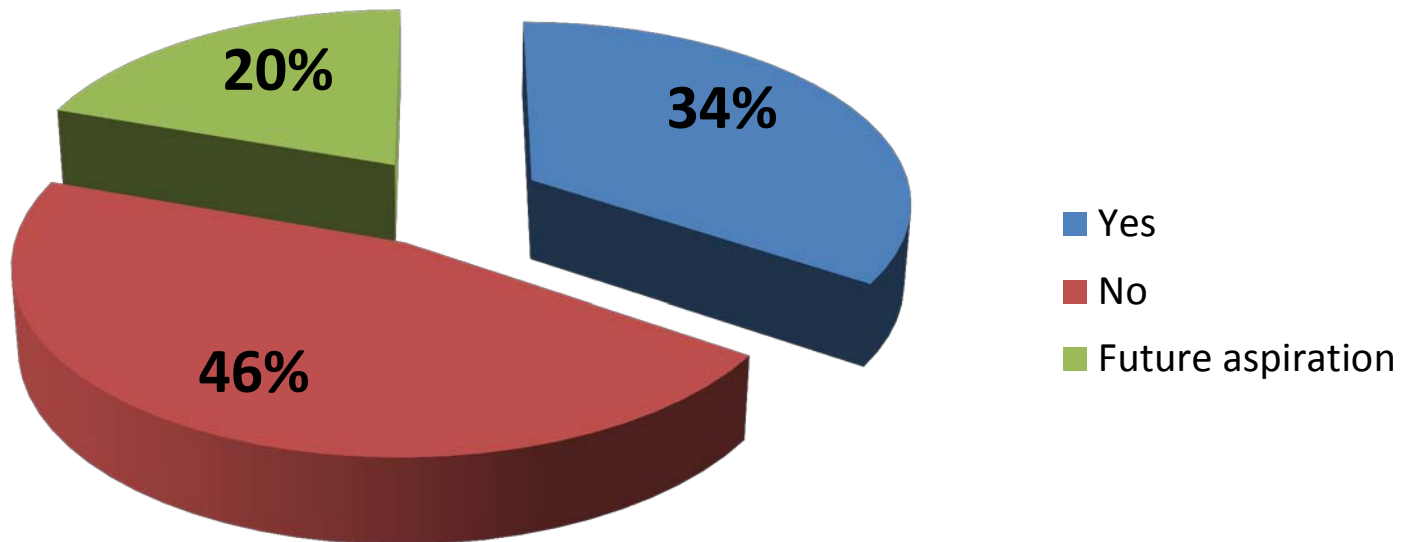


Results: 50ng/mL

Stability of GM (~50ng/mL) over 28 days



Do you perform molecular methods of detection for IA?



Acknowledgements

Shila Seaton

Anthony Christopher

Maila Mutso

Christine Walton

UK NEQAS for Microbiology Team

THANK YOU FOR LISTENING!