

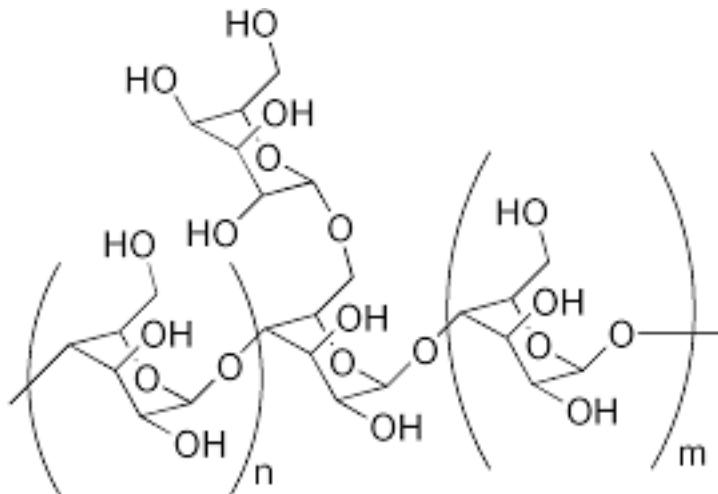
# **Invasive Aspergillosis: EQA of Galactomannan as an Indicator of Disease**

## **UK NEQAS Scientific Meeting 2015**

**Firat Kartal**

## 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

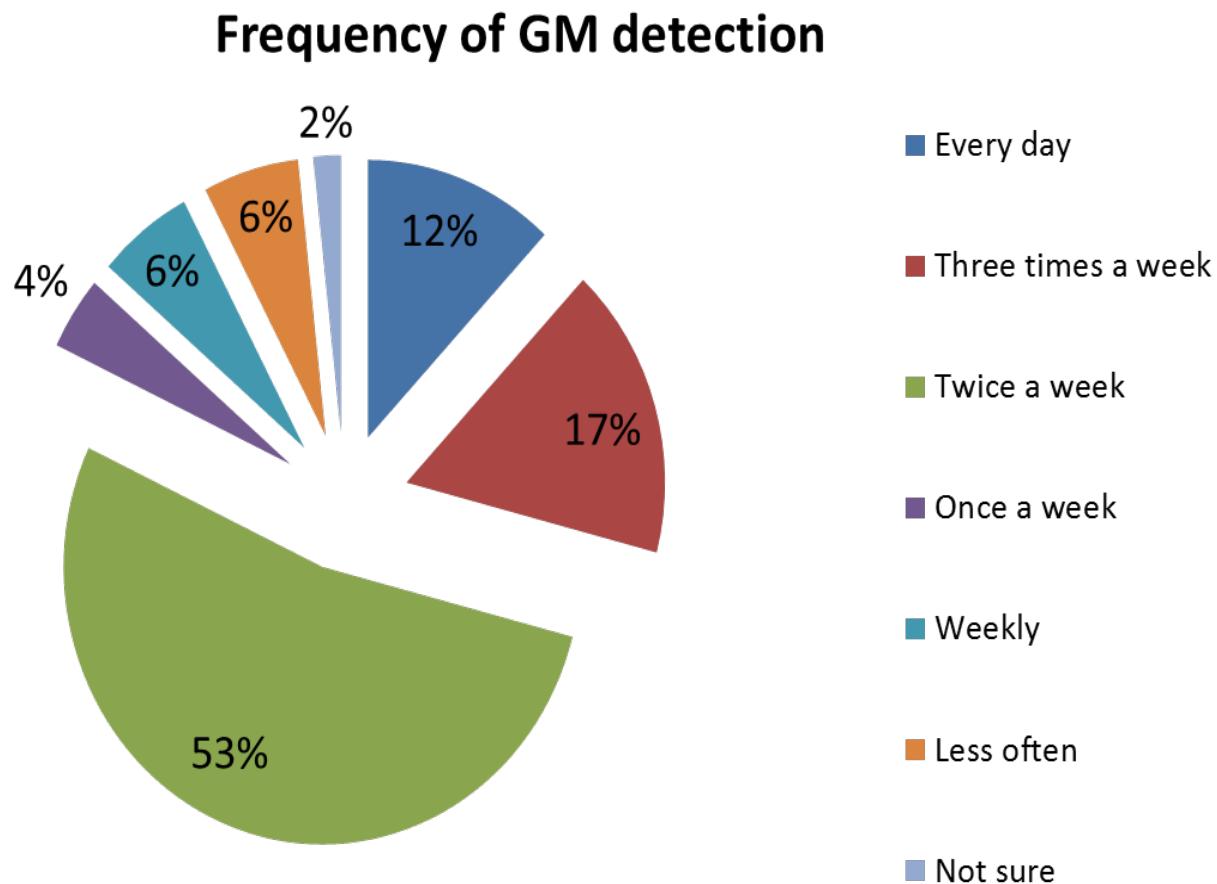
- Galactomannan antigen
- $\beta$ -D glucan antigen
- Cryptococccal antigen
- Aspergillus DNA

## **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?



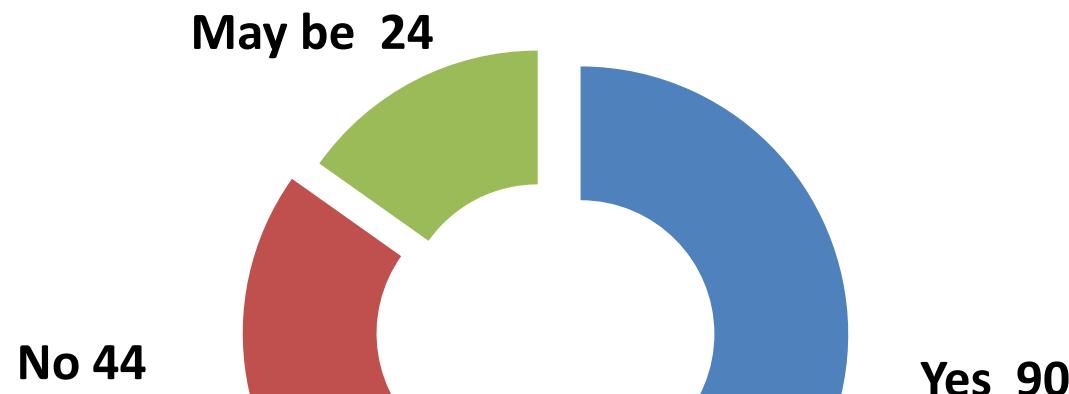
## What type of clinical samples do you test for Galactomannan?

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.

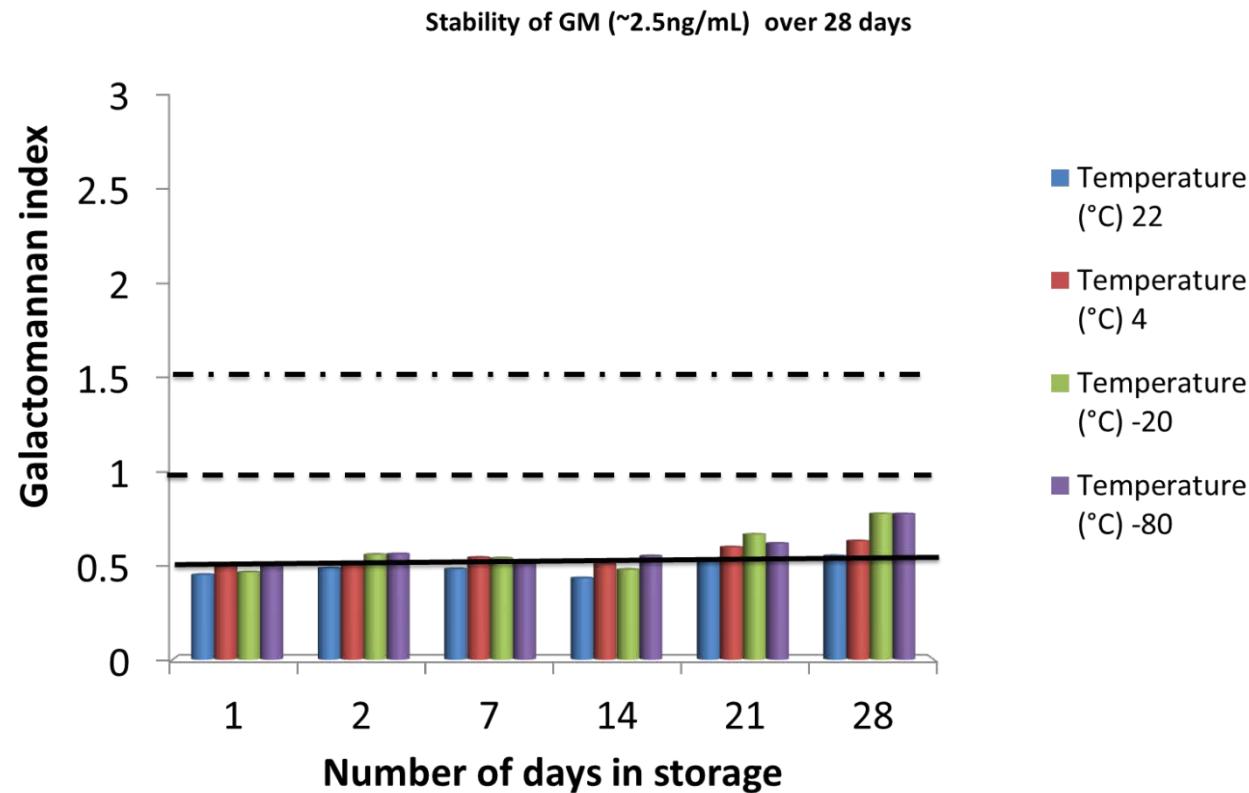
## Method: Sample preparation

- Serial dilutions were prepared (from 1: 10<sup>5</sup> to 1: 5 x 10<sup>6</sup>) 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

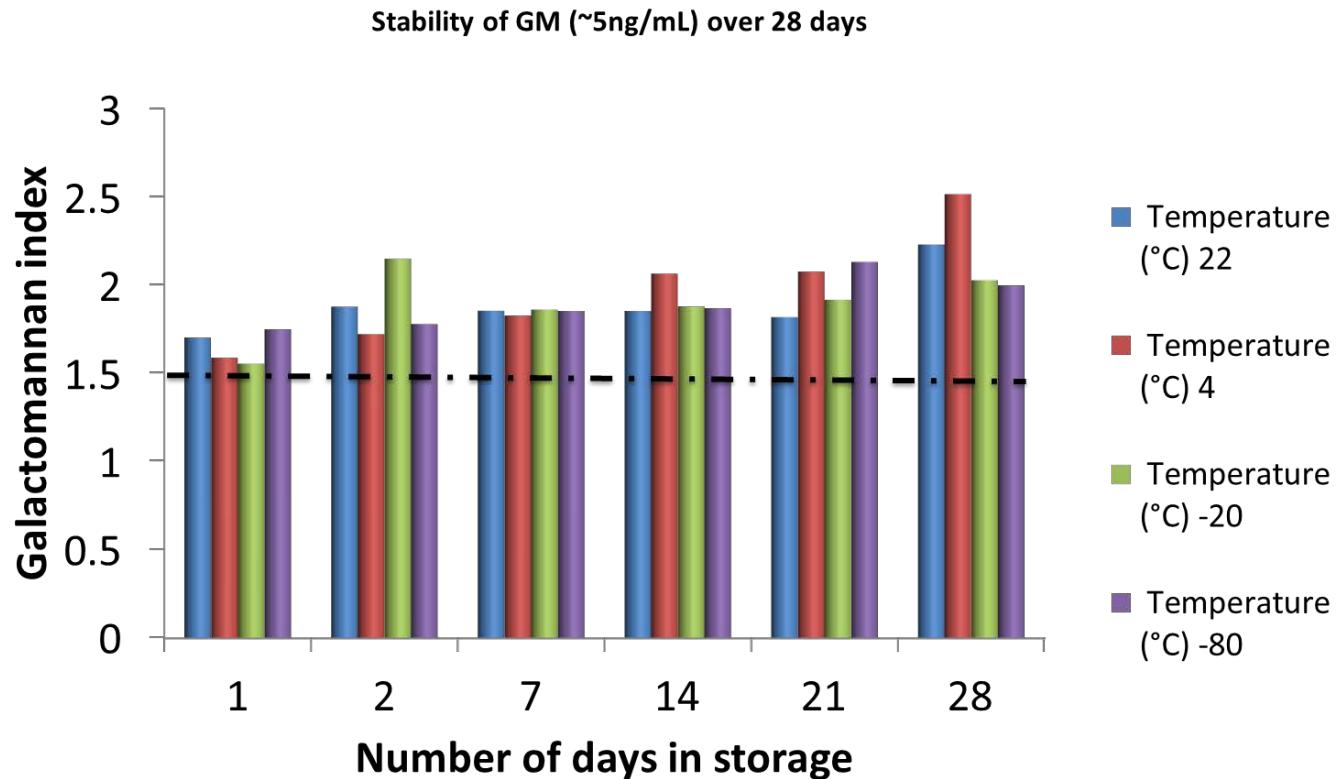
## **Method: Sample testing**

- 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

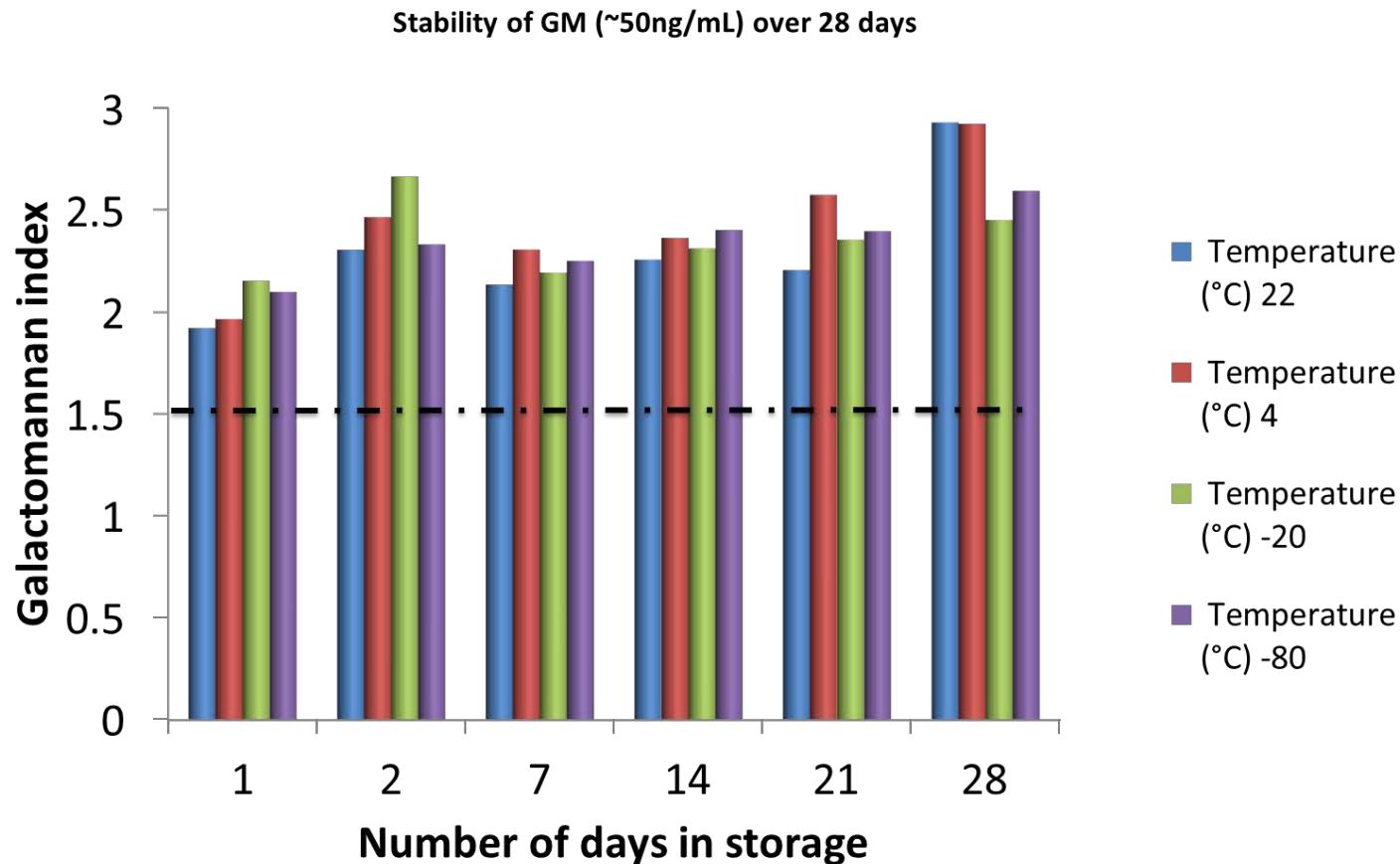
# Results: 2.5ng/mL



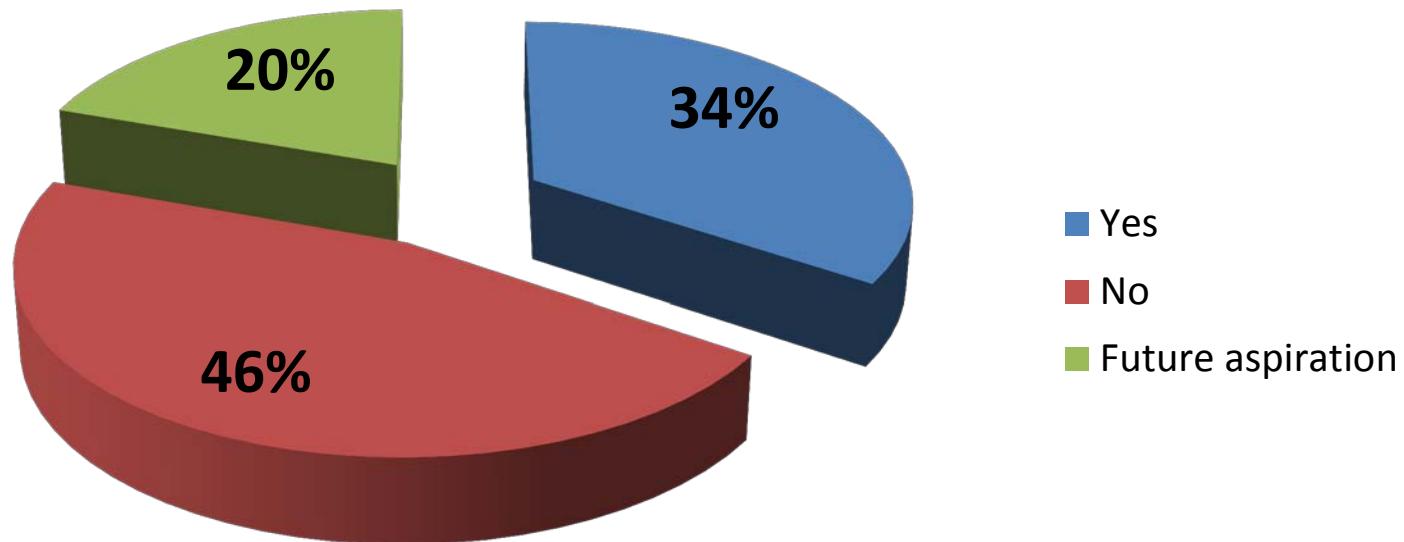
# Results: 5ng/mL



# Results: 50ng/mL



## Do you perform molecular methods of detection for IA?



## **Acknowledgements**

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**THANK YOU FOR LISTENING!**