An Optimised Mucin ELLA Assay for MucilAir™

A Dickie[1,2,3], K Dowers[1], C Roper[1], J Welch[1]
[1] Charles River Preclinical Services, Tranent, Edinburgh EH33 2NE, UK
[2] Scottish Universities Life Sciences Alliance (SULSA), Mayfield Road, Edinburgh EH9 3JR, UK
[3] University of Edinburgh, School of Biological Sciences, Mayfield Road, Edinburgh EH9 3JR, UK

Background

MucilAir™ is a mucus-secreting 3D cell culture airway model. MucilAir™ has numerous potential research applications including safety testing of occupational and environmental chemicals and pharmaceutical development and efficacy testing. In the healthy airway, mucus provides protection from bacteria, viruses and xenobiotics and acts as a lubricant. It is postulated that mucin secretion may be used as an indicator of airway damage. The aim of this project was to develop an assay suitable for measuring mucin lavaged from the surface of MucilAir™. A sandwich enzyme linked lectin assay (ELLA) was used to measure mucin. ELLAs rely on the interaction of lectins with mucin glycoproteins. A sensitive, reproducible assay with a dynamic range appropriate for quantifying mucin over a concentration range of *ca* 0.6-10 μg/mL was required.

Methods

The basic principle of the ELLA assay format is summarised graphically in Figure 1. The six steps involved in the ELLA assay are summarised in Figure 2. The plate is washed after incubations with coating, analyte and detection solutions.

Calibration curves of bovine reference mucin (0-10 μg/mL) were analysed using different sets of conditions and the sensitivity and dynamic range were evaluated in each case. Figure 3 summarises the assay development process.

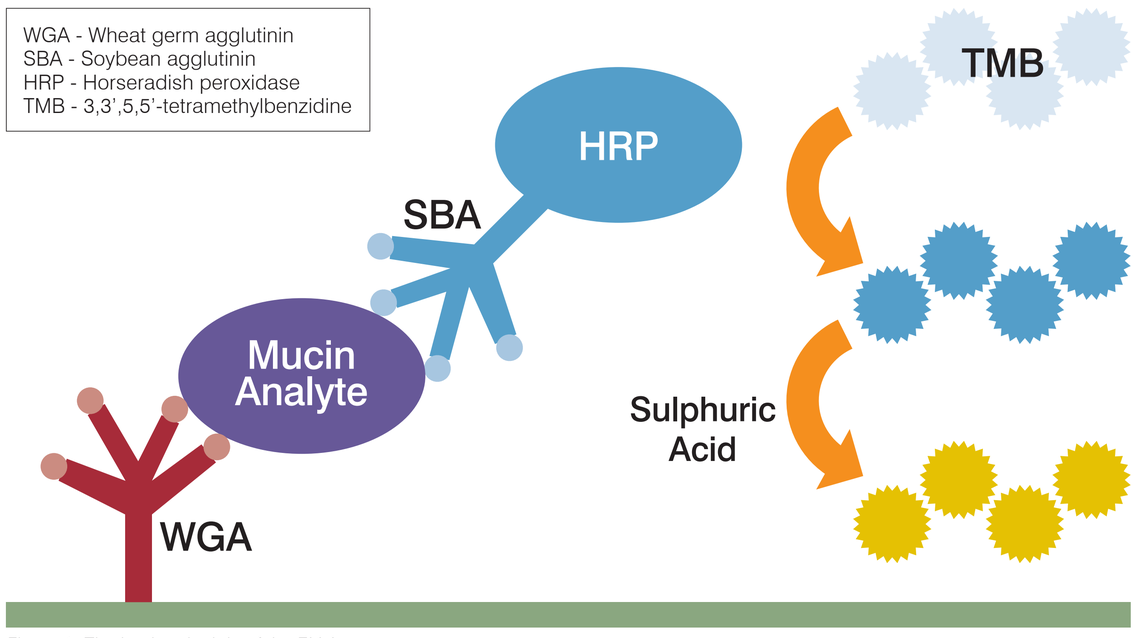


Figure 1. The basic principle of the ELLA

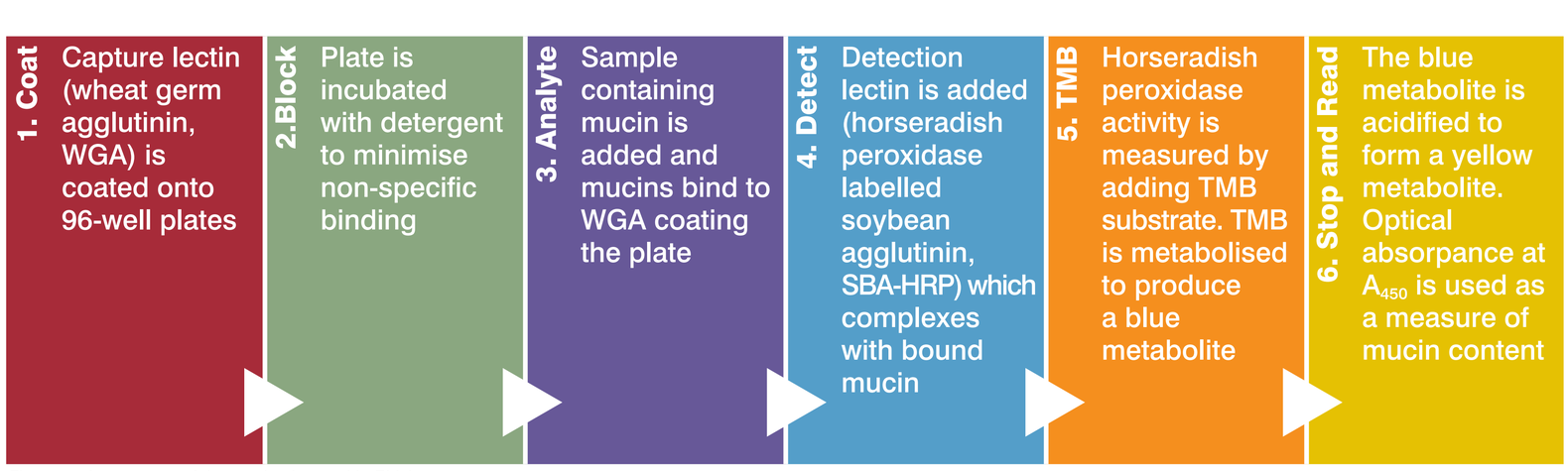


Figure 2. A summary of the methods used in ELLA assays

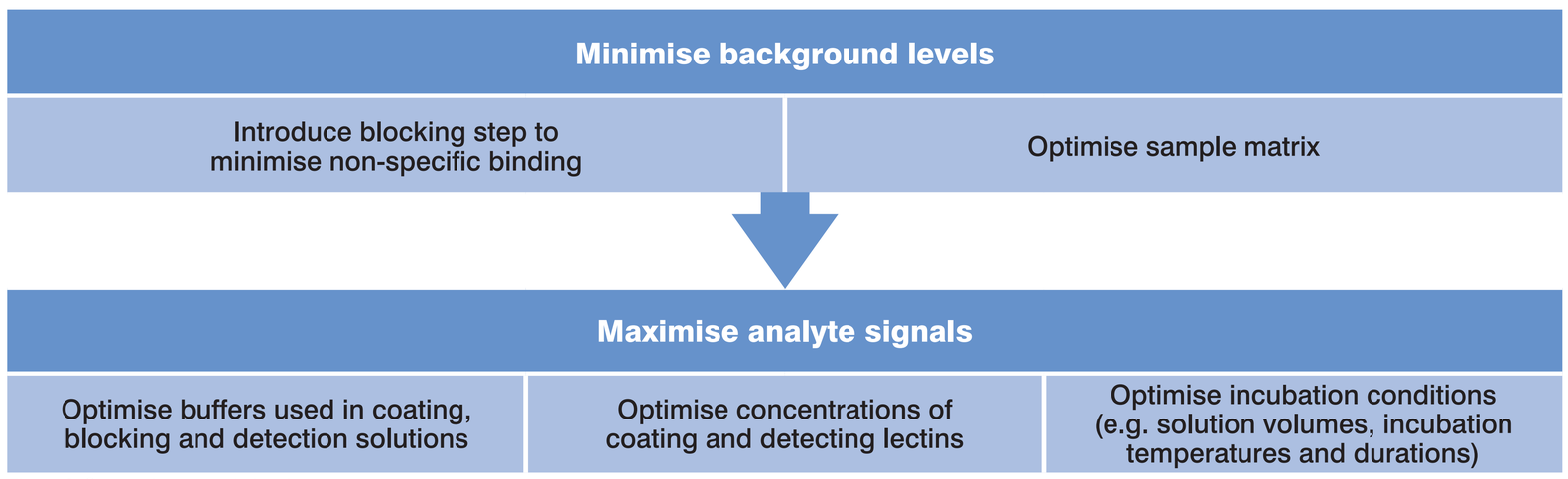


Figure 3. Steps taken to optimise the assay

Figure 4 details the instructions for the optimised method. This method was repeated to assess reproducibility (n = 3). To allow comparison across replicates, optical absorbance values (450 nm; A_{450}) were converted to a percentage of negative control absorbance. Signal to background ratio was calculated as A_{450} (analyte) divided by A_{450} (negative control).

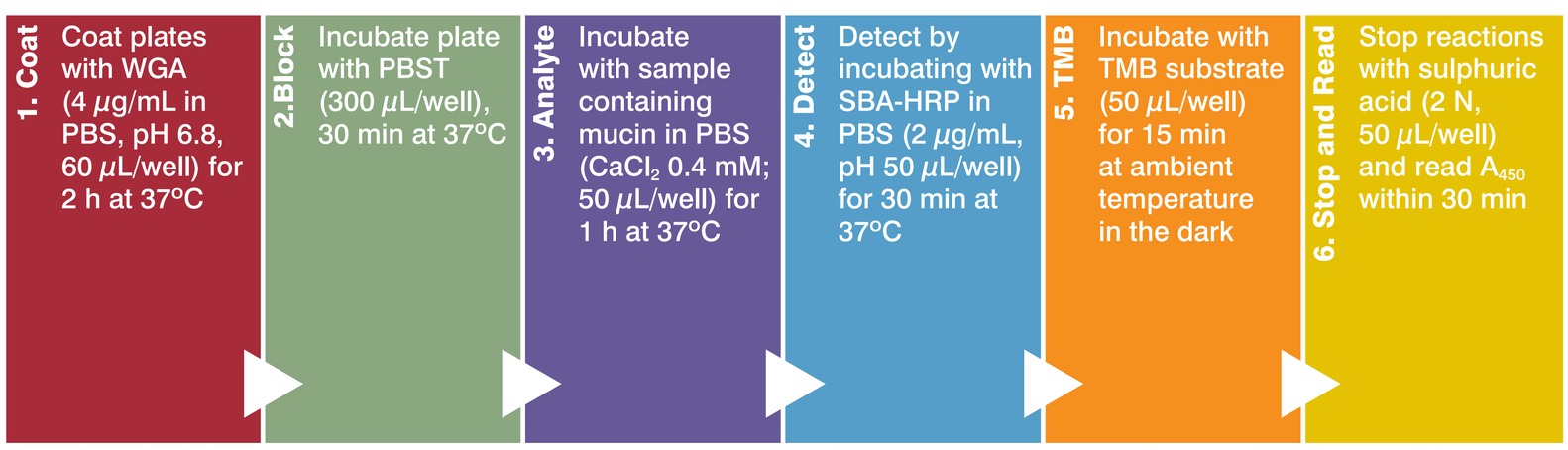


Figure 4. Instructions for the optimised method. The plate is washed with weak detergent after each coating, sample and detection solution incubation using PBST (Tween 20 in PBS; 0.05%, v/v) at 300 µL/well, repeated three times. Prepare TMB substrate according to manufacturer instructions

Results

The optimised ELLA method displayed suitable sensitivity and dynamic range to calculate mucin at concentrations of 0.6-10 μg/mL. Figure 5 shows a representative calibration curve (n = 1) while Figure 6 shows mean results from three occasions of analysis.

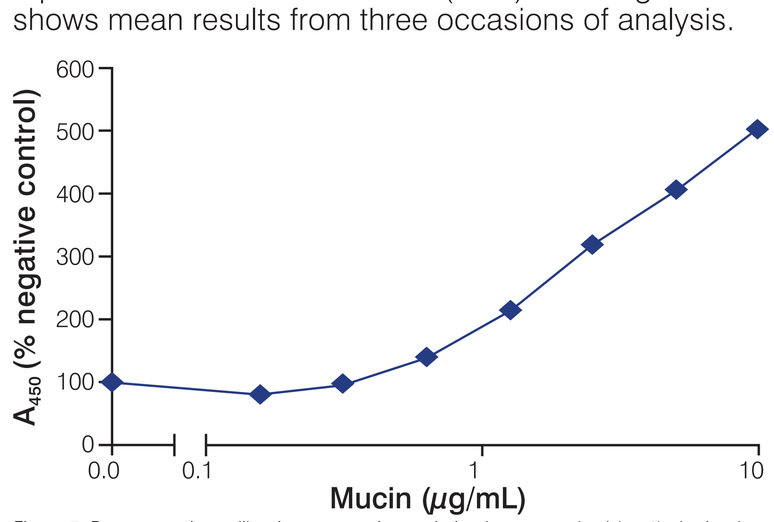


Figure 5. Representative calibration curve using optimised assay method (n=1) obtained from 3 measurements at each mucin concentration. Signal to background ratio = 5.1:1

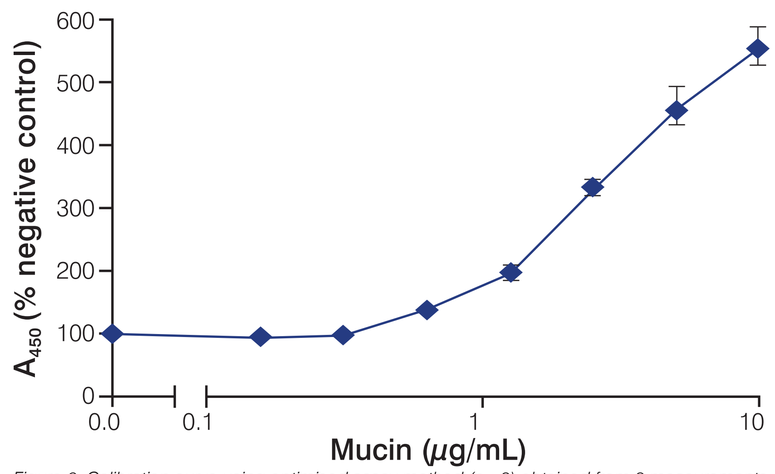


Figure 6. Calibration curve using optimised assay method (n=3) obtained from 3 measurements at each mucin concentration. Signal to background ratio = 5.6:1

The greatest contributions to assay improvement were:

1. Optimising sample matrix (Figure 7). PBS was used instead of HEPES buffered saline (previously used by Epithelix, the manufacturer of MucilAir™).

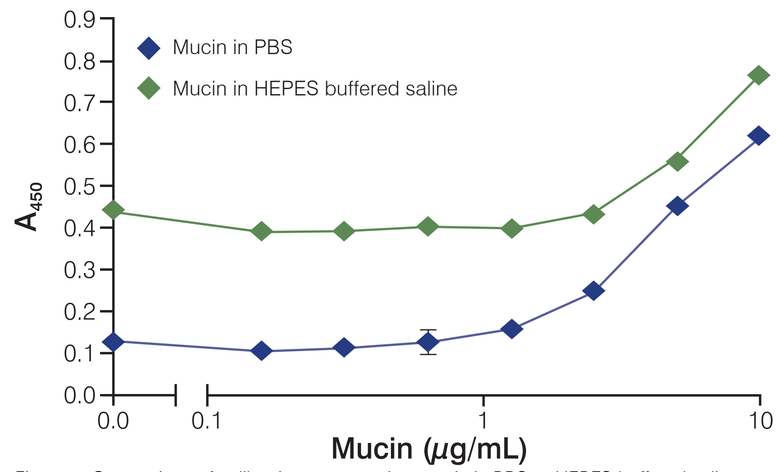
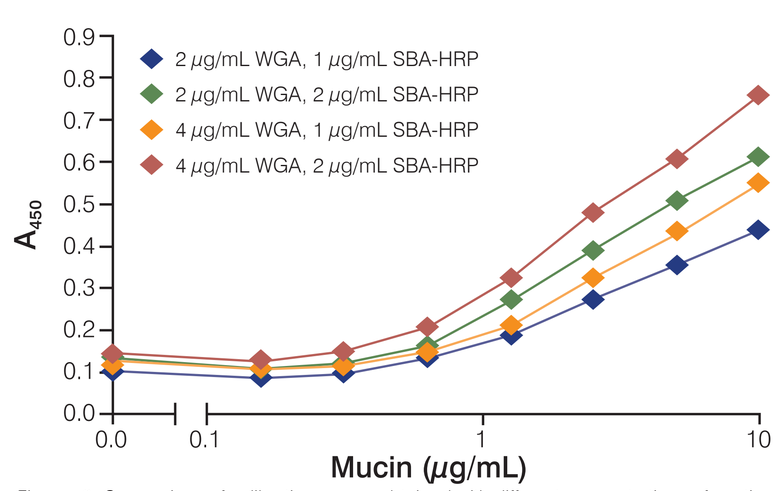


Figure 7. Comparison of calibration curves using mucin in PBS or HEPES buffered saline (0.9% NaCl, 10 mM HEPES, 1.25 mM $CaCl_2$), duplicate measurements at each mucin concentration

2. Optimising concentrations of coating and detection lectins (Figure 8). The optimal combination was 4 μg/mL coating WGA with 2 μg/mL detection SBA-HRP.



Figures 8. Comparison of calibration curves obtained with different concentrations of coating WGA and detection SBA-HRP (individual measurements at each mucin concentration)

Conclusion

In conclusion, this ELLA method was suitably sensitive and reproducible to detect mucin at concentrations anticipated to be extracted from MucilAir™.