A-365 Carbohydrate-binding module proteins to functionalize paper for lateral flow applications

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Introduction

- Lateral flow (LF) tests have employed nitrocellulose membrane since their introduction. Refinement of nitrocellulose and LF production techniques have led to substantial improvements in reproducibility, sensitivity, clarity, and speed of results.
- Recent surge in demand for LF tests during the COVID-19 pandemic has drawn increased attention to the consequences of using non-biodegradable or non recyclable materials in test production, requiring disposal via landfill.
- Nitrocellulose substrate is a critical component for substitution with an equivalent or superior material to address environmental concerns.
- Additionally, nitrocellulose and the specific molecular recognition reagents, typically antibodies, contribute significantly to the test cost, and whilst LF tests are less expensive than most other formats, challenges remain with respect to providing affordable tests with adequate performance globally in low resource environments.
- Despite attempts to substitute cellulose for nitrocellulose, principally for reducing cost and more latterly, environmental impact, cellulose is not widely used due to inferior performance, especially in relation to the clarity and quality of the signal line and reagent requirements.

Methods

- Expression and purification of CBM-streptavidin fusion proteins from E.coli to a purity of >90%.
- Adaptation of in house SARS-CoV-2 antigen assay for use with CBM-streptavidin fusion protein on cellulose material: i) Conjugation of 40nm gold (BBI) with anti-nucleocapsid antibodies (Hytest), ii) Biotinylated anti-nucleocapsid capture antibody was dried down on a separate pad, iii) CBM-streptavidin fusion protein was plotted on Whatman 43 paper at the test line.
- Assay performance was evaluated using SARS-CoV-2 nucleocapsid protein and UV-inactivated SARS-CoV-2 virus (NIBSC) in artificial mucus. Accelerated stability of the CBM-paper system was evaluated at 45°C in a wet and dry assay format.
- For quantitative measurements an in house purpose built lateral flow reader was utilized. It uses reflectance reading, from a controlled diffused light source on the lateral flow strip and detected by a 0.5 MP camera, converting line density into a quantitative unit.

Objective

We demonstrate that cellulose-binding module (CBM)-streptavidin fusion proteins for the immobilisation of capture antibodies on cellulose address the performance limitations, yielding sensitivity, clarity and speed of result matching current clinical and industry requirements for a qualitative SARS-CoV-2 antigen Lateral Flow test.

Results

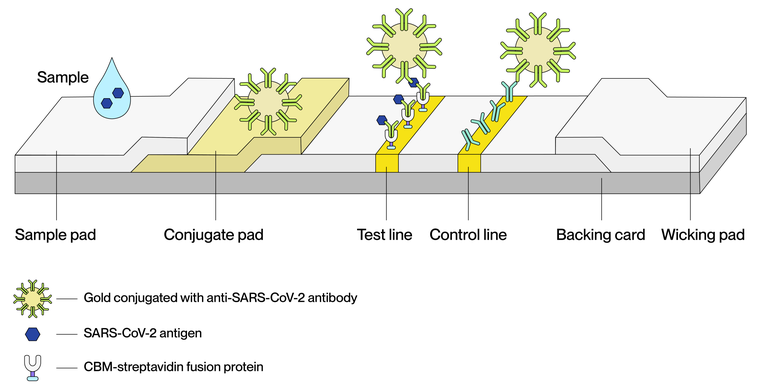
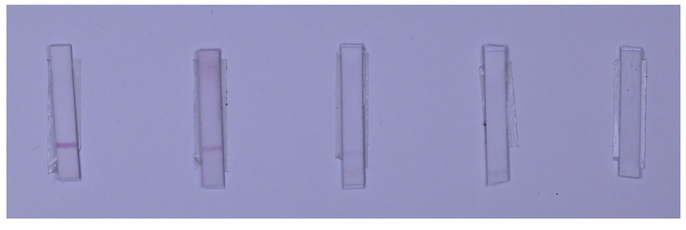


Figure 1. Schematic of CBM-streptavidin—based Lateral Flow test on cellulose material.

Limit of Detection assessment



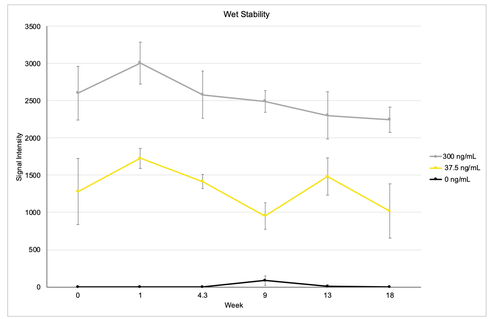
Quantitative measurement

10^5		10^4		10^3		10^2		0	
Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
429.84	60.70	262.78	128.99	105.41	52.28	28.83	19.85	17.53	8.67

Figure 2. Analytical sensitivity evaluation of paper-based assay using inactivated SARS-CoV-2 virus in artificial mucus. Representative visual assessment (top), quantitative measurements (below). Each concentration was measured in triplicates.

Stability assessment

No significant changes in performance were observed after 18 weeks of incubation at 45°C for the wet assay (Wilcoxon signed-rank test). A similar trend is visible for the dry assay format after 4 weeks. These results represent approximate accelerated shelf-life of 16 and 4 months, respectively.



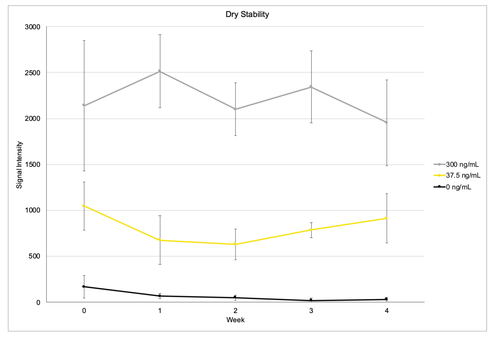


Figure 3. Wet stability (top) and dry stability (bottom) assessments using three different concentrations of SARS-CoV-2 nucleocapsid antigen. X-axis: incubation time in weeks. Y-axis: mean signal intensity of 5 replicates for each time point, with error bars depicting the standard deviation.

Conclusions

- Our results indicate that paper-based tests match analytical sensitivity (10^3 $TCID_{50}$/mL) and speed of current qualitative antigen tests on nitrocellulose (20 minutes), with adequate shelf-life.
- This preliminary investigation demonstrates the viability of using paper as a replacement for nitrocelluloses when coupled with CBM-fusion proteins for antibody immobilisation, offering significantly reduced material cost and environmental impact.

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