Lateral flow test for rapid on-site diagnosis of Foot-and-Mouth Disease SAT 2 virus

K. Quizon, M. Yang

BACKGROUND

Losses due to Foot-and-Mouth Disease (FMD) cost up to $28 billion every year[1]. Outbreak control measures help limit the impact, but only when the virus is known. Further, vaccines are serotype-specific and cannot protect against all 7 FMD virus (FMDV) serotypes[2], much less other vesicular disease viruses like vesicular stomatitis (VSV) or swine vesicular disease (SVDV), which cause the same clinical signs. Therefore, more rapid diagnosis of FMDV and its serotype can help save billions of dollars by informing on appropriate control measures.

Here, a lateral flow test (LFT) using monoclonal antibodies (mAbs) for the SAT 2 FMDV serotype reproducibly provide an accurate diagnosis in as little as 20 minutes and can be used on-site, eliminating the time needed to send samples away to labs for testing.

KEY FINDINGS

- ✓ LFT distinguishes FMDV SAT 2 from other viruses.
- ✓ The LFT needs 32 times less FMDV for detection than a currently used in-lab test, the DAS-ELISA.
- ✓ mAbs tested for the LFT are all highly selective for FMDV SAT 2; 5 of 8 mAbs were specific to SAT 2.

RESULTS AND DISCUSSION

Recognition of FMDV and not other vesicular disease viruses. (Figure 1, A) mAbs were tested for cross-recognition of VSV (serotypes New Jersey, NJ; and Indiana, IND) and SVDV and only recognized FMDV SAT 2.

FMDV SAT 2 serotype-specific. All eight mAbs most strongly recognize FMDV SAT 2. mAbs #5, 13, and 19 crossreact with other FMDV serotypes, but with a much lower affinity. (Figure 1, B)

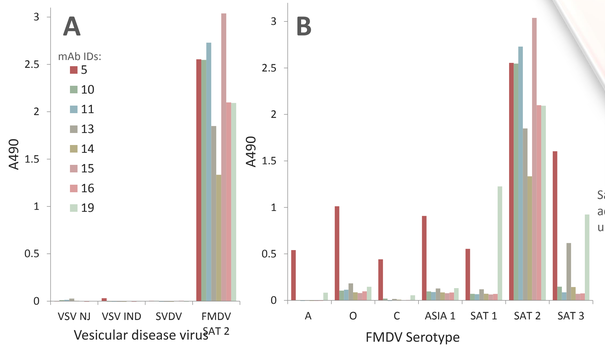

Figure 1. mAb specificity for FMDV SAT 2. (A) mAbs were tested for recognition of VSV NJ and VSV IND, SVDV, and FMDV, and recognized only FMDV SAT 2. (B) mAbs were tested for crossreactivity with other FMDV serotypes; five out of eight were specific to SAT 2 only.

Table 1. Comparison of detection limits between double-antibody sandwich (DAS-) ELISA and LFT by challenge with equal amounts of a two-fold series dilution of FMDV SAT 2. DAS-ELISA positives: A490 ≥0.1.

TCID50/mL (thousands)	379	190	94.8	47.4	23.7	11.9	5.93	2.96	1.48	0.741	0.370	0.185
DAS-ELISA	+	+	+	+	+	-	-	-	-	-	-	-
LFT	+++	+++	+++	+++	+++	+++	+++	++	++	+	-	-

Lower detection limit than DAS-ELISA. When tested with equal amounts of virus, the LFT is able to detect concentrations 32 times lower than the DAS-ELISA, which is currently used to detect FMDV.

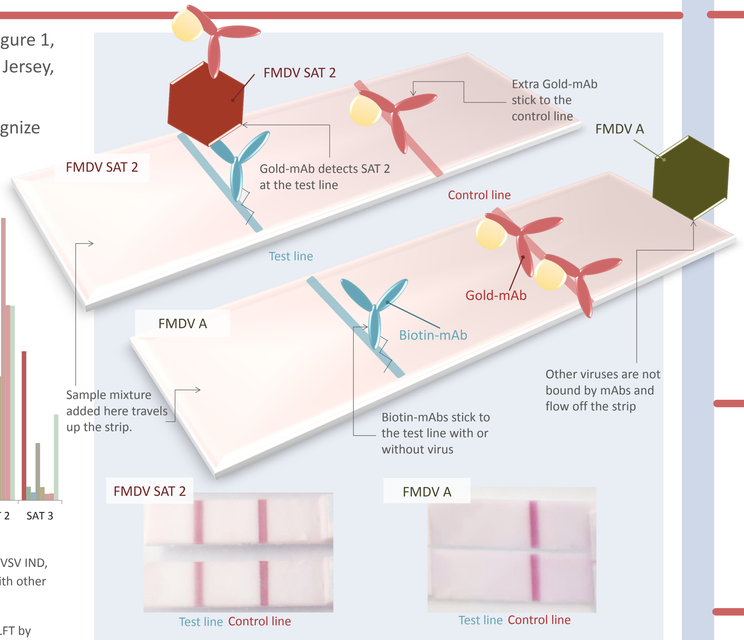

Figure 2. Lateral flow test validation results. (Top) Diagram of mAb interaction with FMDV on the test strip. Gold-mAb is the colour indicator. (Bottom) Lateral flow test strips, with clear distinction between FMDV SAT 2 (two lines) and other viruses (one line; shown: FMDV A).

Identification of FMDV SAT 2 using lateral flow test. (Figure 2) The mAbs were conjugated to gold (Gold-mAb) and biotin (Biotin-mAb). Gold-mAbs serve as the colour indicator at the test line (if SAT 2 is present) and at the control line. Testing shows a positive response (two lines, bottom left) in the presence of FMDV SAT 2 and a negative response (one line, bottom right) with other viruses.

METHODS

Mouse hybridomas were produced and screened for anti-FMDV SAT 2 antibody production using enzyme-linked immunosorbent assays (ELISAs). Positive cultures were alternately subcloned and rescreened until a clonal population was achieved.

FMDV SAT 2 mAbs from pure hybridoma cultures were further tested for cross-recognition of VSV, SVDV, and other serotypes of FMDV. Selective mAbs were conjugated to either gold or biotin and mixed with virus sample before being applied to the test strip.

ACKNOWLEDGEMENTS

The authors recognize VDU for the preparation of viruses and samples used here. This work was supported by the Canadian Food Inspection Agency and the Botswana Institute for Technology Research and Innovation.

Canadian Food Inspection Agency | Agence canadienne d'inspection des aliments

REFERENCES

[1]Knight-Jones, T.J.D, Rushton, J. (2013). The economic impacts of foot and mouth disease – What are they, how big are they and where do they occur? Prev. Vet. Med. 112. 161-173

[2]Yang, M. *et al*., 2015. Development of a multiplex lateral flow strip test for foot-and-mouth disease virus detection using monoclonal antibodies. J. Virol. Methods 221, 119-126.

CONTACT INFORMATION

Kaye Quizon
quizonka@myumanitoba.ca

Dr. Ming Yang
Ming.Yang@inspection.gc.ca