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Slot/Dot Blot protocol.

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Works for me

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XPRIZE Rapid Covid Testing



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ABSTRACT

This is a simple slot/dot blot protocol for detecting antigen on a membrane. Using the method outline here, one can achieve results in under 90 min because it eliminates the need for running a gel, transferring the protein in a transfer apparatus, blocking, secondary antibody incubation and the washes between primary and secondary antibodies.

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Set up Slot/Dot blot apparatus

5m

1. Assemble 3 sheets of cut GB003 blotting paper pre-wetted with transfer buffer (25 mM Tris, 200 mM Glycine, 20%^{5m} methanol) to the dot blot apparatus.
2. Pre-wet a cut sheet of PVDF in 100% methanol for 30 sec and then wash the PDVF in transfer buffer for 2 min.

3. Lay the PVDF membrane on the GB003 blotting paper and seal the slot/Dot blot apparatus.

Add sample 5m

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 1. Apply individual (or pooled) saliva or processed nasal swabs to the slot/dot blot apparatus making note of sample^{5m} location.
 2. Turn on the vacuum source so the sample get drawn onto the membrane.
 3. Disassemble the slot/dot blot apparatus and place the membrane back in 100 % methanol.
 4. Air dry the membrane for 5 minutes.

Incubate with HRP-conjugated antibody, wash and develop 1h

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 1. Once the membrane is dry, add it to 10 ml of TBST (20 mM Tris, 150 mM NaCl, 0.1% Triton X-100, pH 7.5) (or PBST)^{1h} containing HRP-conjugated antibody and incubate with gentle shaking 40 minutes.
 2. Wash the blot 4 times for 5 minutes with 10 ml of TBST
 3. Add ECL or colorimetric substrate, develop for 2-5 min, and image blot.
 4. Record the results.