Parasite Dot-Blot for analysis of VSG expression

This protocol is a derivative of the classical Western blot.

Starting material: 2–3x10^6 cells. Membrane: Hybond-ECL, 60x80 mm (Amersham Cat# RPN68D)

Preparation of membrane

1. Pre-wet membrane for 1 min in distilled water.
2. Discard water and incubate with PBS pH 7.2 for 5 min (this step is critical; if you forget to do it, your result will be a very weak signal or nothing at all).
3. Remove membrane from container and dry it over a paper towel.

Spotting the parasites as a dot onto the membrane

4. Transfer cells to a pre-labeled Eppendorf tube.
5. Spin at RT for 5 min at 6,000 g (setting 7). A tiny white pellet should be visible.
6. Remove medium with a pipet or by vacuum aspiration, leaving behind ~10 µl of medium (be careful, pellet is small and loose).
7. Resuspend pellet in the remaining 10 µl by vortexing the tube.
8. Apply 2 µl of cell suspension onto dry prepared membrane.
9. Let membrane dry for 5 min.

Western blot

10. Block membrane with 3% Milk/PBS/ 0.1% Tween for 45–60 min.
11. Add first antibody, diluted in 3% Milk/PBS. Shake for 1 h at RT (for stronger signal, incubate overnight at 4°C).
12. Wash 3x5 min with PBS/0.1% Tween.
13. Add second antibody, diluted in 3% Milk/PBS. Shake for 1hr at RT.
14. Wash 3x5min with PBS/0.1% Tween.
15. Develop using the ECL system (Pierce). Exposure time should be 1–5 min.

Typical dilutions for rabbit antisera raised against native tryp-derived VSG

VSG (lab name)	VSG (systematic name)	Dilution with our sera
221 (CRD-depleted)	MITat1.2	1:10,000
224	MITat1.3	1:10,000
121	MITat1.6	1:5,000
060	MITat1.1	1:10,000
1.13	MITat1.13	1:120,000
VO2	MITat1.9	1:5,000
1.13 (CRD-depleted)	MITat1.13	1:5,000
V02 (CRD-depleted)	MITat1.9	1:1,000