A Rapid and Effective Tool for Monitoring Monoclonal Antibody Production

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Background

Monoclonal antibodies (Mab) are used in a variety of fields from diagnostics to therapeutics. Manufacturers of *in vitro* diagnostics (IVD) commonly use third-party suppliers to meet their needs for specified monoclonal antibodies. IVD manufacturers must establish QC systems to assure that the product received is suitable for use. As an alternative to laborious time consuming conventional methods such as ELISA or dot immunobinding, we examined the effectiveness and utility of rapid point-of-use immuoanalytical tools created with MedMira's Miriad™ RVF Toolkit to quickly assess the suitability of hybridoma bioreactor supernatants.

Methods

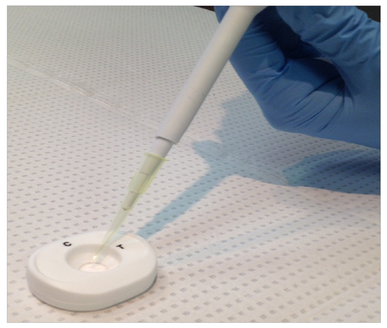
Six supernatants, each containing monoclonal antibodies against unique peptide sequences derived from HIV and HCV proteomes, were obtained from a third-party supplier. Miriad RVF Toolkit, based on MedMira Rapid Vertical Flow (RVF) Technology™, was used according to manufacturer instructions. The Toolkit included pre-assembled test cartridges, protein A/L based gold conjugate caps (InstantGold™ cap), Universal Buffer (UB), and disposable pipettes (Fig. 1a). Although not used here, Miriad RVF Toolkit also includes reagents allowing users to conjugate their own direct labels. Cartridges were prepared by spotting 0.5µL aliquots (1mg/ml, PBS-7.4) of (i) positive control (mouse IgG) and (ii) one or more antigens that were originally used as immunogens during hybridoma development (Fig. 1b). Two sets of cartridges, one each for HIV and HCV antigens, were prepared and allowed to dry at room temperature (RT) for 30 minutes (see Fig. 1c for illustrative example of spotting pattern). Cartridges can be prepared in advance, and stored at RT for up to 12 months in packaging provided with the product. Each test is initiated by priming the appropriately prepared cartridge with 3 drops of UB (Fig. 2a), followed by addition of one drop of sample (Fig 2b). Antigen-specific monoclonal antibody, if present, is captured on the membrane and is subsequently visualized using the InstantGold cap (Figs. 2c-2f). For the results to be considered valid, the positive control spot (C) must be present on all test cartridges.

Fig. 1: Spotting of antigens on the membrane

1a

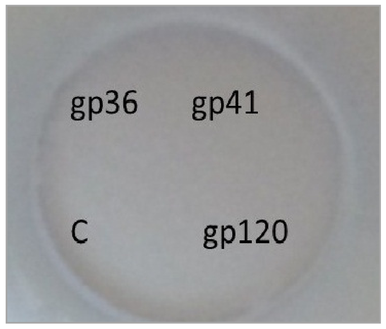
Miriad RVF Toolkit components

1b



Spot the control and antigen, and dry for 30 min.

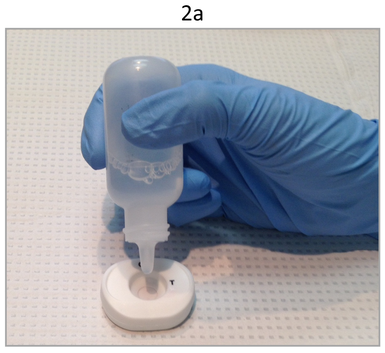
1c



Spotting patterns of HIV antigens and control

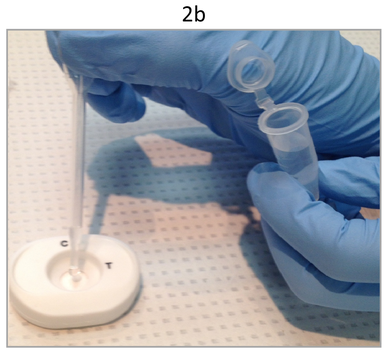
Fig. 2: Procedural steps for performing the test. The completion of steps 2a through 2e requires less than 3 minutes.

2a



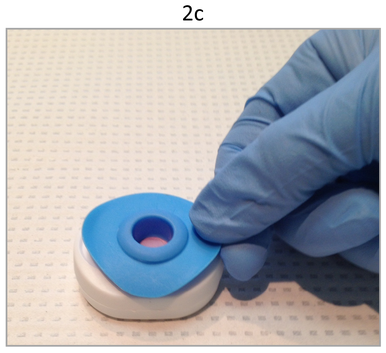
Prime with 3 drops of Universal Buffer

2b



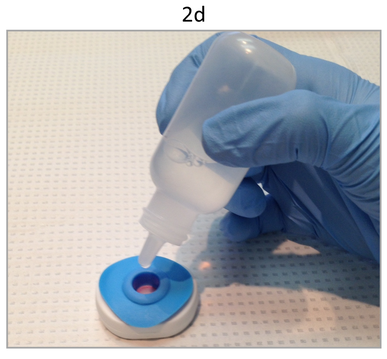
Add 1 drop of sample and allow to absorb

2c



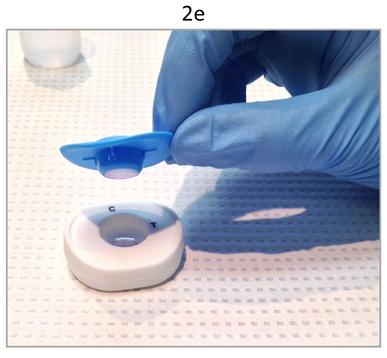
Place the InstantGold cap on the test cartridge

2d



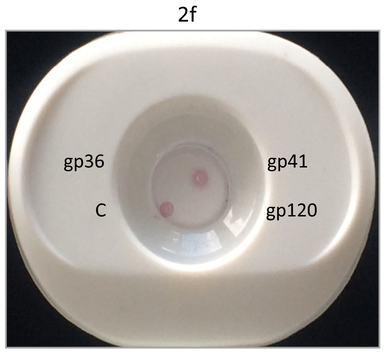
Dispense 12 drops of Universal Buffer and allow to absorb

2e



Remove the cap and read results

2f

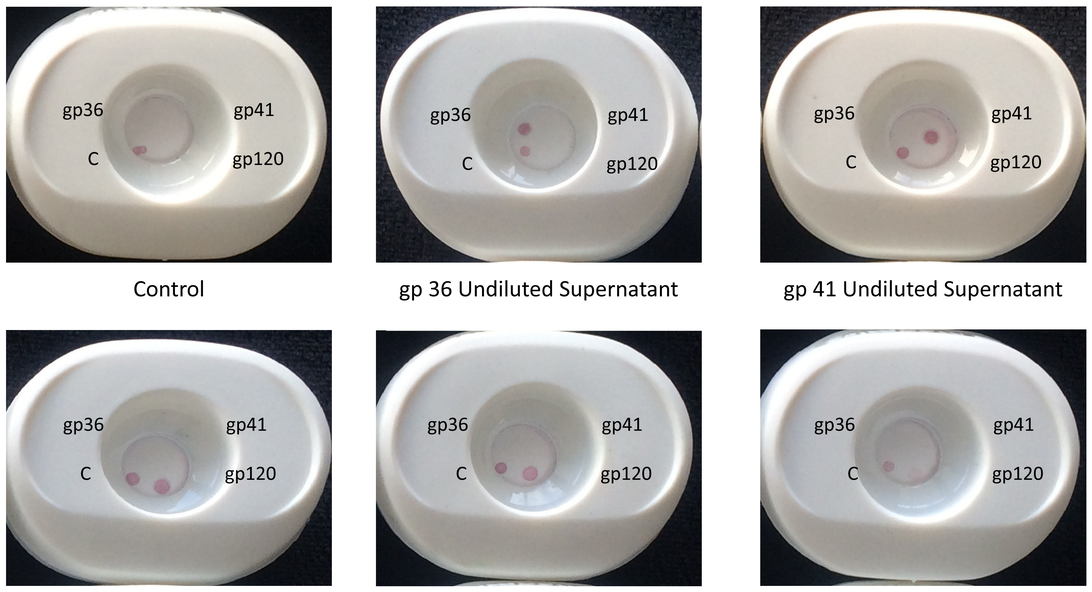


Visual results with gp41 (1:10 Dil.) supernatant

Results

Each cartridge used in this study contained a control spot (mouse IgG) and 3 unique HIV or HCV antigen spots. Hybridoma supernatants received from the supplier were tested at 1:1 (undiluted), 1:10 and 1:100 dilutions in PBS. Representative results are shown in Fig. 3. Valid results were observed for all tests. Each bioreactor supernatant reacted specifically with the immunogen that was used originally to raise the antibody. Visual results were scored on a one to three grading scale, three being the highest (Table 1). Increasing dilutions resulted in decreased reactivity making it feasible to evaluate the potency of supernatants by end point dilutions. For example, two of the supernatants (HCV MDL-1 and MDL-3) became non-reactive at 1:100 dilution, while all others yielded reactive results at 1:100 dilution (Table 1).

Fig. 3: Test results with bioreactor supernatants



Control | gp 36 Undiluted Supernatant | gp 41 Undiluted Supernatant

gp 120 Undiluted Supernatant | gp 120 1:10 Dilution Supernatant | gp 120 1:100 Dilution Supernatant

Table 1: Miriad RVF Toolkit Test Results at Various Dilutions of Supernatant

Capture Peptide	Undiluted	1:10	1:100
HCV MDL-1	3+	1+	—
HCV MDL-3	3+	2+	—
HCV MDL-4	3+	3+	2+
HIV gp36	3+	3+	2+
HIV gp41	3+	3+	2+
HIV gp120	3+	3+	1+

Conclusions

- Testing with the Miriad device was completed in less than one hour following the receipt of antibodies. This included the time needed to prepare the device (Fig. 1b).
- Turnaround time to results could be further decreased to less than 3 minutes by using pre-spotted cartridges. This will allow these devices to be used as an in-processing monitoring tool during antibody production at the point-of-use, i.e., adjacent to a bioreactor.
- Test results can be documented by recording visual scores, or by taking photographs with mobile devices.
- Use of the versatile Miriad RVF Toolkit allowed us to quickly evaluate in-house parameters (antibody titer and specificity) established for the acceptance or rejection of antibodies contained in hybridoma supernatants received from a third-party supplier.

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