

# LATERAL FLOW ASSAY FOR SIMULTANEOUS TYPING OF ABO, RHESUS SUBGROUPS AND KELL

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**Background:** Current simple methods for blood typing, such as slide techniques do not have stable end points, are cumbersome to perform and provide difficulties for documentation. Sophisticated techniques, such as the solid phase techniques, gel techniques and column agglutination techniques do provide more objective interpretation and give stable end points, but are rather slow and need a centrifugation step [1, 2]. All above mentioned techniques have in common that only one parameter at once can be determined.

**Aim:** To develop a blood grouping format on the basis of known lateral flow assays [3], with multi-parameter testing and stable end point, but without centrifugation.

**Material:** A lateral flow device was constructed with a separation membrane equipped in a cassette housing having a central application zone and 2 equidistant detection areas (one at each site of the application zone). In both detection areas, antibody reagents are printed in distinct parallel lines on the separation membrane (anti-A, anti-B, anti-D(VI), anti-D(VI), anti-K at one area; anti-C, anti-Cw, anti-c, anti-E, anti-e at the opposite area). Further, both detection areas contain a control area (ctl.) consisting of a flow control spot (anti-RBC) and an autocontrol spot.

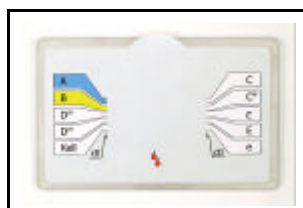


Figure 1a

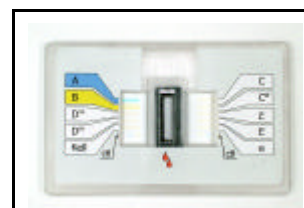


Figure 1b

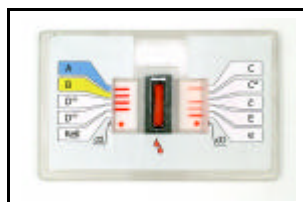


Figure 2

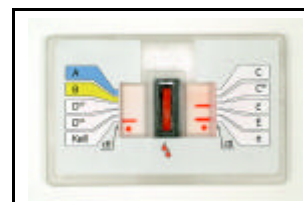


Figure 3

Figure 1: Credit-card sized lateral flow blood grouping device (a) closed device; (b) ready-for-use device after removal of the protective cover

Figure 2: Patient blood AB CcD.Ee

Figure 3: Patient blood O ccddee K

**Method:** 50 µl of anticoagulated whole blood are diluted with 200 µl of a dilution buffer. 100 µl of the resulting suspension are pipetted into the application zone. As soon as the liquid has disappeared from the application zone, 300 µl of a washing buffer are applied into it. In both detection areas, results can be read and recorded after 5 minutes. Positive results are recognized as distinct red bands, negative results are monitored by the absence of the respective band. This result is valid, when the spot for the auto control is negative and the spot for the flow control gives a positive signal (red dot).

**Results:** The bloods of 865 donors, previously typed for the respective blood groups with the Olympus PK-80, have been tested with the new lateral flow test. The results for all antigens were in agreement with those of PK-80. Results were stable 4 weeks after testing. Typical results are illustrated in figures 2 and 3.

**Discussion:** A simple, rapid and flexible method for blood typing with stable and visible end-point for the naked eye is presented. It combines red cell adherence and haemagglutination and provides considerable advantages compared to the state-of-the-art methods:

- 1) Rapid: 10 blood group determinations in 5 minutes
- 2) Simple: 2 pipetting steps for 10 parameters
- 3) No centrifuge
- 4) Suitable for use in non-laboratory situations

## References:

- [1] Sinor LT, Rachel JM, Beck ML, Bayer WL, Coenen WM, Plapp FV: Solid-phase ABO grouping and Rh typing. *Transfusion* 1985; 25, 21-23.
- [2] Lapiere Y, Rigal D, Adam J, Josef, D, Meyer F, Greber S, Drot C. The gel Test: a new way to detect red cell antigen-antibody reactions. *Transfusion* 1990; 30: 109-113.
- [3] Zuk RF, Ginsberg VK, Houts T, Rabbie J, Merrick H, Ullman EF, Fischer MM, Sizto CC, Stiso SN, Litman DJ: Enzyme immunochemistry - a quantitative immunoassay requiring no instrumentation. *Clinical Chemistry* 1985; 31, 1144-1150.

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