

COMPLETE BLOOD GROUP WITH FORWARD AND REVERSE TYPING IN THE SAME LATERAL FLOW DEVICE

I. Aebischer*, K. Löster°, P. Monod*, P. Schwind*, *Medion Diagnostics GmbH, Düringen, Switzerland; °Prisma Diagnostika, Berlin, Germany.

Background:

Lateral flow tests are extensively used in the fields of infectious disease testing, pregnancy testing and also drug screening [1]. Recently, a multi-parameter lateral flow test for the specific determination of ABOD blood groups, Kell and Rhesus subgroups was presented [2]. This test format for forward grouping is independent of centrifugation steps and characterized by a stable end-point within minutes. In many countries the determination of isoagglutinins -the reverse typing- is mandatory in order to complete the ABO blood grouping.

The purpose of this study was to further open up the lateral flow technology to allow a simultaneous detection of isoagglutinins together with the blood group antigens in the same device.

Methods:

Forward Typing: The Forward Typing Area includes a separation membrane with one application zone and one detection area with parallel lines of membrane bound antibodies specific for blood groups A, B and D (principle see figure 1). Method for direct Forward Typing: 100 µl of diluted blood or diluted erythrocyte sediment are pipetted to the application zone, followed by 300 µl of a washing solution. Results can be read after 5 minutes.

Reverse Typing: The Reverse Typing Area contains a separation membrane divided into four distinct application, migration and detection areas each (original principle see figure 2). Method: 25 µl of a 5% suspension of reagent red cells for reverse grouping (Reverse Cyte A1, A2, B, O; Medion Diagnostics, Switzerland) are mixed in separate tubes with 100 µl of plasma each. Suspensions are incubated for 2 minutes at room temperature, then, 50 µl are transferred to the A1, A2, B, O application zones. Results can be read in the detection areas after another 3 minutes.

Positive results in Forward Typing and Reverse Typing are seen as distinct red bands. The absence of a signal is indicating a negative result.

Figure 1

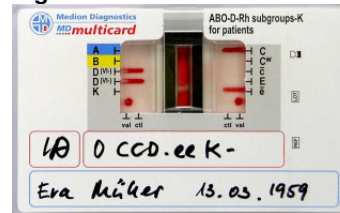


Figure 1: Lateral Flow Forward Typing Assay

Medion multicard with the configuration: A-B-D^(VI)-D^(VI)-K--C-C^w-c-E-e. Positives are recognized as distinct red bands. Results are valid when the val signal is positive (red spot) and the ctl is negative (no signal). Result: Blood group O CC D. ee kk

Figure 2

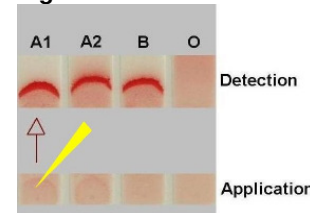


Figure 2: Lateral Flow Reverse Typing: Principle

Prototype strips used for testing a plasma from a donor with blood group O. Plasma is incubated with A1, A2, B and O Reverse-Cyte cells, added to the application zone, and specific reactions are monitored in the detection zone. The presence of isoagglutinins is indicated by the red band in the detection area. Result: Blood group O plasma with anti-A1, anti-A2 and anti-B isoagglutinins.

Figure 3

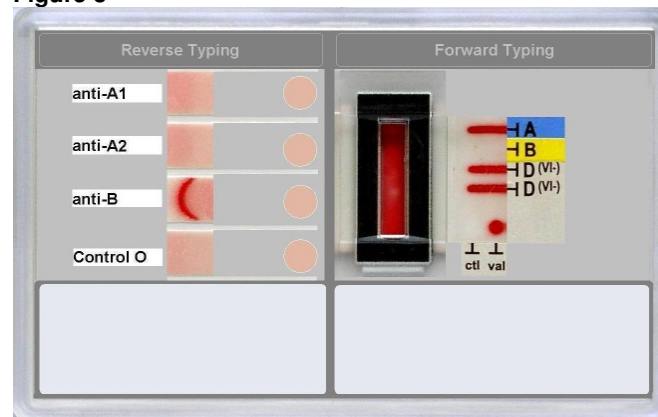


Figure 3: Lateral Flow Forward and Reverse Typing Assay

Design of a combination device for the detection of blood group antigens and isoagglutinins. Results from a donor with blood group A (Rhesus positive) with anti-B isoagglutinin activity are shown.

Results:

Plasmas of various normal donors (n=117), previously tested with the tube technique, have been assessed for isoagglutinin activity with a prototype Reverse Typing method. The sample pool contained ACD or CPDA anticoagulated plasma from blood group A (n=32), B (n=26), AB (n=4) and O (n=55).

The expected isoagglutinin reactivity to Reverse Cyte A1 or B cells was corresponding to the results observed in the forward typing. No discrepancies between the two techniques have been observed. Specificity was shown to be very good as 0 out of 117 plasmas reacted with control cells with blood group O. One plasma out of 55 from the blood group O reacted weakly with the Reverse Cyte A2 cells (1.8%). A similar weak reaction was observed with 4 plasmas out of 26 from the blood group B (15.4%). However, weaker reactions with A2 cells are to be expected as the quantity of A2 antigens on erythrocytes is significantly reduced compared to the quantity of A1 antigens [3]. Interestingly, it was recently reported that in automated systems a significant part of the weak isoagglutinin activity has to be confirmed manually. These findings may indeed be explained by the presence of certain ABO alleles [4].

Conclusions:

The described prototype Reverse Typing test is very robust with a stable end-point. Centrifugation is not required. As a next move Forward Typing and Reverse Typing may be combined in one lateral flow device (see figure 3) to detect blood group antigens and isoagglutinins together.

References:

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2. Löster K, Fleischhauer S, Schwind P. Lateral flow assay for simultaneous typing of ABO, Rhesus subgroups and Kell. *Vox Sang* 2004; 87 (Suppl. 3), 4.
3. Issitt PD, Anstee DJ. Applied blood group serology. Fourth Edition. 1998. Chapter 8. The subgroups of A: A1 and A2. Montgomery Scientific Publications. Durham, NC, USA.
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