

# ABO Forward and Reverse Typing in a Lateral Flow Device with Stable End-Point without Centrifugation

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

## Background:

Lateral flow assays are widely distributed in the fields of infectious disease testing, pregnancy testing and also drug screening [1]. Recently a multi-parameter lateral flow test for the determination of ABOD, Kell and Rhesus subgroups was presented [2]. This test format for forward grouping is independent of centrifugation and shows a stable end-point within minutes. In addition to the forward typing, the determination of isoagglutinins is mandatory in many countries.

## Aim:

The purpose of this study was to further explore the lateral flow technology to determine the isoagglutinins together with the blood group antigens in the same device.

## Methods:

**Forward Typing:** The Forward Typing Area contains a separation membrane with one application zone and a detection area with parallel lines of antibody reagents 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 transferred to the application zone, followed by 300 µl of a diluent. 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.

Figure 1

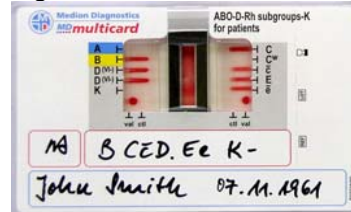


Figure 1: Lateral Flow Forward Typing Assay

Medion multicard with the configuration: A-B-D<sup>(VI-)</sup>-D<sup>(VI-)</sup>-K--C-C<sup>w</sup>-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 B Cc D. Ee kk

Figure 2

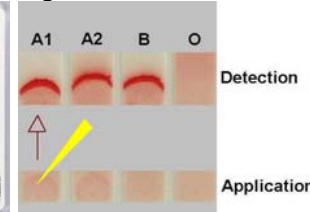


Figure 2: Lateral Flow Reverse Typing: Principle

Prototype strips used for testing a plasma from a donor with blood group O. Plasma is incubated with commercial 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

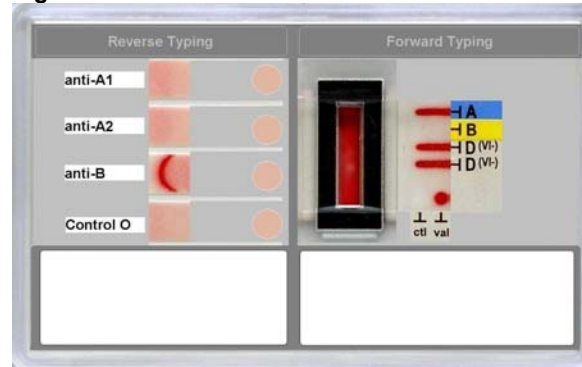


Figure 3: Lateral Flow Forward and Reverse Typing Assay

Design of a possible 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.

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## Results:

The plasmas of 117 normal donors, previously tested with the tube technique, have been characterized for isoagglutinins with the prototype Reverse Typing method. The samples consisted of ACD or CPDA anticoagulated plasma from blood group A (n=32), B (n=26), AB (n=4) and O (n=55). The expected reactivity of the isoagglutinins to Reverse Cyte A1 or B cells was in full agreement with the observed reactivity, in both techniques. Specificity was proven to be very good as no probes reacted with the control cells with blood group O. One plasma out of 55 from the blood group O reacted weakly with the Reverse Cyte A2 cells. A similar weak reaction was observed with 4 plasmas out of 26 from the blood group B. Weak reactions with A2 cells are to be expected as the quantity of the A2 antigens is significantly reduced compared to A1 cells [3]. Recently, it was reported that in automated systems a significant part of the weak isoagglutinin activity has to be confirmed manually and these findings may 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 and does not require centrifugation steps. As a next step, it appears to be feasible to combine Forward Typing with Reverse Typing in one device (prototype see Figure 3) to detect blood group antigens and isoagglutinins together within 5 minutes.

## References:

1. Zuk RF, Ginsberg VK, Houts T, Rabbie J, Merrick H, Ullman EF, Fischer MM, Sizto CC, Stiso SN, Litman DJ: Enzyme immunochromatography - a quantitative immunoassay requiring no instrumentation. *Clinical Chemistry* 1985; 31, 1144-1150.
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.
4. Wagner FF, Blasczyk R, Seltsam A. Nondeletional ABO\*O alleles frequently cause blood donor typing problems. *Transfusion* 2005; 45, 1331-1334.