D^u detection by an automated direct agglutination method that equals detection by indirect antiglobulin test

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INTRODUCTION

Since D^u was described by STRATTON in 1946 as a "new" Rh system antigen, there has been an ongoing scientific debate about the necessity of testing for D^u.

Furthermore D^u detection is dependent on methods and reagents used (e.g. polyclonal vs monoclonal) as there is no clinical significance of immunogenicity of D^u.

We describe a novel agglutination method (ABT) that is at least comparable to IAT, easier to perform and more sensitive.

MATERIALS AND METHODS

We used commercial polyclonal anti-D reagents as well as Rh controls diluted in 3% BSA for IAT testing and antibody diluent for ABT.

Bromelin 0.15% in NaCl/Tween diluent was used for the dilution of donor red cells.

74 EDTA anticoagulated donor samples were initially tested on the Dynatech Microbank System and found to be non-reactive. These samples were tested by a manual indirect antiglobulin method demonstrating a range of 1-3 + reactivity. For the ABT method 60 µl of 1:50 diluted anti-D or Rh control was added to the test wells of a flat bottom 96 well microtiter tray as well as 60 µl of 0.15% Bromelin in NaCl/Tween 20 was added to primary and secondary dilution wells. 70 µl packed cells were added to the primary dilution wells and diluted in several steps with Bromelin solution.

The ABT was performed after addition of sample and reagents. The procedure includes automatic tilting of the tray back and forth as it moves down the track.

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* Footnote
Abbreviations: IAT = indirect antiglobulintest
ABT = automated Bi-directional tilt

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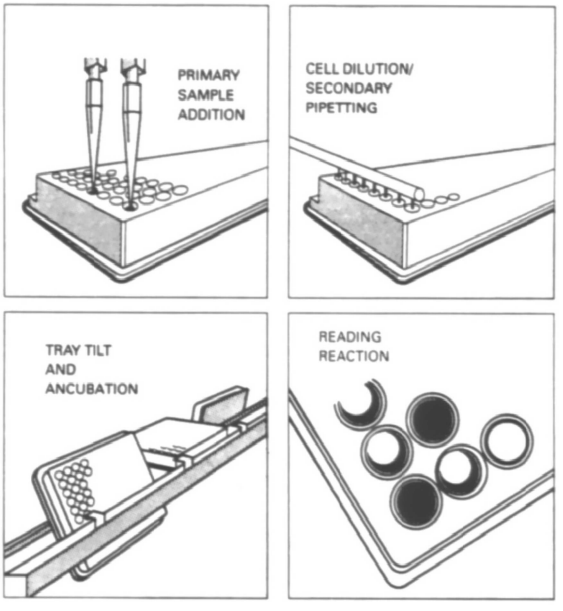
Fig. 1. AGGLUTINATION ASSAY PROCESSING



With this process no centrifugation is required. After the tray proceeds through the series of tilts, any positive reactions will result in a very distinct crescent shaped agglutinate. An agglutination reader at the end of the track then detects either the presence or absence of the crescent.

RESULTS

Table 1 shows the results of a comparison of 74 D^u samples as endpoint result for ABT and IAT. Results demonstrate that anti-D can be diluted to a greater extent in the ABT method than IAT.

Table 1. ENDPOINT COMPARISON STUDY - ANALYSIS OF 74 D^U SAMPLES

ABT Endpoint	No. of Samples Tested	IAT Endpoint (Range)
100	1	50
200	2	50 - 100
400	15	12.5 - 100
800	26	50 - 400
1600	21	25 - 400
3200	7	200 - 800
6400	1	200
12,800	1	400

Similar population distributions are demonstrated with both common D and D^u phenotypes. (Table 2.)

Table 2. POPULATION DISTRIBUTION COMMON D AND D^U PHENOTYPES

	D POSITIVE	D^U POSITIVE	D NEGATIVE
MEAN	250,214	216,202	6799
S. D.	33,350	31,090	1517
% CV	13.3	14.4	22.3
MINIMUM	146,113	125,009	4508
MAXIMUM	314,919	283,606	9645
NUMBER	119	74	40

D mosaics from all categories were also tested to assess specificity when using diluted anti-D in the system. The results indicate that all categories of the D mosaic were detected. (Table 3.)

Table 3. D MOSAIC TESTING

CATEGORY	ABT COUNT
RYR	3857
D II	213868
D III	222892
D III	234337
D IV	235818
D V	232502
D VI	197741
D VI	235152
D VI	200803
D VI	198566
D VI	198235
D VI	255213

SUMMARY

The studies suggest that the ABT method requires less antibody than the manual IAT method to provide D^u detection.
When performing testing with anti-D, no confirmation of D negative results is required.
There is no problem with false positives with the ABT system, as evidenced by performance with D negative samples.
The D mosaic testing indicates that there is no loss in specificity by diluting the anti-D.

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