The use of microtiter techniques for the determination of red blood cell phenotypes in paternity testing

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INTRODUCTION

The use of standard slide or tube techniques for the determination of red blood cell phenotypes is rather cumbersome and requires a large amount of work which can be the reason of clerical errors. In order to avoid these problems, microtiter techniques have been included in our routine paternity tests for the definition of alloantigens of the erythrocyte membrane. The comparison of the results obtained by the microtiter methods and standard techniques is shown in this report.

MATERIAL AND METHODS

<u>Erythrocytes</u>: the red cells to be tested originated from individuals involved in paternity investigations (mothers, children and putative fathers)

<u>Microtiter techniques</u>:
1. For the definition of Rh (C, c, C^w, D, E, e), Ss, K (K, k), Fy (Fy^a, Fy^b), Jk (Jk^a, Jk^b), Lu (Lu^a, Lu^b), Co^b, and Xg^a the commercially available solid phase immunoassay Capture-R™ (Immucor, Norcross, GA/USA) which is performed in microtiter trays (Plapp et al. 1984) has been used according to the manufacturer's specifications with human IgG alloantisera,
2. For the definition of ABO (with A subgroups), MNS, P_1, Le (Le^a, Le^b) and Jk (Jk^a, Jk^b) a liquid-phase microplate method has been used. One drop (50 µl) of a 2% erythrocyte suspension in isotonic saline was mixed with 1 drop (50 µl) monoclonal antibody (for ABO, MN, Le and Jk), alloantiserum (S), xenoantiserum (P_1) or lectin (A subgroups) in U-shaped polystyrol microtiter plates (Greiner #650101, Nürtingen/FRG); after an incubation of 20 - 30 minutes at 22°C, the trays were centrifuged for 1 minute at 110 x g (BCSH Blood Transfusion Task Force and BBTS Working Group 1990).

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RESULTS AND DISCUSSION

1. Solid phase immunoassay Capture-R™

Approximately 2000 samples have been tested in this technique for Fy, Jk, Lu and Co^b; the number of tested samples for Ss, Rh, K and Xg^a are 1200, 200, 150 and 300, respectively. Due to the higher sensitivity of this system for the detection of IgG alloantisera (in comparison to the standard tube techniques with or without indirect anti-humanglobulin-test), it was necessary to dilute the alloantisera used. The dilutions ranged between 1:20 and 1:2 (1:20 - 1:15 for Rh antisera, 1:4 for anti-S, -s, -K, -k, -Fy, -Jk and 1:2 for anti-Lu, $-Co^b$ and $-Xg^a$). The optimal dilution of the alloantisera had to be determined in a preliminary titration of the reagents against the erythrocytes of a heterozygous donor. In all the tests, 14 discrepancies (< 0.1%) in comparison to the standard tube or slide tests were observed. These differences were mainly due to the higher sensitivity of the Capture-R™ technique (Mayr et al. 1989) which gave for instance a much better definition of Fy^x than the conventional tube tests (3 cases). The higher sensitivity of Capture-R™, however, also produced some false positive reactions with antisera containing extraantibodies which did not react in the standard tube test.

2. Liquid-phase microtiter tests

The number of samples tested for AB0, MNS, P_1 and Jk was 500; 100 samples were tested for Le. All reagents were used in the same dilution as in the conventional slide or tube tests.
The phenotyping of AB0 (with A subgroups), MNS, P_1 and Jk also showed a perfect concordance with the results of the standard tests (no discrepancies). The monoclonal antibodies (of different origin) used for the definition of the Le phenotypes did not give clear-cut results, so that the typing for Le has been discontinued for the moment.

The microplate assays offer several advantages: by using Capture-R™, it is possible to perform the tests in a much shorter time (96 reactions in approximately 40 minutes) and the alloantisera can be diluted between 1:20 and 1:2 thus decreasing the costs for these reagents. An analogous saving of time is observed for the liquid-phase technique.
The handling of microtiter trays is much easier than the handling of a large number of tubes and slides; furthermore, all microtiter methods can be rather easily automatized.
The microtiter trays can be stored for a longer period of time, so that all readings can be checked independently by another investigator.
A drawback of the microtiter techniques, however, is the fact that not all reagents working in conventional tests can be employed in these methods; before using them, the sera have to be reevaluated in the relevant microplate technique.
Nevertheless, the advantages prompted us to include microplate techniques in our routine paternity testing; in practice, one series of tests is performed in the conventional tube and slide methods and, for confirmation, the above-mentioned microtiter techniques are used in the second series of phenotypings.

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