Immunocytochemical ABH Blood Group Staining in Vaginal Swabs

R. Scheithauer, I. Hetzler

Dr.med.Richard Scheithauer, Institut für Rechtsmedizin,
Universität Erlangen-Nürnberg, Universitätsstr. 22,
8520 Erlangen, Bundesrepublik Deutschland

INTRODUCTION

In blood grouping of mixed secretions from two or more persons, attributing results to the individual is always a problem. During investigations of sexual assaults, it would be greatly advantageous to be able to determine the blood group of single vaginal epithelial cells, for example discovered on the penis of a suspect.
Although the mixed cell agglutination reaction technique (Cooms et al. 1956) was a possibility, immunostaining (Sternberger et al. 1970) seems to be the method of choice for this purpose. Brinkmann et al. (1986) published an initial report about ABH blood group typing of single cells, with encouraging results.

MATERIAL AND METHOD

In the present investigation, vaginal swabs from 200 gynaecology clinic patients were used. Necessary blood group information could be obtained directly from the patients (n = 57) or through direct (n = 27) or indirect testing (n = 116). 28 of these 116 cases, obviously non-secretors, could not be classified and were not stained. Secretor status was indirectly determined on all swabs tested. Only acid phosphatase negative (sperm negative) swabs were used, or acid phosphatase (Berg 1957) positive swabs, if blood group information of the woman was known (Table 1). Each vaginal swab was spotted onto three separate areas of a glass microscope slide, and incubated in parallel with anti-A-, anti-B-, and anti-H-sera. Immunostaining was performed by a four step technique ((monoclonal anti-A-, anti-B-, anti-H-serum (anti-A and anti-B from Biotest, anti-H from Fresenius), anti-mouse-serum from the rabbit (Bionetics), anti-rabbit-serum from the swine (Dakopatts), peroxidase-anti-peroxidase complex from the rabbit (Dakopatts), AEC as substrate)).

Advances in Forensic Haemogenetics 2
Edited by W. R. Mayr
© Springer-Verlag Berlin Heidelberg 1988

RESULTS AND DISCUSSION

Each case was only stained once, and read by a person not knowing the expected result. Table 2 shows a summary of these results. In 78 % of the cases, the correct blood group was determined from the immunocytochemically stained vaginal cells. In 9 % of the cases, a blood group determination was not possible, because there was an insufficient amount of difference between the cell reactions and background staining. Perhaps this rate could be reduced with improved technique. In 6 % of the cases, there was no staining with anti-A-, anti-B-, or anti-H-serum. In 7 % of the investigated cases, an incorrect blood group was obtained, for unknown reasons.
The best results were obtained from patients between 21 and 40 years and from pregnant patients. In general, more mature vaginal cells with a relatively smaller size nucleus, are better for blood group determinations, rather than the more immature cells with larger nuclei.
Because the swabs investigated were obtained from gynaecology clinic patients, the vaginal cells of many cases showed a higher degree of cytolysis, which obviously diminishes the quality of immunostaining.
The last and probably most interesting aspect to be discussed, is the dependence of the results on secretor status. Table 3 shows the large number of cases of non-secretors which did not react. The investigation of vaginal swabs alone cannot explain this phenomenon. The results of a not yet published study (Scheithauer and Spiegelsberger) will illustrate the reason: All the cell layers of vaginal epithelium of secretors are completely marked, with respect of blood group. However, in non-secretors, only the intermediate and parabasal cell layers are labeled. Therefore, in order to correctly determine ABH blood group in non-secretors, vaginal swabs must contain cells from deeper levels of the mucosa. Hence, it's obviously impossible to determine secretor status from a vaginal swab by ABH staining alone.

LITERATURE

Berg S (1957): Medico-Biological Interpretation in Cases of Sexual Delinquency. J Forens Med 4: 82-89

Brinkmann B, Kernbach G, Rand S (1986): Cytochemical Detection of ABH Antigens in Human Body Fluids. Z Rechtsmed 96: 111-117

Coombs RRA, Bedford D, Rouillard LM (1956): A and B Blood-Group Antigens on Human Epidermal Cells Demonstrated by Mixed Agglutination. Lancet I: 461-464

Scheithauer R, Hetzler I: Immunzytochemische ABO-Blutgruppenbestimmung an Vaginalabstrichen. (Submitted for publication)

Scheithauer R, Spiegelsberger T: AB0-Blutgruppenprägung an weiblichen Genitalorganen. Eine immunhistochemische Studie. (Submitted for publication)

Sternberger LA, Hardy PH Jr, Cuculis JJ, Meyer HG (1970): The unlabeled Antibodyenzyme Method of Immunohistochemistry. Preparation and Properties of soluble Antigen-antibody Complex (Horseradish Peroxidase-Antihorseradish Peroxidase) and its Use in Identification of Spirochetes. J Histochem Cytochem 18: 315-333

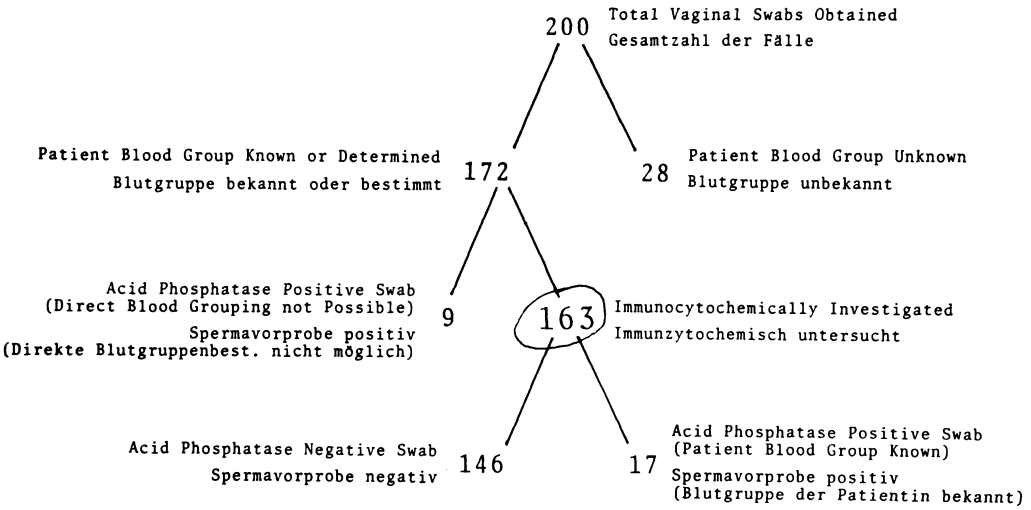

Table 1:
Investigated Cases / Untersuchte Fälle

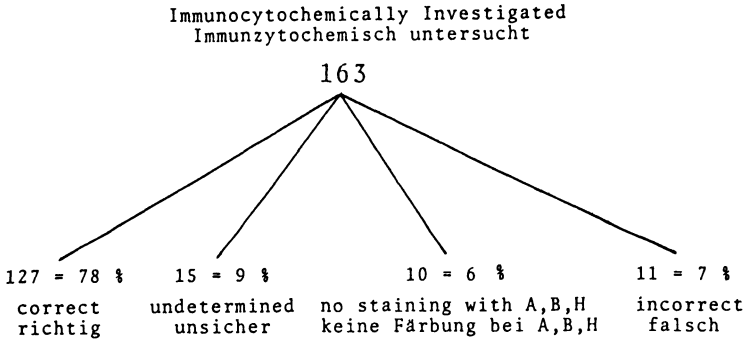

Table 2:
Summary of Results / Gesamtergebnis

	n*	correct richtig	undetermined unsicher	no reaction keine Färbung	incorrect falsch
Secretors Ausscheider	122	100 ≙ 82%	10 ≙ 8%	3 ≙ 3%	9 ≙ 7%
Nonsecretors Nichtaus- scheider	27	16 ≙ 59%	4 ≙ 15 %	6 ≙ 22%	1 ≙ 4%

* Only negative acid phosphatase vaginal swabs used
Nur Fälle mit negativer Spermavorprobe

Table 3:

Results Dependent on Secretor Status
Abhängigkeit der Ergebnisse von der Ausscheidereigenschaft