Absorption-Elution Test for ABO-Determination of Secretor and Nonsecretor Saliva Stains

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Introduction

During the last years sensitive elution tests have been described for the ABO-determination of saliva stains of nonsecretors (LINCOLN 1988, RABL et al. 1990). The object of the present study was to determine the ABO blood group in saliva stains of practical cases and compare the results of the usual inhibition test with those of the absorption-elution test.

Materials and Methods

100 saliva stains of practical cases were tested. The distribution of the ABO-blood groups was as follows:

A_1: 35, A_2: 4, B: 14, O: 39, A_1B: 6, A_2B: 2

Inhibition Test

Equal volumes of saliva stain and anti-AB-Serum (titer of 16) were mixt and left at 4 °C overnight, after which two drops from each tube were titrated using isotonic saline as diluent. Equal volumes of appropiate A_1- or B-indicator cells were added to each tube. The tests were macroscopically read for agglutination after incubation at room temperature for 15 min. The same test was done with Ulex europaeus (titer of 16) using O indicator cells. Results obtained from the stain were compared with those of the unstained material. Reduction in titer of at least three dilutions was necessary to demonstrate the presence of blood group substance.

Absorption-Elution Test

The test was carried out according to the method of KIND and CLEEVELY (1969) with minor changes. An appropiate portion of stain was extracted in a known volume of 5% aqueous ammonia. Aliquots of the extracts were spotted on glass slides, which were left to dry at room temperature. Drops (±30 µl) of undiluted anti-A- and anti-B-sera were added to each slide and carefully spread to cover the area of the dried extract. Absorption took place overnight at 4°C in a humid chamber. The slides were then carefully rinsed with ice-cold isotonic saline for a period of about 30 seconds. Fresh indicator cells were used in a concentration of 0.5% and immediately covered with a coverslide. Elution took place for 15 min at a temperature of 56°C in a suitable moist chamber. After elution the slides were kept at room temperature and were

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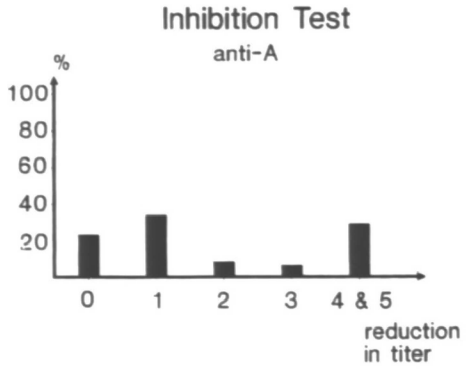

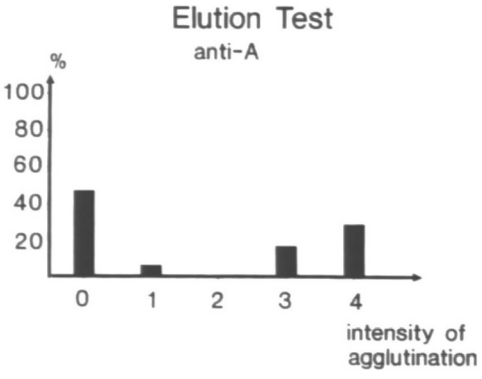

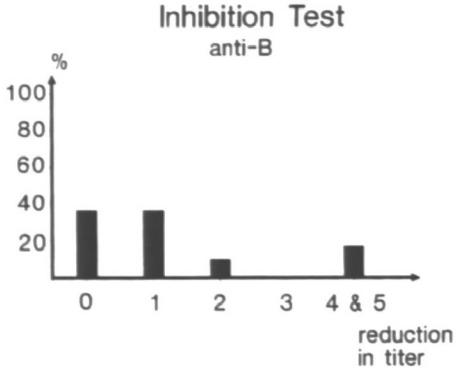

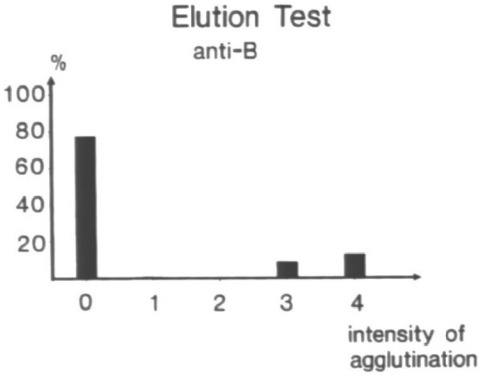

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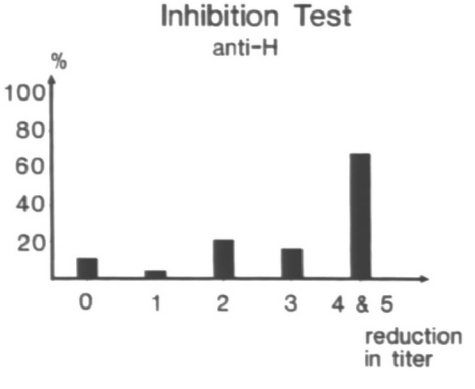

1a

Fig 1a, 1b

1a Results with anti-AB and Ulex europaeus (inhibition test)

1b Results with anti-A and anti-B (elution test)

read under microscope (100 X) within a time period of 10-20 min after completion of the elution procedure. Results were read from negative to 4+.
Some experiments were performed with an anti-H (Ulex europaeus) and O-indicator cells, but reliable reactions could not always be obtained.

Results

The results of anti-A- and anti-B-serum of all saliva stains with the inhibition and the absorption-elution test are shown in Fig. 1a and 1b. Furthermore the results with Ulex europaeus are given for the inhibition test. The inhibition test showed much more "weak" reactions (reduction of 2 titers) than the elution test, which gave in nearly every case clear cut results.
In agreement with the standards in our laboratory, it was established that the saliva stain belonged to a secretor of the concerned blood group when a reduction in titer of 3 or more grades was detected in the inhibition test.
The results show, that with the inhibition test seven A-nonsecretors, four B-nonsecretors, five AB-nonsecretors and fifteen O-nonsecretors were detected. With the absorption-elution-test the ABO-blood group of these 16 A-, AB- or B-nonsecretors could, with one exeption (A-nonsecretor in ABO-elution test 2+), correctly be determined. False positive results were not found.

Discussion

The successful grouping of the A- and B-antigens in the saliva stains shows the high sensitivity of the method. The results of this study are in good agreement with the findings of RABL et al. (1990), who were successful in typing A- and B-nonsecretor saliva stains with a method similar to that used in our labaratory. In agreement to our results, RABL et al. were not successful in grouping O-nonsecretor saliva stains, as in some cases they obtained only negative or weak reactions with Ulex europaeus.
The absorption-elution test is now used in routine cases for ABO-grouping of cigarette ends in our laboratory. In most of these cases the antigens A and B of nonsecretor smokers are detectable, whereas the absorption test gives negative results. The elution test described in this paper can be recommended as a supplementary method, when the absorption test is performed without conclusive results. With this method the saliva stains with an age of up to several months could be successfully typed, as the Type I antigens of the ABO-system, present in body fluids, remain stable for quite a long time. The test needs only minute amounts of saliva stain.

Literature

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