A, B, H- and Lewis grouping of body secretions from a common stain extract

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MATERIALS

Antisera. A, B, H-antisera (Molter, Merz u. Dade) and Lewis-antisera were used for absorption-inhibition tests in dilutions that still gave fairly strong agglutination of NaCl-controls with test erythrocytes. Anti-Le^a and anti-Le^b were employed in parallel from two different manufacturers (Merz u. Dade/Behring).
Since slight titre variations may occur, the titre values of antisera have to be checked occasionally or if new antisera charges are to be used. The following dilutions are generally used:

anti-A	(Molter)	:	1:200
anti-B	(Molter)	:	1:200
Lectin-H	(Merz u. Dade)	:	1:20
anti-Le^a	(Merz u. Dade)	:	1:15
anti-Le^b	(Merz u. Dade)	:	1:25
anti-Le^a	(Behring)	:	1:6
anti-Le^b	(Behring)	:	1:8

A, B, H-antisera for absorption elution tests are diluted 1:8 (Anti-A, anti-B) and 1:2 (Lectin-H) to save material. A monoclonal anti-B was purchased by Biotest and diluted 1:4.

Indicator cells. For agglutination reactions in absorption inhibition tests we used 0,5 % test erythrocytes (Affirmagen, Ortho, H cells were papain-treated), in absorption elution tests 0,2 % test erythrocytes (Affirmagen, Ortho, papain-treated) and for Lewis testing 1,5 % test erythrocytes (Serocyte, Merz u. Dade), being Le^a or Le^b positive.

Secretion stains. All stains were collected from the laboratory staff and stored at room temperature or in the case of semen and vaginal secretion at -20°C. Cigarette tips and stamps were kept in original, semen was provided on squares of viscose material, vaginal secretion, being free of semen contamination was collected on sterile cotton swabs and nasal secretion, sweat and urine stains were made on filter paper.

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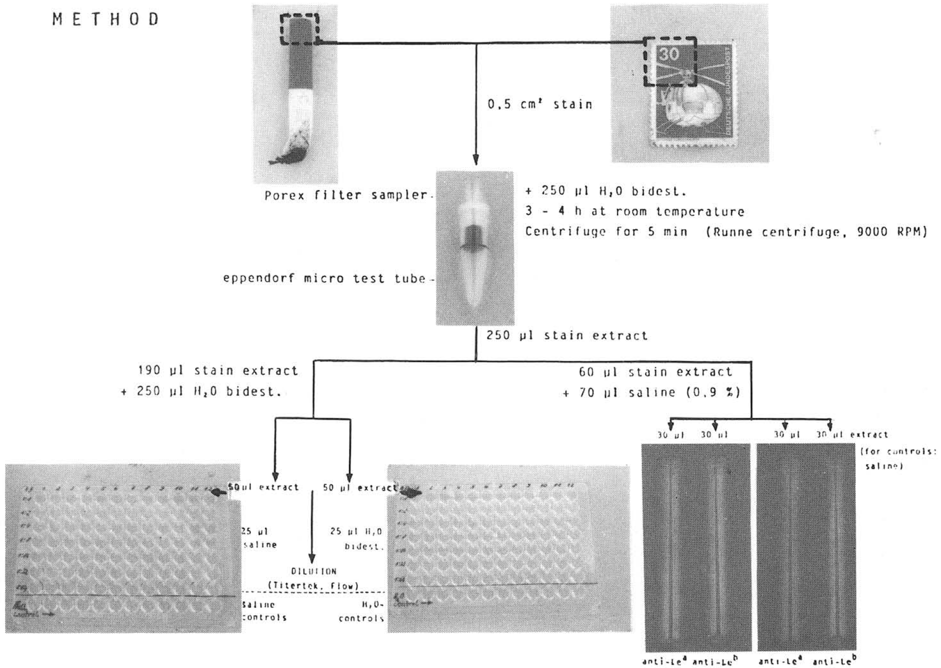

- Dilution of stain extract up to 1:64	- Dilution of stain extract up to 1:64	
	- Fixation of stains onto micro-titer plates by drying	
- Add 25 µl of diluted antisera	- Add 25 µl of diluted antisera	- Add 30 µl of diluted antisera
- Incubation overnight in a humid chamber at 4°C	- Incubation overnight in a humid chamber at 4°C	- Incubation overnight at 4°C (vials covered with a plastic film)
	- Wash 5 times with cold saline	
- Add 25 µl of 0,5 % erythrocytes	- Add 25 µl of 0,2 % erythrocytes	- Add 30 µl of 1,5 % Le^a-positive or Le^b-positive test erythrocytes
	- Elution of antibodies (56°C,15 min)	
		- Centrifugation at low speed (Molter centrifuge, 1500 RPM) after 30 min
- Read agglutination/inhibition of agglutination microscopically after 30 - 60 min	- Read agglutination microscopically after 40 - 60 min	- Read agglutination by eye

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RESULTS

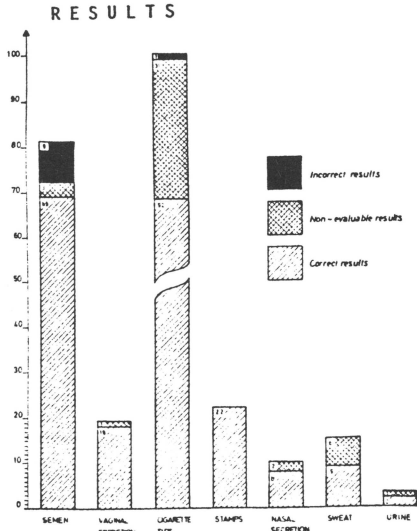

Fig. 1: ABO-grouping in secretion stains.
Absorption inhibition and absorption elution tests are evaluated as a whole. Agglutinations (or inhibition of agglutination) including at least 3 dilution steps were considered as a positive indication of the concerning blood group factor. Weak reactions (below 3 dilution steps) or conflicting results between absorption inhibition and absorption elution tests are classified as non evaluable.

ABSORPTION INHIBITION + ABSORPTION ELUTION	ASe	BSe	ABSe	OSe	Σ	Ase	Bse	ABse	Ose	Σ
Correct results	64	19	5	60	148	15	4	3	6	28
Non-evaluable results	1	3	3	6	13	2	1	1	3	7
Incorrect results	0	0	0	0	0	1	0	2	3	6
Number of stains	65	22	8	66	161	18	5	6	12	41

Fig. 2: Analysis of ABO-grouping results in saliva stains. Classification of results as in Fig. 1. Grouping results in nonsecretors are only related to absorption elution tests

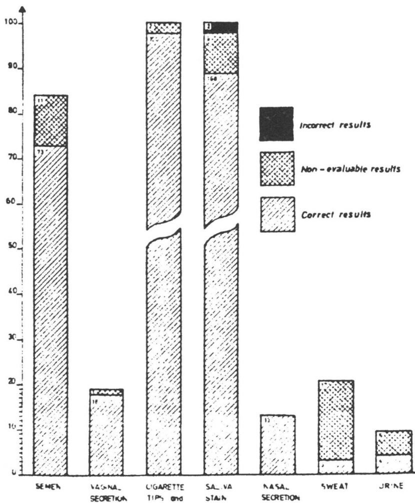

Fig. 3: Lewis grouping in secretion stains
Correct results are defined as definitely positive inhibition reactions, weak and negative inhibition reactions as well as contradictory results of simultaneous tests with two different antisera were summarized as non-evaluable results and false positive inhibition reactions were considered as incorrect results.

		ABSORPTION INHIBITION + ABSORPTION ELUTION			HOLZER-TEST		
		A	B	0	A	B	0
I	AI	5	2	5	6,5	2	8
	AE	6,5	6,5	6,5			
II	AI	5	0	5	7	1,5	7
	AE	6	5	5			
III	AI	5	0,5	5	7	0	8
	AE	6,5	6,5	6,5			
IV	AI	5	0	5	5,5	0	6,5
	AE	6	5,5	6,5			
V	AI	5	0	5	6,5	0	7
	AE	4,5	1	6,5			

Fig. 4: False B-positive reactions in liquid semen of A-secretors.
Absorption inhibition (AI) and absorption elution (AE) results are compared with HOLZER-test. The number of inhibited or agglutinated dilution steps is indicated.

	time	1d			2d			8d			16d		
		AI	AEp	AEm	AI	AEp	AEm	AI	AEp	AEm	AI	AEp	AEm
I	A	5	5	5	5	6,5	6	5	5	6,5	5	6	6
	B	0,5	6,5	0	0	6,5	0	0	6,5	0	0	6,5	0
	0	5	6		5	6,5		5	6,5		5	6,5	
II	A	5	6	6	5	6,5	6,5	5	5	6,5	5	5	6,5
	B	0	3	0	0	6,5	0	0	2	0	0	3	0
	0	5	6,5		5	6,5		5	6,5		5	6,5	

Fig.5: False B-positive reactions in liquid semen from two A-secretors using polyclonal (p) and monoclonal (m) antisera for absorption elution tests (AE). Semen was stored a certain period at room temperature.

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Conclusions

In forensic case-work problems may arise by an uneven concentration of the secretion in a stain, when parts of it are used for different grouping procedures. Therefore we prepare one extract, that is used for A, B, H- and Lewis-determination in an absorption inhibition test and for A, B, H-determination in an absorption elution test. By using two different methods, we are able to control the results of each test.

Correct A, B, H-grouping results were obtained in 85 % of all semen stains and in 95 % of vaginal secretion stains (Fig. 1). The high rate of incorrect results in semen stains is due to false B-positive reactions in A-secretors (Fig. 4). This aberrant blood grouping result did not appear in H-secretors and disappeared when a monoclonal anti-B serum was used (Fig. 5). The false B-positive reaction appeared in semen already after one day and was also found in 16 days old samples. It did not occur in this semen if it was dried up immediately.

Typing of saliva stains on cigarette tips (Fig. 1) was carried out correctly in 74 % of all cases, on prepared saliva stains in 87 % (Fig. 2) and on stamps in approximately 100 %. Incorrect results in prepared saliva stains of nonsecretors are mostly due to false negative reactions (Fig. 2).

In sweat and urine the rate of positive grouping results is insufficient. Investigations should therefore be restricted to the absorption elution test or Lewis grouping so that less water is needed for extraction resulting in higher antigene concentrations. Another possibility may be the concentration of large stains by ultrafiltration.

Lewis typing is also promising. Lewis substances could be successfully determined in 87 % of all semen stains and in 96 % of all saliva stains. A, B, H-secretor or nonsecretor results were confirmed by the typing of Lewis substances. The method has been proved to be successful in forensic case-work.

References

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