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ABH Typing on Formaldehyde Fixed Saliva Stains

REKHA AWASTHI, RAKESH KUMAR GARG
Forensic Science Department, Punjabi University, Patiala, India

FORMALDEHİDDE FİKSE EDİLMİŞ TÜKÜRÜK LEKELERİNDE ABH TİPLEMESİ

Özet

Alınan kişinin sekretör olup olmadığı ve kan grubunun bilindiği, % 10 formaldehidle fikse edilmiş 50 tükürük lekesi, pamuk ve terrycot gibi birbirinden farklı iki substrat üzerinde hazırlandıktan sonra, absorpsiyon-inhibisyon ve absorpsiyon-elüsyon yöntemleriyle ABH-spesifik maddeler açısından incelendi. Absorpsiyon-elüsyon tekniği doğru sonuçlar açısından en yüksek düzeye ulaşırken, %10 formaldehidle fikse edilmiş örneklerdeki suda çözünür maddelerin korunabilirliği açısından, absorpsiyon-inhibisyon yöntemine oranla daha duyarlı ve etkin olduğu saptandı. Uygulamada kullanılan substratların sonuçlar üzerinde kayda değer bir etkisi olmadığı görüldü.

Summary

Fifty saliva stains of known blood group and secretor status were prepared on two different substrates (cotton and terrycot) and examined for the presence of ABH specific substances by absorption-inhibition and absorption-elution after fixation with formaldehyde (10 per cent). The absorption-elution technique showed higher percentage of correct results (after fixation with 10% formaldehyde) as compared to absorption-inhibition and is more sensitive and effective in preserving the water soluble substances. The type of substrates did not influence the results to an appreciable extent.

Key words: *Saliva stains - Formaldehyde fixation - Absorption-inhibition technique Absorption-elution technique - ABH typing*

INTRODUCTION

Saliva stains are occasionally found at the scene of crime and the articles associated with it. It may be observed along with the bite marks on food articles or on the body of the victim itself particularly in sexual offences etc. In addition, saliva may also be observed with chewing-gums thrown by criminals at the scene of crime which may be quite useful for blood group estimation (1). These articles when received in forensic investigations besides being tested for saliva can also be analysed for the presence or absence of the ABH substances which can increase their evidential value. *Yada* et *al* (2, 3) reported the usefulness of formaldehyde and glutaraldehyde fixation in the absorption-elution grouping of dried stains (saliva, semen, vaginal secretion, urine) and pointed out each reagent was quite effective in preserving the water soluble blood group substances present in the stains.

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Sharma et *al* (4) could type the saliva correctly on cigarette butt ends in 92.70 per cent of the sample tested by absorption-inhibition technique. Lipstick samples were correctly typed by absorption-inhibition and absorption-elution technique (5). *Chahal* and *Chattopadhyay* (6) did not find much difference in the results for the detection of ABH substances from lipstick stains by the application of all the three technique. Thus it is apparent that the detection of ABH substances from stain has remained a controversial subject for the past so many years. In addition, bacterial growth and their activity may lead to erroneous results of blood group antigens (7-11). Therefore, on account of the difficulties arising from spurious reactions, both absorption-inhibition and absorption-elution in parallel is emphasised by different workers (12-16). In the present investigation, therefore, it has been thought desirable to undertake the determination of ABH substance from saliva stains on two different substrates by commonly employed methods.

MATERIAL and METHODS

Blood and saliva samples were collected from 50 individuals in serially marked sterilised test tubes from the campus of Punjabi University, Patiala. Blood samples were collected by finger prick method in normal saline while saliva was collected by placing cotton swab under the tongue and after about 5 minutes each individual was asked to squeeze it out into the test tube. The blood samples were analysed immediately for their blood group according to *Dunsford* and *Bowley* (17). Stains of saliva were prepared on two types of cloth pieces, cotton and terrycot purchased from the local market which was thoroughly washed with detergent before stain preparation. Few drops of saliva (5-6) were applied on each type of cloth piece by means of pasteur pipette in stain formation. After this the secretor status from the fresh saliva was determined according to *Race* and *Sanger* (18).

The prepared saliva stains on two types of substrates were examined within a week from the date of their preparation using absorption-inhibition (18) and absorption-elution after fixation with formaldehyde as suggested by *Kind* (19) and *Yada* et *al* (2, 3). Anti-A and anti-B sera were obtained from Haffkeine Institute, Bombay and anti-H was prepared from the seeds of *Ulex europaeus* in the department having titre of 1:128, 1:64, 1:32. Alongwith each saliva stain examined controls were also kept with each test.

Table I. Results of ABO blood groups from fresh blood and sectetor status.

Blood groups	No. Tested	*Secretor status*	
		Secretor	Non-secretor
A	16 (32%)	13 (81.25%)	3 (18.75%)
B	22 (44%)	22 (100%)	
0	9 (18%)	8 (88.00)	1 (11.11%)
AB	3 (6%)	2 (66.66%)	1 (33.33%)
TOTAL	50 (100%)	45 (90%)	5 (10%)

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Table II. Results of secretor status ABO(H) typing in saliva stains (cotton and terrycot) by absorption-inhibition and absorption-elution techniques.

		Secretor status		Absorption-inhibition				Absorption-elution			
				Cotton		*Terrycot*		*Cotton*		*Terrycot*	
BG	ST	SS	No.T	Correct	Incorr.	Correct	Incorr.	Correct	Incorr.	Correct	Incorr.
A	16	S	13	13		13		16*		16	
		NS	3	2	1	2	1				
B	22	S	22	22		22		22		22	
		NS									
AB	3	S	2	2		2		2		2	
		NS	1	1		1		1		1	
O	9	S	8	8		8		8		8	
		NS	1	1		1		1		1	
Total	50		50	49	1	49	1	50		50	

*) Includes three non-secretor samples.
BG=blood group; ST=no.of samples tested; SS=secretor status; No.T.= no.tested; Incorr.=incorrect; S=secretor; NS=non-secretor

RESULTS and DISCUSSION

The results of the fresh blood group and secretor status determination are given in Table I. B blood group is the commonest of all the other types followed by A, O and AB and the frequency of AB blood group is the lowest. The percentage of secretors and non-secretors from fresh saliva has been observed to be 90% and 10% respectively. Similar type of trend of the blood group and secretor status has been found in Northern Indian populations.

The results of blood group specific substances determination from saliva stains on two different types of cloth substrates are given in Table II. It has been observed that the secretor status (ABH) determination from saliva stains showed slightly different results in comparison to fresh saliva. Both the methods employed (absorption-inhibition and absorption-elution) showed similar type of results on two types of clothes whereas absorption-elution technique gave slightly higher percentage of correctly typed results than the absorption-inhibition. In this study, one of the non-secretor sample (A-blood group) has given incorrect typing (B-blood group) by the application of absorption-inhibition method on both the substrates while by using absorption-elution three of the

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samples of non-secretor status has been assigned the correct blood group. This indicates that the application of absorption-elution technique after fixation with formaldehyde becomes more sensitive and even the smallest amount of the blood group substances of the lower order present in non-secretors is also detected (2, 3). Second reason could be ascertained that the bacterial growth and its activity may have lead to the spurious results. On account of these difficulties arising from spurious reactions various workers recommended the application of absorption-inhibition and absorption-elution in parallel for grouping both fluid samples and dried stains and similar type of findings have been made in the present investigation. In the present study it has been observed that the application of absorption-elution method after fixation with formaldehyde (10 per cent) increases the sensitivity and can preserve the water soluble blood group substances of the stains. Therefore, when enough material is available, both the techniques-absorption inhibition and absorption elution-be attempted simultaneously and if concordant results are obtained positive opinion for the presence or absence of ABO(H) substances in saliva stains shall become more relevant.

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Reprints request to :

Dr. R.K. Garg
Department of Forensic Science,
Punjabi University,
Patiala-147002
India