magnification test

GAP

Test run by Regina Demas (Geren) on request of Examiner Joan Faunce in Feb. 1977 to compare incidence of false positives at 30x magnification (vs) 7x magnification. Results indicated that results using the higher magnification should be suspect.

Medical Literature included.

Testing the Use of Bromelated Cells in
Absorption-Elution Typing of Dry Bloodstains

Bromelin is an enzyme derived from the pineapple stem which is used to increase the sensitivity of indicator cells. This experiment was set up to test the necessity of bromelation of the O indicator cells which is presently carried out in this lab.

Weak samples of known blood types were set up (A, B, AB and O). Also weak mixtures of A and O cells, and B and O cells were tested.

The procedure was as described in attached sheet.

Results

Readings were made using a stereo microscope at 30x and 7x magnification (7x is the usual magnification used to read samples).

	30x magnification				7x magnification Examiner A				7x magnification Examiner B			
	A	B	H	Results	A	B	H	Results	A	B	H	Results
1. AB	−	+	−	B	−	+	−	B	−	+	−	B
2. AB	−	+	−	B	−	+	−	B	−	+	−	B
3. AB	−	+	−	B	+	+	−	AB	−	+	−	B
4. AB	−	+	−	B	−	+	+	B	−	+	+	B
5. A+O	−	−	+	O	−	−	+	O	−	−	−	Neg
6. A+O	−	−	+	O	−	−	+	O	−	−	+	O
7. B+O	−	+	+	B	−	+	+	B	−	+	+	B
8. B+O	−	+	+	B	−	+	+	B	−	+	+	B
9. ASe*	−	−	−	Neg	−	−	−	Neg	−	+	−	B
10. ASe*	−	−	−	Neg	−	−	−	Neg	−	−	−	Neg
11. Aext	+	−	+	A	−	−	−	Neg	−	−	−	Neg
12. Aext	+	−	−	A	−	−	+	O	−	−	−	Neg
13. Aext**	+	−	+	A	+	−	+	A	+	−	+	A
14. Aext**	+	−	+	A	+	−	+	A	−	−	+	O
15. Bext	−	−	−	Neg	−	−	−	Neg	−	−	−	Neg
16. Bext	−	−	+	O	−	−	−	Neg	−	−	−	Neg
17. Bext**	−	+	+	B	−	+	+	B	−	+	+	B
18. Bext**	−	+	+	B	−	?	+	?	−	?	+	?
19. ABext	+	+	−	AB	+	+	−	AB	?	+	−	?
20. ABext	+	+	+	AB	+	−	−	A	+	−	−	A
21. ABext**	+	+	−	AB	+	+	−	AB	+	+	−	AB
22. ABext**	+	+	+	AB	+	+	−	AB	+	+	−	AB
23. O ext	−	−	+	O	−	−	+	O	−	−	+	O
24. O ext	−	−	−	Neg	−	−	Dry	——	−	−	Dry	—
25. O ext**	−	−	−	Neg	−	−	−	Neg	−	−	−	Neg
26. O ext**	−	−	−	Neg	−	−	−	Neg	−	−	−	Neg

* Cloth touched by A secretor for control

** stronger extracts

Even numbered samples used bromelated O cells.

CONCLUSIONS

Bromelation of O cells led to a false positive type O result in only one case at 30x and only one case (a different one) at 7x magnification. No true type O readings were picked up with bromelated O cells that were not picked up with nonbromelated O cells.

However, due to the results of this experiment, it was demonstrated the Anti-B used was stronger than the Anti-A. This lead to many false readings. On the weak AB samples, there was agglutination visible at 30x only in the B. At 7x, A was detected on one of the AB samples. Also, in the combination A and O cells, no A was picked up.

It is suggested that bromelation of the A cells be tried on the same samples.

From the results of this experiment it is proven that bromelation of the O cells with the Anti-H currently in use is not necessary.

It should also be noted that there is great danger of incorrect readings if weak samples are read too closely. Under the current conditions, any answer other than an A or AB on weak samples is suspect. It is the opinion of this experimenter that it would be better to call results inconclusive or a sample insufficient than to risk reporting an incorrect blood type.

Regina K. Demas
February 4, 1977

Results

		30x Magnif				7x Magnif Examiner A				7x Magnif Examiner B				Comments
		A	B	H	Result	A	B	H	Results	A	B	H	Results	
(1)	AB	−	$+^{1}$	−	B	−	+	−	B	−	$+^{w}$	−	B	
(2)	AB	−	$+^{1-2}$	−	B	−	+	−	B	−	$+^{w}$	−	B	
(3)	AB	−	$+^{2-3}$	−	B	$+^{w}$	$+^{s}$	−	AB?	−	$+^{s}$	−	B	
(4)	AB	−	$+^{2}$	−	B	−	+	$+^{w}$	B	−	$+^{m}$	$+^{w}$	B	
(5)	A+O	−	−	$+^{1}$	O	−	−	$+^{w}$	O	−	−	−	neg	
(6)	A+O	−	−	$+^{1-3}$	O	−	−	+	O	−	−	$+^{w}$	O	
(7)	B+O	−	$+^{3-4}$	$+^{2-4}$	B	−	+	+	B	−	+	+	B	
(8)	B+O	−	$+^{3-4}$	$+^{3-4}$	B	−	+	$+^{s}$	B	−	+	+	B	
(9)	cloth touched by A Se	−	−	−	neg	−	−	−	neg	−	+	−	B	
(10)		−	−	−	neg	−	−	−	neg	−	−	−	neg	
(11)	A Ext	$+^{2}$	−	$+^{1}$	A	−	−	−	neg	−	−	−	neg	
(12)	A Ext	$+^{1}$	−	−	A	−	−	+	O	−	−	−	neg	
(13)	A Ext stronger	$+^{3}$	−	$+^{1-3}$	A	$+^{s}$	−	+	A	+	−	+	A	
(14)	A Ext	$+^{2-3}$	−	$+^{1-3}$	A	$+^{w}$	−	+	A	−	−	+	O	
(15)	B Ext	−	−	−	neg	−	−	−	neg	−	−	−	neg	
(16)	B Ext	−	−	$+^{1}$	O	−	−	−	neg	−	−	−	neg	
(17)	B Ext stronger	−	$+^{3}$	$+^{3}$	B	−	+	+	B	−	+	+	B	
(18)	B Ext	−	$+^{3}$	$+^{3-4}$	B	−	?	+	?	−	?	+	?	
(19)	AB Ext	$+^{3}$	$+^{4}$	−	AB	+	+	−	AB	?	+	−	?	
(20)	AB Ext	$+^{2}$	$+^{1}$	$+^{1}$	AB	+	−	−	A	+	−	−	A	
(21)	AB Ext stronger	$+^{1}$	$+^{4}$	−	AB	$+^{w}$	$+^{s}$	−	AB	$+^{w}$	$+^{s}$	−	AB	
(22)	AB Ext	$+^{2}$	$+^{4}$	$+^{1}$	AB	$+^{w}$	$+^{s}$	−	AB	$+^{w}$	$+^{m}$	−	AB	
(23)	O Ext	−	−	$+^{1}$	O	−	−	+	O	−	−	$+^{w}$	O	
(24)	O Ext	−	−	−	neg	−	−	Dry	−	−	−	Dry	−	
(25)	O Ext stronger	−	−	−	neg	−	−	−	neg	−	−	−	neg	
(26)	O Ext	−	−	−	neg	−	−	−	neg	−	−	−	neg	

MJ 7x Mag

Read 1-27-77 Joan 7x mag

	MJ A	MJ B	MJ H	MJ 7x Mag	Read A	Read B	Read H	Joan 7x mag
1	−	+	−	B	−	$+^{w}$	−	B
2	−	+	−	B	−	$+^{w}$	−	B
3	$+^{w}$	$+^{s}$	−	AB?	−	$+^{s}$	−	B
4	−	+	$+^{w}$	B	−	$+^{M}$	$+^{w}$	B
5	−	−	$+^{w}$	O	−	−	−	neg
6	−	−	+	O	−	−	$+^{w}$	O
7	−	+	+	B	−	+	+	B
8	−	+	$+^{s}$	B	−	+	+	B+O
9	−	−	−	neg	−	+	−	B−
10	−	−	−	neg	−	−	−	neg
11	−	−	−	neg	forget it	−	−	neg
12	−	−	+	O	−	−	−	neg
13	$+^{s}$	−	+	A	+	−	+	A
14	$+^{w}$	−	+	A	−	−	+	O
15	−	−	−	neg	−	−	−	neg
16	−	−	−	neg	−	−	−	neg
17	−	+	+	B	M-20 dry results just −	+	+	B
18	−	?	+	?	−	?	+	?
19	+	+	−	AB	? −	+	−	?
20	+	−	−	A	+	−	−	A
21	$+^{w}$	$+^{s}$	−	AB	$+^{w}$	$+^{s}$	−	AB
22	$+^{w}$	$+^{s}$	−	AB	$+^{w}$	$+^{M}$	−	AB
23	−	−	$+^{w}$	O	−	−	$+^{w}$	O
24	−	−	Dried	Dry	−	−	Dried	−
25	−	−	−	neg	−	−	−	neg
26	−	−	−	neg	−	−	−	neg

Even #'s w/ Bromelin
Odd #'s w/out Bromelin

30 x mag

20 min readings
~~30 - 1 hr readings~~

	76-10087 Type AB ② L. Hand Insuff.		76-10087 Type AB ② R. Hand		A + O Saline & cells (weak)		B + O Saline & cells (weak)		clot touched by A Se		A ext. weakest		A ext. stronger		B ext. Weakest		B ext stronger		AB ext. weak		AB ext stronger		O ext weakest		O ext stronger	
	①	②	③	④	⑤	⑥	⑦	⑧	⑨	⑩	⑪	⑫	⑬	⑭	⑮	⑯	⑰	⑱	⑲	⑳	㉑	㉒	㉓	㉔	㉕	㉖
BENZIDINE TEST																										
PHENOPHTHALEIN TEST																										
PRECIPITIN TEST																										
A - C	−	−	−	−	−	1+ −	−	−	1½+	−	+2	+1	+3	+2 3	−	−	−	−	+3	+2	+1 3	1+ 2 3	−	−	−	−
B - D	+1	+1-2	+2 3	+2	−	−	+3 4	+3-4	−	−	−	−	−	−	−	+	+3	+3	+4	+2	+4	+4	−	−	−	−
H - E	−	−	−	−	+1	+1 3	+1-2 4	+3 4	−	−	+1	−	+13	+1 (3)	+1	+1	+3	+3	−	+1	−	+1	+1	−	−	−
M - c																										
N - e																										
CONCLUSIONS FROM ABOVE	B	B	B	B	O	O	B w/ H	B w/ H	OK	OK	A	A	A	A	Neg	O	B	B	AB	AB	AB	AB	O	Neg	Neg	Neg
A - C																										
B - D																										
H - E																										
M - c																										
N - e																										

Samples were O cells were bromelated. 1/27/77

	30x mag. Gina	7x mag. MJ	7x mag Joan	
1. AB	B	B	B	missed A
(2.) AB	B	B	B	
3. AB	~~B~~ B*	AB?	B	
(4) AB	B	B	B	
5. A+O	O	O	O	missed A
(6.) A+O	O	O	—	
7. B+O	B (Hpos)	B (Hpos)	B (Hpos)	OK
(8) B+O	B (Hpos)	B (Hpos)	B (Hpos)	OK
9. Cloth touched	neg	neg	B	false agg B ? JF
(10.) By A Se	neg	neg	neg	OK
11. A Ext.	A	neg	neg	missed A MJ JF
(12) A Ext.	A	O*	neg	missed A MJ Brom. Hpos→O missed A JF
13. A Ext	A	A	A	OK
(14) A Ext	A	A	A	OK
15. B Ext	neg	neg	neg	missed B all
(16.) B Ext	O*	neg	neg	missed B Brom Hpos→O Gina
17. B Ext	B	B	B	OK
(18.) B Ext	B	—	—	OK
19. AB Ext	AB	AB	—	OK
(20.) AB Ext	AB	A	A	missed B MJ JF
21. AB Ext	AB	AB	AB	OK
(22) AB Ext	AB	AB	AB	OK
23 O Ext	O	O	O	OK
(24) O Ext	~~neg~~	—	—	OK
25. O Ext	neg	neg	neg	OK
(26.) O Ext	neg	neg	neg	OK

* samples where bromelation lead to a false pos. O type.

Testing the use of formulated cells in Absorption Elution Typing of Dry Bloodstains

Bromelin is an enzyme derived from the pineapple stem which is used to increase the sensitivity of indicator cells. This experiment was set up to test the necessity of bromelation of the O indicator cells which is presently carried out in this lab.

Weak samples of known blood types were set up (A, B, AB and O). Also weak mixtures of A and O cells, and B and O cells were tested.

The procedure was as described in attached sheet.

Results

Readings were made using a stereo microscope at 30x and 7x magnification (7x is the usual magnification used to read samples). Results are in the two sheets provided.

Conclusions

Bromelation of O cells led to a false positive Type O result in only one case at 30x and only one case (a different one) at 7x Magnification. No true type O readings were picked up with bromelated O cells that were not picked up with nonbromelated O cells.

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From the results of this experiment it is proven that bromelation of the O cells with the Anti-H currently in use is not necessary.

It should also be noted

that there is great danger of incorrect reading of weak samples if we read too closely. Under the current conditions, any answer other than an A or AB on weak samples is suspect. It is the opinion of this experimenter that it would be better to call results inconclusive on a sample insufficient than to risk reporting an incorrect blood type.

Regina K. Dumas
February 4, 1977

The Examination And Typing Of Bloodstains In the Crime Laboratory - Brian Culliford

1.4 PRECIPITIN REACTIONS

1.4.1 INTRODUCTION

Since early this century it has been known that it is possible to distinguish the blood of different animals from one another by means of specific precipitating antisera. Before proceeding to group bloodstains in forensic work it is essential to know if the blood is of human origin. On some occasions blood of other animals may be sought and reliable identification methods for this also are required.

The basic technique of bringing into close reacting contact an extract of a bloodstain and suitable antisera has remained the same since the test was originally described. The means of accomplishing this has, over many years, had many variations and only the major ones will be mentioned.

1.4.2 ANTISERA

Whichever method is being used, the whole technique relies on the quality of the antisera used. An antiserum is a biological product and is, as a result, unpredictable in its manufacture. It may turn out to be univalent or polyvalent. If polyvalent serum is required, the antibodies present in different preparations will vary not only between different species but also from batch to batch. The titre of a specific antibody will also vary from one batch of antiserum to another even from the same animal.

There are two basic forms of precipitating antisera depending on the animal in which they are prepared. Those antisera prepared in horses are known as H type and those prepared in rabbits, sheep, goats, etc., are known as R type. Those prepared in chickens are generally of the R type but have certain peculiarities of their own, requiring higher salt concentrations to give good precipitates.

H type antisera produce a flocculent precipitate which is soluble in excess antigen or antibody and, therefore, requires a narrow range of antigen/antibody concentrations. The R type antiserum produces a much more stable precipitate which is not soluble in excess antibody and only partially soluble in excess antigen.

Horses tend to produce more antibodies than rabbits and the antisera at equival produce neater, clearer, cleaner lines than the R type antisera. If the maximu number of antigen/antibody reactions is required, then, the H type is the best. however, the concentration and number of antigens is unknown, the R type shou be used.

The shape and position of the precipitin band depends on the concentrations of antibody and antigen and also their relative diffusion coefficients. Coefficients of diffusion cannot be changed but antigen and antibody concentrations can be changed to improve the shape of the arc obtained. This is a matter of trial and experience.

The specificity of sera is a matter that must be of considerable concern. The precise specificities of the antisera in the conditions in which they are used must be known. Commercially available precipitin sera can be relied on to be reasonably specific. However, two problems occur here. First, these antise are not tissue specific and will give a reaction with most tissues from the anim concerned as well as from the blood. Also they will react with blood and tissue from closely related animals. Anticow for example reacts with deer blood. An human serum reacts with certain anthropoid sera.

Secondly, there is the problem of nonspecific precipitation. This occurs when the concentration of the blood extract is too high. For example, the Metropolit Police Laboratory has found that cow blood at a dilution of 1:400 reacts with ce tain batches of antisera against human and sheep. Obviously, when dealing with stains, this problem is obviated by working at dilutions greater than 1:400.

1.4.3 SPECIFIC ANTISERA

Recently, antiserum of special interest to the forensic scientist has become ava able. This is the anti-human semen serum. Unfortunately, several brands of this antiserum that have been tried reacted with high concentrations of blood an saliva and also with vaginal epithelium from some adult donors. It is, therefor not possible to determine by this method whether there is semen on a vaginal sw

tisera at equivalen
. If the maximum
pe is the best. If,
the R type should

centrations of
s. Coefficients
tions can be
tter of trial and

concern. The
ey are used
relied on to be
t, these antisera
from the animal
blood and tissues
deer blood. Anti-

s occurs when
the Metropolitan
reacts with cer-
en dealing with
han 1:400.

as become avail-
eral brands of
ns of blood and
It is, therefore,
on a vaginal swab.

...t tissue and body fluids can be used to provide tissue specific antisera, such as ...tisaliva, antiurine, antivaginal secretion, antiamniotic fluid, and antistomach ...cretion (to identify vomit).

Until these antisera are available commercially, it is possible to tackle the problem in two ways. The first is to use an immuno-electrophoretic technique using the cross-reactivity of a non-specific, anti-blood serum to detect the common proteins between two body fluids. The second method is to absorb the antiserum with the unwanted antigens, for example absorb anti-human semen serum with vaginal secretion, thus removing the cross-reactivity and making it specific for semen. The tests are then made with the suspect material against the absorbed antiserum.

Since the majority of problems are associated with blood rather than any other body fluid or tissue, the routine laboratory procedure for bloodstains will be described and any other problem can be dealt with as a special project. In all normal routine conditions, commercially available antisera are excellent. In some countries, however, specific local problems may well arise where no suitable antiserum is commercially available. In these circumstances some of the possible solutions have been suggested; this does not invalidate the use of the technique but makes all the more important the need to know well and accurately the attributes of all the antisera that are being used.

1.4.4 METHODS

1.4.4.1 Tube Technique

The original technique used in precipitin tests was the tube technique in which a small quantity of the antiserum, centrifuged or filtered clear, is put into a small glass tube about 2 inches by 1/4 inch. The dilute extract of the bloodstain, also centrifuged or filtered clear, is carefully layered over the top of the antiserum without disturbing the interface thus formed. If a reaction occurs, a white precipitate forms at this interface within 20 to 30 minutes.

Several problems arose with this method, the least of these being the quantity of bloodstain extract and antiserum required. A tube of internal diameter of 2 mm was adopted but the liquids still had to be centrifuged and a number of false negative reactions caused by cloudy solutions still occurred. Then, because of the enormous increase in the number of tests being performed, an electrophoretic method was introduced by Culliford (1964).

1.4.4.2 Electrophoretic Method (Crossed over Electrophoresis)

In this method the two reactants are placed in two wells punched in the gel placed close together along the line of electrophoretic movement—the blood extract in the cathodic well and the antiserum in the anodic well. The antigens in the blood extract are serum albumin and alpha and beta globulins, whereas the antibodies in the antiserum are in the gamma globulins. Under electrophoretic conditions, the movement of the gamma globulins is toward the cathode (because of endosmosis) while all other proteins move toward the anode. When appropriate reactants meet in the area between the wells, a precipitate is formed. (See Figure 1-13.)

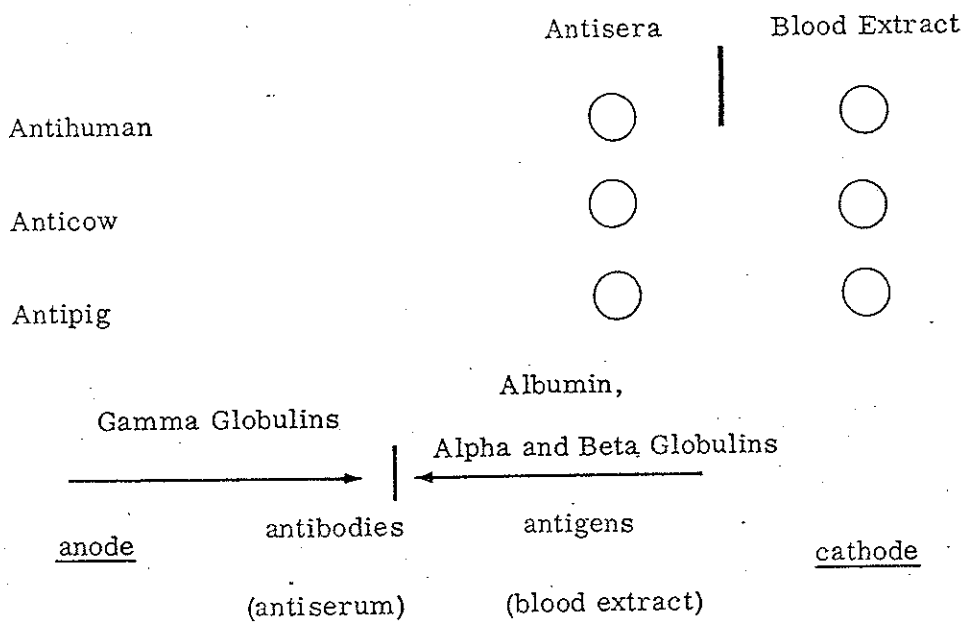

Figure 1-13. The Mechanism of the Electrophoretic Method