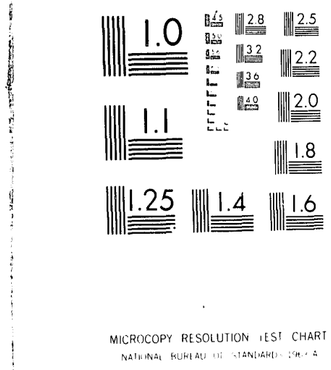
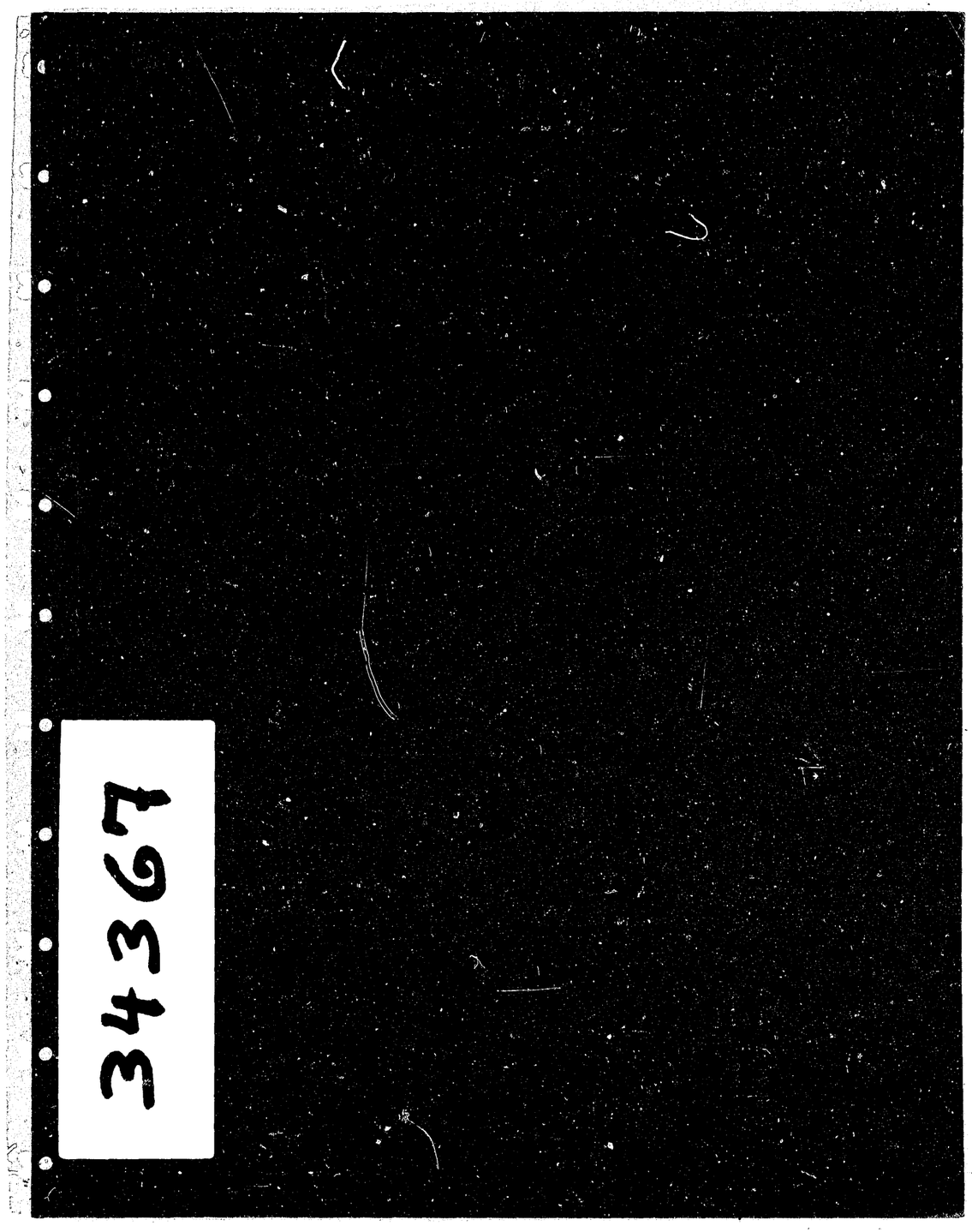




NI-042

FINAL REPORT ON

GRANT NO. NI 042

(Adaptation of Scotland Yard Micro-electrophoresis Identification of Sub-groups in Dried Blood)

I Professor Metzner and Professor Joseph arrived at Scotland Yard October 1st and were immediately put to work in the Biology Laboratory dealing with dried blood. The following blood groups and enzymes were identified for minute quantities of dried blood as follows:

Common ABO System members detectable in dried micro blood quantity

A_1M

A_1N

A_1MN

A_2M

A_2N

A_2MN

A_1BM

A_2BM

A_1BN

A_1BMN

A_2BN

A_2BMN

OM

ON

OMN

ABO	FREQ. %
O	47
A	42
(A_1	34)
(A_2	8)
B	8
AB	3
(A_1B)	1-1/2
(A_2B)	1-1/2

MN	FREQ. %
M	28
MN	50
N	23

GENETIC ENZYMES

PGM	FREQ. %
1	55
2 - 1	37
2	7

AK	FREQ. %
1	93
2 - 1	7

Hp.	FREQ. %
1 - 1	10
2 - 1	56
2 - 2	32
0	2

ADA	FREQ. %
1	90
2 - 1	10

PCE	FREQ. %
U	96
I	4
A	rare
- - - - - - - - - - - - - - - - - - - -	
C_5^-	94
C_5^+	96

6PGD	FREQ. %
A	95
A/C	4
C/C	1

Rh PHENOTYPES

Fisher		English Usage	FREQ. %
R_1	=	CDe	34.89
R_2	=	cDE	2.36
R_0	=	cDe	2.061
R'	=	Cde	0.01
R"	=	cdE	0.14
R_2	=	CDE	0.001
r	=	cde	15.05
R_y	=	CdE	Trace

There are a few other phenotypes for which dried blood techniques have not been developed.

These data represent approximately 40 different genetic blood sub-groups useful in forensic science identification.

Our instructors were Margaret Pereira, department head for ABO and Rh work; Bryan J. Culliford, head of electrophoretic enzyme work; and Bryan Wraxall, assistant to Mr. Culliford.

We were received graciously and had the most professional support during our thirty days in the laboratory. We spent eight hours at the laboratory bench a day.

II GOALS

A. Since the goals of this grant project were to train two professors in micro-dried blood technique, we can say that we have achieved these goals.

B. In order to meet the goals set up for transferring this technology to crime laboratory and medical examiners laboratory personnel, it is necessary to set up a program of training for approximately twenty-one to twenty-four carefully selected personnel from the best crime laboratories in the different geographical areas of the United States.

III PROGRAM IMPACT

A. In order to introduce these techniques to selected personnel, John Jay College of Criminal Justice can make available a bio-chemistry laboratory from August 15th to September 15th, 1970. We have ascertained that there are several inexpensive hotels within five blocks of the college for housing students in this institute on micro techniques in dried blood.

B. Research Aspects. In addition to the transfer of the technology for micro-dried blood genetic identification we would at the same time be training the best people in the country in such a way as to prepare them for carrying on further research on the other known genetic variations in erythrocytes.

C. Furthermore, the same crime laboratory personnel who study these new techniques in the laboratories at John Jay College would be capable of setting up similar courses for training laboratory personnel in their own geographical areas. We would be ready and willing to help them do this and establish the necessary liaison with the universities or medical schools or other crime laboratories in their geographical area to implement such a program.

IV DISSEMINATION

At the time of this course a handbook of the techniques would be available for the students to use in their own laboratories for identification, research, and as a text for use in such courses when they give them in their general geographical area. In the pages that follow in this report we will indicate the general techniques used in the work at Scotland Yard Laboratory. This report does not intend that the listing of techniques for different genetic variations in

dried blood be in such shape that it can be now used as a laboratory manual. However, such a laboratory manual will be prepared for the proposed summer institute in dried blood micro identification of sub-groups.

V PERSONNEL

Personnel for the proposed institute at John Jay College from August 15th to September 15th, 1970 would include Professor Joseph and Professor Metzner who have been at Scotland Yard and a third professor, Bryan Culliford, on the college payroll to be brought in from Scotland Yard Laboratory. Bryan Culliford, was our principal instructor at Scotland Yard Laboratory. He has agreed to come as a visiting professor to help us give the course at the proposed summer institute, August 15th to September 15th, 1970. With three professors we will be able to maintain a ratio in the laboratory of seven to eight students per professor. The plan would be six hours of laboratory work per day plus one to two hours of theory. If the background in blood is very strong in the incoming group, we will be able to dispense with certain basic aspects of the theoretical background. In addition, we will prepare reading references for the prospectiv-students several months before they come to the course.

We feel that we can cover the ABO MN, enzymes, and Rh by manual methods since few or no laboratories in the United States are equipped with the automatic Rh analyzer, we would not teach that technique. However, the manual technique is identical to the automatic and costs very very little.

VI RESOURCES

We have purchased $1,400 worth of equipment suitable for two to three students. We will also provide the necessary UV Spectrophotometer, refrigerators, pumps, constant temperature ovens, the only items we will need support for are enough of chemicals, anti sera, and electrophoretic equipment. We will supply one medical microscope for each student in the laboratory. The laboratory has thirty-six stations, however, by operating on a level of twenty-one to twenty-four students, we will give each of the students greater space to work in. We would need support for nine to eleven additional electrophoresis equipment and power supplies.

FINAL REPORT GRANT NI -69-042

Chapter I

The Individualization by Micro-Analysis of Genetic Sub-Groups In Old Dried Blood

United States Procedures

In the United States of America, Crime Laboratories and Medical Examiners Laboratories that process dried blood for identification purposes are limited in their procedures as follows:

1. Sufficient quantities of dried blood must be available from the victim or culprit.

2. The dried blood must not be more than two weeks old.

3. Identification is limited usually to ABO and occasionally M and N groups. In most laboratories processing for M and N is rarely done. Most laboratories will not process dried blood for ABO. If ABO dried blood is processed, where sufficient quantities are available the only individualization provided tells us whether or not a person is or is not a member of A, B, AB, or O groups. This simply does not identify and individualize.

4. It is not possible in the United States at present to identify the twelve Rh phenotypes in micro-quantities of dried blood.

5. It is also not possible to process old dried blood for various genetic enzymes.

6. Present techniques in the United States used routinely in crime laboratories do not work on semi-micro quantities of blood.

7. Lastly, American techniques do not usually work on old blood stains.

For the above reasons small quantities of dried blood are considered to difficult to process.

It should be pointed out that hematology done in Medical Examiners Laboratories usually involves wet blood. However, micro-quantities of old dried blood are rarely if ever processed for individualization.

Scotland Yard Laboratories Procedures

On the other hand, the Metropolitan Police Forensic Science Laboratory (Scotland Yard Laboratory) London and all the other eight crime laboratories in England all routinely process minute quantities of old dried blood for approximately forty different genetic sub-groups.

This work was developed by Margaret Pereira and Bryan J. Culliford. They have trained 19 year old technicians in the various micro-dried blood technique.

The research required for developing these techniques took 14 years. However, today they are standard laboratory routines. The techniques are called elution-absorption which means extraction and concentration. The enzymes individualization techniques all depend on electrophoresis. The equipment required is relatively simple for both techniques and is already available in more than 50% of the crime laboratories in the United States.

At the Scotland Yard Laboratory and the other English laboratories it is possible to individualize all of the genetic sub-groups for A_1, A_2, B, O, M, N, system as follows:

Common ABO system members detectable in dried micro blood quantity

A_1M, A_1N, A_1MN, A_2M, A_2N, A_2MN, A_1BM, A_2BM, A_1BN, A_1BMN A_2BN, A_2BMN, OM, ON, OMN, BM. BN, BMN.

(NOTE: A_1 A_2 are easily distinguished by the elution-absorption technique).

The probability for any individual for the genetic blood factors involved are found by simply multiplying the frequency for each factor detected for a person whose old dried blood is being examined.

Frequency Distribution ABO MN (Great Britian Population)

ABO	FREQ. %
O	47
A	42
(A_1	34)
(A_2	8)
B	8
AB	3
(A_1B)	1-1/2
(A_2B)	1-1/2

MN	FREQ. %
M	28
MN	50
N	23

Thus for an individual whose blood is A_1 BM we simply multiply the frequency distribution for A_1 B by the frequency distribution for M. Thus: .015 x .28 = .00420.

Next we look at the Rh factor in use in England. The listing below, which are the factors which can be detected on old dried blood are called the phenotypes. There are 10 Rh^+ (plus) and 2 Rh^-(negative). The frequency distribution is shown on the next chart. The Rh^-(negative) each have a frequency distribution of .01 or 1%.

Rh Phenotypes Fisher		English Usage	Freq. %
R_1	=	CDe	34.89
R_2	=	cDE	2.36
R_0	=	cDe	2.061
R'	=	Cde	0.01
R"	=	cdE	0.14
R_2	=	CDE	0.001
r	=	cde	15.05
R_y	=	CdE	Trace

If a person is A_1 BM and has an Rh phenotype of R_2 which has a frequency of .001, he is:

.00420 x .001 = .00000420

This individual is one out of approximately four million people. If this procedure does not provide sufficient individualization then a third routine series of tests for detecting red blood cell enzymes by electrophoresis is used.

(LEAA Research Project 013 by Joseph in 1967 showed that about 50% of the Crime Laboratories in the United States have this electrophoresis equipment).

Electrophoresis is used to identify the following genetic enzymes found in blood. The frequency distribution is found below:

AK	FREQ. %
1	.93
2 - 1	.07

ADA	FREQ. %
1	.90
2 - 1	.10

Hp.	FREQ. %
1 - 1	.10
2 - 1	.56
2 - 2	.32
0	.02

PCE	FREQ. %
U	.96
I	.04
A	rare
C_5^-	94
C_5^+	96

6PGD	Freq.
A	.95
A/C	.04
C/C	.01

The genetic enzymes from micro-quantities of old dried blood provide additional probability information for individualization. If we go back to the hypothetical person who is A_1BM, R_2 and we find that this person PGM has a frequency distribution of .07 multiply his frequency distribution to get:

$$.00000420 \times .07 = .0000000294$$

or one in 2.94 times 10^8. This is almost one in 300 million. Of course if the frequency distribution for individuals are larger than the probability would go down. However, the probability can be further increased if other genetic enzymes are present.

Theoretically it would be possible to approach the probability found in identification by fingerprints.

This is indeed a new powerful tool providing dried blood of the victim or culprit is available. The identification stain size for everything but Rh has been successful on dried blood stains as small as 1/8" in diameter. However, for Rh you need a blood stain on cloth about 1/4" by 1/2".

As previously mentioned the techniques are relatively simple and the equipment is standard. The equipment and chemicals required are found in the appendix of this report.

Furthermore, technicians can be trained to do this work in about four weeks of instruction.

We finally believe that the micro techniques of elution absorption and electrophoresis for dried blood provides a powerful tool for individualization from very small quantities of very old dried blood. Of course all of the techniques also work with small quantities of wet blood if available. Obviously the older the stain the more difficult the procedure. In spite of the belief by many workers in the United States that old dried blood stains including buried stains are suppose to undergo degradation and bacterial action, in England the routine techniques described are successful on old blood stains. One additional techniques distinguishes human fetal blood from adult blood by means of the ultra-violet absorption characteristics.

The next section of this report illustrates typical laboratory procedures that may be applied in our own crime laboratories for various genetic blood sub-groups listed thus far in this report. These are the procedures employed at the Scotland Yard Laboratory.

CONCLUSIONS

A. The goals of this grant project were to train two professors in micro-dried blood technique, we can say that we have achieved these goals.

B. In order to meet the goals set up for transferring this technology to crime laboratory and medical examiners laboratory personnel, it is necessary to set up a program of training for approximately twenty carefully selected personnel from the best crime laboratories in the different geographical areas of the United States. It is hoped that these personnel will in turn disseminate these techniques in their own geographical areas.

C. In order to introduce these techniques to selected personnel, John Jay College of Criminal Justice has made available a bio-chemistry laboratory from August 15th to September 15th, 1970. We have ascertained that there are several inexpensive hotels within five blocks of the college for housing students in this institute on micro techniques in dried blood.

D. Research Aspects. In addition to the transfer of the technology for micro-dired blood genetic identification we would at the same time be training the best people in the country in such a way as to prepare them for carrying on further research on the other known genetic variations in erythrocytes.

Charles Kingston raises several questions about the accuracy of the probability if there should be inter-subgroup blood correlation. Unfortunately, no research has been done on intergroup correlations. This requires a piece of research

On a random sample of blood from several thousand people where individuals of the American Population are tested and any correlations is then detected.

It should be noted that there are more than 287 DNA variations which have been detected in red blood cells. For each of these, there are one or more associated enzymes. Research must be done to find micro-techniques that are simple enough to be used routinely in crime laboratories. This requires the aid of an enzyme chemist. At John Jay we are building a research team to carry on this research beginning Spring 1971.

E. Furthermore, the same crime laboratory personnel who study these new techniques in the laboratories at John Jay College would be capable of setting up similar courses for training laboratory personnel in their own geographical areas. We would be ready and willing to help them do this and establish the necessary liaison with the universities or medical school or other crime laboratories in their geographical area to implement such a program.

After laboratory personnel have been trained in these techniques it may require several months of practice in their own laboratories before they can use them on cases. This will depend upon the time and facilities available.

It is obvious that these British techniques are superior and provide more information than the procedures now followed on micro-techniques for dried blood in the United States.

It is proposed that the National Institute for Law Enforcement support the making of a super-8-millimeter motion picture color film which could be loaned to crime laboratories as a teaching aid.

It is also proposed that a complete laboratory manual be developed and be disseminated to all Crime Laboratories in the United States.

John Jay College of Criminal Justice will be available for consultation by telephone or in person, where distances permit. John Jay College plans to offer a course in these techniques as part of the preparation as the B.S. and M.S. in Forensic Science. It also intends to offer special short course for Crime Laboratory personnel if the demand developes. It is not expected that the implementation in laboratories of the new semi-micro dried blood techniques will be adopted overnight. It may take one to three years. It is hoped that the courses that will be offered by the National Institute of Law Enforcement at John Jay College of Criminal Justice during August 1970 will be the first step in the dissemination of these new powerful techniques.

Determiniation of Haptoglobins in serum by thick starch gel electrophoretic separation

Background:

Haptoglobins are proteins having a strong affinity for hemoglobin.

For practical purposes three groups are used as the basis for most identification: 1-1 2-1 2-2. These constitute about 98% of the population (British) in the following distribution: 1-1 (10%), 2-1 (56%), 2-2 (32%).

Materials: See "Determination of Haptoglobin Groups in Serum."

Tank Buffer

pH - 7.9

NaOH	2.0 gms
Boric Acid	18.6 gms
H_2O dist.	1 liter

Gel Buffer

pH - 8.65

Tris	9.196 gms
Citric Acid	1.05 gms
H_2O dist	1 liter

Whatman 3 MM inserts 5x7 mm. for taking serum samples.

Staining

O-dianisidine or orthotoluidine or benzidine.

Determination of Haptoglobin Groups in Serum

1. Preparation of Plate

A 9" x 6" glass plate is made into a mould by sticking two layers of $\frac{1}{4}$" wide glass strips around the perimeter of the upper surface with M.S.4 silicone grease.

2. Buffers.

Tanks pH 7.9

Sodium hydroxide	2.0 gms.
Boric Acid	18.6 gms.
Distilled Water	1 litre

Gel pH 8.65

Tris	9.196 gms.
Citric Acid	1.05 gms.
Distilled Water	1 litre

3. Preparation of Gel

180 ml of gel buffer is placed in a 1 litre conical flask, and to this is added an appropriate quantity of hydrolysed starch (Connaught Laboratories). The quantity is determined by the amount quoted on the label of the particular batch of starch being used. It is usually between 10 and 12 gms per 100 ml. This mixture is heated over a bunsen with continual shaking. It will become thick and agitation is essential at this point. Subsequently it will become clear and then thinner when boiling will commence. After very brief boiling, a few seconds, remove from the heat and continue agitation by shaking.

De-gassing is effected by connecting the conical flask to a water pump until the mixture boils and becomes quite clear and bubble-free. It is then poured into the mould which has been made as described in Section 1. This mould should be placed on a level surface or a levelling table prior to pouring the starch gel into it.

The mixture is allowed to cool to room temperature and a cover of P.V.C. sheet then put over it.

4. Samples

Blood samples are centrifuged and the serum obtained. A small quantity of haemoglobin "A" must be added to these. If lysis has occurred, sufficient haemoglobin may be present already. The colour of the sample of serum should be deep pink with haemoglobin.

5. Application of Samples

The P.V.C. cover is removed from the gel and a straight edge, such as ruler, placed across the gel parallel to one of the short sides, $2\frac{1}{2}$" from the cathode end. A 7 mm wide piece of razor blade is then inserted vertically into the gel towards the top of the plate. A piece of 3 mm Whatman filter paper approximately 5 x 7 mms is soaked in the serum sample. This is then inserted vertically down the side of the piece of razor blade so that it enters the gel. The razor blade is then withdrawn and a short distance away the next slot is made in the gel and the next sample inserted.

Using these sample sizes the gap between samples should be about ½ cm. When the plate has been filled with samples in this manner it can be transferred to the appropriate tank or tanks.

6. The Run

The gel is connected to the tanks, which have been filled with tank buffer, by means of 6 thicknesses of 3 MM filter paper running the full width of the gel. These should overlap the gel by approximately 1 cm. The P.V.C. cover is replaced over the gel. The power pack, connected to the tanks is then switched on and adjusted to a constant voltage of 170 volts. This is the correct voltage when running in a cold room at 4°C. If the run is being made at room temperature, the voltage should be approximately 120 volts. The run is overnight for 15 - 17 hours.

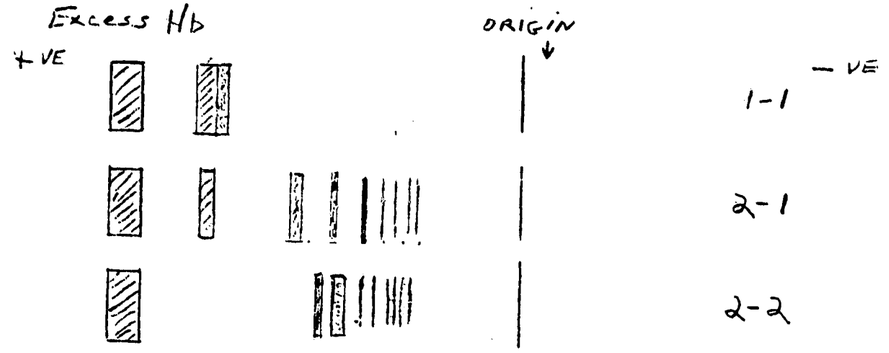
7. Developing the Gel.

The gel is removed from the tanks and the cover is also removed. The filter paper inserts are removed from the gel and one layer of the glass edging also removed. With the aid of a knife or wire the gel is sliced horizontally from the anode to the cathode end, using the single thickness of glass edging as a guide. A piece of filter paper is laid over the surface of the sliced gel and gently easing up one end, the upper half of the gel is removed adhering to the filter paper.

The lower half of the gel is then stained with Orthodianisidine in alcohol acidified with acetic acid, followed by 2% hydrogen peroxide. The bands of haemoglobin and haptoglobin/haemoglobin complex are stained greenish/brown. The gel may be hardened for storage in Alcohol/Acetic/Water in the ratio 50 : 10 : 40.

8. A Final Note

Always record the order in which the samples are placed in the gel. On developing the gel always put the results in the same note-book straight away. These results can then be transferred to the blood sheet on the file.



PREPARATION OF AN AGAR GEL FOR IMMUNOELECTROPHORESIS

Materials Required:

Shandon micro-I.E.A. slide holder

Polythene tape

Eight 3" x 1" glass slides

2% Ionagar gel (stock)

Appropriate buffer

Method

Take eight 3" x 1" glass slides and make sure they all fit into the slide holder. (Slides with unfinished edges are generally too big). Remove slides and mark the appropriate G- number on them with a diamond. Check that there is polythene tape on the back of the slide holder covering the 8 holes. Fill these holes to just over the brim with water and carefully lay the slides in the holder being careful not to get any air trapped under them. Wipe off excess water, and, after checking with a spirit level place on levelling table.

Weigh out 25 grams of stock 2% Ion agar and mix with 25 mls of appropriate buffer giving a final concentration of a 1% gel. Heat slowly over a bunsen and bring slowly to the boil with continuous agitation. Make sure that all the

lumps of gel have dissolved, remove from flame and cool under cold running water. Allow temperature to drop to 45°C., rapidly pour onto the slides and manipulate gel so that it evenly covers the slide holder. Allow to set and store in moist chamber.

System Serum cholinesterase

Method Starch gel electrophoresis

Tank or bridge buffer

Sodium hydroxide	M
Citric acid	0.41M

pH 6

Diluted 1 in 8 for use.

Gel buffer

Succinic acid	0.01M
Tris	0.0184M

pH 6

Sample

Serum on Whatman No.1 inserts.

Conditions of run

30 ma. (constant current) for 17 hours.)

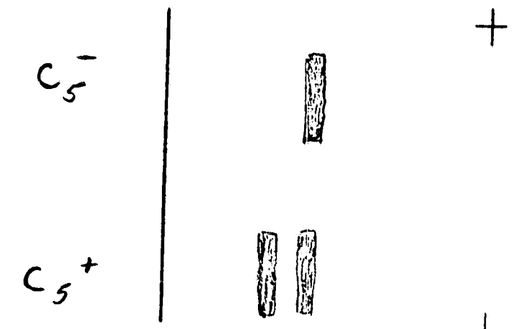
Detection

Reaction mixture:

0.2M Phosphate buffer, pH 7.1	100 ml.
1% α-naphthyl acetate in 50% aqueous acetone	2 ml.
Fast red TR salt	20 mgm.

Pour over sliced gel and develop at room temperature for 2 hours.

Interpretation



Frequencies

C_5^-	95 %
C_5^+	5 %

References

Harris, H., D.A. Hopkinson, Robson, E. and Whittaker, M.
Ann. Hum. Genet., Lond. (1963), 26, 359.

Improved Method for Haptoglobin typing of Bloodstains

Equipment

Shandon Slide Tray

Shandon Kohn Tanks

Power Pack 0 - 60 MA minimum

Shandon Gel Pattern cutter

Materials

2% Ionagar in water

Hirschfeld gel buffer (1) (2) - made up double strength.

Hirschfeld tank buffer (1) (2)

Anti Haptoglobin serum - Dutch Red Cross. Amsterdam.

Preparation of gel

Mix 20 grams of 2% Ionagar in water with 20 mls of gel buffer, melt, cool to 50°C and pour over slide tray containing 8 slides.

When set cut the antigen slots to the pattern as shown in fig. 1. Do not cut antiserum troughs until AFTER the first electrophoretic separation.

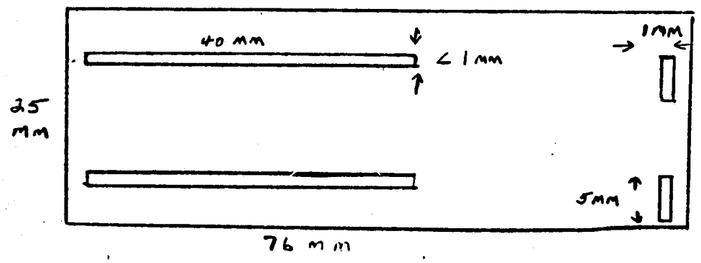


Figure 1

Cut out enough stain material to fill slot and soak in gel buffer for 10 minutes prior to insertion into the gel.

Electrophoresis (1)

160V (7V/cm.) with current 48 Ma. (constant) for 4 to 4½ hours at room temperature. Use gel contact troughs filled with 2% Ionagar in tank buffer.

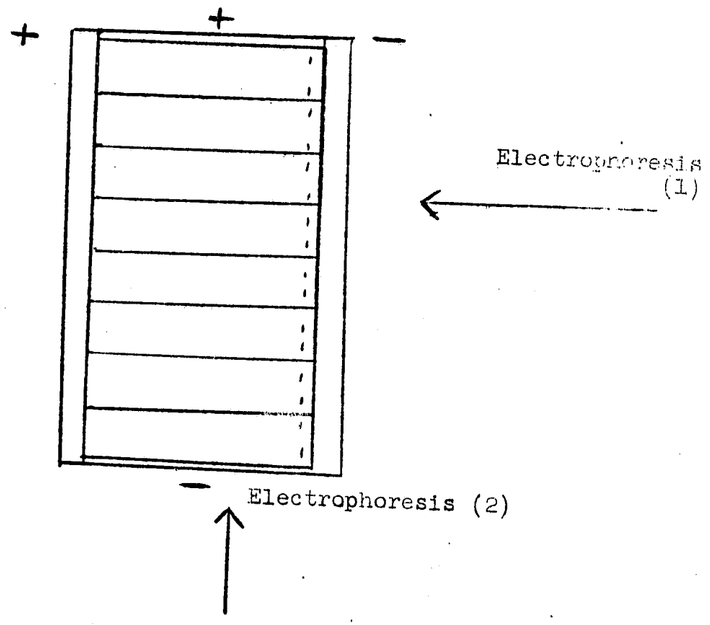
Cut antiserum troughs with Shandon gel cutter and remove waste gel. Fill troughs with anti serum.

Electrophoresis (2)

At right angles to direction of first separation as in Figure 2.

Figure(2)

Tray + Slides



160 V (7V/cm) with constant current 22Ma. for 1 hour at room temperature. Use spontex bridges held in place with a glass plate.

Washing

Place tray in large dish containing 1 M. sodium chloride solution and leave overnight. Place in distilled water for ½ hour before drying.

Remove individual slides, cover with wet Whatman No. 1 and dry at 56°C for ½ hour.

Staining

Stain with Amido Black, differentiate in alcohol/acetic wash.

Time for complete typing - 24 hours.

B.G.D. Wraxall,
Met. Police Laboratory
2, Richbell Place,
Holborn,
London, W.C. 1.

References

(1) Hirschfeld. J. Sc. Tools 7, 2 (1960)

(2) Culliford B.J. & Wraxall B.G.D. Nature 211, 5051 (1966).

Rh - Dry Blood

1. Cut (5) samples of stain from cloth ½" to 1/8".
2. Place in miniature t.t.
3. Add 1 drop appropriate anti-serum to each.

   $\overline{c}$ C Cw D E
4. Add 3 drops saline to each.
5. Cap test tubes - incubate overnight at 37°C.
6. Wash each 10x in saline.

   aspirate saline - add new saline.
7. Finally draw off excess fluid - add 2 full drops of saline.
8. Elute at 60°C - 30 minutes
9. Add appropriate* test cells - cover and incubate at 37°C - 1½ hours.
10. Add 1 large drop 30% bovine albumin. Let drop slide down side of tube forming lager between cells and saline.
11. Reincubate ½ hour at 37°C.
12. Read drops under microscope for agglutinotion.

*Appropriate test cells - blood from person having anti $\overline{c}$ ...

For C use R_1R_1 person

D use R_2R_2

$\overline{e}$ use rr

Cw has to be found.

System Gc Groups

Method Agar Gel Immunoelectrophoresis

Tank or Bridge Buffer - Hirschfeld.

Diethyl Barbituric Acid	2.76 gm
Sodium Veronal	17.52 gm
Calcium Lactate	0.768 gm
Distilled Water	2.1

pH 8.6

Gel Buffer

Diethyl Barbituric Acid	1.106 gm
Sodium Veronal	7.006 gm
Calicum Lactate	1.024 gm
Distilled Water	2 l gm

pH 8.6

Gel

1% agar in gel buffer

Poured on microscope slides in layer 1-2mm thick.

Sample

Serum pipetted into wells punched in gel.

Conditions of run

Electrophoresis:

6-7 V./cm for 2 hours at room temperature.

Immunodiffusion:

Gel trough filled with immune serum.

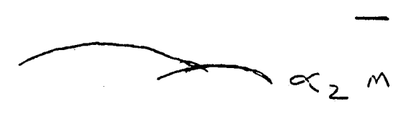
Humidity chamber for 24 hours.

Detection

Precipitates can be viewed directly when illuminated in oblique light against a dark background.

Stain: Amido Black or Ponceau S.

Interpretation



Gc 1-1

Gc 2-1

Gc 2-2

Frequencies

Gc 1-1	54%
Gc 2-1	39%
Gc 2-2	7%
Genes Gc^X Gc^Y G^Z	Very rare

References

Hirschfeld, J. et al. Nature, Lond. 185: 931-932 (19[illegible]

Hirschfeld, J. Thesis. Progr. Allergy 6: 155-186 (19[illegible]

System Adenylate kinase

Method Starch gel electrophoresis

Tank or Bridge Buffer

Citric Acid 0.41M 86.1 g } adjust pH to 7.0
aq. dist 1 l }

Adjusted to pH 7 with sodium hydroxide

Gel Buffer

Histidine 0.005M 0.79 gm./l. Aq. dist.

adjusted to pH 7 with sodium hydroxide

Sample Red cell lysates on Whatman No. 3 inserts.

Conditions of run

10V./cm for 4 hours at room temperature, using metal cooling plates with circulating tap water.

Detection

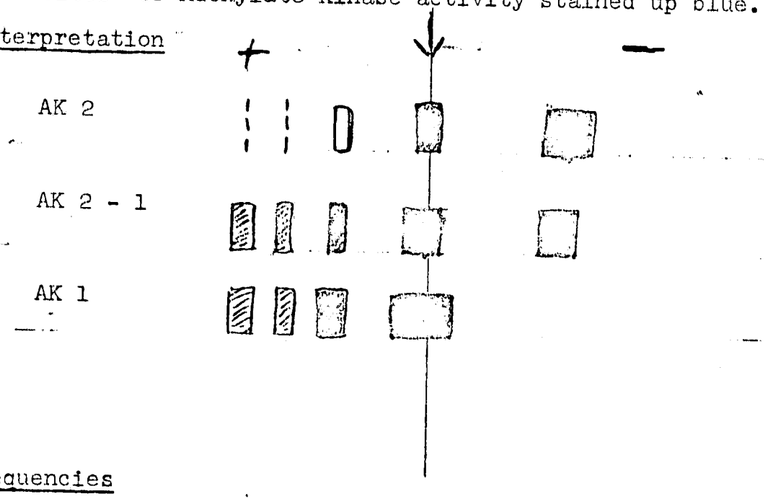
The gel was sliced and covered with a 0.75% agar solution at 45°C, made in:

10 ml	0.1M Tris buffer, pH 8.0, containing;	
18 mgm	Glucose	10 mM
40 mgm	Magnesium Chloride	20 mM
4.9 mgm	Adenosine diphosphate	1.0 mM
3.1 mgm	Nicotinamide adenine dinucleotide phosphate	0.4 mM
1 mg	(a pinch) Phenazine methosulphate	0.012%
1 mg	(a pinch) Tetrazolium salt MTT	0.012%
1 drop	Glucose -6- Phosphate dehydrogenase	0.04 units/ml
1 drop	Hexokinase	0.08 units/ml

Agar overlay was allowed to set and incubated at 37° for 2 hours.

Sites of Adenylate Kinase activity stained up blue.

Interpretation



Frequencies

AK 1	90%
AK 2 - 1	10%
AK 2	Very rare

References

Fildes, R.A. and Harris, H. Nature, 209, 262 (1966)

APPENDIX D

PRECIPITIN REACTION

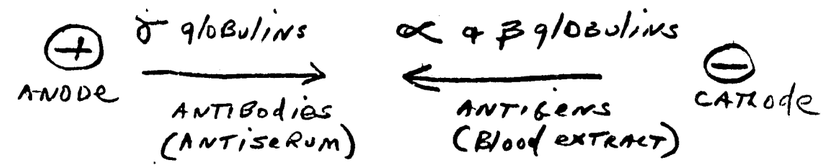
Reaction

This reaction enables the species origin of blood and other body fluids to be determined.

A series of specific antisera are reacted with extracts of material under examination and the formation of a precipitate indicates the site of an antigen and body reactions.

A gel electrophoretic method is used in this laboratory. 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 anti-serum in the anodic well. The antigens in the blood extract are serum albumin and α and β globulins whereas the anti-bodies in the anti-serum are in the γ globulins. Under the conditions employed, when the electrophoresis is performed, the movement of the γ -globulins is toward the cathode, all other proteins move towards the anode. When appropriate reactans meet in the area between the wells a precipitate is formed.

	ANTI. SERA		BLOOD EXTRACT
ANTI HUMAN	O	I	O
" COW	O		O
" PIG	O		O



Apparatus and Reagents

Buffer solutions (Hirschfeld)

Tank buffer

Sodium barbiturate (Veronal)	17.52 g
Diethyl barbituric acid	2.76 g
Calcium lactate	0.77 g
Distilled water	2000 g

pH 8.6

Gel Buffer

Sodium barbiturate (Veronal)	14.012 g
Diethyl barbituric acid	2.76 g
Calcium lactate	2.05 g
Distilled water	2000 g

pH 8.6

Electrophoresis

Tank

1. Make bridges from 3 mm Whatman paper approx. 11½" x 3 3/4" folded in half longitudinally.
2. Add tank buffer until troughs are three quarters full and level. Levelling is achieved by tilting the tank towards the back until the buffer in all sections flows together and then lowering the tank level again.
3. Add minute amount of merthiolate (fungicide) to each trough. (Also known as Thiomersal).

Plates

3" x 2" or 3" x 1" glass microscope slides.

1. Clean with RBS 25 (detergent).
2. Rinse and polish dry.
3. Number slide with glass marking ink in upper right hand corner. This should be consecutive with the previous plate.

Gels

2% stock

Ion agar	10 g
Distilled water	500 ml

1. Mix in cold then bring to boil stirring constantly. Ensure that all crystals are dissolved.
2. Pour into sandwich boxes and store in refrigerator.

0.2% coating

2% stock	10 g
Distilled water	100 ml

Heat and stir until homogeneous mixture obtained.

1% Gel

2% stock	50 g
Gel buffer	50 ml

1. Heat and stir until homogeneous mixture obtained.
2. Pour 7 ml quantities into sterilised test tubes.
3.1 Allow to cool and store corked tubes in racks.

Stain (Amido black)

Naphthalene black 10 B	0.1 g
Methyl alcohol	50 ml
Glacial acetic acid	10 ml
Distilled water	50 ml

Filter stain after making up.

Wash solution

Methyl alcohol	1000 ml
Glacial acetic acid	200 ml
Distilled water	1000 ml

When wash solution becomes discoloured clean by mixing with activated charcoal, (15 - 20 g charcoal/2 litres wash solution), and filtering the mixture. Discard charcoal residue.

Method

1. Melt appropriate quantity of 0.2% agar on water bath.
2. Coat top surface of plate 3" x 2" or 3" x 1" by brushing on lightly with soft haired brush. Wipe edges with tissue and allow coating to set. Normally the preparation of the plates as far as this is done in bulk and they are stored ready numbered and coated. Allow to dry.
3. Melt 7 ml 1% gel (ionagar) in gel buffer. (use water bath and loosen the cork).

4. Place coated plate on horizontal surface with plate number in top right, and pour the melted contents of the test tube onto top surface of plate.
5. Cover with a moisture chamber (sandwich box top) and allow to cool.
6. Place plate on plastic holder, adjust punch over plate and depress punch.
7. Remove plugs of agar from each hole by means of vacuum pipette.
8. Prepare an extract of the blood stained material by adding 1 drop of 50% gel buffer to small quantity of material in a "Lister" tube and prod with a glass rod. With bloodstained material the extract should appear pale "straw" colour, if too strong dilute with 50% buffer.
9. By means of a fine pipette fill three right hand holes of a bank of six with extract (see diagram.).
10. Fill three left hand holes with appropriate antisera, i.e., Anti human + two controls (see diagram). Each bank of six holes represents a single determination.
11. Invert slide onto tank bridges. The inverted number should appear at the bottom right hand side of the plate. The stain extract being nearest to the -ve pole and the antisera nearest to the +ve pole. If inverted the wrong way the reactants will be driven apart, not together.

12. Switch on power pack 100V - 150 v and run for 10-20 minutes.
13. Read plate tiwh the aid of a bench lamp. A fine line of white precipitate in the area between two holes of a pair indicates a positive reaction.
14. Record result in precipitin book: -

Plate No.	Date of run	Signature
e.g. E/784	12-8-66	Brian Smith

Case No. M/126/66

	Jacket front	Jacket back	Trousers leg	Shirt collar
Anti human	+ve	+ve	+ve	+ve
Anti cat	-ve	-ve	-ve	-ve
Anti dog	-ve	-ve	-ve	-ve

Case No: R/196/66 Case No. R/112/66

	Skirt inside	Skirt Outside	Knickers	Skirt
Anti human	-ve	+ve	+ve	+ve
Anti cat	-ve	-ve	-ve	-ve
Anti dog	-ve	-ve	-ve	-ve

15. Place plate in saline (physiological or molar if speed is necessary) merthiolate (fungicide) for 24 hours. Saline removes excess proteins on plate those not involved in the reaction.

16. Transfer to distilled water bath containing 0.01% merthiolate for not less than 2 hours (24 hours is recommended).
17. Remove plate from water, cover surface of gel with piece of damp No. 50 or 54 Whatman filter paper and leave overnight to dry.
18. Remove paper, wash plate under running tap while gently rubbing gel surface with fingers to loosen fragments of adherent paper.
19. Stain in amido black for ten minutes.
20. Transfer to wash solution, examine periodically and remove when background is clear and precipitin bands are clearly stained deep blue/black.
21. Allow plates to dry.
22. Store in cabinet in electrophoresis section on 4th floor.

DIAGRAM SHOWING LAYOUT OF PRECIPITIN PLATE

(Actual Size)

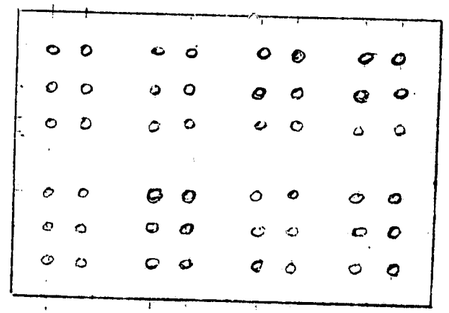
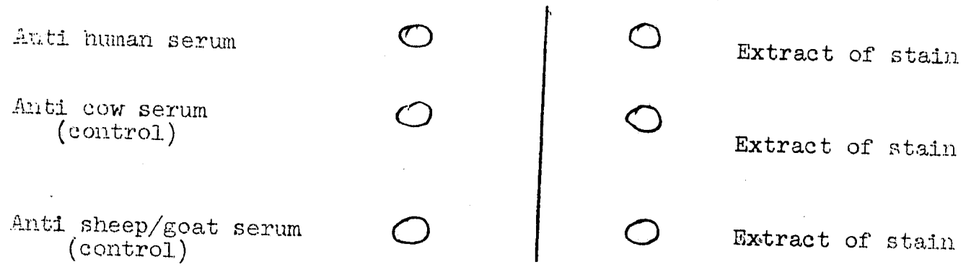


DIAGRAM SHOWING SINGLE BANK OF SIX HOLES
(Much Enlarged)



FLUORESCENCE MICROSCOPE METHOD
FOR DETECTION OF SPERMATOZOA

Reagents

1. Buffer solution.
   Mix 1/15M. KH_2PO_4 and 1/15M. Na_2HPO_4 to give a solution of pH 6.0 approximately.
2. Acridine Orange solution
   0.01% in buffer solution (1).
3. Differentiating solution.
   Aqueous 0.1M $CaCl_2$.

Method

1. Air dry and heat fix slides in usual way.
2. Place in A.O. solution for 4 minutes.
3. Drain briefly onto filter paper and pass into buffer solution for 1 minute.
4. Drain onto filter paper and pass into differentiating solution for 3 minutes.
5. Rinse in buffer solution and mount in this liquid under a coverslip.
6. Examine under fluorescence microscope using X40 objective.

System Haptoglobins

Method Starch gel electrophoresis

Tank or Bridge Buffer

Boric Acid	0.3 M	18.6 gm
Sodium hydroxide	0.5 M	2.0 gm / 1 l. aq dist
pH 7.9		

Gel Buffer

Tris	0.076 M	9.136 gm
Citric Acid	0.005 M	1.05 gm / 1 l. aq dist-d
pH 8.65		

Sample

Blood serum on Whatman 3 MM inserts.

Conditions of run

130V for 17 hours at room temperature.

or 170V for 17 hours at 4°C.

Detection

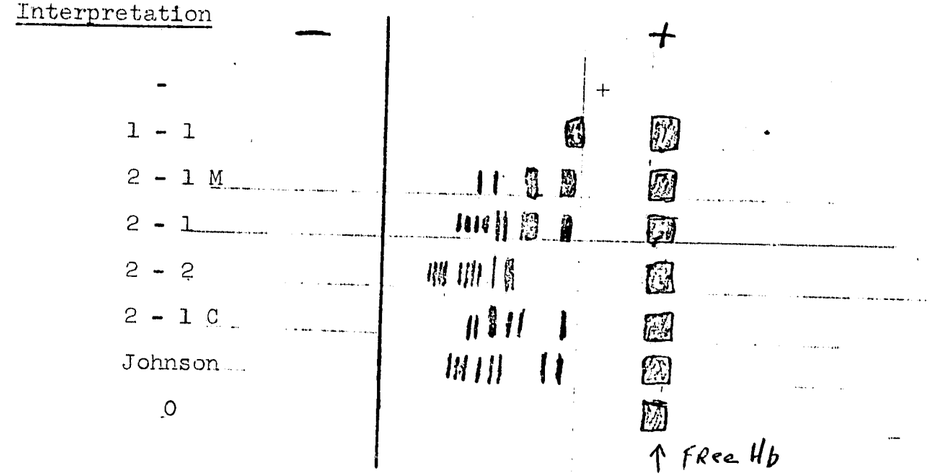
Reaction mixture: - O-dianisidine (Ortho-Tolidine or Benzidine) in 50/50 Ethyl Alcohol (95%) - Glacial acetic acid solution. (Knife point of O-dianisidine in about 20 ml of Alcohol/acetic acid solution), - and,

2% Hydrogen peroxide solution.

Gel is sliced in half and lower half, flooded with O-dianisidine solution, followed by 2% Hydrogen peroxide solution.

Results develop at room temperature.

Interpretation



Frequencies

1 - 1	10%
2 - 1	56%
2 - 2	32%
0	2.7%
2 - 1 M	less than 1% (As high as 10% in Negroe populations)
2 - 1 C Johnson	Very rare

References

1. Smithies, O. Biochem. J. 61, 629 (1955)
2. Smithies, O. Nature 175, 307 (1955)
3. Smithies, O.& Walker, M.F. Nature 176, 1265 (1955)
4. Poulik, M.D. Nature 180, 1477 (1957)

Preparation of a Starch Gel

Materials Required

One 9" x 6" Glass Plate

Four 9" x ¼" Glass strips

Four 5½" x ¼" Glass strips

MS4 silicone grease

Starch - Hydrolysed

Buffer

Method

Lightly grease 4 glass strips with silicone grease and stick to glass plate forming 2½ mm deep mould. Lightly grease other strips and place on top of previous strips forming ½ cm deep mould. Enough grease should be used to make a watertight mould only, special attention being given to the corners.

Weigh out accurately the amount of Hydrolysed starch (as stipulated on the bottle) required for 180 mls. of buffer. The amount needed varies from batch to batch but always use 190 mls of buffer for a 9" x 6" plate.

Measure 180 mls of the appropriate buffer (P.G.M., Hp. etc. as found in the bottles stored in the coldroom) and mix with the starch in a 1 litre conical flask. Heat gently over a bunsen, using a cloth wrapped around the neck of the flask, continuously swirling the liquid throughout the whole heating process.

THIS IS IMPORTANT OR YOU WILL END UP WITH BURNT STARCH.

After about 2 minutes the gel will thicken (keep the

gel moving) and then thin out as it boils. When it is gently boiling remove from heat, continue swirling for one minute and apply vacuum by means of a water pump. When the gel has finished frothing remove vacuum and pour gel into mould. The bench is sufficiently level so a levelling table is not required.

Allow gel to solidify then cover with P.V.C. sheet.

The Preservation of Starch Gels

Flood the gels with a solution of alcohol acetic wash containing 5% Glycerol.

Diffusion is as follows:

45% alcohol
40% water
10% acetic acid
5% glycerol

In this dissolve 0.5% sodium azide. The gels are allowed to soak in this for 24 hours. The wash solution is poured off, the gels placed on a siliconed glass sheet and covered with a hardened filter paper, the edges of which should overlap the gel and should be weighted down on to the siliconed plate. Left at room temperature for a few day, the gel dries down to a thin pliable film which can be peeled from the siliconed plate and sealed between pieces of polythene film.

The same method can be used for drying agar gels into pliable films. In this instance the gels are placed on the siliconed plate without covering.

System — Red cell phosphogluconate dehydrogenase.

Method — Starch gel electrophoresis.

Tank or Bridge buffer

Phosphate buffer 0.01M pH 7.0

Gel buffer

As for tank buffer.

Sample

Red cell lysate on Whatman NO. 17 filter paper inserts.

Conditions of run

10V./cm. for 4 hours using cooling plates.

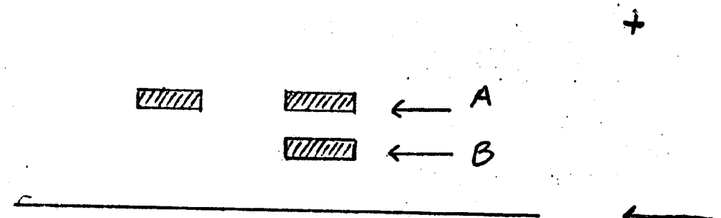
Detection

Agar overlay method:

Agar 100 mgm	
0.1M Tris buffer at pH 8.0	10 ml
N.A.D.P.	2 mgm
Sodium - 6 - phosphogluconate	10 mgm
Phenazine methosulphate	0.4 mgm
Tetrazonium salt 'MTT'	2.0 mgm

Pour over sliced starch gel at 45°C and incubate at 37° for 1 hour.

Interpretation



Frequencies

A band only	140/150
A + B band	10/150
B band only	Very rare (not yet found)

References

Fildes R.A. and Parr C.W. Nature 200, 890 (1963)

Thin-layer starch gel method for Enzyme Typing of Bloodstains

B.G.D. Wraxall & Bryan J. Culliford

Metropolitan Police Forensic Science Laboratory, London

Introduction

The basic method of starch gel electrophoresis, developed by Smithies (Smithies, 1955), has been used in both routine and research establishments for the determination of various protein and enzyme allelomorphs. With appropriate modifications of buffers it is used successfully in this laboratory for the determination of protein and enzyme types. However, the quantity of bloodstained material available for enzyme type determinations is often very small and in such cases the original method of Smithies is inadequate. The difficulty has been overcome by the development of a micro-technique requiring a minimal quantity of blood or bloodstained material.

The method using this micro-technique for the determination of Phosphoglucomutase (PGM) types in bloodstains (Culliford, 19[illegible]) is described below.

Materials

A. Two glass plates 22 cm. x 15 cm. x 3-6 mm. thick.
B. Two glass strips 22 cm. x 5 mm. x 1 mm. thick.
C. Two glass strips 14 cm. x 5 mm. x 1 mm. thick.
D. Two glass strips 22 cm. x 5 mm. x 3 mm. thick.
E. Four glass strips 14 cm. x 5 mm. x 3 mm. thick.
F. Two foam plastic (Spentex) buffer bridges 14 cm. wide to fit the tank being used.

Hydrolysed starch, buffers, etc. Shandon Kohn tanks and Vokam power peaks were used.

Method

The glass strips B & C are lightly greased with M.S.4 silicone grease and stuck by means of the grease around the perimeter of one of the glass plates A to form a shallow mould 1 mm. thick. The required amount of hydrolysed starch (as recommended by the manufacturers for the particular batch) is added to 50 mls. of gel buffer. The starch and buffer are then heated until boiling, degreased using a vacuum pump and poured into the mould at one end. By drawing a bevelled perspex starch-gel scraper (Shandon Scientific Co.) across the mould resting on the glass strips B, excess gel is removed. The purpose of this operation is to produce and even layer of starch gel 1 mm. thick. This can be done only by starting with an excess and proceeding as described. If a simple straight edge is used in place of the suggested gel scraper a thicker uneven gel is produced. All the failures so far recorded from people starting to use this technique have been due to a gel that is too thick. One millimeter should be considered a maximum not a minimum thickness.

The origin is placed one third of the length of the plate (i.e. 7-8 cm.) from the cathode. Slots approximately 1 cm. long are cut in the gel using pieces of razor blade broken to this length; the inserts for lysates are pieces of cotton thread of the same length. When dealing with crusty bloodstains or stains on solid objects such as glass or metal, a piece of thread is soaked in gel buffer and the stain swabbed from the surface. In the case of bloodstains

garments a thread is carefully removed and allowed to soak in gel buffer for five minutes prior to insertion into the gel. In the case of weak bloodstains or stains on thin material several threads should be used.

The spontex bridges are placed across the ends of the plate over-lapping the gel by about 2 cm. and are held in contact with the gel by the second glass plate A.

Electrophoresis is carried out for 17 - 20 hours at 2° - 4° C, using a voltage of 100 v. (4 V/cm. across the gel). This should produce a current of 8 ma. for a gel 1 mm. thick; if the current for a 22 cm. x 14 cm. gel is higher than 10 ma. that indicates that the gel is too thick.

On completion of electrophoresis the two glass strips D, and two of the glass strips E, are greased and placed on top of the strips B & C respectively. The remaining two glass strips, E, are used to block off an area running from the origin to 3 cms. from anode. The reaction mixture is prepared (Culliford, 1968), and poured onto the starch gel surface between the inner glass strips; there is no need to slice the gel horizontally as in the method described by Smithies (1955) or Hopkinson (Hopkinson & Harris 1965). The gel is covered with the second glass plate (A) and incubated in the dark at 37°C for 1 - 2 hours. The zones of PGM activity appear as distinct blue bands.

This method has been successfully applied to the determination of PGM types and to Adenylate Kinose types, (Culliford & Wraxall, to be published). The determination of Haptoglobin types in serum

has so far, however, not been successful.

This is now the routine method for enzyme type determination in this laboratory.

References

Culliford, B.J., 1968, J. For. Sci. Soc., 7, N.3.

Hopkinson, D.A., & Harris, H., 1965, Nature, 208, 410-412.

Smithies, O., 1955, Biochem J., 61, 629-641.

Complete ABO - MN

Typing - whole blood

Absorption - elution method. (whole blood)

1. 2 drops blood in centrifuge tube.
2. add saline (normal) to 3/4 full.
3. Spin at 3000 rpm 5 minutes.
4. aspirate super natant
5. add saline to 3/4, spin, aspirate repeat third time thus three washings.
6. Set up acetate sheet as indicated.

Use following serum types on drop in each space vertically. Abscissa serum types added horizontally one drop each.

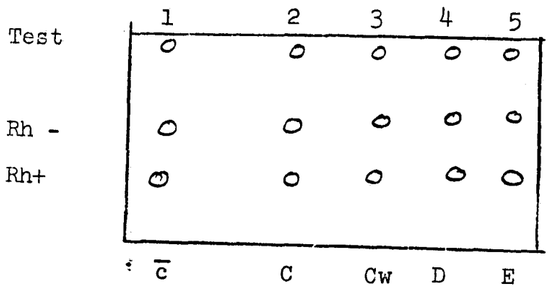
	A_1M	BN	ON	OHN	A_2M	One Test Sample
A	4	-	-	-	4	4
A_1	4	-	-	-	-	3
O	4	4	-	-	4	4
B	-	4	-	-	-	4
H	-	2	4	4	3	-
M	4	-	-	4	4	4
M	4	-	-	4	4	4
N	-	4	4	3	-	-
N	-	4	4	4	-	-

Procedure is same as in thread technique.

A_1B M Example is Dr. Alexander Joseph's blood.

Rh - Whole Blood

Set up small blood sample test tubes ($\frac{1}{4}$" diam.) in rack as per diagram.



1. Add anti serum $\bar{c}$ to row 1.
2. Add anti serum C to row 2 → etc.
3. Incubate at 37°C for 1 hour.
4. Remove all tubes except D.
5. D incubates $1\frac{1}{2}$ hours, then add 30% albumin - 1 drop.
6. C- incubates 2 hours.
7. After incubation add about $\frac{1}{2}$" saline to all tubes.
8. Look for agglutination at bottom of tubes.
9. Check with microscope - after picking up 1 drop of each on slide.
10. Examine all test samples.

Sample: Dr. Alexander Joseph's blood.

	1	2	3	4	5
Test	-	+	-	-	-
Rh-	-	-	-	-	-
Rh+	+	+	+	+	+

Interpretation:

$CD\bar{e} = R_1R_1+$

Pseudo Cholinesterase Determination of Dibucaine Number.

(The degree of inhibition of dibucaine on the hydrolyses of benzoil choline chloride solution by serum cholinesterase under standard conditions and expressed as % is termed the dibucaine number of the serum source of the enzyme.)

Rationale:

Cholinesterase and psuedo - cholinesterase are normally in the body. The former is found in r b c s, the latter in blood serum.

The genetic types of individuals for psuedo cholinesterase reflect modifications of the enzyme molecule.

Detection of types is based on dibucaine inhibition of activity of the enzyne in comparison with an uninhibited reaction.

Measurement is on log scale because we are interested in absorbance. This is true for all enzyme reactions.

System — Serum cholinesterase.

Method — Agar gel diffusion

Gel buffer

Tris 0.1M

Adjusted to pH 7.4 with hydrochloric acid.

Gel

Two plates set up:

(i) Control

DIFCO Special Agar Noble 1.5%

Gel Buffer

(22) Inhibitor

As control plus 10^{-7}M R02-0683

(Dimethyl Carbamate of 2 hydroxy - 5 Phenyl Benzyl Trimethyl Ammonium Bromide)

Sample

Serum diluted with gel buffer, diluted 1 in 8 for inhibitor plate and 1 in 32 for control. Samples pipetted into wells cut in gel.

Conditions of run

Incubate at 37°C for 17 hours.

Detection

Reaction mixture:

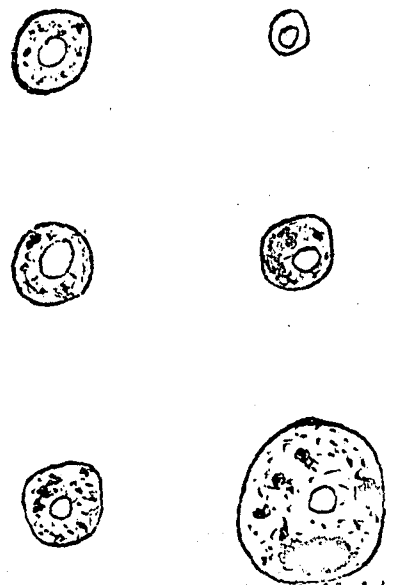
0.2M Phosphate buffer, pH 7.1 100 ml.

1% α-naphthyl acetate in 50% aqueous acetone 2 ml.

Fast red TR salt 20 mgm

Pour over sliced gel and develope at room temperature for 2 hours.

<u>Interpretation</u>



usual type

Intermediate type

atypical type

Control Plate

Inhibitor Plate

<u>Frequencies</u>

Usual type	96%
Intermediate type	4%
Atypical type	1 in 2500

<u>References</u>

1. Harris, H., Robson, E.B., The Lancet, Aug. 3rd, 1963, pg. [illegible]
2. Lehmann, H., Davies, D., Med. Science and the Law, Vol. [illegible] (1962)

Calculated group frequency % (if only 1st. 4 sera used)	Genetic and antigenic constitution	Short symbols: much used	Short symbols: Wiener & Wexler (1950)	Calculated genotype frequency per cent
15·1020	cde/cde	rr	rr	15·1020
2·0609	cDe/cde	R_0r	R^0r	1·99[illegible]
	cDe/cDe	R_0R_0	R^0R^0	0·06[illegible]
0·9376	cdE/cde	$R''r$	$r''r$	0·9235
	cdE/cdE	$R''R''$	$r''r''$	0·0141
14·9769	cDE/cDE	R_2R_2	R^2R^2	1·9[illegible]6
	cDE/cdE	R_2R''	R^2r''	0·3353
	cDE/cDe	R_2R_0	R^2R^0	0·7[illegible]
	{cDE/cde,	R_2r	R^2r	10·[illegible]7
	{cDe/cdE	R_0R''	R^0r''	0·[illegible]
0·7644	Cde/cde	$R'r$	$r'r$	0·7[illegible]
	Cwde/cde	R'^wr	r'^w	0·0[illegible]
34·8899	CDe/cDe	R_1R_0	R^1R^0	2·[illegible]22
	{CDe/cde	R_1r	R^1r	31·6759
	{cDe/Cde	R_0R'	R^0r'	0·[illegible]95
	CwDe/cDe	$R_1^wR_0$	$R^{1w}R^0$	0·0664
	{CwDe/cde	R_1^wr	$R^{1w}r$	1·0[illegible]19
	{Cwde/cDe	R'^wR_0	r'^wR^0	0·0[illegible]00
0·0234	cdE/Cde	$R''R'$	$r''r'$	0·0234
	CdE/cde	R_yr	r^yr	0·000[illegible]
	CdE/cdE	R_yR''	r^yr''	0·0[illegible]
	Cwde/cdE	R'^wR''	r'^wr''	0·0[illegible]
13·4179	CDe/cDE	R_1R_2	R^1R^2	11·5[illegible]
	cDe/CDE	R_0R_z	R^0R^z	0·[illegible]
	{CDe/cdE	R_1R''	R^1r''	0·[illegible]
	{cDE/Cde	R_2R'	R^2r'	0·[illegible]
	{CDE/cde	R_zr	R^zr	0·[illegible]
	{CdE/cDe	R_yR_0	r^yR^0	0·[illegible]
	cDE/CDE	R_2R_z	R^2R^z	0·[illegible]7
	{cdE/CDE	$R''R_z$	$r''R^z$	0·[illegible]58
	{CdE/cDE	R_yR_2	r^yR^2	0·[illegible]
	CwDe/cDE	$R_1^wR_2$	$R^{1w}R^2$	0·3[illegible]
	{CwDe/cdE	R_1^wR''	$R^{1w}r''$	0·[illegible]7
	{Cwde/cDE	R'^wR_2	r'^wR^2	0·0200
0·0097	Cde/Cde	$R'R'$	$r'r'$	0·[illegible]
	Cwde/Cde	R'^wR'	r'^wr'	0·[illegible]
	Cwde/Cwde	$R'^wR'^w$	$r'^wr'^w$	0·00[illegible]
18·5073	CDe/CDe	R_1R_1	R^1R^1	16·[illegible]
	CDe/Cde	R_1R'	R^1r'	0·[illegible]
	CDe/CwDe	$R_1R_1^w$	R^1R^{1w}	1·[illegible]
	{CwDe/Cde	R_1^wR'	$R^{1w}r'$	0·[illegible]
	{Cwde/CDe	R'^wR_1	r'^wR^1	0·[illegible]
	CwDe/CwDe	$R_1^wR_1^w$	$R^{1w}R^{1w}$	0·016[illegible]
	Cwde/CwDe	$R'^wR_1^w$	r'^wR^{1w}	0·00[illegible]
0·2101	CDe/CDE	R_1R_z	R^1R^z	0·[illegible]
	{Cde/cDE	$R'R_z$	$r'R^z$	0·0[illegible]
	{CdE/CDe	R_yR_1	r^yR^1	0·[illegible]
	CDE/CDE	R_zR_z	R^zR^z	0·0[illegible]
	CwDe/CDE	$R_1^wR_z$	$R^{1w}R^z$	0·[illegible]
	CdE/CDE	R_yR_z	r^yR^z	0·[illegible]
	{CdE/CwDe	$R_yR_1^w$	r^yR^{1w}	0·[illegible]
	{Cwde/CDE	R'^wR_z	r'^wR^z	0·00[illegible]
0·0000	CdE/Cde	R_yR'	r^yr'	0·0[illegible]
	CdE/CdE	R_yR_y	r^yr^y	0·0[illegible]
	CdE/Cwde	$R_yR'^w$	$r^yr'^w$	0·0000

System Haptoglobins

Method Starch gel electrophoresis

Tank or Bridge Buffer

Boric Acid	0.3 M	18.6 gm
Sodium hydroxide	0.5 M	2.0 gm / 1 l. aq. dist.
pH 7.9		

Gel Buffer

Tris	0.076 M	9.136 gm
Citric Acid	0.005 M	1.05 gm / 1 l. aq. dist.
pH 8.65		

Sample

Blood serum on Whatman 3 MM inserts.

Conditions of run

130V for 17 hours at room temperature.

or 170V for 17 hours at 4°C.

Detection

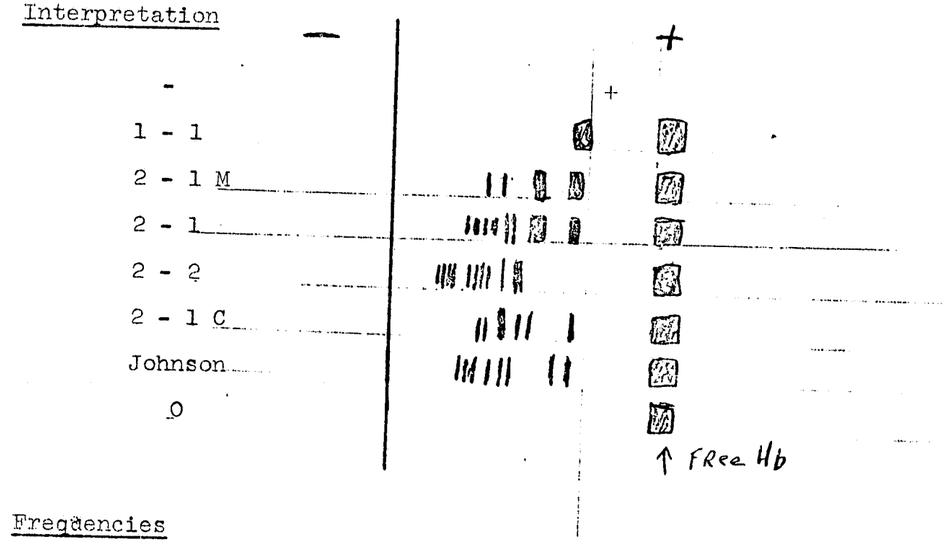
Reaction mixture: - O-dianisidine (ORTHO-Tolidine or benzidine) in 50/50 Ethyl Alcohol (95%) - Glacial acetic acid solution. (Knife point of O-dianisidine in about 20 ml of Alcohol/acetic acid solution), - and,

2% Hydrogen peroxide solution.

Gel is sliced in half and lower half, flooded with O-dianisidine solution, followed by 2% Hydrogen peroxide solution.

Results develop at room temperature.

Interpretation



Frequencies

1 - 1	10%
2 - 1	56%
2 - 2	32%
0	2.7%
2 - 1 M	less than 1% (As high as 10% in Negroe populations)
2 - 1 C, Johnson	Very rare

References

1. Smithies, O. Biochem. J. 61, 629 (1955)
2. Smithies, O. Nature 175, 307 (1955)
3. Smithies, O.& Walker, M.F. Nature 176, 1265 (1955)
4. Poulik, M.D. Nature 180, 1477 (1957)

System Haemoglobin

Method Cellulose Acetate Paper Electrophoresis.

(ii) Kohn

Tanker or Bridge buffer

Anodic

Tris	12.6 gm
E.D.T.A.	1.25 gm
Boric Acid	0.95 gm
Distilled water	500 ml

pH 9.1

Cathodic

Sodium diethylbarbiturate	2.57 gm
Diethylbarbituric Acid	0.46 gm
Distilled water	500 ml

pH 8.6

Cellulose Acetate Paper buffer

Cathodic and Anodic buffers are mixed in equal proportions.

Sample

Red cell lysate painted on buffered cellulose acetate strip in this band.

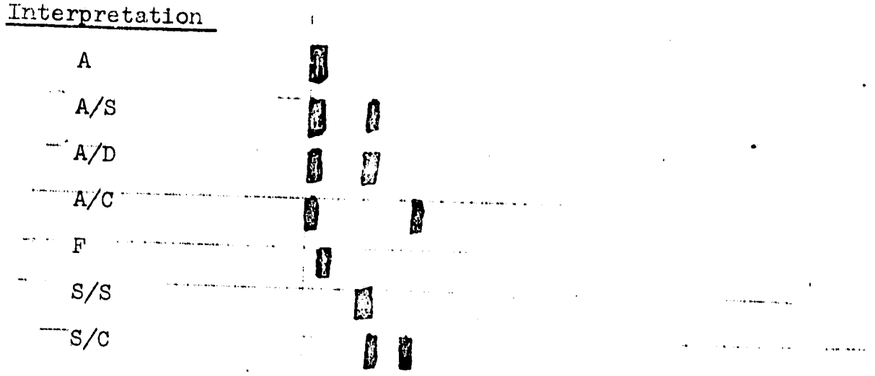
Conditions of run

Kohn system: 150V. to 200V. for 1 to 1½ hours at 4°C.

Detection

Staining with Ponceau S.

Interpretation



Frequencies

A	Normal
F	All children and Thalassaemics
AS and SS	10% - 15% Negroes
AC and CC	3% Negroes
D	Very rare
E	

References

1. Lehmann, H. and Ager, J.A.M. The Assoc. of Clin. Pathologists, Broadsheet NO. 33 (New Series), May [illegible]

2. Culliford, B.J. Journal of the Forensic Science Soc. Vol. 4, No. 3, March, 1964.

3. Graham, J.L. and Grunbaum, B.W. The American Journal of Clin. Path. Vol. 39, NO. 6 567 (1963)

4. Kohn, J. Shandon, Instrument Applications, NO. 11 (19[illegible]

Improved Method for Haptoglobin typing of Bloodstains

Equipment

Shandon Slide Tray

Shandon Kohn Tanks

Power Pack 0 - 60 MA minimum

Shandon Gel Pattern cutter

Materials

2% Ionagar in water

Hirschfeld gel buffer (1) (2) - made up double strength.

Hirschfeld tank buffer (1) (2)

Anti Haptoglobin serum - Dutch Red Cross. Amsterdam.

Preparation of gel

Mix 20 grams of 2% Ionagar in water with 20 mls of gel buffer, melt, cool to 50°C and pour over slide tray containing 8 slides.

When set cut the antigen slots to the pattern as shown in fig. 1. Do not cut antiserum troughs until AFTER the first electrophoretic separation.

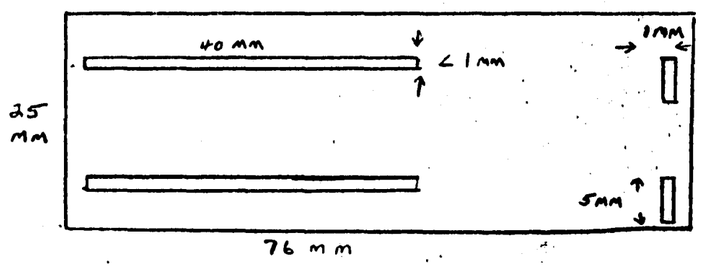


Figure 1

Cut out enough stain material to fill slot and soak in gel buffer for 10 minutes prior to insertion into the gel.

Electrophoresis (1)

160V (7V/cm.) with current 48 Ma. (constant) for 4 to 4½ hours at room temperature. Use gel contact troughs filled with 2% Ionagar in tank buffer.

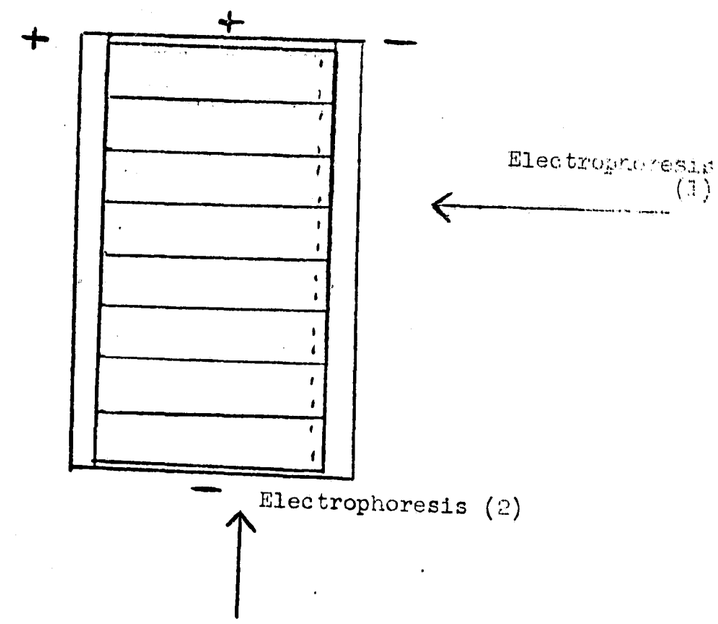
Cut antiserum troughs with Shandon gel cutter and remove waste gel. Fill troughs with anti serum.

Electrophoresis (2)

At right angles to direction of first separation as in Figure 2.

Figure(2)

Tray + Slides



160 V (7V/cm) with constant current 22Ma. for 1 hour at room temperature. Use spontex bridges held in place with a glass plate.

Washing

Place tray in large dish containing 1 M. sodium chloride solution and leave overnight. Place in distilled water for ½ hour before drying.

Remove individual slides, cover with wet Whatman No. 1 and dry at 56°C for ½ hour.

Staining

Stain with Amido Black, differentiate in alcohol/acetic wash.

Time for complete typing - 24 hours.

B.G.D. Wraxall,
Met. Police Laboratory,
2, Richbell Place,
Holborn,
London, W.C. 1.

References

(1) Hirschfeld, J. Sc. Tools 7, 2 (1960)

(2) Culliford B.J. & Wraxall B.G.D. Nature 211, 5051 (1966).

System — Serum cholinesterase.

Method — Agar gel diffusion

Gel buffer

Tris 0.1M

Adjusted to pH 7.4 with hydrochloric acid.

Gel

Two plates set up:

(1) Control

DIFCO Special Agar Noble 1.5%

Gel Buffer

(22) Inhibitor

As control plus 10^{-7}M R02-0683

(Dimethyl Carbamate of 2 hydroxy - 5 Phenyl Benzyl Trimethyl Ammonium Bromide)

Sample

Serum diluted with gel buffer, diluted 1 in 8 for inhibitor plate and 1 in 32 for control. Samples pipetted into wells cut in gel.

Conditions of run

Incubate at 37°C for 17 hours.

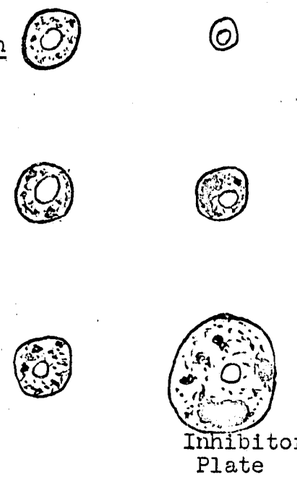
Detection

Reaction mixture:

0.2M Phosphate buffer, pH 7.1	100 ml.
1% α-naphthyl acetate in 50% aqueous acetone	2 ml.
Fast red TR salt	20 mgm

Pour over sliced gel and develope at room temperature for 2 hours.

Interpretation



Control plate

Inhibitor Plate

usual type

Intermediate type

atypical type

Frequencies

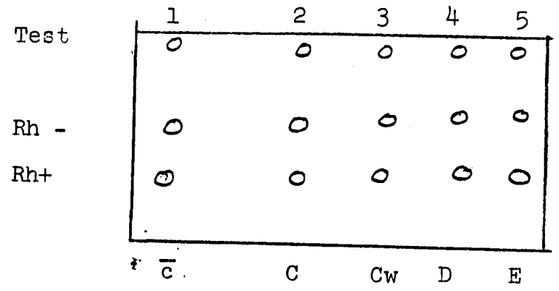
Usual type	96%
Intermediate type	4%
Atypical type	1 in 2500

References

1. Harris, H., Robson, E.B., The Lancet, Aug. 3rd, 1963, pg. 218.
2. Lehmann, H., Davies, D., Med. Science and the Law, Vol. 2 No.3 (1962)

Rh - Whole Blood

Set up small blood sample test tubes ($\frac{1}{4}$" diam.) in rack as per diagram.



1. Add anti serum $\bar{c}$ to row 1.
2. Add anti serum C to row 2 ⟶ etc.
3. Incubate at 37°C for 1 hour.
4. Remove all tubes except D.
5. D incubates $1\frac{1}{2}$ hours, then add 30% albumin - 1 drop.
6. C- incubates 2 hours.
7. After incubation add about $\frac{1}{2}$" saline to all tubes.
8. Look for agglutination at bottom of tubes.
9. Check with microscope - after picking up 1 drop of each on slide.
10. Examine all test samples.

Sample: Dr. Alexander Joseph's blood.

	1	2	3	4	5
Test	-	+	-	-	-
Rh-	-	-	-	-	-
Rh+	+	+	+	+	+

Interpretation:

$CD\bar{e} = R_1R_1+$

Complete ABO - MN

Typing - whole blood

Absorption - elution method. (whole blood)

1. 2 drops blood in centrifuge tube.
2. add saline (normal) to 3/4 full.
3. Spin at 3000 rpm 5 minutes.
4. aspirate super natant
5. add saline to 3/4, spin, aspirate repeat third time thus three washings.
6. Set up acetate sheet as indicated.

Use following serum types on drop in each space vertically. Abscissa serum types added horizontally one drop each.

	A_1M	BN	ON	OHN	A_2M	One Test Sample
A	4	-	-	-	4	4
A_1	4	-	-	-	-	3
O	4	4	-	-	4	4
B	-	4	-	-	-	4
H	-	2	4	4	3	-
M	4	-	-	4	4	4
M	4	-	-	4	4	4
N	-	4	4	3	-	-
N	-	4	4	4	-	-

Procedure is same as in thread technique.

A_1B M Example is Dr. Alexander Joseph's blood.

Pseudo Cholinesterase Determination of Dibucaine Number.

(The degree of inhibition of dibucaine on the hydrolyses of benzoil choline chloride solution by serum cholinesterase under standard conditions and expressed as % is termed the dibucaine number of the serum source of the enzyme.)

Rationale:

Cholinesterase and psuedo - cholinesterase are normally in the body. The former is found in r b c s, the latter in blood serum.

The genetic types of individuals for psuedo cholinesterase reflect modifications of the enzyme molecule.

Detection of types is based on dibucaine inhibition of activity of the enzyne in comparison with an uninhibited reaction.

Measurement is on log scale because we are interested in absorbance. This is true for all enzyme reactions.

END