MANE-001
Human Genetics

Indira Gandhi
National Open University
School of Social Sciences

Block

7

PRACTICAL IN HUMAN GENETICS

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BLOCK 7 PRACTICAL IN HUMAN GENETICS

The anthropologists are interested more in populations than in individuals. To characterize populations and to study relations between them traits are needed. During 19th century, anthropometry – the measurement of man – provided scientific methods and techniques for taking various measurements and observations on the living man and the skeleton. A new dimension to population variation studies was provided when scientific study of human blood started in the beginning of the 20th century with the discovery of the ABO blood groups. Since then there have been many discoveries of the typology of blood and the multiple markers present in it. In contrast to the traditional anthropometry studying visible characteristics, the blood group and other genetic markers are used to study invisible characteristics that are not affected by environment and therefore serve as excellent tools for research in physical anthropology. Genetic characteristics belong to a number of genetic systems and those of any one system are inherited independently of those of all other systems.

The number of genetic systems discovered by serological and electrophoretic techniques is large and covers different categories such as blood groups, serum protein and erythrocyte polymorphisms and a battery of DNA markers. In fact, for describing and classifying populations, these blood characteristics have now long surpassed, and indeed superseded, studies of anatomical features, including anthropometry, in their usefulness to anthropologists. The basic unit of study in population genetics is allele frequency rather than phenotype or genotype frequencies, and accordingly a population is now characterized by a set of allele frequencies obtained from the study of different genetic systems. Therefore, allele frequency data are essential prerequisite for studying genetic variation in humans. In Unit 1, calculation of gene (allele) frequencies in ABO, A_1A_2BO and RH D blood group systems are given, followed by the protocols of studying ABH secretion in saliva. In Unit 2, techniques of studying traditional genetic traits viz., phenylthiocarbamide (PTC) tasting ability, colour blindness and haemoglobin types are given in detail. Finally, in Unit 3, protocol of DNA extraction from leucocytes is given along with methods of estimation of its quantity and quality.

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UNIT 1 SEROLOGY

Practical is an important component in the field of human genetics. To understand population variation, a number of genetic markers play a significant role, of which the blood group polymorphisms are the classic examples. For the calculation of allele frequencies, several methods are available such as gene counting (for codominant alleles), square root (for a pair of dominant and recessive alleles) and maximum likelihood (for more than two dominant and recessive alleles etc.) In this Unit you will learn two aspects: (1) how to calculate allele frequencies in ABO, A_1A_2BO and RH D blood group systems and (2) protocol of the determination of ABH substances from the saliva using aborption-inhibition test.

1) Calculation of gene (allele) frequencies in ABO, A_1A_2BO and RH D blood group polymorphisms

Karl Landsteiner, an Austrian scientist, discovered the ABO blood group system in 1900. Depending on the presence or absence of antigens and antibodies in the blood, human beings are classified into four groups namely A, B, AB and O. The antigens are designated as A and B and the antibodies as anti-A and anti-B. The former are found on the surface of the red blood cells (RBCs) while the latter are found in the plasma. Group A person carries antigen A on the RBCs and antibody anti-B in the plasma. Group B person carries antigen B on RBCs and antibody anti-A in the plasma. Group O individuals possess both the antibodies and lack any antigen while group AB individuals carry both the antigens but lack any antibody.

Bernstein (1924) showed that the four ABO blood groups were inherited in Mendelian fashion by means of three allelic genes viz., *A* (now designated *ABO*A*), *B* (*ABO*B*) and *O* (*ABO*O*), the former two being codominant and the latter recessive. Dungern and Hirszfeld (1911) described two subgroups of A i.e., A_1 and A_2 and Thomsen et al. (1930) put forward the four allele theory of inheritance of the A_1A_2BO blood groups to include these subgroups. The alleles involved are *A1, A2, B* and *O*, now designated as *ABO*A1, ABO*A2, ABO*B* and *ABO*O,* respectively, the former three being dominant over *ABO*O*; *ABO*A1* is dominant over *ABO*A2*.

The RH (rhesus) blood group system was discovered in 1940 by Landsteiner and Wiener. The two phenotypes (blood groups) of the system are RH D + and RH D – based on, respectively, the presence or absence of the RH D antigen (earlier referred to as Rh factor). A pair of alleles, one dominant (*RH*D*) and one recessive (*RH*d*), determine the antigens of this blood group system.

The serological techniques of typing ABO, A_1A_2BO and RH D blood group systems from blood samples are based on the principle of agglutination i.e. a reaction between known antibody such as anti-A and an unknown antigen such as A. Positive result, seen as clump(s) of red cells, demonstrates the presence of the corresponding antigen and a negative result demonstrates lack of it. After having identified serologically the blood group types in these genetic systems in a population, the next step is the calculation of allele frequencies, from the observed phenotype (blood group) data, so that it can be characterized genetically.

Calculation of allele frequencies in ABO system

When anti-A and anti-B have been used

Bernstein's method

Bernstein (1930) gave the following formulae for the estimation of allele frequencies in the ABO system.

$$p = 1 - \sqrt{e + f}$$

where, p=frequency of allele A, q=frequency of allele B and r = frequency of allele O and c, e and f denote the proportions of the blood groups A, B and O, respectively, in the population.

In practice $p+q+r$ will not be equal to 1. The deviation from unity is referred to as D and corrected (adjusted) estimates of the allele frequencies are obtained as follows (Bernstein, 1930).

$$p_c = \mathrm{p}\left(1 + \frac{\mathrm{D}}{2}\right)$$

where, p_c= corrected frequency of allele A, q_c= corrected frequency of allele B and r_c= corrected frequency of allele O.

Example: Calculation of allele frequencies in the ABO blood group system in the Koraga tribe of Karnataka state (Chandrasekar, 2000).

Phenotype	Observed number	Phenotype proportions	Proportions of observed frequency (Observed number/Total)	Expected number	$\div^2$
A	20	c	0.187	17.38	0.3950
B	37	e	0.346	34.51	0.1797
O	48	f	0.449	50.28	1.6416
AB	2		0.019	4.81	0.1039
Total (N)	107		1.001	106.98	2.3202

=

= 0.1097

$$q = 1 - \sqrt{c + f}$$

=

= 0.2047

$$r = \sqrt{f}$$

=

= 0.6855

$p+q+r = 0.1097+0.2047+0.6855 = 0.9999$

Calculation of adjusted (corrected) allele frequencies using correction factor (D):

$$D = 1 - (p + q + r)$$

= 1– 0.9999

= 0.0001

=

= 0.1097(1.00005)

= 0.1097

=

= 0.2047(1.00005)

= 0.2047

=

= (0.6855 + 0.00005) (1 + 0.00005)

= (0.68555) (1.00005)

= 0.6855

Therefore, adjusted allele frequencies in the Koraga tribe of Karnataka state are as follows, which incidentally are the same as the original estimate.

$p_c = 0.1097$

$q_c = 0.2047$

$r_c = 0.6855$

Total = 0.9999

Calculation of expected numbers

A =

$= [(0.1097)^2 + (2 \times 0.1097 \times 0.6855)] \times 107$

= 17.38

B =

$= [(0.2047)^2 + (2 \times 0.2047 \times 0.6855)] \times 107$

= 34.51

O = × N

$= (0.6855)^2 \times 107$

= 50.28

AB= $(2p_cq_c) \times N$

$= (2 \times 0.1097 \times 0.2047) \times 107$

= 4.81

where, N is the total number tested.

Calculation of goodness of fit $\div^2$

$$\div^2 = Ó \frac{(\text{Expected number} - \text{Observed number})^2}{\text{Expected number}}$$

$\div^2$ for A $= (17.38\text{-}20)^2 / 17.38$

$= 0.3950$

$\div^2$ for B $= (34.51\text{-}37)^2 / 34.51$

$= 0.1797$

$\div^2$ for O $= (50.28\text{-}48)^2 / 50.28$

$= 0.1039$

$\div^2$ for AB $= (4.81\text{-}2)^2 / 4.81$

$= 1.6416$

$Ó\div^2$ $= 2.3202$

Degree of freedom (d.f.) is calculated as follows.

Number of blood groups in ABO system (4) – number of alleles of the system (3).

Thus d.f. = 4 – 3 = 1

From the $\div^2$ distribution table, the p (probability) for $\div^2$ value of 2.3202 at d.f.=1 is found out to be $0.20>p>0.10$, which is statistically non-significant as it is >0.05. This demonstrates that the the Koraga tribe of Karnataka state is in Hardy-Weinberg (genetic) equilibrium for the distribution of ABO blood groups.

Yasuda's method

Yasuda (1984) gave a simple yet efficient method for calculation of allele frequencies in ABO system which give estimates which are as good as those obtained from the maximum likelihood method. In his method, firstly, there is no need to calculate proportions of different phenotypes (blood groups) observed, and rather phenotype numbers are used as such in the formulas listed below. Secondly, there is no need to apply the correction factor D to obtain the adjusted values of allele frequencies as they always add to unity. Thirdly, unlike Bernstein (1930) method, this method considers phenotype AB.

Yasuda (1984) gave the following formulae for the estimation of allele frequencies in the ABO system.

$$\left[1/2(AB) + A + O - \sqrt{O(A + O)}\right] / T$$

$p\ (ABO^*A) =$

q (ABO^*B) =

r (ABO^*O) = 1 – p – q

where, A, B, O, AB refer to observed phenotype numbers in respective blood group and T to total number tested.

Using the above phenotype data, the allele frequencies in the ABO system in the Koraga tribe of Karnataka state have been calculated as follows.

p (ABO^*A) =

= 0.11092

q (ABO^*B) =

= 0.20678

r (ABO^*O) = 1 – 0.11092 – 0.20678

= 0.6823

p (ABO^*A)+ q (ABO^*B)+ r (ABO^*O)

= 0.11092+0.20678+0.6823

= 1

There is some discrepancy between the results obtained by Bernstein (1930) and Yasuda (1984) formulae but since the values of allele frequencies in the ABO system obtained by the latter formulae give near maximum likelihood estimates, the calculations should preferably be done following Yasuda (1984).

The expected numbers in the ABO system are calculated using the following formulae provided by Yasuda (1984).

A= (p^2+2pr)T

B= (q^2+2qr)T

O= (r^2)T

AB= $(2pq)$T

where, p= frequency of the allele A, q= frequency of the allele B, r = frequency of the allele O and T is the total number tested.

Thus using the above allele frequency data on ABO system, the expected numbers in the Koraga tribe of Karnataka state have been calculated as follows.

A = $[(0.11092)^2 + 2\ (0.11092)\ (0.6823)] \times 107$

= 17.5124

$B = [(0.20678)^2 + 2\ (0.20678)\ (0.6823)] \times 107$

$= 34.7670$

$O = (0.6823)^2 \times 107$

$= 49.8123$

$AB = [2\ (0.11092)\ (0.20678)] \times 107$

$= 4.9083$

Calculation of goodness of fit $\div^2$

$$\div^2 = Ó \frac{(\text{Expected number} - \text{Observed number})^2}{\text{Expected number}}$$

$\div^2$ for A = $(17.5124\text{-}20)^2 / 17.5124$

$= 0.3533584$

$\div^2$ for B = $(34.767\text{-}37)^2 / 34.767$

$= 0.1434201$

$\div^2$ for O = $(49.8125\text{-}48)^2 / 49.8125$

$= 0.0659504$

$\sqrt{f + c + a} - \sqrt{f + a}$ $\div^2$ for AB = $(4.9083\text{-}2)^2 / 4.9083$

$= 1.7232461$

Ó $\div^2$ $= 2.285975$

Degree of freedom (d.f.) is calculated as follows.

Number of blood groups in ABO system (4) – number of alleles of the system (3).

Thus d.f. =1.

From the $\div^2$ distribution table, the p (probability) for $\div^2$ value of 2.285975 at d.f.=1 is found out to be $0.20>p>0.10$, which is statistically non-significant as it is >0.05.This demonstrates that the the Koraga tribe of Karnataka state is in Hardy-Weinberg (genetic) equilibrium for the distribution of ABO blood groups.

Calculation of allele frequencies in the A_1A_2BO system

When anti-A, anti-B and anti-A_1 have been used

Bernstein's method

Bernstein (1930) gave the following formulae for the estimation of allele frequencies in the A_1A_2BO system.

$p_1 =$

p_2 =

$q = \sqrt{f + e} - \sqrt{f}$

$r = \sqrt{f}$

where, p_1=frequency of allele *ABO*A1*, p_2 = frequency of allele *ABO*A2*, q=frequency of allele *ABO*B* and r=frequency of allele *ABO*O,* and c, a, e and f denote, respectively, the proportions of the blood groups A_1, A_2, B and O in the population.

In practice p_1+p_2+q+r will not equal 1. The deviation from 1 is referred to as D and an improved (adjusted) estimate of the allele frequencies is calculated as follows.

Example: Calculation of allele frequencies in the A_1A_2BO blood groups among the Koraga tribe of Karnataka (Chandrasekar, 2000)

Phenotype	Observed number	Phenotype proportions	Proportions of observed frequency (Observed number/total)
A_1	20	c	0.1869
A_2	0	a	0.0000
B	37	e	0.3458
O	48	f	0.4486
A_1B	1		
A_2B	1		
Total (N)	**107**		

p_1 =

=

= 0.1274

p_2 =

$= \sqrt{0.4486 + 0} - \sqrt{0.4486}$

$= 0$

$q = \sqrt{f + e} - \sqrt{f}$

$= \sqrt{0.4486 + 0.3458} - \sqrt{0.4486}$

$= 0.2215$

$r = \sqrt{f}$

r =

$= 0.6698$

$p_1 + p_2 + q + r = 0.1274 + 0 + 0.2215 + 0.6698 = 1.0187$

Calculation of adjusted (corrected) allele frequencies using correction factor (D):

$D = 1 - (p_1 + p_2 + q + r)$

$= 1 - 1.0187 = -0.0187$

$1 - D = 1 - (-0.0187)$

$= 1.0187$

=

$p_{2c} = \frac{p_2}{1 - D}$

=

=

$r_c = \frac{r}{1 - D}$

=

Adjusted (corrected) allele frequencies

$p_{1c} = 0.1251$

$p_{2c} = 0.0000$

$q_c = 0.2174$

$r_c = 0.6575$

Total = 1.0000

Yasuda's method

Yasuda (1984) gave a simple yet efficient method for calculation of allele frequencies in A_1A_2BO system which gives estimates which are as good as those obtained from the maximum likelihood method. In his method, firstly, there is no need to calculate proportions of different phenotypes (blood groups) observed, and rather phenotype numbers are used as such in the formulas listed below. Secondly, there is no need to apply the correction factor D to obtain the adjusted (corrected) values of allele frequencies as they always add to unity. Thirdly, unlike Bernstein (1930) method, this method considers phenotypes A_1B and A_2B.

Yasuda (1984) gave the following formulae for the estimation of allele frequencies in the A_1A_2BO system.

$$p_1\,(ABO^*A_1) = \frac{\left[1/2(A_1B) + A_1 + A_2 + O - \sqrt{(A_2 + O)(A_1 + A_2 + O)}\right]}{T}$$

$q\,(ABO^*B) =$

$p_2\,(ABO^*A2) =$

$r\,(ABO^*O) =$

where, A_1, A_2, B, O, A_1B and A_2B refer to observed phenotype numbers of the respective blood group and T to total number tested.

$p_1\,(ABO^*A_1) =$

$= 0.106248$

$q\,(ABO^*B) =$

$= 0.206777$

$p_2\ (ABO*A2) =$

$= 0$

$r\ (ABO*O) = \left[\sqrt{48/(0+48)}\right][1 - 0.106248 - 0.206777]$

$= 0.686975$

$p_1\ (ABO*A1) + q\ (ABO*B) + p_2\ (ABO*A2) + r\ (ABO*O)$

$= 0.106248+0.206777+0+0.686975$

$=1$

There is some discrepancy between the results obtained by Bernstein (1930) and Yasuda (1984) formulae but since the values of allele frequencies in the A_1A_2BO system obtained by the latter formulae give near maximum likelihood estimates, the calculations should preferably be done following Yasuda (1984).

The expected numbers in the A_1A_2BO system are calculated using the following formulaue provided by Yasuda (1984).

$A_1 = (p_1^2+2p_1r+2p_1p_2)T$

$A_2 = (p_2^2+2p_2r)T$

$B = (q^2+2qr)T$

$O = (r^2)T$

$A_1B = (2p_1q)T$

$A_2B = (2p_2q)T$

$\left[1 - \sqrt{48/(0+48)}\right][1 - 0.106248 - 0.206777]$

where, T is the total number tested.

Thus using the above allele frequency data on A_1A_2BO system, the expected numbers in the Koraga tribe of Karnataka state have been calculated as follows.

$A_1 = [(0.106248)^2+2(0.106248)(0.686975)+2(0.106248)(0)]107$

$= 16.82774$

$A_2 = [(0)^2+2(0)(0.686975)]107$

$= 0$

$B = [(0.206777)^2+2(0.206777)(0.686975)]107$

$= 34.97377$

$O = [(0.686975)^2]107$

$= 50.49698$

$A_1B = [2(0.106248)(0.206777)]107$

$= 4.701515$

$A_2B = [2(0)(0.206777)]107$

$= 0$

Calculation of goodness of fit $\div^2$

$$\div^2 = Ó \frac{(\text{Expected number} - \text{Observed number})^2}{\text{Expected number}}$$

$\div^2$ for $A_1 = (16.82774\text{-}20)^2 / 16.82774$

$= 0.5980145$

$\div^2$ for $A_2 = (0\text{-}0)^2 / 0$

$= 0$

$\div^2$ for B $= (34.97377\text{-}37)^2 / 34.97377$

$= 0.117391$

$\div^2$ for O $= (50.49698\text{-}48)^2 / 50.49698$

$= 0.1782738$

$\div^2$ for $A_1B = (4.701515\text{-}1)^2 / 4.701515$

$= 2.9142124$

$\div^2$ for $A_2B = (0\text{-}1)^2 / 0$

$= \infty$

$Ó \div^2 = 3.8078917$

Degree of freedom (d.f.) is calculated as follows.

Number of blood groups in A_1A_2BO system (6) – number of alleles of the system (4).

Thus d.f.= 2.

From the $\div^2$ distribution table, the p (probability) for $\div^2$ value of 3.8078917 at d.f.=2 is found out to be $0.20>p>0.10$, which is statistically non-significant as it is >0.05. This demonstrates that the the Koraga tribe of Karnataka state is in Hardy-Weinberg (genetic) equilibrium for the distribution of A_1A_2BO blood groups.

Calculation of allele frequencies in the RH D System

When anti-D has been used

The square root is the method used in the calculation of allele frequencies in genetic systems involving a pair of dominant and recessive alleles such as RH D blood group system (as also PTC tasting ability and ABH secretion system, among others).

$RH^*d = \sqrt{a}$

$RH^*D = 1 - RH^*d$

where, a is the phenotype proportion of RH D – subjects in the population.

Example: Calculation of RH D allele frequencies in the Koraga tribe of Karnataka state (Chandrasekar, 2000)

Phenotype	Observed number	Phenotype proportion (observed number/ total)
RH D +	85	0.86734
RH D –	13	0.13265
Total	98	1.00000

Since in RH D system, genotype of only RH D– phenotype is known viz., *RH*dd* (which can also be written as RH^*d^2), first the frequency of the recessive allele is calculated as follows.

*RH**d =

=

= 0.3642

*RH*D* = 1- *RH*d*

=1-0.3642

= 0.6358

*RH*d* + *RH*D* = 0.3642+0.6358 = 1

$\sqrt{0.13265}$

PRACTICE

i) Calculate the allele frequencies in ABO system from the following phenotype data.

Phenotype	Observed number
A	29
B	39
O	61
AB	3
Total (N)	132

ii) Calculate allele frequencies in A_1A_2BO system from the following phenotype data.

Phenotype	Observed Number
A_1	29
A_2	0
B	39
O	61
A_1B	1
A_2B	1
Total (N)	132

ii) Calculate allele frequencies in RH D system from the following phenotype data.

Phenotype	Observed number
RH D +	113
RH D -	19
Total	132

2) **Detection of ABH substances in saliva by absorption-inhibition technique**

The ABH antigens present on red cell surface which are alcohol soluble, can also exist in a water soluble form in body fluids and constitute the ABH secretor system which is genetically independent of the ABO blood groups. The term "ABH secretor" refers to secretion of such antigens in saliva, sweat, tears, semen and breast milk. If you are an "ABH secretor", you will secrete antigens according to your ABO blood group type i.e., group O individuals will secrete H antigen, group A will secrete A and H antigens, group B will secrete B and H antigens and group AB will secrete A, B and H antigens. About two third people in India are ABH secretors.

The ABH secretion is controlled by a pair of alleles, *Se* and *se*; the former being dominant over the latter. *Se* causes secretion in saliva and other fluids of the antigen(s) corresponding to the individual's ABO group while *se* in the homozygous condition determines non-secretion. Hetrozygotes (*Se se*) are secretors. Presently, the secretor (*Se*) locus is withdrawn and renamed fucosyl transferase 2 (*FUT2*) locus (McAlpine et al., 1989). Under the new nomenclature, secretors are renamed *FUT2 SEC* and non-secretors are renamed *FUT2 NON SEC*; the alleles *Se* and *se* are renamed as *FUT2*SEC* and *FUT2*QO,* respectively.

Absorption-inhibition test

The ABH secretor status from saliva is determined by using another serological technique viz., the absorption-inhibition test. The principle of the technique is that if ABH antigens are present in saliva, they will absorb (bind to) the corresponding antibodies added in the form of titrated (diluted) antisera in the initial stage of the test, thereby inhibiting them from agglutinating red cells possessing the same antigens added in the final stage of the test.

Procedure

The subject should wash his mouth with water before the collection of saliva sample. Ask the subject to keep a small cotton swab below the tongue and to remove it after 2 min and squeeze into a 10 ml glass test tube to collect about 0.5 ml saliva.

Plug the test tubes containing saliva samples with cotton. Take a medium glass beaker, place some cotton on its base and fill it with water to a quarter and place the test tubes, held together using rubber bands around them, in it. Keep the beaker in a bigger glass beaker with water level a little higher than the former beaker. Boil on heater for 30 min to inactivate the salivary enzymes. Take out the test tubes and after cooling at room temperature, centrifuge for 10 min at 2000-3000rpm. Transfer the supernatant saliva samples into eppendorf tubes and store them in a refrigerator till their typing for ABH substances.

Titration of the test sera (anti-A, anti-B and anti-H)

Unlike simple agglutination test used in conventional blood grouping, where high titre of commercially available antisera is a non-issue, in the absorption-inhibition test it is. This is because if titration and accordingly dilution of respective antisera is not done, excess of antibodies in test sera will lead to agglutination in all cases, irrespective of ABH secretor status of the individual. In fact, in the absorption-inhibition test just enough antibodies which can be absorbed by antigens in a drop of saliva (if the subject is secretor) are required and this can be achieved by first determining the titre of the antisera and thereafter diluting it with normal saline in proportion to the obtained titre value.

Titration can be carried out either on porcelain tiles or in glass tubes and agglutination reaction can be visualized with the naked eye. Take a clean porcelain tile, and place one drop of normal saline in each cavity, excluding the first cavity. Add one drop of specific test serum (antisera) each in the first two cavities. Mix by gently gyrating the contents thoroughly. Take out one drop of contents from the second cavity and pour it into third cavity. Likewise, mix the contents in the third cavity and place one drop into to the next cavity i.e. fourth cavity, and so on. Now we have 1:1, 1:2, 1:4, 1:8, 1:16, 1:32, 1:64 etc. of antibody to saline ratio in cavities 1,2,3,4,5,6,7 etc. Thereafter,, to each cavity add one drop of subject's own or any corresponding 5% red cell suspension. Gyrate gently the contents and incubate for 10-15 min at room temperature. Note the least positive visible reaction cavity; titre of the test sera is taken as one dilution before it. If the titre obtained is say1:16, dilute the test sera with normal saline by mixing one drop of test sera with 15 drops of normal saline for use in the absorption-inhibition test. The glass tube method of titration is essentially similar to the tile method.

Testing the saliva for A, B and H substances

When ABO group of the subject's blood is known, the presence of corresponding ABH antigens in the saliva can be determined using absorption-inhibition test as follows.

Take one drop of boiled and centrifuged saliva and to this add one drop of appropriate diluted (titrated) test serum (antisera). Rotate the tile occasionally in a circular fashion, place it in a covered petri dish to prevent evaporation and incubate for about 1h at room temperature. Add one drop of 5% suspension of subject's red cells in the cavities, rotate in circular fashion to mix thoroughly and leave for 30 min at room temperature. Observe for the agglutination; the absence of agglutination is recorded as ABH secretor and the presence of agglutination as ABH non-secretor. For AB blood group subjects, test the saliva sample separately for the presence of A and B substances using titrated anti-A and anti-B, respectively.

PRACTICE

Examine the ABH secretor status of 10 subjects provided.

Suggested Reading and References

Bhasin, M.K. and Chahal, S.M.S. 1996. *A Laboratory Manual for Human Blood Analysis.* Delhi: Kamla-Raj Enterprises.

Chandrasekhar, A. 2000. *Genetic structure of Koraga tribe, Karnataka*. Paper presented at the 25th Annual conference of the Indian Society of Human Genetics (ISHG) held at Nagpur, January 9-10.

Yasuda, N. 1984. *A note on gene frequency estimation in the ABO and ABO-like system*. Jpn. J. Hum. Genet. 29: 371-380.

Sample Questions

1) Describe the procedure to calculate allele frequencies for ABO and Rh D blood group systems from the given phenotype frequencies.

2) Write down the protocol for the detection of ABH substances from saliva.

UNIT 2 GENETICAL TRAITS

In this unit the detailed techniques and protocols for studying the variation in the following traits, commonly used in human population genetics surveys, are given.

a) Phenylthiocarbamide (PTC) tasting ability by serial dilution method.

b) Colour blindness by Ishihara colour plates chart test.

c) Haemoglobin typing by agarose gel electrophoresis.

d) Demonstration of sickle cell haemoglobin from human blood.

a) Phenylthiocarbamide (PTC) tasting ability by serial dilution method

Phenylthiocarbamide (PTC), also referred to as phenylthiourea, is a crystalline organic compound. A dimorphism with regard to ability to taste this chemical substance is observed in the humans. Some individuals are unable to find any taste of it while others find it very bitter. Those who are sensitive to the taste of PTC are called 'tasters' while the others who are not are called 'non tasters'. PTC tasting ability is a genetic trait controlled by a pair of alleles *T* and *t*, the former being dominant over the latter. Individuals with genotypes *TT* and *Tt* are tasters and those with genotype *tt* are non-tasters for PTC.

Technique

A quantitative threshold method given by Harris and Kalmus (1949) is the most widely used protocol for segregating PTC tasters from non-tasters. In this method, first a stock solution is prepared by dissolving 1.3 g of phenylthiocarbamide in 1litre of boiled tap water and is designated as solution No. 1. From the stock solution serial dilutions as shown in table 2.1 are made as follows. From stock solution (solution No.1) take 50 ml and add 50ml of boiled tap water and mix thoroughly and label it as solution No. 2. From this solution take 50ml and add 50 ml of boiled tap water to make solution No.3. Likewise, a series of serially diluted solutions from solution No.1 to 14 is prepared.

Table 2.1: Preparation of solutions for PTC tasting ability test

Solution No	PTC (mg/l)
1	1,300
2	650
3	325
4	162.5
5	81.25
6	40.63
7	20.32
8	10.16
9	5.08

10	2.54
11	1.27
12	0.64
13	0.32
14	0.16

Procedure

The subject should be asked to wash mouth before tasting the solutions. The determination of the taste 'threshold' proceeds in two stages. In stage one, starting from the higher dilution (i.e. solution Nos. 14, 13) and working up, the subject is given a few ml in a tumbler till the subject first perceives a definite taste. This gives an approximate value for the threshold. Majority of the individuals experience a bitter taste at the back of the tongue immediately after savouring a particular PTC solution. However, some subjects never experience such a taste and they are referred to as 'no-tasters'. In stage two, the subject is presented with eight tumblers, four tumblers are filled with few ml of water (control) and the remaining four are filled with few ml of the solution determined in stage one**.** The tumblers are then arranged randomly. The subject is told that four of the tumblers contain the solution and other four contain water. The subject is then asked to taste and separate them into two groups of four each. The quantity of fluid is not limited and tumblers are refilled during the test, if desired. If the two groups of four are correctly separated, the test is repeated with the next lower concentration and so on, until the subject can no longer discriminate correctly. The lowest concentration at which a completely correct answer is given is taken as the threshold. If, on the other hand, the subject is unable to separate the two groups accurately, the test is repeated in the same manner with increasing concentrations of PTC till a concentration is reached where a completely correct answer is given.

Precautions

Boiled tap water from the local source should be used both for making the solutions and for control. The solutions are stored in bottles at room temperature. Distilled water was ruled out because of its unaccustomed taste for the subject.

Practice

Find the PTC taste threshold values for 20 individuals provided by using serial dilution method.

b) **Colour blindness by Ishihara colour plates chart test**

Colour blindness is the failure to distinguish certain colours. A better wording for colour blindness is colour vision deficiency because majority of the colour blind people can see colours, barring red, green or blue. Individuals with normal colour vision can differentiate colours by the addition of the three primary colours viz., red, green and blue. Sometimes, an individual's power of perceiving one of these colours is either below normal or totally lost, and rarely a person may lose colour sense completely.

Approximately twenty characters of man exhibit sex-linked inheritance and their genes are mostly located on X rather than Y chromosome, the former being much bigger chromosome than the latter. It has been established that colour vision defect is inherited as X-linked trait with the normal colour vision dominating over colour vision defect. The most popular example of sex linked inheritance is red-green (R-G) colour blindness which may be of two main types as follows.

Protan (red blind) type with subtypes

(a) Absolute/strong (Protanopia)

(b) Partial/ mild (Protanomalia)

Deutan (green blind) type with subtypes

(a) Absolute/ strong (Deuteranopia)

(b) Partial/ mild (Deuteranomalia)

In Protanopia the visible range of the spectrum is shorter at the red end compared with that of the normal, and that part of the spectrum which appears to the normal person as blue-green, appears to those with Protanopia as grey. The whole visible range of the spectrum in protanopia consists of two areas which are separated from each other by this grey part. Each area appears to those with protanopia as one system of colour with different brightness and saturation within each area, the colour in one area being different from that of other. The red with a slight tinge of purple colour which is the complementary colour of blue-green appears also as grey.

In Deuteranopia, that part of the spectrum which appears to the normal person as green appears as grey, and the visible range of the spectrum is divided by this zone into two areas, each of which appears to be of one system of colour. The visible range of spectrum is not contracted, in contrast to protanopia. Purple-red which is the complementary colour of green appears also as grey.

In Protanomalia and Deuteranomalia, there is no part of the spectrum which appears grey, but the part of spectrum which appears to those with Protanopia is grey, appears to those with Protanomalia as a greyish indistinct colour, and likewise, the grey part of the spectrum seen by the person with Deuteranopia appears to those with Deuteranomalia as an indistinct colour close to grey. Consequently, one of the peculiarities of red-green deficiencies is that blue and yellow colours appear to be remarkably clear compared with red and green colours. The application of this peculiarity to the test for colour vision deficiencies is the distinguishing feature of this series (Ishihara, 1960).

A very rare congenital colour vision deficiency is Total Colour Weakness, in which colour sensitivity to red and green as well as to yellow and blue is very low and only the clear colours can be perceived but, except for the colour sensitivity, there is no abnormality in the visual functions. Another very rare group of individuals who suffer from Total Colour Weakness show a complete failure to discriminate any colour variation, usually with an associated impairment of central vision with photophobia and nystagmus.There may be extremely rare cases, who fail in the appreciation of blue and yellow who may be termed Tritanomalia (if partial) and Tritanopia (if absolute). Ishihara plates are not designed for the diagnosis of such cases.

The inheritance pattern of red-green colour blindness in man is diagrammatically presented in Fig. 2.1.

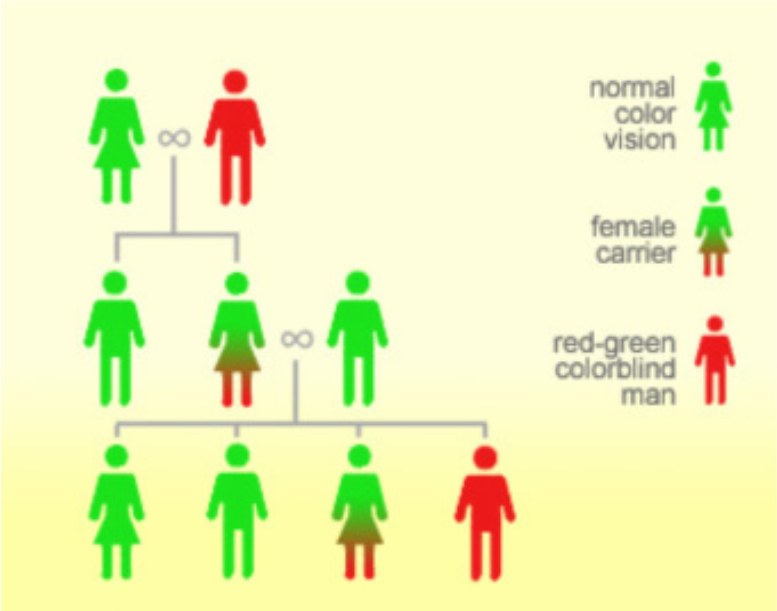

Fig. 2.1: Red-Green Colour Blindness Inheritance Pattern (Source:colblindor.com)

When a normal woman marries a colour blind man all her sons and daughters have normal colour vision. But when her daughters are married to a man with normal colour vision some colour blind sons are found. It means that a woman with normal colour vision whose father is colour blind gives birth to children of which about half of the sons are expected to be colour blind and other half to have normal colour vision.

Technique to detect colour blindness

The test that is widely used to detect colour vision deficiency is the Ishihara test. In this test the Ishihara charts are arranged as a book (Fig.2.2) which consists of a set of cards on which figures are printed in coloured dots of varying sizes against a background of dots of a different colour. The colours are so chosen that individuals with defective colour vision either misread, or are unable to read the figures. The test has a series of 38 plates (Fig.2.3 shows some of the plates) to assess quickly and exactly colour vision deficiency. Of these plates, Nos. 1-25 are having numerals meant for literates and the remaining plates from Nos. 26-38 are having winding lines meant for illiterates.

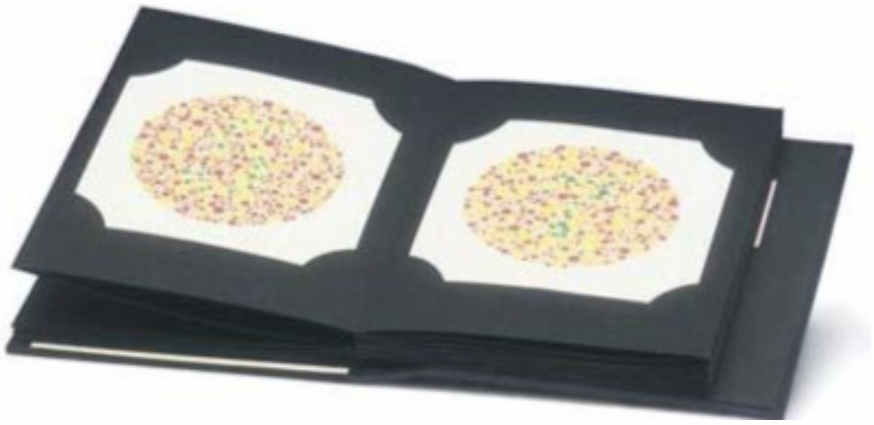

Fig. 2.2: Ishihara-Test Book (Source: www.trishir.com)

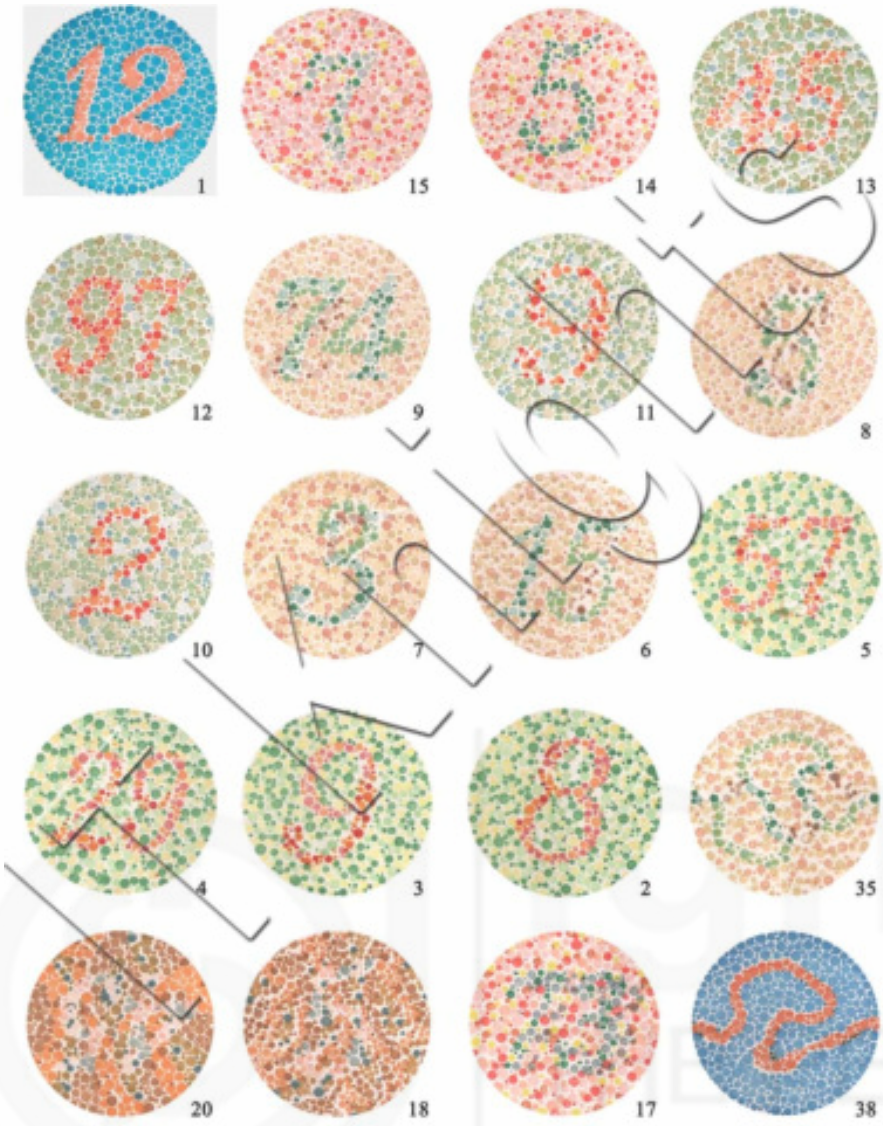

Fig.2.3: Ishihara colour blindness test plates (Source: filebuzz.com).

The first 21 plates (Nos. 1-21) in the 38 plate version of Ishihara test, charts are designed to distinguish normal colour vision individuals from red-green blind individuals. The next four plates (Nos. 22-25) are intended to separate colour vision individuals so detected into Protanopes (strong red blind) and protanomals (mild red blind) and deuteranopes (strong green blind) and deuteranomals (mild green blind).

Procedure

The plates are held at a distance of about 75cm at right angles to the line of vision of the subject in a room which is well lit by daylight The subject is asked to read numerals in plates Nos. 1-25 and each reading should be completed within three seconds. The numerals identified by the subject are matched with the standard table (Table 2.2) to assess the visual defect of the subject. If 17 or more plates are read correctly then the subject is regarded as of normal colour vision. If 13 or less plates are read correctly the subject is regarded as of deficient in red-green colour vision. But in case of plate Nos. 18, 19, 20 and 21, only those subjects who read the numerals as 5, 2, 45 and 73, respectively and read them easier than those on plates 14, 10, 13 and 17 are recorded as of deficient colour vision.

Table 2.2: Description of Ishihara plates (after Ishihara, 1960).

Plate No.	Normal colour vision person	Person with Red-Green (R-G) Deficiencies				Person with Total Colour Blindness and Weakness
1	12	12				12
2	8	3				X
3	6	5				X
4	29	70				X
5	57	35				X
6	5	2				X
7	3	5				X
8	15	17				X
9	74	21				X
10	2	X				X
11	6	X				X
12	97	X				X
13	45	X				X
14	5	X				X
15	7	X				X
16	16	X				X
17	73	X				X
18	X	5				X
19	X	2				X
20	X	45				X
21	X	73				X
		Protan		**Deutan**		
		Strong	Mild	Strong	Mild	
22	26	6	(2) 6	2	2 (6)	X
23	42	2	(4) 2	4	4 (2)	X
24	35	5	(3) 5	3	3 (5)	X
25	96	6	(9) 6	9	9 (6)	X

The mark X shows that the plate cannot be read. Blank space denotes that the reading is indefinite. The numerals in parentheses show that they can be read but they are comparatively unclear.

For the subjects who are unable to read the numerals in plate Nos. 1-25, plates Nos. 26-38 are used and the subject is asked to trace the winding lines between

the two X's on the plate with a soft brush. The tracing of each plate should be done within ten seconds. A detailed account of these is as follows (Bhasin and Chahal, 1996).

Plate Nos. 26 and 27. In tracing the winding line between the two X's, the normal person trace along the purple and red lines. In protanopia and strong protanomalia only the purple line is traced, and in case of mild protanomalia both lines are traced but the purple line is easier to follow. In deuteranopia and strong deuteranomalia only the red line is traced, and in case of mild deuteranomalia both lines are traced but the red line is easier to follow.

Plate Nos. 28 and 29. In tracing the winding line between the two X's the majority of those with red-green deficiencies trace along the line, but the majority of the normal and those with total colour blindness and weakness are unable to follow the line.

Plate Nos. 30 and 31. In tracing the winding line between the two X's, the normal trace the bluish green line, but the majority of those with colour vision deficiency are unable to follow the line or follow a line different from the normal line.

Plate Nos. 32 and 33**.** In tracing the winding line between the two X's**,** the normal trace the orange line, but the majority of those with colour vision deficiencies are unable to follow the line or follow a line different from the normal one.

Plate Nos. 34 and 35. In tracing the winding line between the two X's, the normal trace the line connecting the bluish-green and yellowish green, those with red-green deficiencies trace the line connecting the bluish green and purple, and those with total colour blindness and weakness are not able to trace the line.

Plate Nos. 36 and 37. In tracing the winding line between the two X's, the normal trace the line connecting the purple and orange, those with red-green deficiencies trace the line connecting the purple and bluish green, and those with total colour blindness and weakness are not able to trace the line.

Plate No. 38. In tracing the winding line between the two X's, both the normal and those with colour vision deficiencies are able to trace the line.

Precautions

The test should not be performed in direct sunlight or electric light as that may produce discrepancy because of an alteration in the appearance of shades of colour. When not in use, keep the book of test plates closed as undue exposure to sun light may cause fading of the colour of the plates. Change the order of the plates, if it is suspected that there is a deliberate deception on the part of the subject.

Practice

Assess the colour vision deficiency of congenital origin using Ishihara plates in 20 subjects provided.

C) Haemoglobin typing by agarose gel electrophoresis

Electrophoresis

Electrophoresis is a biochemical technique to separate charged colloidal particles (molecules) such as various blood proteins (including enzymes) and nucleic acids, based on their mobility in an electric field. In nature some molecules are positively charged and some are negatively charged, depending on net ionic charge present on them. In electrophoresis, charged ions (cations and anions) migrate towards oppositely charged electrodes (respectively, anode and cathode). Migration rates of ions during electrophoresis depend on their net ionic charge and molecular weight. To apply this technique, two simple requirements must be met. Firstly, the particles to be studied must either be charged or capable of accepting a charge. Many biological compounds like proteins, amino acids, nucleotides and phosphosugars meet this requirement. Secondly, the medium in which the electrophoresis is performed must be capable of carrying an electric current.

Electrophoresis can be carried out either in vertical (Fig.2.4) or horizontal (Fig.2.5) positions. It is obvious that the vertical position allows longer separations to be carried out, however the horizontal position is most commonly come across and almost always used for electrophoresis of proteins and enzymes in starch and agarose gel.

Electrophoresis can be carried out one dimensionally or two dimensionally. In routine, proteins and nucleic acids can be separated through one dimensional electrophoresis, whereas two dimensional gel electrophoresis is commonly used to analyze proteins (Bhasin and Chahal, 1996). To carry out electrophoresis the supporting medium may be one of several types: liquid e.g. a dense sucrose solution, solid e.g. paper or cellulose acetate sheets or gel e.g. polyacrylamide, starch or agarose (a seaweed polysaccharide).

Fig. 2.4: Vertical Electrophoresis Equipment (Source: scientiis.com)

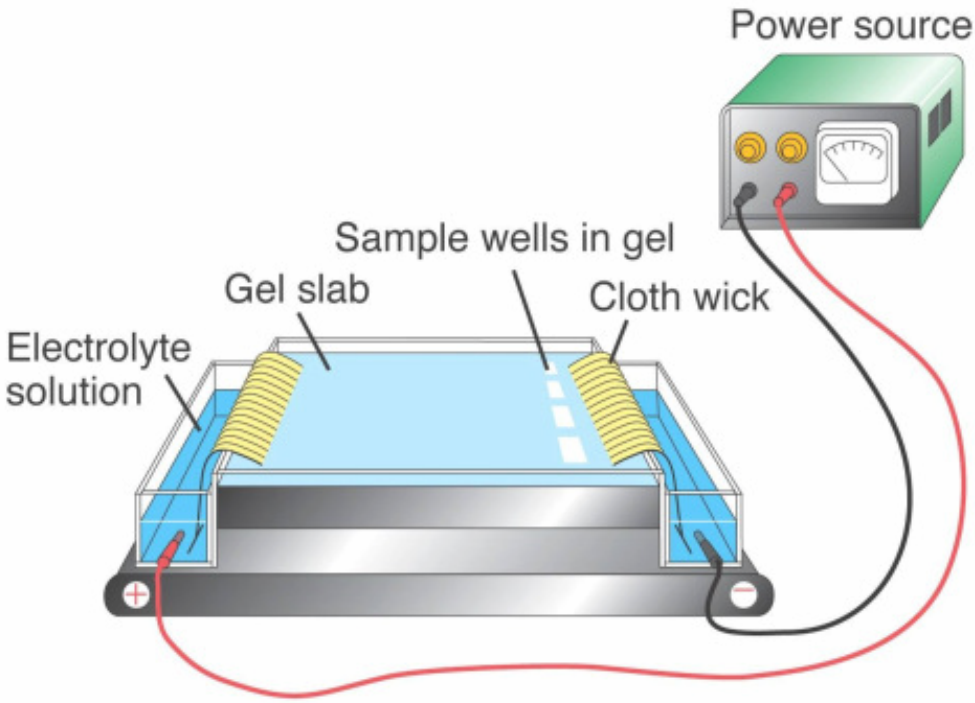

Fig. 2.5: Horizontal Electrophoresis Equipment (Source: mun.ca)

Haemoglobin Electrophoresis

Haemoglobin (HB) is an iron-containing and oxygen carrying protein of the red blood cells. HB is a tetramer that consists of two á-like and two â-like globin subunits.

There are four kinds of normal haemoglobin.

Embryonic haemoglobin (HB Gower I and HB Gower II),

Fetal haemoglobin (HBF),

Adult haemoglobin (HBA), and

Adult haemoglobin A_2 (HBA2).

Embryonic hemoglobins HB Gower I and HB Gower II are normally supplanted by fetal haemoglobin (HBF) and later by adult haemoglobins HBA and HBA2. Under normal conditions, the red cells of an adult human contain approximately 98% HBA, about 2.0% HBA2 and trace HBF.

Haemoglobin variants and thalassaemias constitute haemoglinopathies which are the inherited (genetic) disorders of haemoglobin in man.The most frequent mutations of haemoglobin which affect the beta globin gene result in variants such as sickle cell haemoglobin (HBS), haemoglobin C (HBC), haemoglobin D (HBD) and haemoglobin E (HBE). In people of India, HBS (sickle cell haemoglobin), followed by HBE and HBD are frequently encountered haemoglobin variants.

Hemoglobin electrophoresis is an experiment for identifying different types of hemoglobins present in the blood. Haemoglobin molecules are negatively charged and therefore migrate towards anode (positive electrode) in this experiment. In the following, haemoglobin typing by agarose gel electrophoresis is described.

Preparation of haemolysate

About 3-5 ml venous blood is collected into vacutainers containing EDTA as an anticoagulant. Plasma is separated by centrifugation at 2,000–3,000rpm for 10 minutes and removed using a pippete. Wash the remainder (red cells) with 0.9% NaCl solution thrice to remove traces of plasma. Add an equal quantity of distilled water and stir the contents to cause haemolysis using vortex. Add 0.4 volumes of carbon tetrachloride / toluene, vortex and centrifuge for 20 min at 3,000rpm. The supernatant above the stroma layer is haemolysate and it was removed with a pipette into an eppendorf tube and labelled. The remainder was discarded. Haemolysates were stored in a freezer (-20°C).

Procedure for Agarose gel electrophoresis of haemoglobin

The following are required for this laboratory technique.

Stock buffer (Citrate buffer, pH 5.9.)

Dissolve 73.5 g tri-sodium citrate (0.25 M) in approximately 700 ml distilled water. Using 0.5 M citric acid adjust pH to 5.9. Make the solution up to 1 litre with distilled water and store at 4^0C in a refrigerator..

Working buffer

1:5 dilution with distilled water, pH 5.9 - 6.0.

Gel preparation

Prepare gel using 1 per cent agarose in working buffer. When agarose grains are fully dissolved and the solution becomes transperant, pour on levelled thin glass plate and allow to gel for 15 minutes at room temperature. Cool for at least 15 minutes at 4°C in a refrigerator before use.

Staining solution

Dissolve 20 mg bromophenol blue in 200 ml distilled water containing 2ml glacial acetic acid. (Since haemoglobin is naturally coloured red, its bands after electrophoresis can be visualized without the use of any stain and read; however, for keeping the records staining is required).

Equipment

Power supply capable of delivering 50-100 mA, Horizontal electrophoresis tank and gel slot / comb i.e., a device for making sample application wells.

Method

Using the gel slot, 5 slots are made on the gel, mid-way between anode and cathode.

Using a pipette enough haemolysate is delivered to fill each slot on the gel.

700 ml of the working buffer is poured into the electrophoresis tanks.

Wicks made from Whatman No. 3 chromatographic paper are placed along the bridges of the electrophoresis tank. The gel is placed along the wicks and is held in position by wicks in order to ensure contact with the buffer.

A constant voltage of 40 V (approximately 30 mA) is applied across the gel.

Electrophoresis is run at 4^OC for 3 hours.

The gel is stained for 20 minutes with the bromophenol blue stain and then rinsed with distilled water. Results are read with reference to standard electrophoretic patterns of normal haemoglobin (HBA), sickel cell trait (HBAS) and sickle cell disease (HBSS) available in the literature (Figs. 2.6, 2.7). The gels may be dried in incubator for keeping the record.

Phenotype

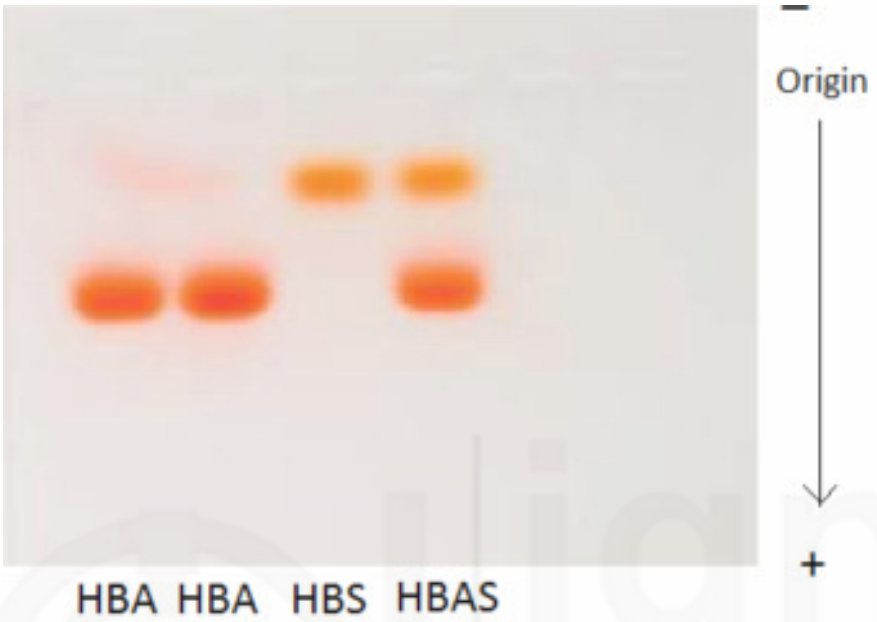

Fig. 2.6: Electrophoretic patterns of normal haemoglobin (HBA), sickle cell trait (HBAS) and Sickle cell disease (HBS)

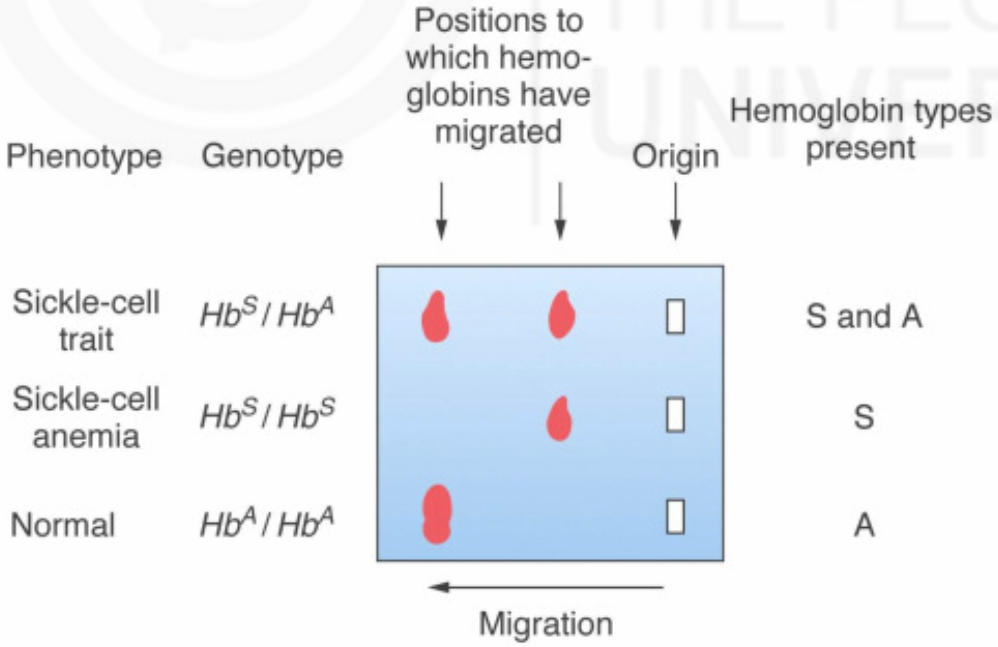

Fig. 2.7: Diagramatic representation of different phenotypes of normal (HBA) and abnormal (HBAS, HBS) haemoglobins (Source: mun.ca)

PRACTICE

Examine the two human blood samples provided for haemoglobin electrophoresis.

d) Demonstration of Sickle Cell Haemoglobin from Human Blood

Haemoglobin is a complex protein made up of four protein globulin chains combined with the iron pigment *haeme*. The normal haemoglobin is mainly composed of Haemoglobin A (HBA) with little foetal haemoglobin (HBF) (0.5%) and Haemoglobin A2 (HBA2) (1.5-3%). Haemoglobin A is composed of two alpha chains and two beta chains. HBA2 consists of two alpha chains and two delta chains and HBF contain two alpha chains and two gamma chains. There are hundreds of abnormal haemoglobins of which sickle cell haemoglobin S (HBS), haemoglobin C (HBC) and Haemoglobin E (HBE) are the most frequent ones. In the following, detection of sickle cell haemoglobin is described.

Sickle Cell Haemoglobin S (HBS)

Sickle cell anaemia is a genetically-inherited condition that is characterized by the presence of abnormal haemoglobin HBS in red blood cells due to which they attain sickle shape, under oxygen tension conditions such as exercise or high altitude. This genetic disorder affects millions of people throughout the world, predominantly among individuals with sub-Saharan African ancestry, people of Spanish-speaking regions in South America, Cuba and Central America and populations inhabiting, Saudi Arabia, India and Mediterranean countries such as Turkey, Greece and Italy.

Technique for the detection of sickle cell haemoglobin from human blood

Different tests are available for detection of sickle cell haemoglobin from human blood but Bisulfite method making use of sodium metabisulphite ($Na_2S_2O_5$) is the most common and simple method and is described below.

Slide test with sodium metabisulphite: Dissolve 0.2 g of sodium metabisulphite in 10 ml of distilled water. Stir until the chemical is completely dissolved. Store in a bottle with dropper cap; use same day.

One drop of blood is mixed with 1 drop of fresh 2% sodium metabisulphite solution on a microscope slide using applicator stick. Place a cover slip over the mixture, press to spread and seal the edges with wax/vaseline mixture or with nail varnish to create oxygen tension. Keep at room temperature for at least 30 minutes so that bisulphite solution removes the oxygen. Examine under a microscope with the dry objective; if HBS is present in the red cells they will acquire the shape of a sickle. Occasionally the preparation may need to be kept for several hours before examination. In this case the slide should be kept in a moist covered petri dish to avoid drying.

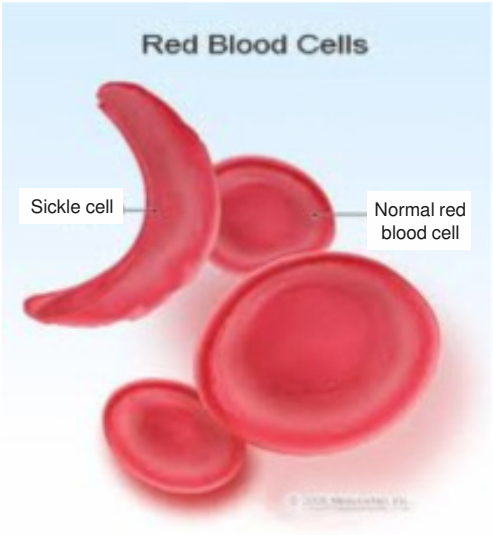

Fig. 2.8: Blood film showing normal red blood cells and sickle cells (Source: medicinenet.com)

Suggested Reading

Bhasin, M.K. and Chahal, S.M.S. 1996. *A Laboratory Manual for Human Blood Analysis*. Delhi: Kamla-Raj Enterprises.

Sample Questions

1) What is PTC? Explain the technique for PTC tasting ability through serial dilution method.
2) Define colour blindness? Describe the procedure to detect colour blindness by using Ishihara plates.
3) Discuss the technique to detect the sickle cell haemoglobin from human blood.
4) What is electrophoresis? Write an account on Haemoglobin Electrophoresis.

UNIT 3 MOLECULAR TRAITS

In this unit the protocol of DNA extraction from leucocytes by salting out method and its quantity and quality checks is given.

Deoxyribonucleic acid (DNA) is the blueprint for all biological products such as proteins and enzymes and is the primordial genetic material. It carries genetic information from one generation to the next. It is present in all the nucleated cells. Study of DNA helps us in understanding evolution and the manifestation of diseases and deformities. In humans, extraction of DNA is done from leucocytes as red blood cells are anucleated and therefore do not contain DNA.

DNA extraction from human leucocytes by salting out method

DNA Extraction

There are three major methods of DNA extraction: 1. Enzymatic method, 2. Phenol chloroform method and 3. Salting out method. As the last method is rapid, and inexpensive giving good DNA yield, in the following, protocol of the salting out method given by Miller et al. (1988) is described below:

Collect 5-10 ml of venous blood into sterile and clean tubes containing ethylene diamine tetraacetic acid (EDTA) as anticoagulant. Centrifuge the tubes at 2500 rpm for 20 minutes.

Pipette out the supernatant (plasma) and transfer the Buffy coat which is in between the plasma and red cells into another tubes. Resuspend the buffy coats in 15 ml of polypropylene centrifugation tubes with 3ml of nuclei lysis buffer (10mM Tris- HCl, 400mM NaCl and 2 mM disodium ethylene diamine tetraacetic acid (Na_2EDTA), pH 8.2).

The lysates are to be digested overnight at 37°C with 0.2 ml of 10% sodium dodecyl sulphate (SDS) and 0.5 ml of protease K solution (1 mg of protease K in 1% SDS and 2mM Na_2EDTA). Once the digestion is completed, add 1ml of saturated NaCl (approximately 6M) to the tubes and shake them vigorously for 15 seconds and then centrifuge the tubes for 15minutes at 2500 rpm. The precipitated protein pellet are left at the bottom of the tube. Transfer the supernatant containing the DNA to another 15 ml of polypropylene tubes. Afterwards add 2 volumes of absolute ethanol (room temperature) and the tubes are inverted several times until the DNA precipitated. Remove the precipitated DNA strands with a plastic spatula or pipette and transfer in to 1.5 ml of microcentrifuge tubes containing 100-200 µl of TE buffer (10mM Tris-HCl, 0.2mM Na_2EDTA, pH 7.5). Allow the DNA to dissolve at 37°C for 2 hours before quantitating.

Estimation of quantity and quality of DNA

Determination of DNA Concentration by Spectrophotometry

Prior to any analysis, DNA should be quantified and checked for purity and integrity (no shearing). Based on its structure, DNA absorbs light in the ultraviolet range, specifically at a wavelength of 260nm. A value of 1 at optical density

(OD) 260 is equal to 50ng/µl double stranded DNA, therefore to calculate the concentration of DNA, the following formula can be used.

Concentration of DNA = OD at 260nm x 50ng/µl x dilution factor.

Procedure

Dilute 2µl of DNA sample with 200 µl of Milli Q (demineralized) water (dilution factor 100). Set spectrophotometer to auto zero with the Milli Q (demineralized) water. Measure Optical Density (OD) of the diluted DNA aliquot at 260 nm and 280nm using quartz crystal cuvette.

Quality Assessment

A ratio of OD values at 260nm and 280nm i.e. 260nm/280nm indicates the purity of the extracted DNA sample. If the ratio is within a range of 1.6 to 2.0, the DNA is considered as clear and free from contaminants like residual protein and RNA. An OD ratio less than 1.6 indicates the residual proteins (or phenol contamination in organic method) while a ratio of more than 2.0 indicates residual RNA contamination.

DNA quality check using electrophoresis

Electrophoretic analysis of DNA using agarose gel can confirm DNA integrity. DNA quality can be checked in 1% agarose gel by adding required quantity of agarose to 1X Tris-Acetate-EDTA (TAE) buffer to make a mixture. The mixture is heated in a microwave oven until agarose grains dissolve completely. Cool the mixture to ~60^{o}C and add ethidium bromide to make a final concentration of 0.001µg/ml. Alternatively SafeView may be used as a stain for DNA. Pour the mixture into a tray in which a comb is fixed to make wells. After gel formation, place the tray in a tank containing 1X TAE buffer for submerged gel electrophoresis and remove the comb with care to avoid rupture of wells. Mix 1µl of each DNA sample with 1µl of loading dye (i.e. a mixture of bromophenol blue and glycerol) and load the mixture into the wells using micropipette. Subject the gel to electrophoresis at 100V for 30 min and thereafter visualize DNA bands using gel documentation (GelDoc) system.

Reference

Miller, S.A., Dykes, D.D. and Polesky, H.F. 1988. *A simple salting out procedure for extracting DNA from human nucleated cells.* Nucleic Acids Research, 16:1215.

Sample Question

1) Explain the protocol to extract DNA from human blood through salting out method.