B.Sc. ZOOLOGY LAB MANUAL

3rd Semester

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PREFACE TO THE FIRST EDITION

This is the first edition of Lab Manual for UG Zoology Third Semester (NEP). Hope this edition will help you during practical. This edition mainly tried to cover the whole syllabus. Some hard core instrument based topic are not present here that will be guided by responsive teachers at the time of practical.

ACKNOWLEDGEMENT

We are really thankful to our students, teachers and non teaching staffs to make this effort little bit complete.

Mainly thanks to Director and Principal Sir to motivate for making this lab manual.

MAJOR (MJ)

MJ-3P: Genetics (Practical) Credit 01

i. Study of mode of inheritance in human by Pedigree charts - Autosomal (dominant & recessive), X-linked (recessive & dominant) and Y- linked trait.
ii. Identification of Chromosomal aberration in *Drosophila* and Human from photograph.
iii. Chi square analysis for Mendelian ratio test.
iv. Solve the problems related to sex-linked genes and their inheritance in humans (Haemophilia, Colour blind, Sickle cell anaemia and Thalassemia).
v. Solve the problems related to two factor crosses, three factor crosses and gene interactions.

MJ-4P: Animal Physiology (Practical) Credit 01

1. Preparation of temporary mounts: Squamous epithelium.
2. Preparation of stained blood film to study various types of blood cells. Enumeration of red blood cells and white blood cells using haemocytometer. Calculation of total count and differential count from blood.
3. Estimation of bleeding time and clotting time.
4. Estimation of haemoglobin using Sahli's haemoglobinometer
5. Preparation of haemin crystals.
6. Recording of blood pressure using a sphygmomanometer.

MJ-3P: Genetics (Practical)

Study of mode of inheritance in human by Pedigree charts - Autosomal (dominant & recessive), X-linked (recessive & dominant) and Y- linked trait.

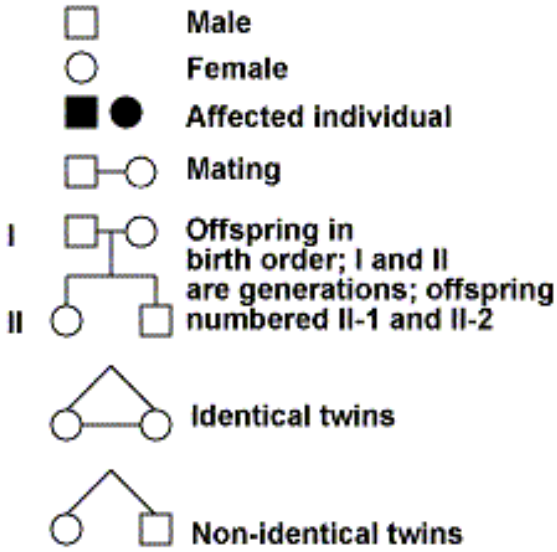
Pedigree analysis of some human inherited traits

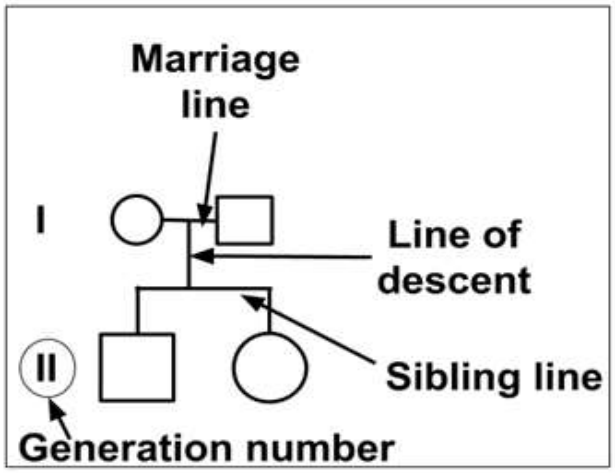
Pedigrees are used to **analyze** the pattern of **inheritance** of a particular trait throughout a family. **Pedigrees** show the presence or absence of a trait as it relates to the relationship among parents, offspring, and siblings. It is usually undertaken if families are referred to a genetic counsellor following the birth of an affected child. The **pedigree analysis** chart is **used** to show the relationship within an extended family. Males are indicated by the square shape and females are represented by circles. It is very important tool for studying human inherited diseases. These diagrams make it easier to visualize relationships with in families, particularly large extended families. Pedigrees are often used to determine the mode of inheritance (dominant, recessive, etc.) of genetic diseases. **Pedigree** analysis is therefore an **important** tool in both basic research and genetic counseling. Each **pedigree** chart represents all of the available information about the inheritance of a single trait (most often a disease) within a family

Key terms

Term	Meaning
Pedigree:	Chart that shows the presence or absence of a trait within a family across generations
Genotype:	The genetic makeup of an organism (ex: TT)
Phenotype:	The physical characteristics of an organism (ex: tall)
Dominant allele:	Allele that is phenotypically expressed over another allele
Recessive allele:	Allele that is only expressed in absence of a dominant allele
Autosomal trait:	Trait that is located on an autosome (non-sex chromosome)
Sex-linked trait:	Trait that is located on one of the two sex chromosomes
Homozygous:	Having two identical alleles for a particular gene
Heterozygous:	Having two different alleles for a particular gene

Reading a pedigree





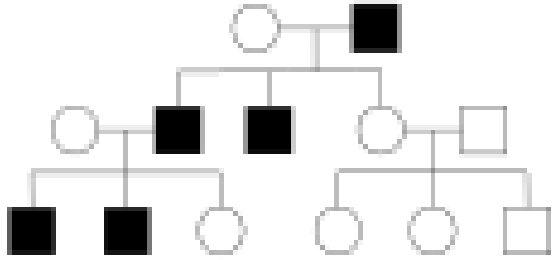
Pedigrees represent family members and relationships using standardized symbols.

By analyzing a pedigree, we can determine **genotypes**, identify **phenotypes**, and predict how a trait will be passed on in the future. The information from a pedigree makes it possible to determine how certain alleles are inherited: whether they are **dominant**, **recessive**, **autosomal**, or **sex-linked**.

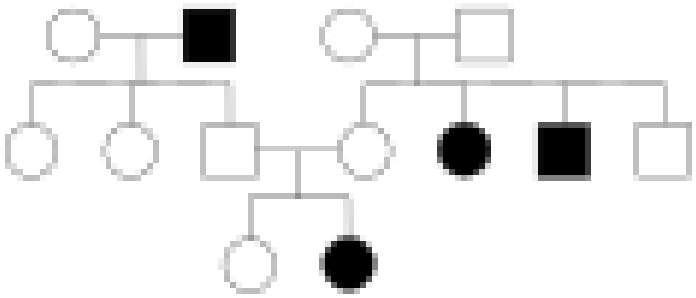
To start reading a pedigree:

1. **Determine whether the trait is dominant or recessive.** If the trait is dominant, one of the parents *must* have the trait. Dominant traits will not skip a generation. If the trait is recessive, neither parent is required to have the trait since they can be heterozygous.

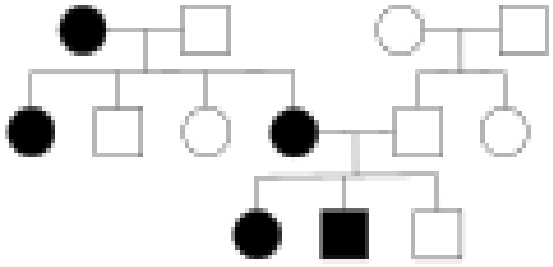
2. **Determine if the chart shows an autosomal or sex-linked (usually X-linked) trait.** For example, in X-linked recessive traits, males are much more commonly affected than females. In autosomal traits, both males and females are equally likely to be affected (usually in equal proportions).



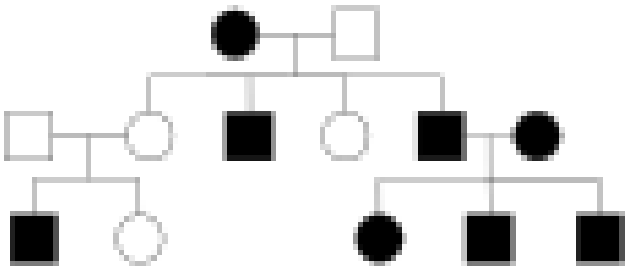
In a Y-linked disorder, only males can be affected. If the father is affected all sons will be affected. It also does not skip a generation.



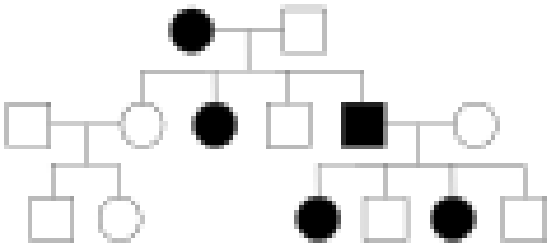
In an Autosomal Recessive Disorder, both parents can not express the trait, however, if both are carriers, their offspring can express the trait. Autosomal recessive disorders typically skip a generation, so affected offspring typically have unaffected parents. With an autosomal recessive disorder, both males and females are equally likely to be affected.



Autosomal Dominant disorders don't skip a generation, so affected offspring have affected parents. One parent must have the disorder for its offspring to be affected. Both males and females are equally likely to be affected, so it is an autosomal disorder.

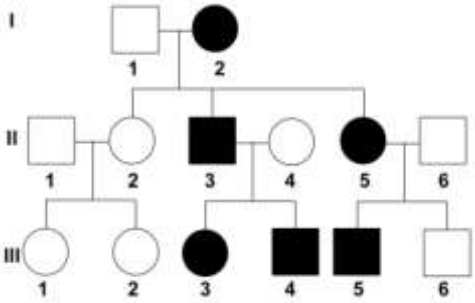


In a X-linked Recessive Disorder, males are more likely to be affected than females. Affected sons typically have unaffected mothers. The father also must be affected for daughter to be affected and the mother must be affected or a carrier for the daughter to be affected. The disorder is also never passed from father to son. Only females can be carriers for the disorders. X-linked recessive disorders also typically skip a generation.



In a X-Linked Dominant disorder, if the father is affected all daughters will be affected and no sons will be affected. It doesn't skip a generation and if the mother is affected she has a 50% chance of passing it onto her offspring

Example: Autosomal dominant trait



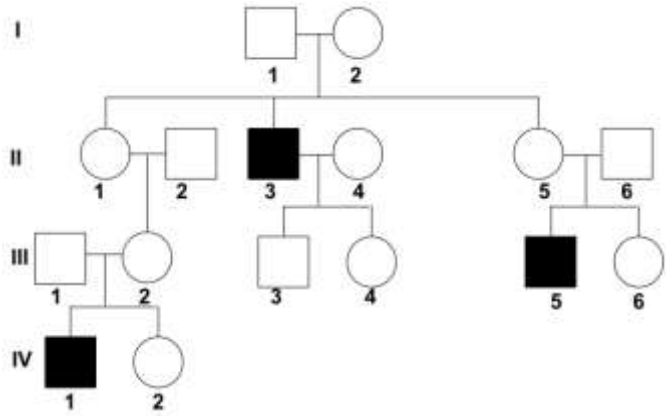
Freckles are small brown spots on your skin, often in areas that get sun exposure. In most cases, **freckles** are harmless. They form as a result of overproduction of melanin, which is responsible for skin and hair color (pigmentation). Overall, **freckles** come from ultraviolet (UV) radiation stimulation. Since freckles are dominant to no freckles, an affected individual such as I-2 must at least have one **F** allele.

The diagram shows the inheritance of freckles in a family. The allele for freckles (**F**) is dominant to the allele for no freckles (**f**). At the top of the pedigree is a grandmother (individual I-2) who has freckles. Two of her three children have the trait (individuals II-3 and II-5) and three of her grandchildren have the trait (individuals III-3, III-4, and III-5).

What is the genotype of 1-2?

The trait shows up in all generations and affects both males and females equally. This suggests that it is an autosomal dominant trait. Unaffected individuals must have two recessive alleles (**ff**) in order to not have freckles. If we notice, I-2 has some children who do *not* have freckles. In order to produce children with a genotype of **ff**, I-2 must be able to donate a **f** allele. We can therefore conclude that her genotype is **Ff**.

Example: X-linked recessive trait

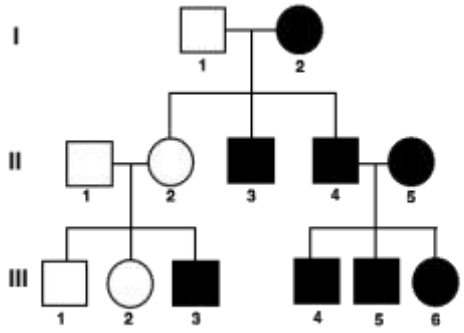


The diagram shows the inheritance of colorblindness in a family. Colorblindness is a recessive and X-linked trait (X^b). The allele for normal vision is dominant and is represented by X^B. In generation I, neither parent has the trait, but one of their children (II-3) is colorblind. Because there are unaffected parents that have affected offspring, it can be assumed that the trait is recessive. In addition, the trait appears to affect males more than females (in this case, exclusively males are affected), suggesting that the trait may be X-linked

What is the genotype of Generation III - 2?

We can determine the genotype of III-2 by looking at her children. Since she is an unaffected female, she must have at least one normal vision allele X^B. Her two genotype options are then X^BX^B or X^BX^b. However, her son (IV-1) is colorblind, meaning that he has a genotype of X^bY. Because males always get their X chromosome from their mothers and Y from their

fathers), his colorblind allele must come from III-2. We can then determine that III-2's genotype is X^BX^b, so she can pass the X^b her son.



ii. Identification of chromosomal aberration in Drosophila and man from photograph

Chromosomal abnormalities occur when there is a defect in a chromosome, or in the arrangement of the genetic material on the chromosome. Very often, chromosome abnormalities give rise to specific physical symptoms, however, the severity of these can vary from individual to individual.

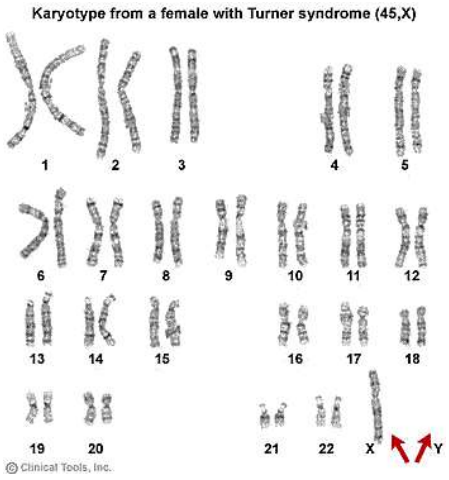
Abnormalities can be in the form of additional material which may be attached to a chromosome, or where part or a whole chromosome is missing, or even in defective formation of a chromosome. Any increases or decreases in chromosomal material interfere with normal development and function.

There are two main types of chromosomal abnormality which can occur during meiosis and fertilization: numerical aberrations and structural aberrations.

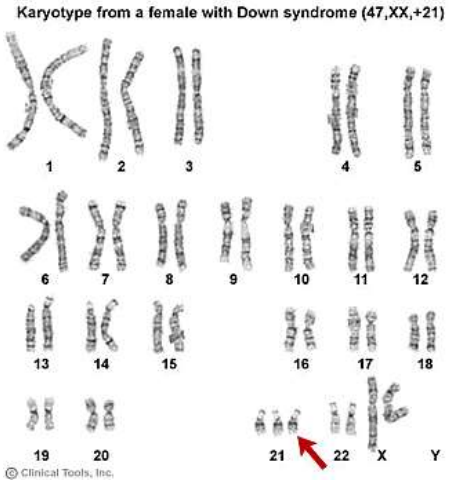
Numerical Aberrations

These are usually caused by a failure of chromosome division, which results in cells with an extra chromosome or a deficiency in chromosomes.

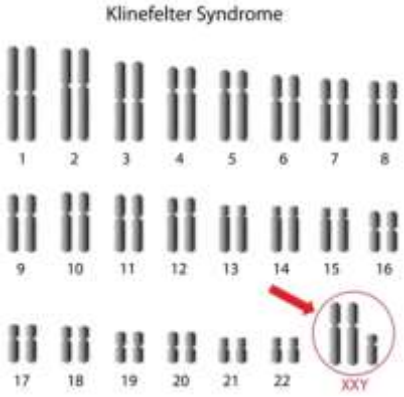
Gametes with these anomalies can result in conditions such as Down syndrome (who have 47 chromosomes instead of 46), or Turner syndrome (45 chromosomes). Common types of numerical aberrations are: triploidy, trisomy, monosomy and mosaicism.



Karyotype of Turner syndrome (45 chromosomes instead of 46)



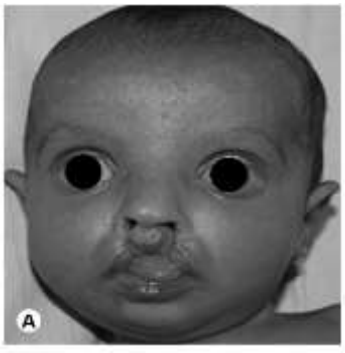
Karyotype of Down Syndrome (47 chromosomes instead of 46)

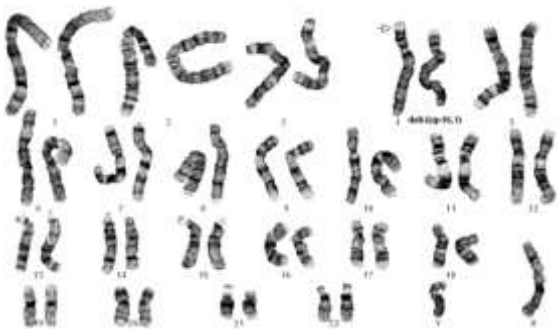


Structural Aberrations

These occur due to a loss or genetic material, or a rearrangement in the location of the genetic material. They include: deletions, duplications, inversions, ring formations, and translocations.

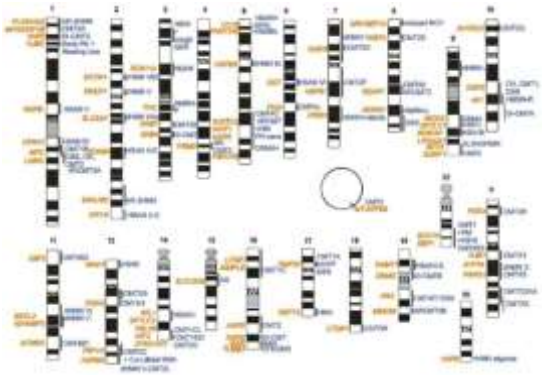
- Deletions: A portion of the chromosome is missing or deleted. Known disorders include Wolf-Hirschhorn syndrome, which is caused by partial deletion of the short arm of chromosome 4; and Jacobsen syndrome, also called the terminal 11q deletion disorder.





Wolf-Hirschhorn syndrome

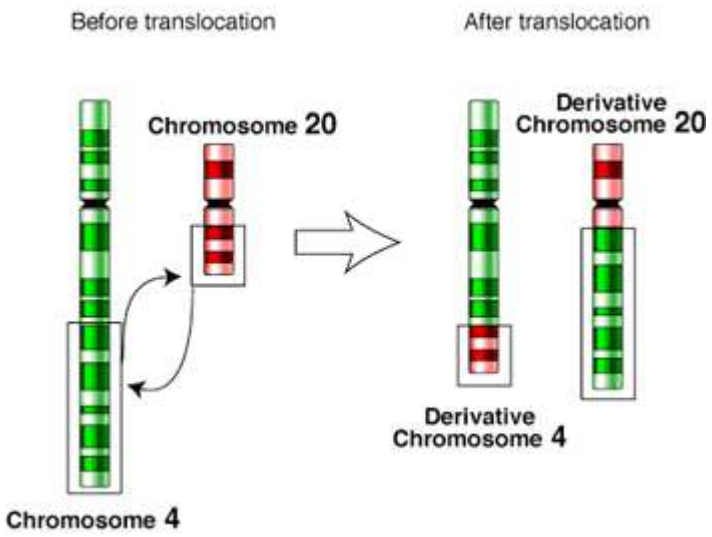
- Duplications: A portion of the chromosome is duplicated, resulting in extra genetic material. Known disorders include Charcot-Marie-Tooth disease type 1A which may be caused by duplication of the gene encoding peripheral myelin protein 22 (PMP22) on chromosome 17.



- Translocations: When a portion of one chromosome is transferred to another chromosome. There are two main types of translocations. In a reciprocal translocation, segments from two different chromosomes have been exchanged. In a Robertsonian translocation, an entire chromosome has attached to another at the centromere; these only occur with chromosomes 13, 14, 15, 21 and 22.
- Inversions: A portion of the chromosome has broken off, turned upside down and reattached, therefore the genetic material is inverted.
- Rings: A portion of a chromosome has broken off and formed a circle or ring. This can happen with or without loss of genetic material.
- Isochromosome: Formed by the mirror image copy of a chromosome segment including the centromere.

Structural aberrations also include some disorders which are characterized by chromosomal instability and breakage. One example, is the creation of a fragile site on the X Chromosome - Fragile X syndrome. Boys are worse affected by this because they only have one X-Chromosome but even in girls, Fragile X syndrome can cause learning difficulties.

Most chromosome anomalies occur as an accident in the egg or sperm, and are therefore not inherited. The anomaly is present in every cell of the body. Some anomalies, however, can happen after conception, resulting in mosaicism (where some cells have the anomaly and some do not). Chromosome anomalies can be inherited from a parent or be "de novo". This is why chromosome studies are often performed on parents when a child is found to have an anomaly.



Translocation - showing a portion of one chromosome transferred to another chromosome

iii. Chi square analysis for Mendelian ratio test.

This is a statistical test performed to determine how closely the observed ratio fits the This expected ratio. Chi-square ($\chi 2$) test was used in testing statistical hypothesis in the year 1900 by Karl Pearson.

Statistical Hypothesis: Any kind of statement about a statistical population or statistical value are called statistical hypothesis.

Test of Hypothesis: A test of hypothesis is a method which specifies whether to accept or reject the hypothesis under consideration under the preview of a set of rules for decision. Test of hypothesis deals only with the acceptance and rejection of null hypothesis.

Null Hypothesis: According of R. A. Fisher, "Null Hypothesis is the hypothesis which is to be tested for possible rejection under the assumption it is true.

" Null hypothesis is denoted by H_o and is tested against an alternative hypothesis.

Alternative Hypothesis: The negative form of null hypothesis is known as the alternative hypothesis. Alternative hypothesis is denoted by H_a.

Level of significance: The maximum probability with which a null hypothesis is rejected is called the level of significance of the statistical test. Generally, level of significance is considered at 1% or 5% level or any other level depending upon the consequences of statistical decision.

Degrees of freedom: This is the values of a sample which are freely variable without disturbing the mean. This parameter is used to determine whether a chi-square value is statistically significant or not.

If the data is given in the form of a series of variables in a row or column then the degrees of freedom (df) is calculated by the formula.

df = n-1, where 'n' is the number of items in the series. Chi-square value against particular degrees of freedom is obtained from the probability chart published by Yates & Fisher.

Type of Chi-squares: There are three types of Chi-square analysis. These are- (i) Goodness of fit, (ii) Contingency Chi-square, (iii) Homogeneity Chi-square.

Goodness of fit: Common type of Chi-square analysis is known as Pearsonian Chi-square test. This his is also known as test for goodness of fit, because the test is performed to determine whether the observed value is in good agreement with the expected values.

This is determined by the following formula:

$$\chi^2 = \sum \frac{\left[(O - E) - \frac{1}{2}\right]^2}{E}$$

Where O = observed value

E = Expected value

$\frac{1}{2}$ = Yates correction

*Yates correction means reduction of 0.5 from absolute difference between observed and expected (+ or -) frequencies is generally applicable in Monohybrid cross.

Null hypothesis for Goodness of fit: Null hypothesis for Goodness of fit can be studied very well with the ratio obtained from Mendelian monohybrid and dihybrid crosses e.g.

(i) Mendelian monohybrid test cross ratio 1:1

(ii) Mendelian monohybrid F_2, ratio 3:1

(Iii) Mendelian dihybrid test cross ratio 1:1:1:1

(iv) Mendelian dihybrid F_2, ratio 9:3:3: 1

Some Examples of Goodness of Fit:

Example1: In a cross between tall and dwarf garden pea plants 350 tall and 110 dwarf pea plants were obtained in F_2, Test the goodness of fit of these data to a 3: 1 ratio, using the chi-square test considering probability at 5% level.

Observed (O)	Expected (E)	(O–E)	$(O-E)-\frac{1}{2}$	$\{(O-E)-\frac{1}{2}\}^2$	$X^2=\frac{[(O-E)-\frac{1}{2}]^2}{E}$
Tall = 350	345	5	5 – 0.5 = 4.5	20.25	$\frac{20.25}{345}=0.058$
dwarf = 110	115	–5	|–5| –0.5 = 4.5	20.25	$\frac{20.25}{115}=0.176$
Total = 460					$\Sigma = 0.234$

Solution : (a) Here Null hypothesis Ho = 3 : 1

(b) Alternative hypothesis Ha = 1 : 1

(c) Formula : $\chi^2 = \Sigma \frac{\left[(O-E)-\frac{1}{2}\right]^2}{E}$

Here degrees of freedom 2 – 1 = 1

At 5% level of significance the table value is 3·84

Inference: Since the calculated value is 0.234 and table value is 3.84, therefore difference between the observed and expected values are insignificant, so the null hypothesis is accepted. In this case the data has good fit to the Mendelian 3:1 ratio.

Example-2: In a cross between long wing and vestigial wing Drosophila, 510 long wing and 490 vestigial wing Drosophila were obtained in F_2. Test goodness of fit of these data to a 1:1 ratio, using the X^2 test considering 5% level of significance.

Solution : (i) Null hypothesis Ho = 1:1

(ii) Alternative hypothesis Ha = 3:1

(iii) Formula : $\chi^2 = \Sigma \frac{\left[(O-E)-\frac{1}{2}\right]^2}{E}$

Here degrees of freedom 2-1=1

At 5% level of significance the table value is 3.84

Observed (O)	Expected (E)	(O–E)	$(O-E)-\frac{1}{2}$	$\{(O-E)-\frac{1}{2}\}^2$	$X^2=\frac{(O-E)^2}{E}$
Long Wing = 510	half of 1000 = 500	10	$(10)-\frac{1}{2}=9.5$	90.25	$\frac{90\cdot25}{500}=0.180$
Vestigial Wing = 490	= 500	−10	$(-10)-\frac{1}{2}=9.5$	90.25	$\frac{90\cdot25}{500}=0.180$
Total = 1000					$\Sigma = 0.360$

Inference: The difference between the observed and expected values are insignificant at 0.05 probability. Hence the Null Hypothesis is accepted.

Example-3: From a cross between Grey body-Long wing *Drosophila melanogaster* and Ebony body-vestigial wing flies, the following progenies were obtained in F_2, Grey long = 380: Ebony 135 Vestigial = 125, Ebony-vestigial = 40. Test the goodness of fit of the data to a 9:3:3:1 ratio using x^2 test considering probability at 5% level.

Observed (O)	Expected (E)	(O – E)	$(O-E)^2$	$\chi^2=\frac{(O-E)^2}{E}$
Grey long–380	382.5	382.5 – 380 = –2.5	6.25	$\frac{6\cdot25}{382\cdot5}=0.016$
Ebony–135	127.5	135–127.5 = 8.5	68.0	$\frac{68\cdot0}{127\cdot5}=0.533$
Vestigial–125	127.5	127.5–125 = –2.5	6.25	$\frac{6\cdot25}{127\cdot5}=0.049$
Ebony–vestigial–40	42.5	42.5–40 = –2.5	6.25	$\frac{6\cdot25}{42\cdot5}=0.147$
Total–680				$\Sigma = 0.745$

Solution: Here, Null Hypothesis H_o 9: 3:3:1

Alternative hypothesis H_a = 1:1:1:1

Here, degrees of freedom = 4-1 = 3

At 5% level of significance the table value is 7.82

Inference: Difference between observed and expected is insignificant at 5% level of significance and hence the Null Hypothesis is accepted.

Example-4: From a cross between heterozygous tall plant with round seed Coat pea plant and homozygous dwarf plant with wrinkled seed the following phenotypes were obtained in F_2. Tal-round = 200, Tall-wrinkled = 190; dwarf-round = 180 and dwarf-wrinkled = 170. Test the goodness of fit considering suitable null hypothesis. Consider the level of significance at 5%.

Observed (O)	Expected (E)	Obs–Exp. (O – E)	$(O - E)^2$	$\chi^2 = \frac{(O-E)^2}{E}$
Tall-round = 200	$\frac{740}{4} = 185$	(200–185) = 15	225	$\frac{225}{185} = 1.216$
Tall-wrinkled = 190	= 185	(190–185) = 5	25	$\frac{25}{185} = 0.135$
Dwarf-wrinkled = 180	= 185	(185–180) = –5	25	$\frac{25}{185} = 0.135$
Dwarf-wrinkled = 170	= 185	(185–170) = –15	225	$\frac{225}{185} = 1.216$
rotal–740				Σ=2.702

Solution : Here Null Hypothesis is 1 : 1 : 1 : 1 and alternate hypothesis is 9 : 3 : 3 : 1

Here degrees of freedom = 4 – 1 = 3

At 5% level of significance the table value is 7·82

Inference : Difference between observed and expected is insignificant at 5% level of significance and hence the Null hypothesis is accepted.

(ii) Contingency Chi-square: In many experiments, it becomes necessary to compare one set of observation recorded under a particular condition to those of identical nature but under different conditions. For this studies it can be identified whether the both the results are dependent (contingent upon) or independent of conditions under which the observation were made. Therefore, the test is called **contingency test**.

Formula of contingency Chi-square: Common formula for contingency Chi-square is as follows -----

$$\chi^2 = \frac{N\left\{(ad - bc) - \frac{N}{2}\right\}^2}{R_1 \times R_2 \times C_1 \times C_2}$$

Where 'a', 'b', 'c' and 'd' are the frequencies for the four cell contingency table, where 'a' &'b' are in one row and 'c' & °d' in the other row. On the other hand, 'a' and 'c', 'b' and 'd' are in two columns.

The R_1 denotes the total of a + b ; R_2 denotes the total of c + d. Likewise a + c is denoted by C_1; b + d is donoted by C_2.

$N = a + b + c + d = R_1 + R_2 = C_1 + C_2$

In this case the degress of freedom = $(R - 1)(C - 1) = 1 \times 1 = 1$

Example : Among 60 males and 50 females, 25 males and 20 females were with attached ear lobes. Statistically probe whether attached ear lobe has any relation with the sex.

Null hypothesis: Attached ear lobe character is not a sex-linked character.
Alternative hypothesis: The character is sex linked.

Calculation :

Ear lobe	Male	Female	Total	
Free ear lobe	35 (a)	30 (b)	65	(a + b) R_1
Attached ear lobe	25 (c)	20 (d)	45	(c + d) R_2
	a + c = 60	b + d = 50	a + b + c + d = 110	
	C_1	C_2		

$$\chi^2 = \frac{N\left\{|ab - bc| - \frac{N}{2}\right\}^2}{R_1 R_2 C_1 C_2}$$

$$= \frac{110 \times \left\{|700 - 750| - \frac{110}{2}\right\}^2}{65 \times 45 \times 60 \times 50}$$

$$= \frac{110 \times \{|-50| - 55\}^2}{65 \times 45 \times 60 \times 50}$$

$$= \frac{110 \times \{|-5|\}^2}{65 \times 45 \times 60 \times 50} = \frac{^{11}\cancel{55}\cancel{110} \times \cancel{25}}{65 \times 45 \times \cancel{60}_{12} \times \cancel{50}_{\cancel{25}}}$$

$$= \frac{11}{65 \times 45 \times 12} = \frac{11}{35100}$$

$$= 0{\cdot}0003$$

Calculation :

$ad = 35 \times 20 = 700$

$bc = 30 \times 25 = 750$

$R_1 = a + b = 65$, $C_1 = a + c = 60$

$R_2 = c + d = 45$, $C_2 = b + d = 50$

$N = a + b + c + d = 110$

Degrees of Freedom (df) : $(R-1)(C-1) = (2-1)(2-1) = 1 \times 1 = 1$

Table value at 5% level of significance at df is 3.84

Inference: The relationship between the attached ear lob and sex of human being is insignificant and hence the null hypothesis is accepted.

(iii) **Homogeneity Chi-square**: Homogeneity Chi-square is done when different results are obtained of similar kind of experiments. For example, results obtained from a number of Mendelian monohybrid or dihybrid crosses are taken together for X^2 analysis first individually are between the individual X^2 data and pooled data can be observed.

Working Principle:

(1) Calculate the X^2 of individual experiments.

(2) Make the summation of all X^2 values along with df.

(3) Make the X^2 of the summed individual data.

(4) Draw inference on the basis of null hypothesis considered from the results of homogeneity Chi-square.

Example: From five sets of monohybrid crosses between tall and dwarf pea plants following results were obtained in F_2.

Table : A

	1st set	2nd set	3rd set	4th set	5th set
Tall	55	40	44	41	31
Dwarf	15	10	12	11	9

Solution : **Null hypothesis** : The results conform 3 : 1 principle.

Alternative hypothesis : The results do not conform 3 : 1 principle.

Table : B

	Observed	Expected	Obs–Exp.	$(O-E)^2$	$\frac{(O-E)^2}{E}$	χ^2	df
1st	55	52·5	2·5	6·25	$\frac{6\cdot25}{52\cdot5}=0\cdot119$	0·476	2–1
set	$\frac{15}{70}$	17·5	–2·5	6·25	$\frac{6\cdot25}{17\cdot5}=0\cdot357$		=1
2nd	40	37·5	2·5	6·25	$\frac{6\cdot25}{37\cdot5}=0\cdot016$	0·516	2–1
set	$\frac{10}{50}$	12·5	–2·5	6·25	$\frac{6\cdot25}{12\cdot5}=0\cdot500$		=1
3rd	44	42	2	4	$\frac{4}{42}=0\cdot095$	0·380	2–1
set	$\frac{12}{56}$	14	–2	4	$\frac{4}{14}=0\cdot285$		=1
4th	41	39	2	4	$\frac{4}{39}=0\cdot102$	0·409	2–1
set	$\frac{11}{52}$	13	–2	4	$\frac{4}{13}=0\cdot307$		=1
5th	31	30	1	1	$\frac{1}{30}=0\cdot033$	0·133	2–1
set	$\frac{9}{40}$	10	–1	1	$\frac{1}{10}=0\cdot100$		=1

Summed X^2 $\Sigma X^2 = 1.914$

Data	χ^2	df	Table value
Total	1.914	4	
Sum Data	1.989	1	
Homogenity	Difference between the two is 0.075	3	at 5% level of significance table value 7.82

Inference: The difference is insignificant and null hypothesis is accepted.

Sex-Linked Inheritance Problem Set

The study of inheritance of genes located on sex chromosomes was pioneered by T. H. Morgan and his students at the beginning of the 20th century. Although Morgan studied fruit flies, the same genetic principles apply to humans. Since males and females differ in their sex chromosomes, inheritance patterns for X-chromosome linked genes vary between the sexes. Our objective is to understand the principles that govern inheritance of genes on sex chromosomes.

Problem 1: Hemophilia in humans

Hemophilia in humans is due to an **X**-chromosome mutation. What will be the results of mating between a normal (non-carrier) female and a hemophilac male?

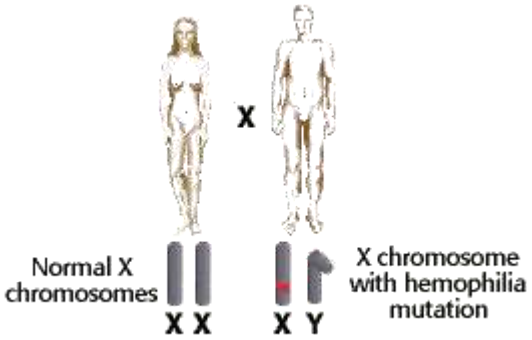
A. half of daughters are normal and half of sons are hemophilic.
B. all sons are normal and all daughters are carriers.
C. half of sons are normal and half are hemophilic; all daughters are carriers.
D. all daughters are normal and all sons are carriers.
E. half of daughters are hemophilic and half of daughters are carriers; all sons are normal.

Answer: B. all sons are normal and all daughters are carriers.
(Daughters inherit a normal allele from their mother and the hemophilia allele from their father. Sons inherit the normal allele from their mother.)

Genotypes and phenotypes of parents

The eggs of the mother will all contain the normal **X** chromosome.

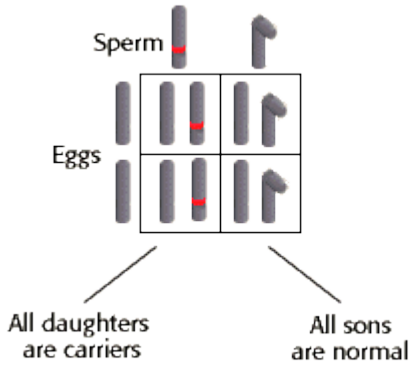
The sperm of the father will contain either the **X** chromosome with the mutation causing hemophilia or the **Y** chromosome.



Genotypes and phenotypes of offspring

All of the daughters inherit an **X** chromosome with the mutation from their father, and will be carriers.

All the sons inherit a normal **X** from the mother.



Problem 2: Red-green color blindness in humans

A human female "carrier" who is heterozygous for the recessive, sex-linked trait causing red-green color blindness (or alternatively, hemophilia), marries a normal male. What proportion of their male progeny will have red-green color blindness (or alternatively, will be hemophiliac)?

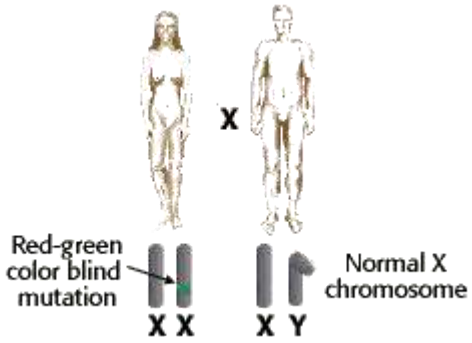
A. 100%
B. 75%
C. 50%
D. 25%
E. 0%

Answer: C. 50%
(Half the sons would be expected to inherit the allele from their mother and be afflicted because they are hemizygous. Half the daughters would be carriers like their mothers.)

Genotypes and phenotypes of parents

The eggs of the mother will contain either a normal **X** chromosome or an **X** chromosome with the mutation causing red-green color blindness.

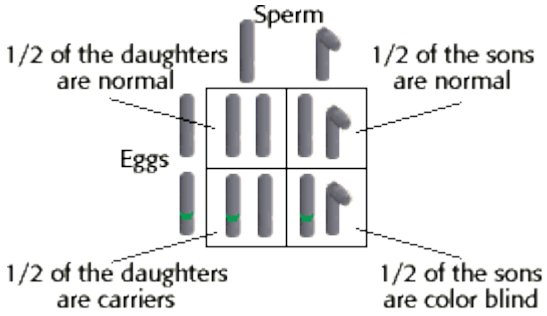
The sperm of the father will contain either the normal **X** chromosome or the **Y** chromosome.



Genotypes and phenotypes of offspring

None of the female children would be red-green color blind, but half would be "carriers."

Half of the sons would inherit the allele from their mother and be afflicted.



Problem 3: Tracing the inheritance of the human Y chromosome

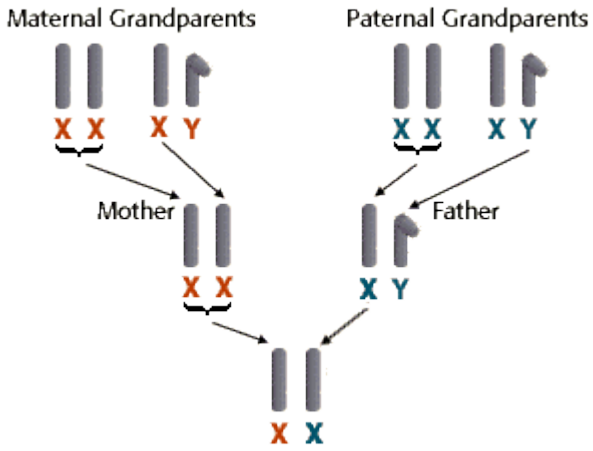
Women have sex chromosomes of **XX**, and men have sex chromosomes of **XY**.

Which of a man's grandparents could not be the source of any of the genes on his **Y**-chromosome?

A. Father's Mother.
B. Mother's Father.
C. Father's Father.
D. Mother's Mother, Mother's Father, and Father's Mother.
E. Mother's Mother.

Answer: D. Mother's Mother, Mother's Father, and Father's Mother.
(The Y chromosome is inherited solely from father to son in each generation.)

X chromosome inheritance



The **X** chromosomes are inherited from either maternal grandparent and paternal grandmother The diagram shows how the **X** and **Y** chromosomes are inherited from the maternal and paternal grandparents to the parents to the son. The **Y** chromosome is passed strictly from the father to male children in each generation. No male from the father's side of the family can be a source of genes on a women's **X** chromosome.

Problem 4: Tracing the inheritance of the human X chromosome

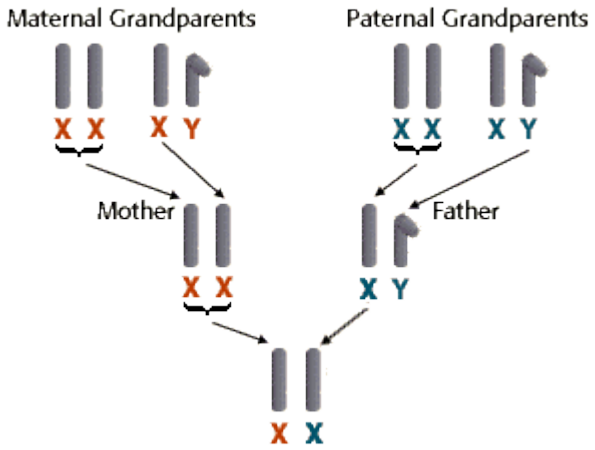
Women have sex chromosomes of **XX**, and men have sex chromosomes of **XY**.

Which of a women's grandparents could not be the source of any of the genes on either of her **X**-chromosomes?

A. Mother's Father.
B. Father's Mother.
C. Mother's Mother.
D. Father's Father.
E. Mother's Mother and Mother's Father.

D. Father's Father.
(The father's father contributes only the Y chromosome to his sons, and subsequently to his grandsons.)

X chromosome inheritance



The **X** chromosomes are inherited from either maternal grandparent and paternal grandmother The diagram shows how the **X** and **Y** chromosomes are inherited from the maternal and paternal grandparents to the parents to the son. The **Y** chromosome is passed strictly from the father to male children in each generation. No male from the father's side of the family can be a source of genes on a women's **X** chromosome.

Problem 5: Offspring of human females who are carriers for X-linked traits

A human female "carrier" who is heterozygous for the recessive, sex-linked trait red color blindness, marries a normal male.

What proportion of their female progeny will show the trait?

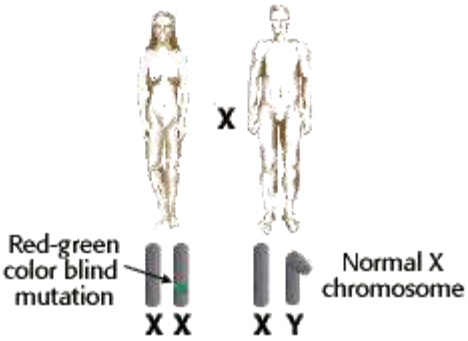
A. All
B. 1/2
C. 1/4
D. 0
E. 3/4

Answer: D. 0
(Half the sons would be expected to inherit the color blindness allele from their mother. Half the daughters would be carriers like their mothers.)

Genotypes and phenotypes of parents

The eggs of the mother will contain either a normal **X** chromosome or an **X** chromosome with the mutation causing red-green color blindness (i.e. She is a "carrier".)

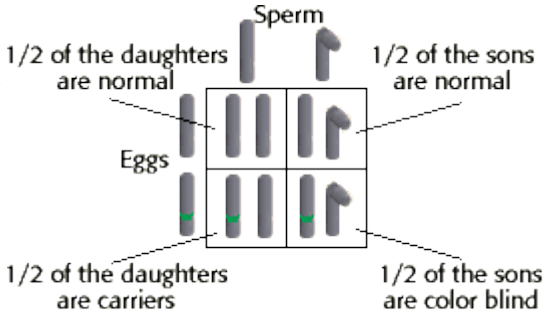
The sperm of the father will contain either the normal **X** chromosome or the **Y** chromosome.



Genotypes and phenotypes of offspring

The eggs of the mother will contain either a normal **X** chromosome or an **X** chromosome with the mutation causing red-green color blindness (i.e. She is a "carrier").

The sperm of the father will contain either the normal **X** chromosome or the **Y** chromosome.



Problem 6: Crossing a white-eyed female and red-eyed male fly

In a cross between a white-eyed female fruit fly and red-eyed male, what percent of the female offspring will have white eyes? (White eyes are X-linked, recessive)

A. 100%
B. 25%
C. 50%
D. 75%
E. 0%

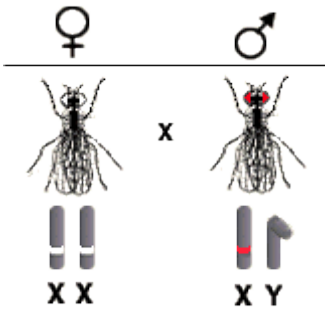
Answer: E. 0%
(All of the females are red-eyed and heterozygous. All of the males are white-eyed and hemizygous.)

Tutorial:

Genotypes and phenotypes of parents

The female parent must be homozygous because she has the recessive white-eyed phonotype.

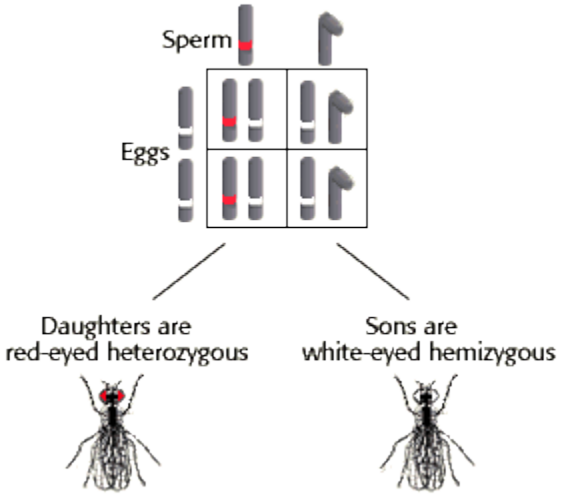
The male parent is hemizygous, red-eyed.



Genotypes and phenotypes of offspring

All of the females eggs will contain an X chromosome with the white-eye mutation.

The sperm will contain either a normal X chromosome or a Y chromosome.



Problem 7: Test cross of a red-eyed female fly

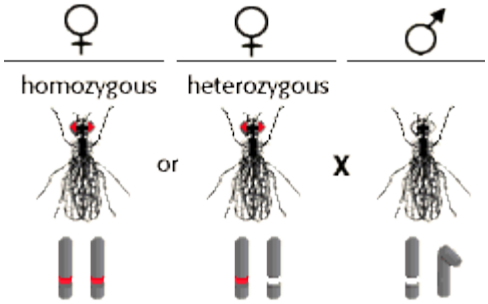
A female Drosophila of unknown genotype was crossed with a white-eyed male fly, of genotype $X^{w}Y$(w = white eye allele is recessive, w+= red-eye allele is dominant.) Half of the male and half of the female offspring were red-eyed, and half of the male and half of the female offspring were white-eyed. What was the genotype of the female fly?

A. $X^{w+}Y$
B. $X^{w+}X^{w+}$
C. $X^{w}X^{w}$
D. $X^{w}Y$
E. $X^{w+}X^{w}$

Answer: E. $X^{w+}X^{w}$

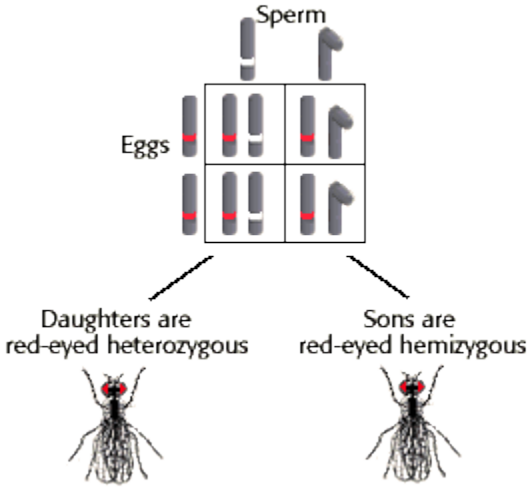
(Female must be heterozygous because she can transmit either the red eye or white eye allele to her sons and daughters.)

Possible Female Genotypes



Genotypes and phenotypes of offspring if the female is homozygous

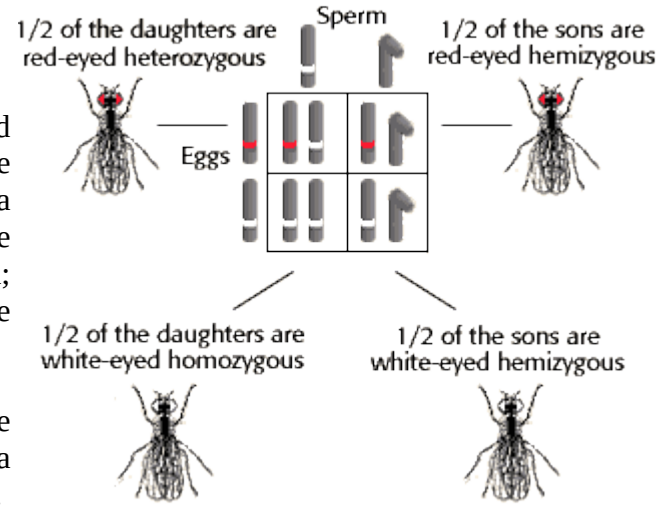
All of the male and female offspring of a homozygous female would be red-eyed.



Genotypes and phenotypes of offspring if the female is heterozygous

Half of the male and half of the female offspring of a heterozygous female would be white-eyed; the other half would be red-eyed.

Therefore the female must be a heterozygous, $\mathbf{X}^{w+}\ \mathbf{X}^{w}$.



Summary

We had a cross between a female Drosophila of unknown genotype with a white-eyed male fly. The result of the cross was that half of the male and half of the female offspring were red-eyed, and half of the male and half of the female offspring were white-eyed. What was the genotype of the female fly? The female fly had to be heterozygous, $\mathbf{X}^{w+}\ \mathbf{X}^{w}$.

Problem 8: Predicting the offspring of a homozygous red-eyed female fly

In a cross between a pure bred, red-eyed female fruit fly and a white-eyed male, what percent of the male offspring will have white eyes? (white eyes are **X**-linked, recessive)

A. 100%
B. 75%
C. 50%
D. 25%
E. 0%

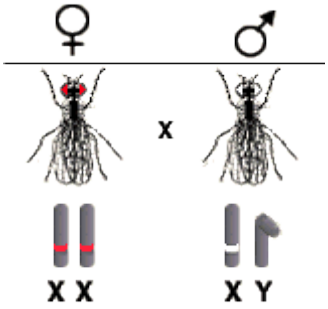
Answer: E. 0%
(All of the males and all of the females are red-eyed.)

Tutorial:

Genotypes and phenotypes of parents

The pure bred female is homozygous for the normal **X** chromosome.

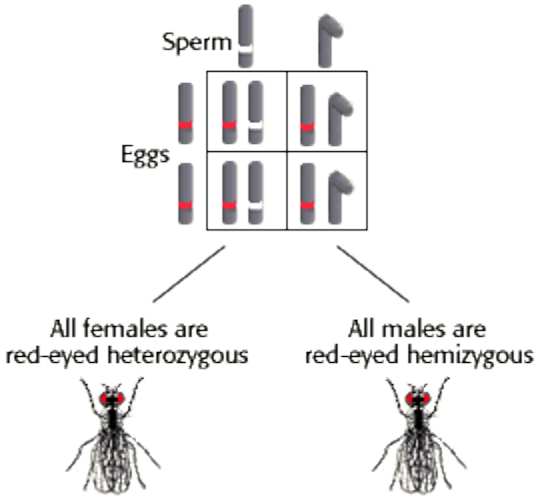
The white-eyed male is hemizygous for the **X** chromosome with a white eye mutation.



Genotypes and phenotypes of offspring

Both male and female children will inherit a normal **X** chromosome from the female.

None of the male or female offspring will have white eyes.



Problem 9: Predicting genotype when phenotype is known

What is the genotype of a red-eyed, yellow-bodied female fruit fly who is homozygous for the eye color allele?
Red eyes (w+) and tan bodies (y+) are the dominant alleles. (Both traits are **X** chromosome linked).

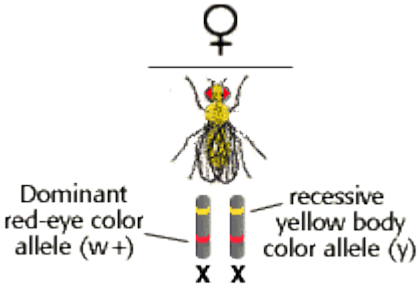
A. $X^{w+y}\ X^{w+y}$
B. $X^{w+y}\ Y$
C. $X^{wy+}\ X^{wy+}$
D. $X^{wy+}\ Y$
E. $X^{w+y}\ X^{w+y+}$

Answer: **A.** $X^{w+y}\ X^{w+y}$
(The female is homozygous for dominant eye color (given), and homozygous for recessive body color allele.)

Tutorial:

Possible female genotypes

Since the female fly has a yellow body, and the tan body color is the dominant allele, she will be



homozygous recessive for yellow body color.

Therefore, the female fly will be homozygous for both traits.

The genotype is $\mathbf{X}^{w+y}\ \mathbf{X}^{w+y}$.

Problem 10: Another white-eyed female x red-eyed male fly cross

A white-eyed female fruit fly is crossed with a red-eyed male. Red eyes are dominant, and **X**-linked. What are the expected phenotypes of the offspring?

A. All of the females will have red eyes; half of the males will have red eyes, and half of the males will have white eyes.
B. All of the females and all of the males will have white eyes.
C. All of the females will have red eyes; all of the males will have white eyes.
D. All of the females and all of the males will have red eyes.
E. All of the females will have white eyes; half of the males will have red eyes, and half of the males will have white eyes.

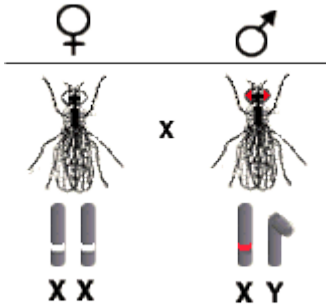
Answer: C. All of the females will have red eyes; all of the males will have white eyes.
(All of the females are red-eyed and heterozygous. All of the males are white-eyed and hemizygous.)

Tutorial:

Genotypes and phenotypes of parents

The female parent must be homozygous because she has the recessive white-eyed phonotype.

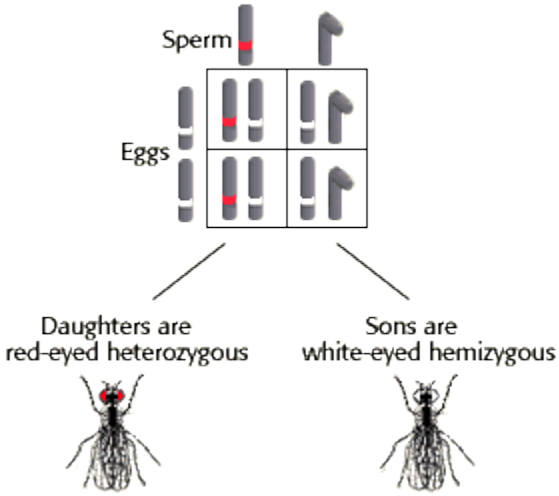
The male parent is hemizygous, red-eyed.



Genotypes and phenotypes of offspring

All of the females eggs will contain an **X** chromosome with the white-eye mutation.

The sperm will contain either a normal **X** chromosome or a **Y** chromosome.



Summary

We use a punnett square to predict the outcome of this cross. Female offspring receive an **X** chromosome from both the sperm and egg. All females receive the dominant, red-eyed allele from their fathers and the recessive, white-eyed allele from their mothers.

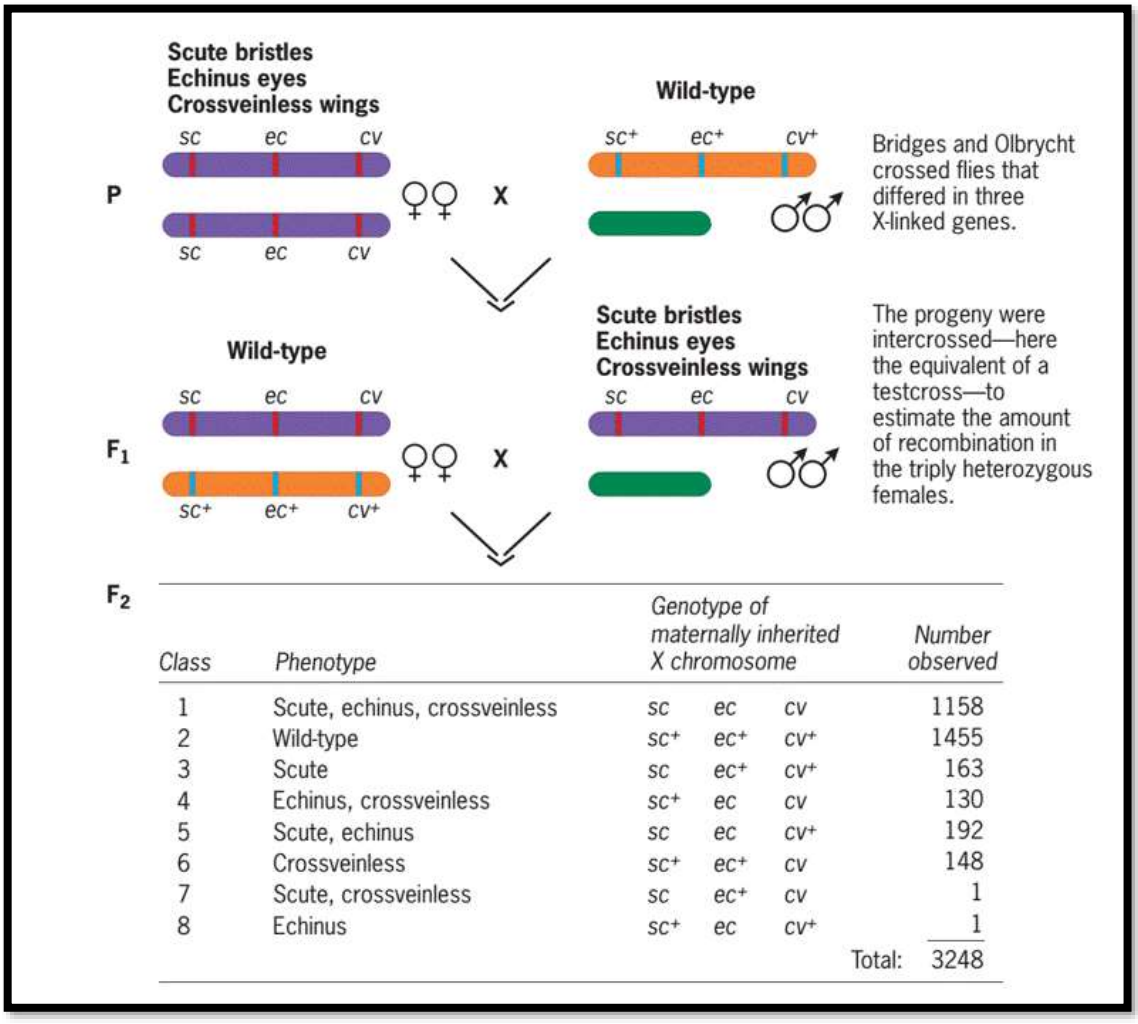
Three point test cross: gene order, map distance, inference and coefficient of coincidence and level of inference

Three point test cross

Three point test cross in ***Drosophila***:

- Wild-type Male *Drosophila* was crossed with female *Drosophila* homozygous for three recessive X-linked mutations—**scute (sc)** bristles, **echinus (ec**) eyes, and **crossveinless (cv)** wings to obtain F1 progeny.
- Wild Male Drosophila= (***sc+, ec+, cv+)***
- Mutated female Drosophila = (***sc, ec, cv***)
- Then F1 progeny were intercrossed to produce F2 flies, which are then classified and counted.
- The F1 males carried the three recessive mutations on their single X chromosome. Thus, this intercross was equivalent to a testcross with all three genes in the F1 females present in the homozygous form.
- The F2 progeny flies from the intercross comprised eight phenotypically distinct classes, two of them are parental and six recombinant.

Class	Phenotypes	Characters	Genotypes	Counts
1.	Scute, echinus, crossveinless	Parental	Sc, ec, cv	1158
2.	Wildtype (non scute, non echinus, crossvein)	Parental	Sc+, ec+, cv+	1455
3.	scute	Recombinant	Sc, ec+, cv+	163
4.	Echinus, crossveinless	Recombinant	Sc+, ec, cv+	130
5.	Scute, echinus	Recombinant	Sc, ec, cv+	192
6.	Crossveinless	Recombinant	Sc+, ec+, cv	148
7.	Scute, crossveinless	Recombinant	Sc, ec+, cv	1
8.	echinus	Recombinant	Sc+, ec, cv+	1
Total				**3248**



Class	Phenotype	Genotype of maternally inherited X chromosome			Number observed
1	Scute, echinus, crossveinless	sc	ec	cv	1158
2	Wild-type	sc+	ec+	cv+	1455
3	Scute	sc	ec+	cv+	163
4	Echinus, crossveinless	sc+	ec	cv	130
5	Scute, echinus	sc	ec	cv+	192
6	Crossveinless	sc+	ec+	cv	148
7	Scute, crossveinless	sc	ec+	cv	1
8	Echinus	sc+	ec	cv+	1
				Total:	3248

Gene order:

- The parental classes were by far the most numerous (1158+1455=2613). The less numerous recombinant classes each represented a different kind of crossover chromosome.
- To figure out which crossovers were involved in producing each type of recombinant, we must first determine how the genes are ordered on the chromosome.
- There are three possible gene orders :

1. ***sc—ec—cv***
2. ***ec—sc—cv***
3. ***ec—cv—sc***

- Four of the recombinant must have come from a single crossover in one of the two regions of the genes. The other two recombinant must have come from double crossing over—one exchange in each of the two regions. Because a double crossover switches the gene in the middle with respect to the genetic markers on either side of it, it is used for determining the gene order.
- Again, intuitively, double crossover occur much less frequently than a single crossover. Therefore, among the six recombinant classes, **the two rare ones must represent the double crossover chromosomes**
- From the given example, the double crossover must have occurred in class 7 ***(sc ec+cv***) and class 8 (***sc+ec cv+),*** each containing a single recombinant F2 progeny.
- Comparing these rare recombinant to parental class 1 (***sc ec cv***) and class 2 ***(sc+ec+Cv+),*** the echinus allele has been switched with respect to scute and crossveinless.
- Consequently, the echinus gene must be located between the other two.
- Therefore the correct gene order is **sc–ec–cv.**

Map distance:

- It is the distance between each pair of gene and it is obtained by estimating the average number of crossovers.
- Total map distance between these three genes is map distance between ***sc*** and ***ec*** plus map distance between ***ec*** and

i. Map distance between sc and ec:

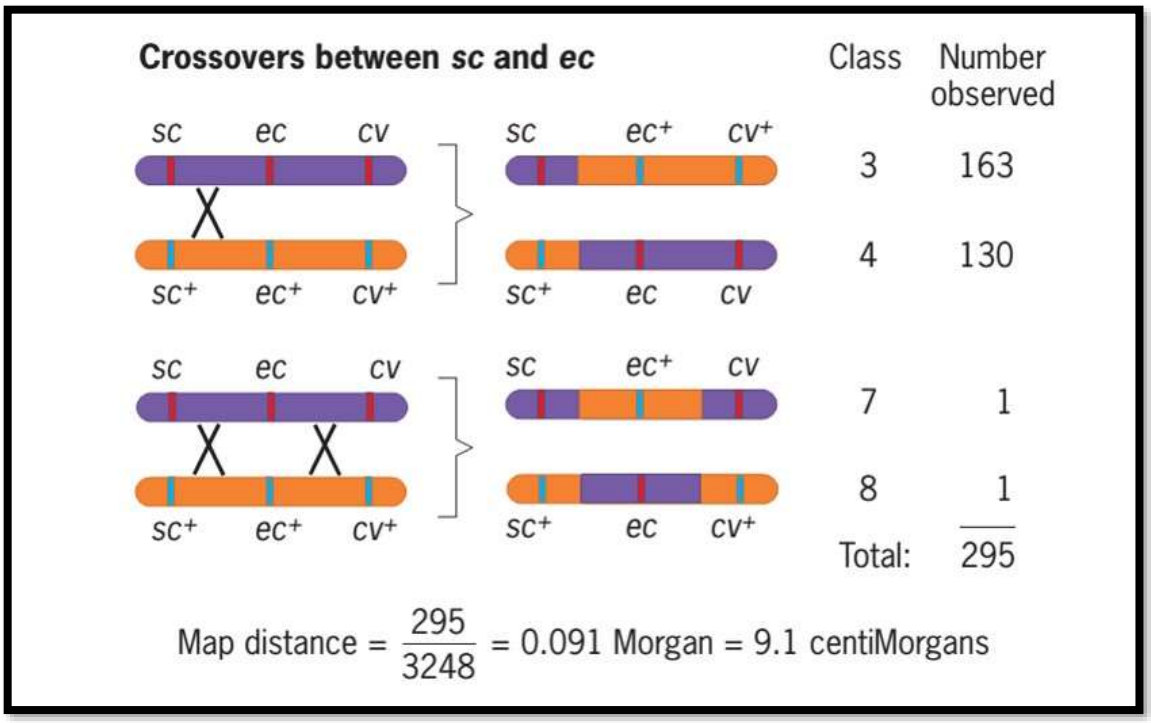
- We can obtain the length of the region between ***sc*** and ***ec*** by identifying the recombinant classes that involved a crossover between these genes.
- There are four such classes: class 3 (***sc ec+cv+),*** class 4 (***sc+ec cv***), class 7 (***sc ec+cv***), and class 8 (***sc+ec cv+).***
- Classes 3 and 4 involved a single crossover between sc and ec, and classes 7 and 8 involved two crossovers, one between ***sc*** and ***ec*** and the other between ***ec*** and

- We can therefore use the frequencies of these four classes to estimate the average number of crossovers between ***sc*** and ***ec:***
- Average crossover between ***sc*** and ***ec*** =(163+130+1+1) /3248

=0.091 **Morgan**

=9.1 centiMorgan or Map unit

- Thus, in every 100 chromosomes coming from meiosis in the F1 females, 9.1 had a crossover between ***sc*** and ***ec***.
- The distance between these genes is therefore 9.1 map units.

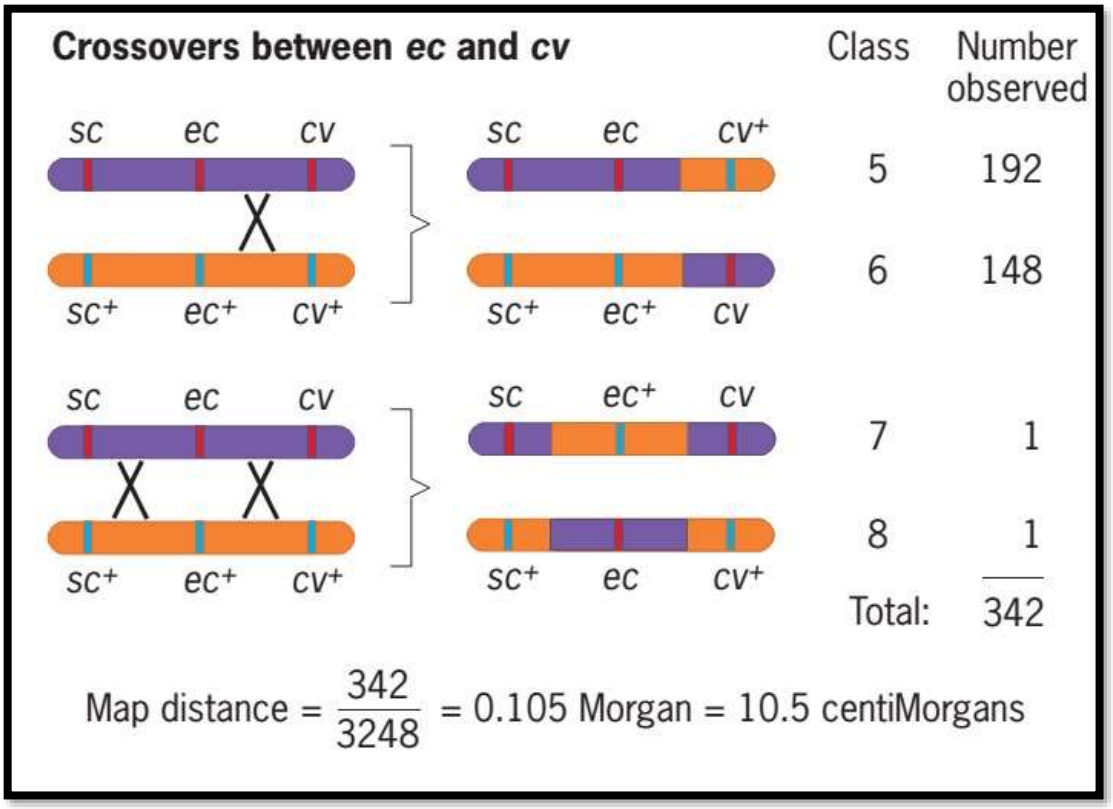


ii. Map distance between ec and cv:

- In a similar way, we can obtain the distance between ***ec*** and ***cv***.
- Four recombinant classes involved a crossover in this region: class 5 (***sc ec cv+),*** class 6 (***sc+ec+cv),*** class 7 and class 8.
- The **double recombinants are also included here** because one of their two crossovers was between ***ec*** and ***cv***.
- The average cross between ***ec*** and ***cv*** =(192+148+1+1)/3248

=**0.105 morgan**

= 10.5 centiMorgans or map unit



Total map distance:

- Combining the data for the two regions, the map is ***sc—9.1— ec—10.5— cv***
- Thus map distances between ***sc*** and **cv**= 9.1 cM +10.5 cM =19.6 Cm

Alternative way of calculating map distance:

- Directly calculating the average number of crossovers between these genes:
- Recombination frequency (RF)= Non–crossover + Single crossover + Double crossover

= (0)*(1158+1455)/3248 + 1 (163+130+192+148)/3248 + 2 (1+1)/3248

= 0 + 0.195 + 0.0006

= 0.196 Morgan

= 19.6 CentiMorgan

Inference and coefficient of coincidence:

- **Inference** is the phenomenon of inhibition of crossover of by another crossover nearby.
- For example, the crossover frequency between ***sc*** and ***ec*** in **region I** was (163 +130 +1+1)/3248 =0.091, and crossover frequency between ***ec*** and ***cv*** in **region II** was (192+148 +1 +1)/3248 =0.105. TSKhaddar
- If we assume both crossover are independence of each other, the expected frequency of double crossovers in the interval between ***sc*** and ***cv*** would be 0.091 *0.105 = 0.0095.
- But **actual observed frequency of double crossover** is (1+1)/3248 = **0006**
- Double crossovers between ***sc*** and ***cv*** were much less frequent than expected.
- The result suggest one crossover inhibited the occurrence of another nearby, a phenomenon called **interference**
- The extent of the interference is measured by the **coefficient of coincidence (C)**.
- **Coefficient of coincidence** is the ratio of observed frequency to double cross to expected frequency to double cross.
- **C**= (observed frequency of double crossovers)/(expected frequency of double crossovers)

=0.0006/0.0095

C=0.063

Level of inference (1-C):
Level of inference = 1-C
=1-0.063
=0.937

Because in this example the coefficient of coincidence is close to zero, its lowest possible value, interference was very strong (I is close to 1).

cases:

i. if a coefficient of coincidence equal to 1; no interference between crossover at all which means the crossovers occurred independently of each other.

ii. If a coefficient of coincidence is equal to 0; very strong inference between crossover therefore double cross do not occur.

iii. ** map distance less than 20cM has very strong inference. Thus, double crossovers seldom occur in short chromosomal regions.

iv. The strength of interference is therefore a function of map distance

MJ-4P: Animal Physiology Credit 01

1. Preparation of temporary mounts: Squamous epithelium

Preparation of temporary slide of animal tissues and their study.

Principle

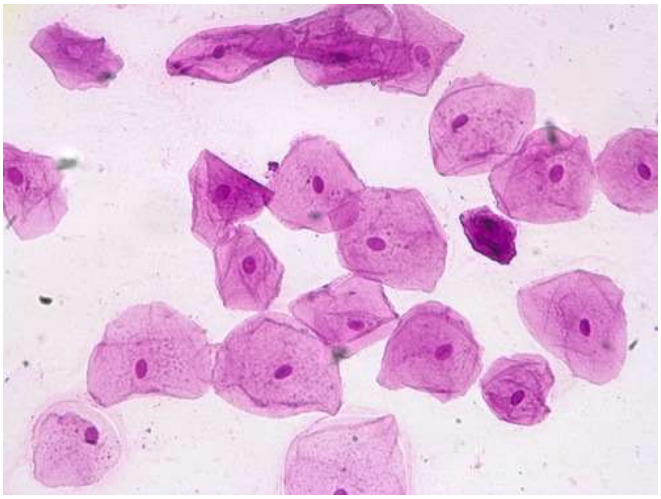
Group of similar type of cells that perform a specific function, is called a tissue. Tissues are organised in a specific proportion and pattern to form different organs. There are four basic types of tissues : (i) Epithelial, (ii) Connective, (iii) Muscular and (iv) Nervous. The epithelium or epithelial tissue provides a covering or lining for some parts of the body. Connective tissues have special function of linking and supporting other tissues or organs of the body. Muscular tissue plays an active role in all movements of the body. Nervous tissue controls the body's responsiveness to changing conditions within and outside the body. Requirement: Live material/concerned tissue, beakers, glass slides, coverslips, watch glasses, dropping bottle, dropper, required stain, glycerine, NaCl solution (0.9% w/v), needle, forceps, brush, toothpick, water, wash-bottle, dissecting tray, microscope.

I. EPITHELIUM OR EPITHELIAL TISSUE

Procedure

- Rinse your mouth well with water.
- Gently scrap the inside of your cheek with the broad end of a clean toothpick. Discard this material.
- Scrap again, and spread these cells gently on a clean slide. Add a drop of 0.9% NaCl solution or physiological saline and a drop of methylene blue with the help of a dropper. • After two minutes, remove the excess stain and saline using the edge of a filter paper. Now, put a drop of glycerine on the cells.
- Place a coverslip over the tissue and gently press it with the back of a pencil to spread the cells.
- Examine the slide under the low power of microscope.
- Draw a labelled diagram of your preparation.

Observations



Discussion

Epithelial tissue (epithelium) forms the covering tissue of the body. It covers the body surface and lines the body cavities and hollow visceral organs. It may be single or multi-layered. The lower most layer normally rests upon a non-cellular basement membrane. It is protective/sensory/absorptive/and secretory in nature and also helps in exchange and movement of materials inside the body.

2. Preparation of stained blood film to study various types of blood cells. Enumeration of red blood cells and white blood cells using haemocytometer. Calculation of total count and differential count from blood.

Connective tissue is the tissue that connects, separates and supports all other types of tissues in the body. Like all tissue types, it consists of cells surrounded by a compartment of fluid called the extracellular matrix (ECM). However connective tissue differs from other types in that its cells are loosely, rather than tightly, packed within the ECM. Based on the cells present and the ECM structure, there are two types of connective tissue:

Connective tissue proper; further divided into loose and dense connective tissues

Specialised connective tissue; reticular, blood, bone, cartilage and adipose tissues

Blood is the specialized connective tissue within the circulatory system that transports blood cells and dissolved substances throughout the body via blood vessels.

Procedure

• Clean the slides so that it become free from grease, finger prints, etc.

• Clean the tip of your middle finger with rectified spirit and prick with a sterilised needle or lancet available at the medical store.

• When a drop of blood appears on the finger tip, wipe it away with cotton dipped in rectified spirit. • Press the finger tip to get the next drop of blood and touch it with the clean surface of slide (placed on working-table) (Fig 5.1 (a)) about 1 cm away from the right side edge (this be named as the first slide).

• Hold the narrow edge of another slide (2nd slide) at about 450 angle to the 1st slide and to the left of the drop of blood (Fig. 5.1(b)).

• Pull to the right until the 2nd slide touches the blood. Wait for 2-3 seconds till the blood spreads along the line of contact. Now push the 2nd slide towards the left in a steady but brisk movement. Take care to keep the edge pressed uniformly against the surface of the 1st slide. Keep pushing until the other end of the slide is reached (Fig. 5.1 (c)). This method spreads the blood thinly (also called a blood film) over the surface of the slide but does not run over the cells and crushes them. You may make 3 or 4 such film (smear) preparations.

• Once the uniform smear is made, air dry the slide for about 10 minutes.

• Mark with a wax pencil the region of the smear that is to be stained.

• Cover the region marked with wax pencil with few drops of Leishman's/ Geimsa/Wright's stain. Leave the stain over the smear for 2 to 3 minutes. Now, add an equal amount of distilled water gently with a dropper and leave it for 2 to 4 minutes. Repeat this process till its colour becomes light violet. Air dry the slide thoroughly and mount with a cover slip, using a drop of glycerine.

• Observe the slide under a compound microscope first under low magnification and then at higher magnification.

Observations and Results

A. **RBCs (erythrocytes)** are biconcave, circular and non-nucleated. The various types of white blood corpuscles (WBC's), the eosinophils, the basophils and neutrophils are also observed. Also observe the blood platelets

RBCs are biconcave as the central part is lighter.

B. Shape of WBC is irregular.

Mainly two types of WBC are found-

(a) Granulocytes: Granules are found in these cells. These cells are of the following types:

• Eosinophils or Acidophils: Its nucleus is trilobed and twisted into an 'S' shape.

Basophils – Its nucleus is bilobed.

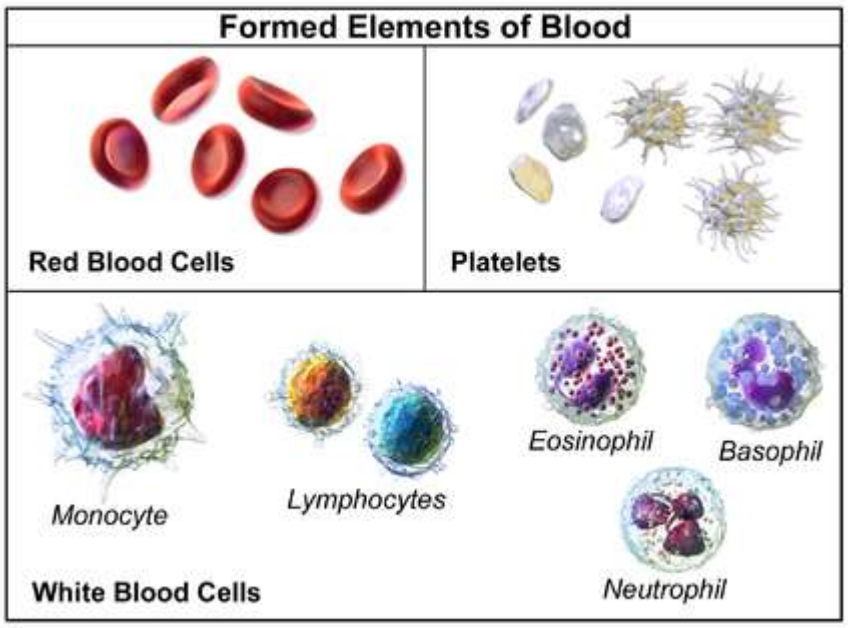
• Neutrophils – Its nucleus is multilobed (2-5 lobes).

(b) Agranulocytes: Its cytoplasm is without granules and nuclei are nonlobed. These are of the following types.

• Monocytes – These are large in size with kidney shaped nucleus.

• Lymphocytes – They are slightly bigger than RBC with spherical nucleus.

C. Platelets have no nuclei. They appear in clusters as violet granules.



Discussion

Different types of corpuscles present in the blood perform different functions. Haemoglobin present in the RBCs help in exchange of oxygen and carbon dioxide. Monocytes and lymphocytes participate in destroying harmful microorganisms that invade our body. Platelets help in blood clotting activity which to a certain extent prevents blood loss during injury. The total counts of RBCs and WBCs and the differential counts of various WBCs are of great medical significance for diagnostic purpose.

3. Estimation of bleeding time and clotting time

In the lab, the protocol for estimating bleeding time (BT) involves making a small, standardized incision on the forearm and measuring the time it takes for bleeding to stop. Clotting time (CT) is determined by measuring the time it takes for blood to clot in a test tube containing anticoagulants.

Bleeding Time (BT):

1. **Equipment:** A sterile lancet or blade, timer, filter paper, antiseptic.
2. **Procedure:**
    i. Clean the patient's forearm with an antiseptic.
    ii. Make a standardized incision (e.g., with IVY method) on the volar forearm.
    iii. Use a timer to record the time the incision was made.
    iv. Gently blot the blood with filter paper every 30 seconds until bleeding stops.
    v. Calculate the bleeding time by multiplying the number of blots by 30 seconds.
    vi. **Normal Bleeding Time:** Typically 2-7 minutes.

Clotting Time (CT):

1. **Equipment:** Non-heparinized test tubes, timer, water bath.
2. **Procedure:**
    i. Collect a blood sample into a non-heparinized test tube.
    ii. Incubate the tube in a water bath at a specific temperature.
    iii. Periodically invert the tube to check for clot formation (e.g., every 30 seconds).
    iv. Record the time it takes for the blood to clot.
    v. **Normal Clotting Time:** Usually 6-15 minutes.

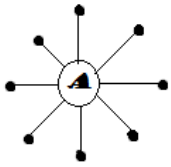
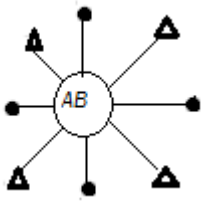
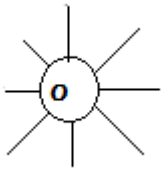
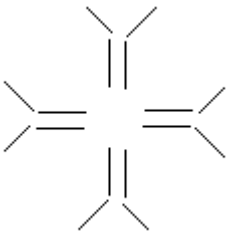
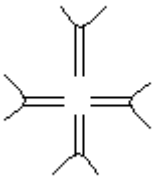
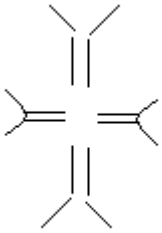
Important Considerations:

- **Aseptic Precautions:** Ensure proper hygiene and sterilization to prevent infection.
- **Standardization:** Use consistent methods and equipment to ensure accurate results.
- **Interpretation:** Abnormal bleeding or clotting times may indicate underlying medical conditions like platelet dysfunction or deficiencies in clotting factors.

Determination of human blood group

Principle:

In 1900 Karl Land Steiner grouped human blood into 4 group based on presence of two antigens on the surface of RBCs. This groups are designated as A,B, AB & O. This are commonly called ABO Blood group. This blood group system is determined on the basis of presence or is absence of antigen on RBC on antibody in blood group is designated as a blood group. B antigen is present outer membrane of RBC and anti-a antibody present blood plasma. This blood group is designated by B+ blood group. A,B both antigen is present outer membrane of RBC, this blood group is called AB+ blood group. A, B both antigen is absent outer membrane of RBC, this blood is called O+.
In addition to antigen on ABO system the red cells of 80-85% also have an addition antigen called RH+ antigen or Rh factor.

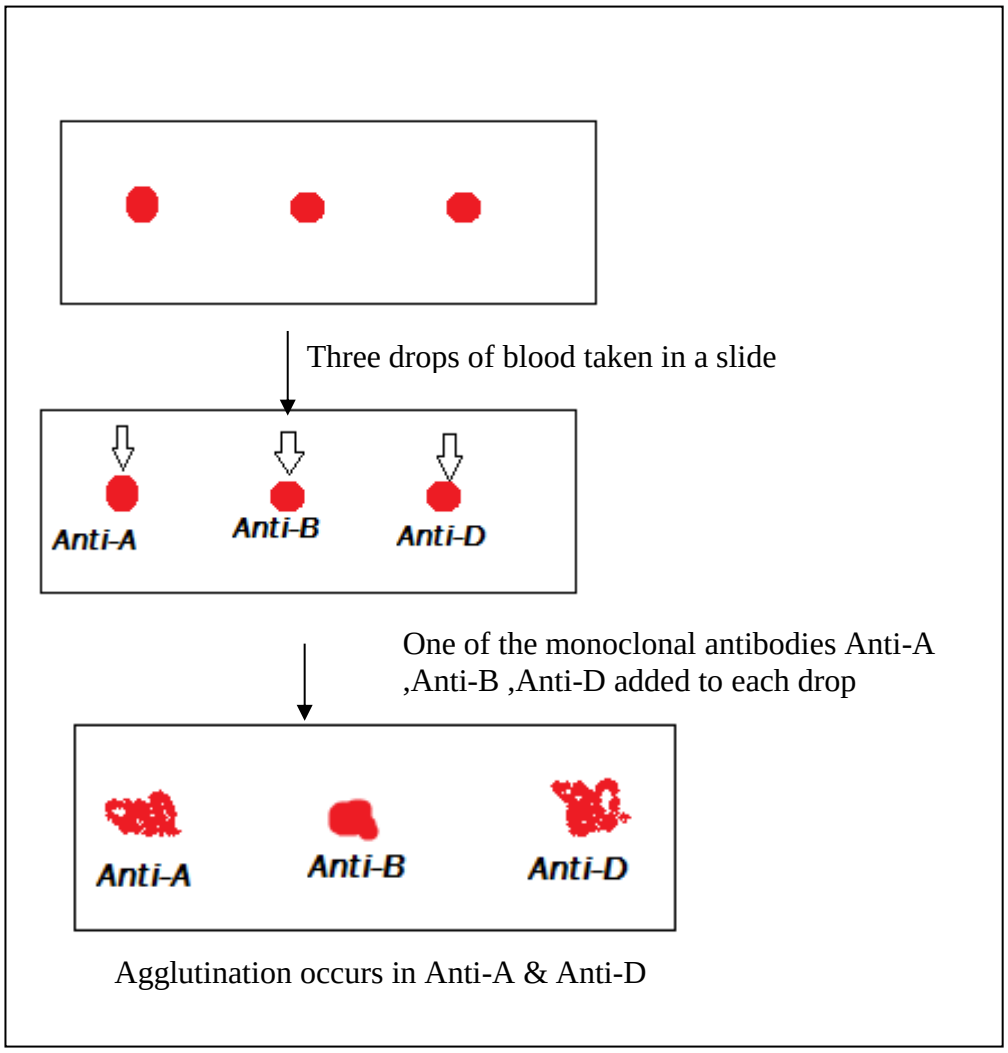
RBC TYPE	GROUP-A	GROUP-B	GROUP-AB	GROUP-O
				
ANTIBODY IN PLASMA	Anti-B 	Anti-A 	None	Anti-A Anti-B 
ANTIBODY IN RBC	A	B	AB	None

In 1940 Karl Land Steiner and Weiner reported that Rabbit sera contains antibodies against of Rhesus monkey that agglutinates RBC of some human being. This antigen is latter name as Rhesus factor (Rh Factor).
During RH determination blood Anti-D serum is mixed with blood and if there is agglutination then the blood is (Rh)(D) is agglutination then the blood is does not agglutinate the blood not have (Rh).

Material required:

1. Rectified spirit
2. Cotton
3. Sterile needle
4. Human blood

5. Glass slide
6. Anti-A(monoclonal)
7. Anti0B(monoclonal)
8. Anti-C(monoclonal)
9. Glass rod



So, blood group is "A"

Procedure:

1. Three area on a slide are marked and labelled them A,B and.
2. The finger tip is punctured by a sterile needle and three drops of blood is taken in a clean glass slide.
3. One drop of Anti-a is added to area 'A' are drop of anti 'Rh' is added to area 'B' and one drop of Anti Rh is added to area D.
4. With separate application glass rod blood is mixed reagent well.
5. After a few seconds the drop are examined.

Observation:

1. Agglutination occurs when reaction is made with Anti-A serum.
2. Agglutination does not occurs when reaction is made with Anti-B serum.
3. Agglutination occurs when tested with Anti-D serum.

Conclusion

On the basis of the above observation it can conclude that my blood group is A positive.

Precaution:

1. The blood should be mixed with antigen quickly, otherwise it will gel coagulated quickly.
2. The slide used should be dust free.
3. The drop of the blood and the drop of anti sera should be equal or else we can not conform if agglutination has occurred or not.

ENUMERATION OF RED BLOOD CELLS AND WHITE BLOOD CELLS USING HAEMOCYTOMETER

Introduction

Red blood cells (RBCs) are the round shape, biconcave discs, present in the blood that helps in the transport of gases throughout the body. The biconcave shape helps the RBCs in rendering the red cells quite flexible so that they can easily pass through the capillaries. On an average, the size of the Red Blood Cells (RBCs) is 7.2 – 7.4 mm (microns). The mature RBCs are non-nucleated cells with an Iron-containing pigment known as Hemoglobin which helps in the transport of oxygen from the lungs to tissues and carbon dioxide from tissues back to the lungs for excretion. The Average lifespan of Red Blood Cells (RBCs) is 100 – 120 days.

Aim

The purpose of performing Total Red Blood cell count is to know whether or not you are suffering from Erythrocytosis or Polycythemia (i.e. the increase in the no. of Red Blood Cells to more than 6.5 million/mm^3) or Erythrocytopenia or Erythropenia (i.e. the Decrease in the no. of Red Blood Cells to less than 3.5 million/mm^3).

Principle

Very large numbers of Red Blood Cells are present in the Blood Specimen. Practically, counting this amount of Red cells directly under the microscope is highly impossible. So, the Red Blood cells are counted by using a special type of chamber, designed for the counting of blood cells in the specimen, known as Hemocytometer or Neubauer's chamber.

For this, the blood specimen is diluted (usually in 1:200 ratio) with the help of RBC diluting fluid (commonly the Hayem's Fluid) which preserve and fix the Red blood cells. The Hayem's

fluid is isotonic to the Red blood cells and does not cause any damage to it. The Normal Saline solutions can also be used for this but it causes the slight creation of red blood cells and allows rouleaux formation which may cause the errors in results.

After diluting the specimen, the content is charged on Hemocytometer / Neubauer's chamber and the cells are counted in the areas specific for RBC count.

Nowadays, two types of RBC Diluting fluid are commonly used in Laboratories –

- Hayem's RBC Diluting fluid
- Formalin Citrate diluting fluid

The composition of Hayem's diluting Fluid

COMPONENTS	**QUANTITY**
Mercuric Chloride	0.25 grams
Sodium sulfate	2.5 grams
Sodium chloride	0.5 grams

The Final pH of the solution (at 25°C) varies from 5.8 – 6.0 which depends on the composition and companies who manufacture it.

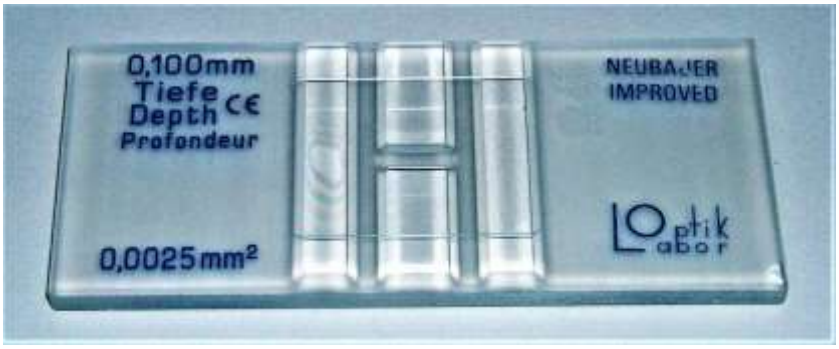
The composition of Formalin Citrate diluting fluid

COMPONENTS	**QUANTITY**
Trisodium citrate	3 grams
Formalin	1 ml
Distilled water	99 ml

This diluting fluid is commonly used because it is cheaper than the Hayem's fluid. However, Hayem's diluting fluid gives the better results.

Hemocytometer

This is a special type of glass chamber that is used for the cell counting, especially for Blood cells. Nowadays, most commonly Improved Neubauer's Chamber is used and in some laboratories, other types of chambers are also employed like Burkers chamber, Levy's chamber and Fusch – Rosenthal chamber etc.

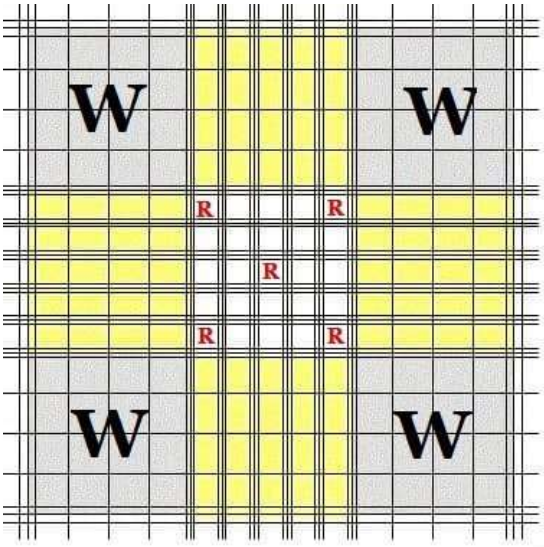


HEMOCYTOMETER

The Neubauer's Chamber has ruled the area of total 9 square mm and the depth is 0.1 mm as when the coverslip is placed on the surface of the counting chamber, the space between the bottom of the cover glass and the base of grooved area measures 0.1 mm in depth.

The central 1 square is highly ruled which is divided into 25 squares. Each square of the Central square is further subdivided into 16 small squares.

For RBC count the cells are counted in the 5 squares of the Central square as 4 Corner squares of the Central square (divided into 25 squares) and 1 central square of the Larger Central Square (divided into 25 squares).



R – RBC AREAS W – WBC AREAS

Each square of the Central Square (divided into 25 squares) contains 16 small squares so the total no. of the area to be counted for RBC Count –

16 × 5 = 80 small squares

Two Method has been developed for the Manual Estimation of Total Red Blood Cell Count Using Hemocytometer / Neubauer's chamber –

- ***Microdilution Method***
- ***Macrodilution Method***

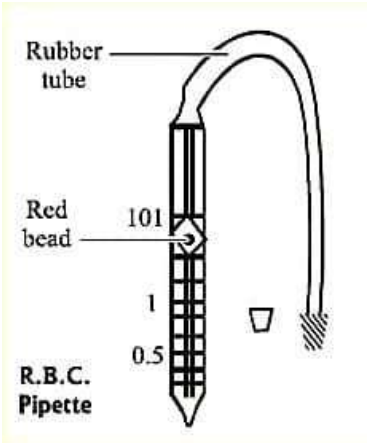
MICRODILUTION METHOD FOR THE ESTIMATION OF TOTAL RBCs USING HEMOCYTOMETER

Materials Required for the Total Red Blood Cell (RBC) Count by Microdilution Method –

- Blood sample (Capillary blood or EDTA anticoagulated specimen)
- RBC diluting fluid (preferably Hayem's fluid)
- Gauze piece or Cotton
- RBC pipette
- Hemocytometer a.k.a. Neubauer's Chamber
- Coverslip
- Microscope

A Brief Introduction to RBC Pipette

RBC pipette is a graduated pipette that gives the dilution of 1:100 and 1:200. It has two markings at the bottom as 0.5 and 1 and the top of the pipette is marked 101. It has a round shape bulb which contains the Red bead to mix the blood specimen and the diluting fluid. On the top, a rubber tube is attached to the pipette for sucking the blood specimen and diluting fluid.



RBC PIPETTE

When blood is sucked up to 0.5 mark and the diluting fluid up to 101 marks, gives the 1:200 dilution of Blood: Diluting fluid and When the Blood is sucked up to 1 mark and the diluting fluid up to 101, gives the 1:100 dilution of Blood: Diluting fluid which is commonly used in anemic patients. After sucking the Specimen & Diluting fluid, the content is gently mixed by rotating the pipette on its long axis to ensure thorough mixing of blood and diluting fluid.

Procedure of the Total Red Blood Cell (RBC) Count by Microdilution Method

⇒ Fill the RBC pipette up to the 0.5 mark with the blood specimen and wipe out the pipette externally to avoid false high results.

⇒ Fill the same pipette with the RBC diluting fluid (preferably Hayem's Fluid) up to the mark 101.

⇒ Be cautious that there should be no air bubble in the pipette bulb.

⇒ Mix the Blood and Diluting fluid in the pipette by rotating the pipette (horizontally) between your palms.
⇒ Take out the Neubauer's chamber / Hemocytometer from its case and clean it using a swab or gauze piece. Similarly, clean out the cover glass and place it over the grooved area of Hemocytometer.
⇒ Now, put the RBC pipette, mix the solution present in it again and then discard 1-2 drops from the pipette before charging the chamber.
⇒ Gently press the rubber tube of the RBC pipette, so that the next drop of fluid is in hanging position.
⇒ Touch the Tip of the pipette with the hanging drop against the edge of the coverslip making an angle of 45° approximately.
⇒ Allow a small amount of fluid from the pipette to fill into the chamber which occurs by the Capillary action. Do not overcharge the chamber and there should be no air bubble in the Chamber.
⇒ After charging, wait for 3-5 min so that the cells settle down in the chamber & then focus the chamber under the microscope to calculate Red Cells.

MACRODILUTION METHOD FOR THE ESTIMATION OF TOTAL RBCs USING HEMOCYTOMETER

Materials Required

- Blood sample (Capillary blood or EDTA anticoagulated specimen)
- RBC diluting fluid (preferably Hayem's fluid)
- Hb pipette or Micropipette (0.02 ml or 20 μl)
- Hemocytometer / Neubauer's Chamber
- Gauze piece or Cotton swab
- Graduated Pipette (5 ml)
- Test tubes
- Cover Slip

Procedure

⇒ Take 3.98 ml of RBC diluting fluid in a Clean, Dry and Grease free Test tube.
⇒ Now add 0.02 ml or 20 μl of Blood Specimen to the tube containing diluting fluid with the help of micropipette or RBC pipette.
⇒ Mix well for few minutes and ready your Hemocytometer / Neubauer's Chamber.
⇒ Take out the Neubauer's chamber / Hemocytometer from its case and clean it using a swab or gauze piece. Similarly, clean out the cover glass and place it over the grooved area of Hemocytometer.
⇒ Now, take out the RBC pipette and fill it with the Diluted Specimen, mix the solution well and then discard 1-2 drops from the pipette before charging the chamber.
⇒ Gently press the rubber tube of the RBC pipette, so that the next drop of fluid is in hanging position.
⇒ Touch the Tip of the pipette with the hanging drop against the edge of the coverslip making an angle of 45° approximately.

⇒ Allow a small amount of fluid from the pipette to fill into the chamber which occurs by the Capillary action. Do not overcharge the chamber and there should be no air bubble in the Chamber.

Using Micropipette instead of RBC pipette for charging the Hemocytometer

⇒ You can also use a micropipette instead of RBC pipette for charging the Hemocytometer. So, with a micropipette, carefully draw up around 20 μl of the diluted specimen. Press the knob of the pipette to make a hanging drop at the tip of the micropipette.

⇒ Now gently place the pipette tip against the edge of the cover glass and if required slowly expel the more liquid until the counting chamber is full. This process occurs by Capillary action, but care should be taken not to overfill the chamber. A volume of 10 μl is sufficient to fill out the one counting chamber.

⇒ After charging, wait for 3-5 min so that the cells settle down in the chamber.

Counting the red blood cells under microscope

⇒ Focus the ruling using the 10x Objective lens and then Count the RBCs in 5 small squares of the central square as described above, using the 40x Objective lens.

⇒ Count the cells which are lying on the right and lower lines of the 5 small squares but not the opposite line. In case of marginal cells, count the cells on 'L' line that is either on Right and Lower lines or Left and Upper lines.

Calculations

⇒ After counting the cells under the microscope, we know the No. of RBC in 5 squares of the central square. Let's consider it as 'N' no. of cells.
⇒ Now, the volume of the fluid inside the chamber is the product of Area and depth of the Hemocytometer / Neubauer's chamber.
⇒ The central area is the 1 sq. mm which is divided into 25 parts so the area is
25 squares = 1 sq. mm
⇒ Out of these 25 squares, the RBCs are counted in 5 squares. So the Area of 5 small squares is 5/25 i.e. 1/5
⇒ The depth of the Hemocytometer is 0.1 mm as described above in a short description of Hemocytometer.
⇒ Now Apply the Following formula to get the Total Red Blood Cell Count –
Total RBC Count = N × Dilution / Area × Depth
N × 200 (or 100 as the dilution is made) / (1/5 × 0.1)
Total RBC count = N × 10,000 / mm^3

Using the Above formula we can calculate the Total No. of Red Blood Cells present in the Blood Specimen.

NORMAL VALUES OF RED BLOOD CELLS
In Males – 4.8-5.5 million/mm^3
In Females – 4.5-5 million/mm^3

PRECAUTIONS
⇒ Use of Mouth pipettes (RBC pipette) is banned in many countries. However, in case you have to use it, be cautious that you should not intake the diluting fluid or Specimen.
⇒ Accurately measure the amount of specimen and Diluting Fluid to avoid any error in the results.
⇒ In case you are performing this test by Microdilution method, mix the specimen and diluting fluid appropriately by gently rotating in between your palms.
⇒ Carefully charge the Hemocytometer or Neubauer's chamber that it should not be overcharged and do not contain any air bubble in it.

Estimation of haemoglobin using Sahli's haemoglobinometer

INTRODUCTION
Hemoglobin (Hb or Hgb) is a red color pigment present in red blood cells (RBCs) comprises Fe2+ and Globin protein. It is Hemoglobin in RBCs that carries the oxygen from the lungs to the tissues and CO2 from body tissues to the lungs for excretion.
Hemoglobin (Hb or Hgb) is responsible for the appearance of Red color RBCs and blood. Hemoglobin is a chromoprotein consisting of Globin molecule attached to 4 red colored Heme molecules. Hemoglobin synthesis requires the coordinated production of Heme and Globin. Heme is a prosthetic group that medicates reversible binding of oxygen by hemoglobin. Globin is the protein that surrounds and protects the Heme molecule.

The Estimation of hemoglobin in the blood is commonly prescribed in various physiological and pathological conditions and as both diagnostic and prognostic test especially in case of suspected Anemia which can be caused by various factors.

Nowadays in many laboratories, the Hemoglobin estimation is done by using Automatic Hematology Analyzers but still in many other labs the following method is Commonly used to determine the Hemoglobin concentration in patient's blood.

- Sahli's Method a.k.a. Acid Hematin Method
- Cyanmethemoglobin Method (CMG) a.k.a Drabkin's Method

Principle

The principle of Sahli's Method or Acid hematin method is quite easy that when the blood is added to N/10 Hydrochloric acid (HCl), the hemoglobin present in RBCs is converted to acid hematin which is a dark brown colored compound. The color of the formed acid hematin complex corresponds to the Hemoglobin concentration in the blood and is matched with the standard which is a reference brown glass given in the Sahli's apparatus by diluting with N/10 hydrochloric acid or distilled water until the color of acid hematin complex match with the color of the standard.

Reagents required

- N/10 hydrochloric acid (It is prepared by diluting concentrated hydrochloric acid 0.98 ml in distilled water and volume is made up 100 ml).
- Distilled water

Apparatus & equipments required

- Sahli's Apparatus
    - Hemoglobin pipette (0.02 ml or 20 µl capacity)
    - Sahli's graduated Hemoglobin tube
    - Thin glass rod Stirrer for Hemoglobin Tube
    - Sahli's Comparator box with brown glass standard

- Spirit swab
- Blood Lancet
- Dry cotton swab
- Pasteur pipette

Procedure

⇒ N/10 Hydrochloric acid is taken in Hemoglobin tube (has two graduations – one side gm/dl, and other side shows the Hb %age), up to the mark 20 – the lowest marking (yellow marking).

⇒ For capillary blood draw, boldly prick the tip of the middle or ring finger with the help of Blood lancet or pricking needle. Wipe out the first drop of blood and suck the blood from the second drop in Hb pipette up to the mark of 20 µl. Fill the Hb pipette by capillary action.
⇒ Wipe out the surface of the pipette with the help of tissue paper/ cotton so that excess blood may not be added to the Hb tube.
⇒ Dispense the blood into N/10 hydrochloric acid taken in the hemoglobin tube, rinse the pipette with the same solution and mix properly with the help of stirrer.
⇒ Place the tube at room temperature for 10 minutes for complete conversion of hemoglobin into acid hematin.
⇒ After the reaction completes, place the Hb tube in the column in Sahli's Comparator box and start diluting the dark brown coloured compound (Acid Hematin) formed in the Hb tube using the N/10 HCl or distilled water by adding drop by drop of it into the solution and mix with the help of stirrer after each addition.
⇒ This process is done until the endpoint comes matching the color of standard with the color of the test.
⇒ Once the color is matched with the standard brown glass, lift the stirrer up and note down the reading in Sahli's Hb tube by taking the lower meniscus in consideration.
⇒ Now add one more drop of distilled water and mix it properly with the help of stirrer. If color is still matching with the standard add another drop till it matches with the standard and note down the reading and, if it gets lighter after adding the first extra drop, it shows reading taken before dilution was correct. Note down that reading as the final result.
⇒ Reading of this method is expressed in Hemoglobin gm/dl (gram/100 ml) of blood.

Precautions

⇒ Sahli's apparatus especially the Hemoglobin pipette and Sahli's Hemoglobin tube should be clean and dry before use.
⇒ Suck the blood exactly up to the mark of 20 µl (0.02 ml) and air bubbles should not be present in the pipette with blood.
⇒ Mix well the acid and blood and wait for at least 10 minutes after adding the blood in acid.
⇒ Add distilled water drop by drop and mix well after each dilution. Avoid over dilution of the content.
⇒ The matching of color should be done against the natural source of light or electrical tube light (white light) to avoid any visual errors.
⇒ Blood sample and N/10 HCl acid should be taken in an accurate and precise amount in the Hb tube.
⇒ The Hb pipette should be wiped off properly in order to avoid the excess addition of

Advantages

⇒ It is the simple and easy method and may be done at any place because apparatus can be picked up anywhere.

Disadvantages

⇒ Visual intensity may be different for different individuals by this method, we are not able to measure the inactive hemoglobin.
⇒ This method estimates only oxy Hemoglobin. Carboxyhemoglobin and methemoglobin cannot be estimated.

⇒ The endpoint disappears soon so it is difficult to know the actual endpoint and also the Proper stable standard is not available
⇒ The resulting solution is not a clear solution but a suspension due to the action of hydrochloric acid on the proteins and lipids.

Normal values of hemoglobin

- Adult Male: 14-16 gm/dl
- Adult Female: 13-15 gm/dl
- Newborn: 16-18 gm/dl

Clinical significance of hemoglobin estimation

Hemoglobin estimation gives a brief idea of the pathological conditions to the physician so that your physician can easily understand the cause of pathology and prescribe an effective treatment for it.

Raised Hemoglobin Content

- Polycythemia Vera
- Associated with Hypoxia
- Cyanotic Congenital Heart disease
- High Altitudes
- Heavy smoking
- Methemoglobinemia
- Elevated erythropoietin levels
    - Tumors of Kidney, Liver, CNS, Ovary etc.
    - Renal Diseases (Hydronephrosis & Vascular impairment)
- Adrenal hypercorticism
- Therapeutic androgens
- Relative causes of high hemoglobin content
    - Dehydration – Water deprivation, Vomiting, Diarrhea
    - Plasma loss – Burns, Enteropathy

Reduced Hemoglobin Content

Low Hemoglobin value means anemia caused by the following conditions

- Leukemia
- Tuberculosis
- Iron deficiency anemia
- Parasitic infections severely in hookworm infection
- Sickle cell anemia
- Thalassemia
- Aplastic anemia
- Hemolytic anemia
- Loss of blood

Preparation of haemin and haemochromogen crystals

Introduction

Red blood cells of human beings contain a pigment called haemoglobin, which provides red colour to the blood. Haemoglobin is a conjugated chromoprotein and is composed of two parts; non-protein haem and the protein globin. Haem, also known as ferroprotoporphyrin, is made up of four pyrrole rings (tetrapyrrole) which hold iron in the ferrous state. The purpose of this exercise is to enable you to understand how haem component of the haemoglobin present in blood can be converted into specific crystals for the identification.

Materials required

Glass slides, Cover slips, Cotton, Pricking needles, Dropper, Spirit lamp, Compound microscope, Match box, 90% alcohol/Spirit, Takayama's reagent.

Preparation of Takayama's reagent: Add 3 mL of 10% NaOH, 3 mL of Pyridine, and 3 mL of saturated glucose solution in 7 mL of distilled water.

Principle

Hemochromogen, also spelled as Haemochromogen, is a compound of heme with globin modified by the action of alkali. The crystals of haemochromogen are prepared by the heating of blood with Takayama's reagent (the reagent has obtained its name from Masao Takayama who introduced the reagent in Japan in 1912). Heating ruptures the red blood cells and releases the haemoglobin. The protein also gets denatured but remains attached to the haem which is called ferrohaemochrome. During the process, the ferrous form of iron is converted to ferric form due to the presence of NaOH in Takayama's reagent. This oxidized haem is known as alkaline haematin. Haematin combines with pyridine, a nitrogenous compound present in Takayama's reagent, to form insoluble coloured crystals. These are called pyridine haemochromogen which appear as pink-coloured crystals. Saturated glucose solution in the reagent acts as a reducing agent in the reaction, which reduces the solubility of haemochromogen and forms numerous crystals. This property of haem present in human blood is used for identification of blood stains.

Haemoglobin + Heat + NaoH → Haem + Globin

Haem +Glucose+ Pyridine+ Takayama' Reagents → Pyridine Haemochromogen

Procedure

1. Take a cotton swab, soak in the 90% alcohol/spirit and sterilize the tip of your finger.
2. Prick the fingertip with the help of sterilized pricking needle.
3. Place a small drop of blood in the center of a clean slide.
4. Spread the blood drop a little with the help of a needle.
5. Add 2-4 drops of Takayama's reagent on the blood and place a cover slip taking care that bubbles do not appear.
6. Heat the material gently over low flame for 10-20 seconds.
7. Remove the slide from the flame, and then add 1-2 drops of Takayama's reagent from the side of the cover slip.
8. Keep the slide aside at room temperature for 4-5 minutes and examine under the microscope at low (x10) and then at high (x40) magnification power.

Observations

Pink needle-shaped crystals of pyridine haemochromogen appear which confirms the presence of hemoglobin.

Haemochromogen Crystals of Human Blood

Discussion

Haemochromogen crystals are used in forensic cases and medico-legal practices to distinguish blood stains from other red-coloured marks. The crystals can be prepared from fresh as well as dried blood, and thus, it also helps to identify old blood stains. In addition, minute traces of blood are enough to form the crystals.

Precautions

• Prepare Takayama's reagent afresh.
• Use Takayama's reagent carefully as pyridine is a noxious compound and can cause harmful effects if inhaled, swallowed or absorbed through the skin.
• Sterilize the finger with alcohol before pricking.
• Discard the first drop of blood.
• Avoid overheating of the slide. During summers, heating can be skipped.
• Keep the slide undisturbed at the time of cooling.

RECORDING OF BLOOD PRESSURE USING A SPHYGMOMANOMETER

Blood pressure is the force of blood against the walls of the arteries. Blood pressure is recorded as two numbers, the **systolic** pressure (the pressure when the heart beats) over the diastolic pressure (the pressure when the heart relaxes between beats).

Normal systolic pressure is 120 mmHg (millimetres of mercury) and the diastolic pressure is 80 mmHg, that would describe the blood pressure as '120 over 80', written 120/80.

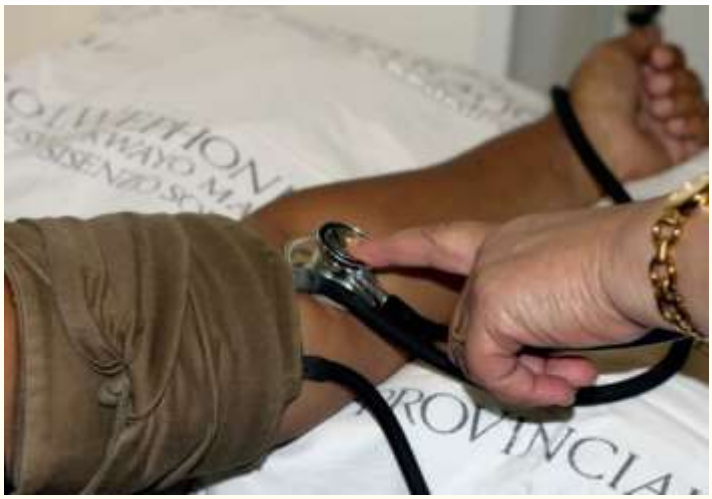


Figure 1. Sphygmomanometer (wall-mounted)

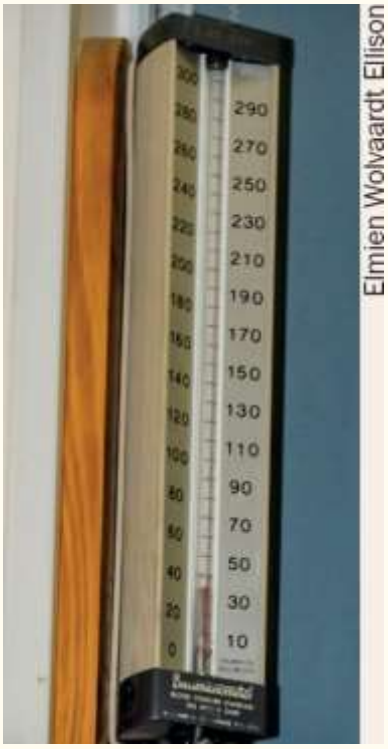


Figure 2. The arm is supported on a level surface. The cuff is around the upper arm and the stethoscope is over the brachial artery, in the bend of the elbow

Blood pressure may vary according to whether the patient is lying down, sitting or standing. It is normally recorded with the patient sitting.

Required apparatus

- sphygmomanometer
- blood pressure cuffs: small, medium, large
- stethoscope

- chair
- patient's care notes or observation chart
- alcohol wipe

Procedure

- Ask the patient to loosen any tight clothing or remove long-sleeved garments so that it is possible to access the upper arm. Do not use an arm that may have a medical problem.
- Place the cuff around the upper arm and secure.
- Connect the cuff tubing to the sphygmo-manometer tubing and secure.
- Rest the patient's arm on a surface that is level with their arm.
- Place the stethoscope over the brachial artery (in the bend of the elbow) and listen to the pulse
- Pump up the cuff slowly and listen for when the pulse disappears. This is an indication to stop inflating the cuff.
- Start to deflate the cuff very slowly whilst watching the mercury level in the sphygmomanometer.
- Note the sphygmomanometer reading (the number the mercury has reached) when the pulse reappears: record this as the systolic pressure.
- Deflate the cuff further until the pulse disappears: record this reading as the diastolic pressure.
- Record these two measurements, first the systolic and then the diastolic (e.g., 120/80), in the patient's notes or chart.
- Tell the patient the blood pressure reading.
- Disinfect the stethoscope drum and ear pieces with the alcohol wipe.
- Wash and dry your hands.
- Report an extremely low or high reading to the clinically qualified person in charge of the patient's care.