UNIT 4 SEROLOGY AND DERMATOGLYPHICS

ABO BLOOD GROUP

Introduction

Human blood holds an extremely important position in our body system. Blood groups are immunological characters, determined by the presence of an antigen on the red blood cell, which are strongly inherited, hence come under the classificatory categories, which are inherited characters. Blood groups differ between individuals in a population. Population also differs in the frequency of different blood group. These differences in the frequency of blood group are characteristics of the population and thus are anthropologically valuable. Fifteen different blood group systems are known (such as ABO, MN, Rh, Lutheran, Kell, Duffy, Kidd, Lewis, Diego, etc) each controlled by a separate locus, and at each locus multiple alleles are known to be present.

What is the basis of classifying blood in different blood groups?

It is the antigen – antibody reaction. Antigens are proteins which excite the production of antibodies and antibodies are substances in the serum or plasma which are very specific to an antigen reaction.

What does Blood grouping imply?

It is process of testing the unknown red cells with known antiserum.

Now how do you identify the antigens?

Different antigens on the red cells are specific to the antibodies (proteins in serum) which when react with the antigens cause agglutination of the red blood cells.

What do you understand by agglutination?

Agglutination refers to clumping, clustering or bundling together of red blood cells.

ABO blood group has naturally occurring antibodies in their serums; while in others like MN and Rh, it can be produced through immunization. As mentioned earlier the basic principle of blood grouping is an antigen-antibody reaction. A particular antigen reacts only with its specific antibody and not with others. The reaction is an observable phenomenon in the form of agglutination.

ABO BLOOD GROUP SYSTEM

As you already know A, B, AB and O are commonly known blood groups. How do we differentiate between them and create an identity for them? The presence or absence of blood group antigens A and B on the red cells forms the basis of classification of ABO blood group. If antigen –A , is present on the red blood cells then there are anti-B antibodies in the serum; similarly, if there is antigen – B, on the red blood cells then, anti-A antibodies are found in the serum. If, in a person both antigen A and antigen B are present on the red cells, then neither antibody in the serum is found and people who do not contain either of the antigens on the red blood cells have blood group O and thus have both the anti-

A and anti-B antibodies in their serum. This means that type A blood group indicates the presence of antigen A, while type B blood group shows the presence of antigen B. Type AB blood group, as has been mentioned earlier, has both the antigens A and B, while type O blood group has no antigens. In the ABO system, antibodies are there in the serum right from the time of birth. Individuals with Group A blood group have anti-B in their plasma, those with group B blood group have anti-A, AB individuals have neither, while O individuals have both the antibodies.

The antigen-antibody reactions hold an important criterion for determining the mode of blood transfusion. For example, all the people belonging to blood group A can take blood from A blood group people, but not from other blood groups, like B or AB blood group. In blood group O, no antigen is present on the red blood cells; therefore it can be transfused to persons with other blood groups.

ABO blood group system holds an important position as far as its applications are concerned. It enjoys wide field of application like in ethnic diversity, blood transfusion, paternity diagnosis, genetic counseling and also in forensic investigations including medico-legal angle and detection of drugs in blood. Furthermore, different blood groups show certain level of association with particular disease, such as Blood group A shows an association with cancer of the stomach.

Let us understand how blood groups are inherited. The ABO blood group system is controlled by a single locus with three alleles viz. A, B and O. They hold responsibility for the production of antigen-A, antigen –B, and neither antigen, respectively. Alleles A and B are both dominant hence are referred as co-dominant, while allele O is recessive to both. There are two subtypes of the group A, designated as A_1 and A_2 and, therefore, A is replaced by two alleles, A_1 and A_2. A_1 is dominant over A_2. It is because of $A_1$and A_2 which is two sub-types of group A, the system ABO Blood group has been designated as Blood group A_1A_2 BO. The four alleles give rise to ten genotypes and six phenotypes as given below:

Phenotypes	Genotypes
A_1	A_1A_1, A_1A_2, A_1O
A_2	A_2A_2, A_2O
B	BB, BO
A_1B	A_1B
A_2B	A_2B
O	OO

As mentioned earlier let us look at the antigen and antibodies in the ABO blood group system again

Blood group	**Antigen**	**Antibody**
A	A	anti - B
B	B	anti - A
AB	A,B	-
O	-	anti - A, anti - B

The fundamental principle behind ABO blood grouping is that, an unknown blood sample or red cells are agglutinated, when treated by anti-A serum, the cells are classified as belonging to group A; if there is reaction with anti-B serum, the cells are classified as blood group B; if there is reaction with anti - A as well as with anti-B serum, the cells are said to be belonging to group AB. When there is no reaction with either anti-A or anti-B serum, the cells are classified as group O. We can understand this from the following table:

Reaction in the ABO blood group system

Determination of A_1A_2BO Blood group using Anti- A, Anti- A_1, Anti- A_2, Anti- B, Anti- AB and Anti- H

Anti sera					Blood group
Anti A	Anti B	Anti AB	Anti A_1	Anti H	
+	-		+		A_1
+	-	-		+	A_2
-	+				B
-	-	-		+	O
+	+	+	+		A_1B
+	+	+	-	+	A_2B

ABO Grouping Technique involves the following steps:

1) Preparation of normal saline: Dissolve 8.5 to 9.0 gms of Sodium Chloride in 100 cc of distilled water, which will make 8.5 to 9.0 % of normal saline. (8.5 gms is ideal as it allows for increased concentration of salt as a result of evaporation of water). This solution is called isotonic with respect to red blood cells of the human body.

2) Blood collection and making 2% red cell suspension: Clean the finger from which the blood is to be taken with cotton swab soaked in methanol.

   Prick the finger with a new disposable lancet.

   a) Collect the blood in the micro tube which already has 24 drops of normal saline solution.

   b) Centrifuge the tube which has distilled water and a drop of blood.

   c) Take the supernatant with the pipette.

   d) Pour 24 drops of normal saline into the tube.

   e) Centrifuge it twice. There is a risk of washing away the antigen on the surface of the red blood cells, if centrifuged more than thrice.

   Did you realise how you made 2% red cell suspension?

   Each drop of blood contains half a drop of cells. Now, when you add 1 drop of blood to 24 drops normal saline solution it makes 2% cell suspension. $^1/_2$ drop in 25 drops makes it 2 drops in 100 drops i.e. 2%.

3) Mix the blood with the respective serum: Take a grooved slide in which the groove resembles the bottom of the tube, this aids in the agglutination.

a) Clean the groove with spirit and dry it.

b) Put a drop of anti-A serum in one groove and a drop of Anti-B serum in another groove.

c) Add a drop of the prepared 2% cell suspension in each groove

Take a cleaned and dried dumble-shaped glass stud and stir the mixture of blood and sera in a circular motion.

4) Determination of blood by examining the agglutination: Examine the groove for agglutination.

If there is a positive reaction or agglutination when treated with anti –A serum, it is blood group A. Similarly when treated with anti –B serum, it is blood group B (if agglutination is noticed). If the agglutination is observed when treated with anti-A and anti-B serum, it is blood group AB. If no agglutination is observed with either serum, it is blood group O.

Rh blood group

Landsteiner and Weiner deserve the credit of discovering Rh factor in 1940. How did they give the name Rh to the blood group?

They injected the blood of rhesus monkey, in a rabbit and found antibodies formed in the rabbit. These antibodies agglutinated the red blood cells of all the rhesus monkeys. This occurred because the monkey's erythrocytes bear a particular antigen designated as Rh. Human beings that produce anti-Rh antibodies are Rh^+ (Rh positive) and those who do not are Rh^-. There are very few people who are Rh^- (Rh negative). Antigen D most commonly referred to as Rh^+ blood group antigen is frequently concerned with the problems of blood transfusion and sometimes with those of pregnancy.

The blood group Rh can be analysed by two methods

Slide Test Method

Rapid Test Tube Method

Slide Test Method

a) Put a drop of anti-D serum on a warm (47 degrees Celsius) slide.

b) Add 1 drop of 40% red cell suspension in normal saline to be tested.

c) Mix the contents systematically with the applicator stick.

d) Move it back and forth for about 2 minutes.

e) Use a hand lens to check the agglutination.

Rapid Test Tube Method

a) Take a clean dry test tube and put a drop of anti-D serum.

b) Add 5% red blood cell suspension to the test tube.

c) Mix the contents in the tube thoroughly and centrifuge for 2 minutes at 1000 rpm.

d) Care should be taken so that there is no hemolysis as it can be misinterpreted as negative result.

e) Suspend the cells by gentle shaking.

f) Use hand lens to see if agglutination is present or not and accordingly record the result.

Anti - D	Blood group
+	Rh^+
–	Rh^-

PRACTICE 1

Analyse the blood sample for ABO and Rh blood group for eight subjects.

Introduction

The blood groups are identified in ABO group on the basis of presence or absence of antigen A and B on red blood cells. They are identified on the principle of agglutination reaction between the unknown blood and the sera of known antibody. Suppose the unknown red blood cells get agglutinated by anti-A sera then the blood group is A, if the unknown red blood cells get agglutinated by anti-B sera then the blood group is B, if it reacts with both the sera, then the blood group is AB and if no agglutination occurs with any sera, it is blood group O.

When the unknown blood is treated with anti-D and agglutination takes place then the blood is Rh^+ (Rh positive) and if no agglutination takes place, then the blood is Rh^- (Rh negative).

Material

The following apparatus and reagents are required for blood collection and analysis of ABO and Rh blood group:

Apparatus

1) Beakers
2) Centrifuge
3) Cotton
4) Droppers
5) Eppendrof tubes
6) Forceps
7) Funnel
8) Glass marking pencil
9) Lancet
10) Leucoplast
11) Measuring cylinder
12) Ordinary test tubes
13) Porcelain tiles
14) Petri dishes
15) Scissors
16) Slides
17) Stirrer
18) Test tube stand

Reagents

1) Anti- A
2) Anti- B
3) Anti- AB
4) Anti- D
5) Anti- H
6) Distilled water
7) EDTA
8) Methanol
9) Normal saline solution (0.9%)

Method

Blood sample collection

Collect the blood samples in the eppendrof tubes which contain EDTA. The blood samples are transferred to the ordinary test tube with saline water for testing ABO blood groups and Rh factor, then the blood samples from the eppendrof are transferred to the slide for analysis.

a) Cleanse the subjects' left ring finger with cotton swab soaked in methanol.
b) Prick the finger with sterilized disposable lancet and collect few drops of blood in the eppendrof. The ordinary test tube for ABO blood group contains 3-4 ml of normal saline solution.
c) Now analyse the blood

There are two methods for blood analysis

Slide Method

a) 10% suspension of cells in physiological saline is prepared.
b) Place a drop of anti-A on one side or one cavity of the tile
c) Place a drop of anti-B on the other side
d) To each half of the slides add a drop of 10% red cell suspension.
e) Mix the cells and serum with clean corner of the slide and then mix it to smooth suspension
f) Shake the slide back and forth and gently rock.

Allow it to stand for 2-3 minutes shaking it gently occasionally to ensure thorough mixing.

Cell Suspension Method

a) Centrifuge the blood samples with normal saline solution for about 2-3 minutes at 2000 rpm.
b) Discard the supernatant through the dropper.
c) Add normal saline to the RBC button
d) Shake it thoroughly so that the cells are suspended in the saline
e) Centrifuge and repeat the step

f) Prepare 5% cell suspension i.e., 18 drops of saline and one drop of RBC for subsequent analysis.

g) Pour RBC suspension of the subject with the help of a dropper, in the different grooves of the porcelain tile.

h) Put a drop of antiserum in each drove

i) Allow it to coagulate for the analysis.

Analysis

The analysis of the blood group is based on agglutination reaction between antisera and antigen present in the body.

a) If the red blood cells carry the corresponding antigen to the known antibody, note that agglutination would take place.

b) No agglutination indicates the absence of particular antigen

See the table below to understand it better

Unknown red cell samples

	1	2	3	4
Anti- A	+	-	+	-
Anti- B	-	+	+	-
Blood group	A	B	AB	O

Here + denotes complete agglutination and – represent no reaction i.e. no agglutination.

Rh factor is based on agglutination between the antigen on RBC and anti- D.

A) Transfer the blood sample on the slide using a micro dropper;

B) Pour a drop of anti- D using a micro dropper on the blood sample;

C) Allow it to agglutinate for analysis;

D) If agglutination of the cells is noticed, it indicates blood group is Rh^+ (Rh positive); and

E) If no agglutination of the cells takes place, it indicates the blood group is Rh (Rh negative).

Precautions

a) Use cotton swab with methanol to clean the finger before the prick.

b) Dispose off lancet after single use.

c) Use separate droppers for separate suspensions.

d) Collect the blood properly in the eppendrof tube without any loss.

e) After you collect the blood in eppendrof gently mix with EDTA properly.

f) Put the blood samples thus collected in the ice box as soon as possible.

g) Test the blood within 24 hours of collection.

h) Clean all the glassware to be used.

i) Pour few drops of EDTA in the Eppendrof tubes.

j) Take equal quantity of serum and cell suspension for I deal results.

Practice 1

Subject	Anti A	Anti B	Anti D	Blood group
1				
2				
3				
4				
5				
6				
7				
8				

DERMATOGLYPHICS

Cummins and Midlo (1962) coined the term Dermatoglyphics which is derived from two Greek words 'Dermato' which means skin and 'glyphics' meaning carving. Basically, it is the science of epidermal ridge pattern on human fingers, palms, toes and soles. Print of the palm is termed as palmar print and that of feet is referred to as plantar print. If you want to study extensively the skin pattern of any area, it is best studied in detail when the print of whichever area you want is taken following standard method. Before we learn the methods to take print, let us get familiar with certain terms that will help in understanding the objective better.

Ridges: The ridge patterns have an identity of their own. Did you notice that the palmar and plantar surfaces and fingers in man lack hair and sebaceous glands with plenty of sweat glands. That is, the ridged skin with sweat is structural specialisation among human beings. The ridges on our skin or epidermal ridges form a regular pattern on the phalanges of the digits, palms and soles. Every individual possesses distinct features of ridges and their pattern in fingers, palms and soles and remain stable all through life. From the anthropological perspective, it holds an important position as they are one of the anthropological characters that are relatively stable for a population. Not only does it provide invaluable information in forensic anthropology, but an association with genetic conditions and chromosomal aberration has also been observed. The evolutionary relationship with the hand and foot prints of primates with humans holds important comparative information too.

Source: www.barcode.ro

Ridge Configurations: The epidermal ridges or the ridges on the skin are not just random. They form a definite local pattern on the terminal segments or phalanges of the digits and also on the palm and sole. This holds an extremely important position in personal identification, inheritance, racial variation and other biological aspects of dermatoglyphics as mentioned earlier due to immense variation of the configuration. The configuration present on distal phalanges of finger and toes, depending upon their construction are termed as arches, loops and whorls by Galton (1892). Except for plain arches all other configurations appear to be composed of abruptly curved ridges. Actually, plain arches have

parallel ridges and is a special form of open field too. The middle and proximal segments of the digits seldom exhibit true patterns because of configuration which could be open field or erratic arrangement of ridges called vestiges.

Triradius: Triradius as the name indicates is the meeting point of three systems of parallel ridge systems which are called radiants; hence it holds a distinct landmark in parallel ridge system. There are four digital triradii, positioned at the base of the digits II, III, IV and V. When referred from radio-ulnar order it is called as a, b, c and d. The two distal radiants encompass the digital area of each distal triradius. Whereas, the proximal radiant heads towards the interior of the palm, and when fully traced this radiant shapes the palmar main line. Subsequently, the genesis of the four main lines are the four digital triradii point which are assigned A, B, C and D. The margin between the thenar and hypothenar eminences, is the axial triradii also called as't'-triradius. Sometimes, the axial triradius is positioned more toward the centre of the palm. There may be more than one of this triradius, and in rare cases, there may be none. The distal radiant of this triradius, when fully traced, is its main line, designated as 'T'.

Source: www.multiperspectivepalmreading.com

Core: It constitutes the main focus of the pattern as, when the ridges of the pattern diffuse around this centre they take the shape of an island, a short straight ridge, a hook shaped ridge, a circle etc. Sometimes two or more short straight ridges form the core of a pattern.

Source: www.webopedia.com

Finger Topography

Did you realise that there is a pattern on the ball of the fingers? Notice that these ridges in the larger area run parallel to each other, whereas in smaller area they bend to form discontinuity. These are called patterns. As mentioned earlier Galton differentiated the prints or configuration on fingers as whorl, loop and arch. But then Henry proposed composites to be included in these three as the fourth one. Often whorls and composites are combined and Galton's three fold classification is ensued. Let us briefly understand the patterns:

Fingertip patterns representing an arch (A), loop (B), and whorl (C). Adapted from Holt (*Holt SB. Quantitative genetics of finger-print patterns.* Br Med Bull 1961*;*17*:*247*–50)*

Arches: These are most uncomplicated of all the patterns and are commonly referred to as patternless configurations. There are two types of arches:

Plain arch (A): There is no triradius which is connected with the ridges, and ridges flow from one margin to the other slightly bowing, distally characterised by three arch curves.

Source: www.students.stedwards.edu

Tented Arch (TA): It appears to have triradius on which the ridge overtops moving from one margin to another in the form of a tent.

Source: www.policensw.com

Loops: In this the ridges of the loop form head of the loop by curving around only one extremity of the pattern, whereas on the opposite extremity it is said to be open as ridges flow to the margin of the digit. Loops are known to have only one triradius and the extremity in which this triradius lies is a close area. Loops turn at an angle of 180 degrees and are generally associated with one triradius.

Ulnar loop (UL) as the name suggests opens to the ulnar side and then the triradius is on the radial side. Subsequent loops which are on the toes and soles are called **Fibular loops (FL)** which naturally opens to the fibular side and in this case triradius is on tibial side.

Radial loop (RL) similarly opens to the radial side and in this case triradius is on the ulnar side. In this case the corresponding loops on toes and soles are called **Tibial loop (TL)** which as the name indicates open to the tibial side and triradius is on fibular side.

Distal loops open to the distal side. A loop on palm or sole generally opens in the direction of finger or toes i.e., distal side.

Proximal loops are the loops that open on the wrist side as on the thenar area of the palm.

Whorls (W) are the patterns with two triradii with ridges forming circuit around the core.

True Whorls are the ones which have single core and at times double core too. In this the ridges go round 360 degrees.

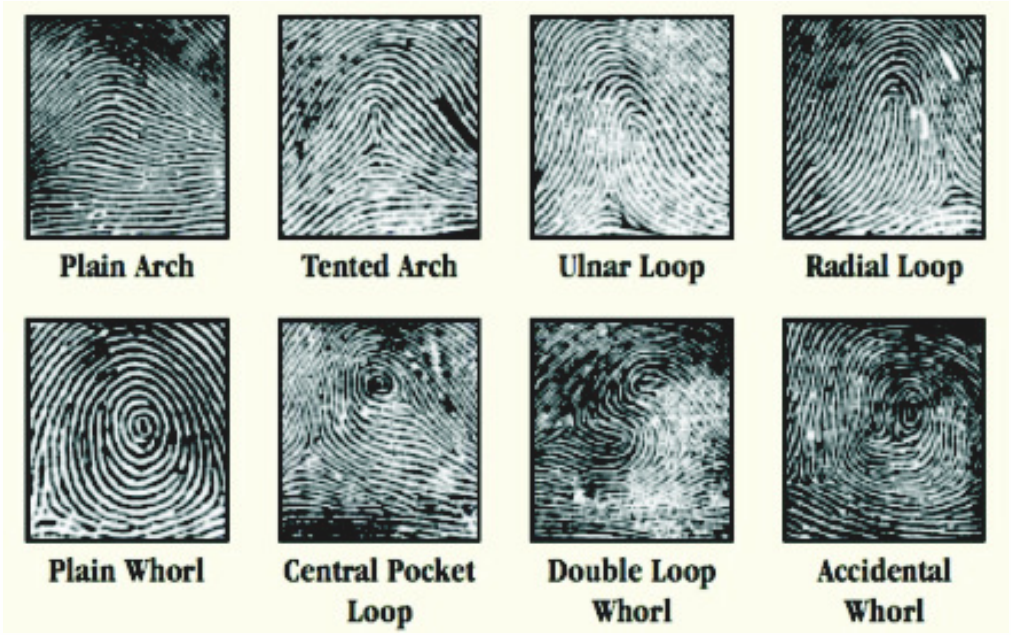
Double loop type of Whorl:

Central Pocket Loop (CPL) is a whorl, distinguished, as it bears a small loop within a loop.

Twin Loop (TL) as the name suggests is a type of whorl where two loops open to opposite direction.

Lateral Pocket Loop (LPL) is a whorl where in two interlocked loops open to the same margin.

Superwhorl (SW) is a condition when the pattern has three loops and three triradius.



Source: www.viewzone.com

Twin Loop

Source:www.fotolibra.com

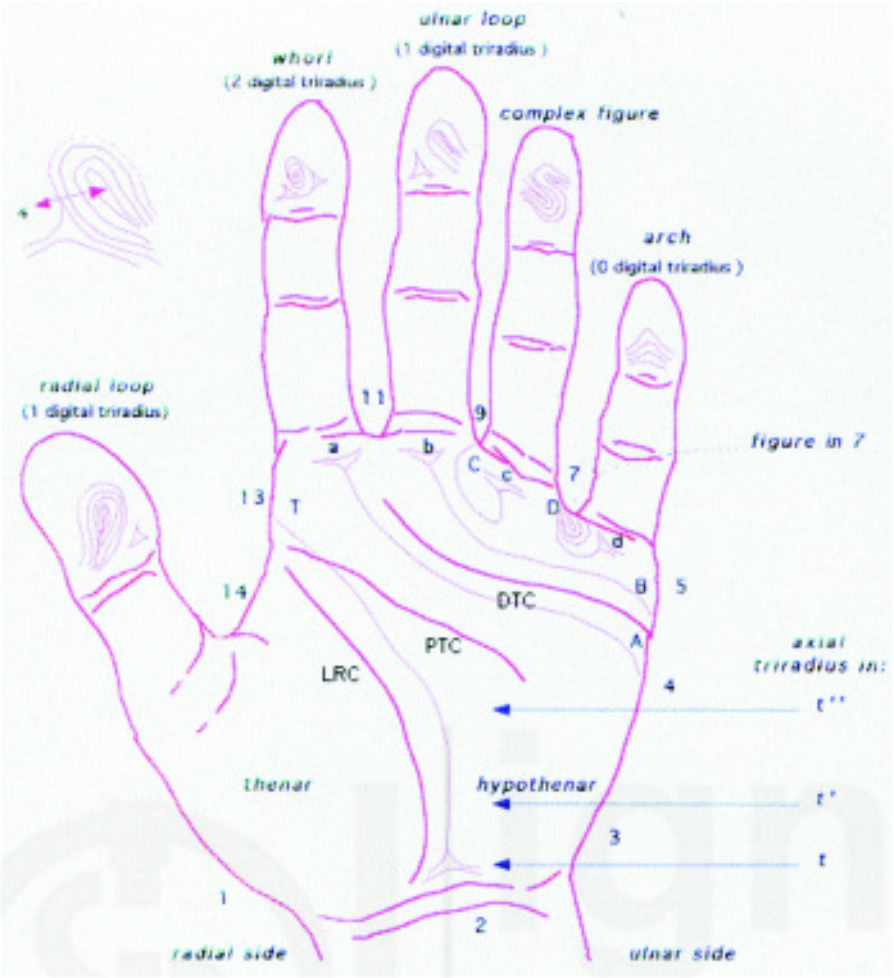
Palmar Topography

Look at your palms carefully. The proximal area of the palm is bound by a bracelet crease, very much like the name, and on the other side are the metacarpo-phalangeal creases. There are about six elevations around the hollow of the palm, varying in prominence. Four of these are interdigital pads, lying in the proximal to the interdigital intervals and are numbered accordingly as I, II, III and IV. The remaining two out of six are the thenar eminence occupying a large area of the proximodigital quadrant at the base of the thumb. These are bound by the radial longitudinal crease which is popularly called as the life line. Opposite to this, the hypothenar eminence is distinct; lying is a more elongated elevation, in the ulnar portion of the palm. Notice the shape of the palm, it runs in four anatomical directions proximal, distal, radial and ulnar.

Numbering the Palmar Area and the Main Lines

The margin of the palmar area is divided into 14 points and intervals. The number sequence begins with the proximal part of the thenar eminence. On the radial side of the axial triradius and at the base of the thumb, number 1 is given; this area continues around the proximal, ulnar, distal and radial borders of the palm. Number 2 position is allotted to axial triradius which is a point. The approximate midpoint of the ulnar margin is designated as 4; the digital areas are represented by 6,8,10 and 12. The interval between the points 4 and 6 is numbered 5, which is further divided into 5' which is the proximal half, and 5'' which is the distal half. Here the rule is that, each of the marginal areas of the palm are numbered following the principle that points are given even numbers, 2, 4,6, 8,10 and 12 beginning as mentioned above from the base of the palm behind the thumb (No.1), moving from ulnar to radial side, and odd numbers 1,3,5,7,9,11 and 13 are given to intermediate areas as shown in Figure.

It is the radiants of the triradius that traces the lines in the form of loops and whorls which are called as the Type line. One can identify the pattern on the basis of these lines. The longest radiant of digital triradius is the main line, hence the designation of main lines as D, C, B, A and T lines (see Figure).



Source: www.atlasgeneticsoncology.org

PRACTICE 1

Record the finger and palm prints of eight subjects

Material used: Magnifying Glass

Inking Plate (Metal or ¼" Glass) 6" wide x 14" long

Card Holder

Hardwood stand 2' length x 1' height and width

Cleaning Fluid or Cream

Paper Towels

Roller

Inking Plate Cleanser

Printer Ink/Stamp Pad Ink (heavy black paste),

Note: Printing ink or ordinary ink or infact any other coloured inks are not advised for fingerprinting work. The reason being they are too light, thin and do not dry quickly.

Finger Print Method

a) Clean the hand of the subject and dry with clean towel.

b) Smear the ink over the fingers.

c) Hold the terminal knuckle of the finger and roll it from radial to ulnar side. The thumb should be rolled from opposite side.

d) The ideal finger prints should be square in shape. The triradii should be visible in the print. One triradius for loop, two triradius for whorl and three triradius on a super whorl.

Palmar Print Method

a) Hold the wrist of the subject and place the hand on the inked slab uniformly.

b) Lift it up slowly from the ulnar end of the palm.

c) Place the palm on the paper.

d) Press the interdigital areas and hollow in the centre of the palm.

e) Remove the palm from the paper slowly without any jerk pressing the centre of the palm.

f) Roll the palm on the ulnar end.

g) Take care that there is uniformity in the print including that of the hollow in the centre and ulnar end of the palm.

Practice 1

Recording the pattern from finger prints and Palmar prints of eight subjects

PALMAR DERMATOGLYPHICS

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S.No				Name		Age	Sex	Date of Printing Date of Analysis		
D	C	B	A	Axial Triradius	Hypothenar	Thenar/I	II	III	IV	MLI

FINGERBALL DERMATOGLYTPHICS

Discipline of Anthropology, IGNOU.

Name
Age
Sex
Date of Printing
Date of Analysis

Pattern Typing (RIGHT HAND)

I	II	III	IV	V

Pattern Typing (LEFT HAND)

I	II	III	IV	V

Reference

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