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# FORENSIC SCIENCE UNIT – I

## Forensic Science



*As Per Updated Syllabus*

**FORENSIC SCIENCE UNIT – I**

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**FORENSIC SCIENCE: DEFINITION, HISTORY & DEVELOPMENT, SCOPE, ETHICS IN FORENSIC SCIENCE**

In the era of technology, criminals are committing crime by using technology. So it is almost impossible to solve those types of crime without using science or technology. Forensic Science has become an integral part of the Criminal Justice System by recognizing and gathering evidences from crime scene and thereafter analyzing them scientifically in the laboratory. This could help in deciding the guilt or innocence of a suspect. Forensic Science has developed itself to operate within the reality determined by the Criminal Justice System. To maintain this relationship between Forensic Sciences and the Criminal Justice System communication is very important. The focus has been shifted from the quantity to the quality and makes it possible for the Forensic Scientist to answer honestly the questions that have been posed to him. The reforms are needed in the law and legal system to define these changes in a new reality. Forensic Science, being a discipline that comes first in contact with different types of problems, has an important part to play in defining this reality.

In other words, to apply the scientific methods and techniques so as to help in conducting criminal investigations that will contribute in solving the legal problems.

Like the pathologists who testify the causes of death and engineers testify the causes of damage from equipment failure, fires, or explosions; Forensic scientists present Expert Testimony to the court of Law.

**Introduction to Science**

The word 'science' originated from the Latin word scientia, meaning knowledge. It refers to a system of acquiring knowledge and uses these observations and experimentation to explain phenomena of nature. Sometimes it becomes difficult to describe science as what public think about science and scientists is not exactly correct.

Their belief about the scientists is that they are the people who are capable of using complex methods and even secret means to, discover or invent something for the benefit of humanity. Scientific experiments are supposed to yield results or conclusions which frame absolute 'truth' which is one of the prime objectives of science. So science may be defined as

"Areas of human endeavor which tries to establish and understand the connections among human phenomena, with objects around us and processes that are subject to observations, measurements and experimentations."

Or

It is a systematic creativity which builds and organizes knowledge in the form of testable explanations and predictions or in the simple words the type knowledge that can be rationally explained and consistently applied.

It is different from other disciplines with regard to its approach which is theoretical as well as empirical in nature.

**1. Basic Sciences**

Pure or Basic sciences are different from applied sciences which are the application of research to human requirements. Science communities have been debating on the following questions for the last few decades about the worth of different type of sciences:

- Is it valuable to pursue sciences for the sake of simply gaining knowledge? or
- Does this knowledge only have worth if it can be applied to solve a specific case/problem to improve our lives?

The questions focus on the differences between two types of sciences: basic and applied.

- Basic sciences or pure sciences include Biological, Physical and Chemical Sciences which are the study of the natural world, or research performed solely to expand the knowledge base.

- Applied sciences refer to the disciplines dealing with the art or science of applying scientific knowledge to solve real-world problems.

Some of us may perceive applied science as "useful" and basic science as "useless." But many scientists think that a basic understanding of science is necessary before an application is developed therefore, applied science relies on the results generated through basic science.

Another category of the scientists think that it is more appropriate to move on from basic science and instead to find solutions to actual problems. Both approaches are valid. It is true that there are problems that demand immediate attention; however, few solutions would be found without the help of the wide knowledge foundation generated through basic science. The following few examples shows how the basic and applied science can work together to solve practical problems occurred after the discovery of DNA structure, which led to an understanding of the molecular mechanisms governing DNA replication. Strands of DNA are unique in every human being, are found in our cells where they provide the instructions necessary for life. During DNA replication, it makes new copies of itself shortly before a cell division take place. To understand the mechanisms of DNA replication, scientists developed a laboratory technique that is now used to identify genetic diseases, pinpoint individuals who were present at a crime scene, and also help in solving paternity/ maternity disputes. Without basic science, it is unlikely that applied science would exist.

Similarly another example of the link between basic and applied research is the Human Genome Project, a study in which each human chromosome was analyzed and mapped to determine the precise sequence of DNA subunits and the exact location of each gene. As the gene is the basic unit of heredity; complete collection of genes of an individual is his or her genome. To have a better understanding of human chromosomes, there is a need to study other less complex organisms. The Human Genome Project relied on basic research carried out with simple organisms and, later, with the complex human genome. The data so generated eventually become important to achieve the end goal of applied research in the form of finding cures and early diagnoses for genetically-related diseases.

At the same time, there are differences between forensic science and basic sciences. The differences are attributable in part to the fact that forensic science has some unique objectives, and in part to its continuous and necessary interaction with the legal system. In most scientific investigations of crime, different forms of the scientific methods have been used.

Forensic science is basically the application of the scientific methods and techniques of sciences such as physics, chemistry, and biology to the matters of the law; which includes a variety of different activities and specialties.

## **2. The Scientific Methods:**

To attain the 'truth' is one of the basic aim of science, and thus one of the base for using the scientific methodology, this ideal perhaps is difficult to achieve sometime in actual practice. Actually, Science is concerned with natural phenomena and is that area of human endeavor which tries to unite and understand the connections of things/ environmental conditions and processes that are subject to observations, measurements, and experimentation. In addition, science can be distinguished from other disciplines on the basis of its combined theoretical and empirical approaches, which are usually called the scientific method.

The scientific method may be characterized as consisting of several separate steps in the form of theory, hypothesis, observations (collection of data), conjecture and testing. Most scientists do follow this general procedure. The significance of the observations made is considered (conjecture), until some sensible explanation consistent with all the data-a hypothesis-is arrived at. Experiments are designed in such a way to test the hypothesis are then conceived and carried out. The new data thus



obtained are used to refine the primary hypothesis if necessary. A modified hypothesis which has been verified by a good deal of rigorous testing may be called 'a theory'. Sometimes, theories which have been extensively tested and verified by many scientists working independently may come out to be-regarded as 'natural laws'.

Forensic scientists engaged in reconstruction (i.e. sequences of steps) of events follow the essential principles of the scientific method sketched above. In an attempt to reconstruct the events which took place at a crime scene, for example, the first step is careful observations and collection of all the known facts in the form of observations and clue materials. Different hypotheses can then be checked to see how well all of them relate to the facts of the case. As additional facts are revealed by further observations or by experimental testing, it may then become possible to arrive at a theory of what took place and how?

The scientific methods are not strict set of rules by which all scientists proceeds, rather, this is a particular way of going further to gather information about the natural world and attempting to organize and understand it. Elements of both inductive and deductive logic are used while applying the scientific methods.

- 1. Deductive Logic:** In deductive logics or reasoning, links premises with conclusions. If all premises are true, the terms are clear, and follow the rules of deductive logic, then the conclusions reached are valid. Consider the following example of deductive logic. The red pigment carrier of oxygen in the blood of all mammals is hemoglobin. As human are mammals; therefore, hemoglobin is present in human blood. The conclusion here is a logical consequence of the two premises. Mathematical proofs are therefore examples of deductive reasoning/logic. The conclusion is created on the facts present in the premises reductively.
- 2. Inductive Logic:** In the scientific experiments, deductive logic would not be sufficient alone. It is also necessary to go beyond the facts to draw conclusions that may have predictive value. This type of reasoning is known as inductive reasoning or logic, which leads to the development of hypotheses. The conclusion drawn has not been proved to be true, although in some cases it may be regarded as true from a practical point of view. A conclusion may be based on a huge experience that it is unlikely to be false.

For example, that fingerprints are individual, but this has never been rigorously proved. The conclusion is reached through inductive logic, and is based upon observations of the millions of scientists. The reasoning behind the conclusion runs are as follows:

Ten of thousands of fingerprints have been collected and catalogued in national files. Thus far no two people have been found to have the same fingerprints; therefore, fingerprints are (probably) individual.'In order to prove this conclusion rigorously, by deductive logic, it would be necessary to compare the fingerprints of every person living on this planet earth! If two sets were found to be matched, then the conclusion reached would be inductively false.

Since it would be impossible to conduct the examination of every person's fingerprints living on the earth, we must be satisfied our self with the conclusion reached inductively, by extrapolation from inadequate experience with a few million examples thought to act as representative. Even though the inductive conclusion has not been rigorously proved, it seems quite unlikely that it is false. It is, therefore, will be a probable conclusion. Such conclusions may be drawn from analogies or informed conjecture which is based upon a large amount of representative data. To be useful practically, it is required only that they ascertain to be correct most of the time.

### **Hypothesis**

Science begins simply with inquisitiveness about some of the natural phenomenon. Then a scientist makes observations on this phenomenon and formulates an 'educated guess' to clarify the

observations made. This guess is called a hypothesis and its plural is hypotheses. Since the hypothesis describes an explanation for some of these phenomenon and made it possible to make some predictions based upon it. In order to be within the jurisdiction of science, a hypothesis must be able to create forecasts which can be tested with scientific experiments.

Hypotheses or theories remain outside the realm of science which are not subject to scientific experimental testing, but those which can be tested with scientific experiments the truth of their predictions are called Scientific hypotheses. True hypotheses always produce true predictions. The distress is that false hypotheses can also sometimes yield true and false predictions as well. Therefore, scientific experiments which prove that a prediction is true do not certainly demonstrate that the hypothesis from which the prediction was derived is true. They just lend a support to the hypothesis.

The scientific experiments which scientists conduct to test any hypotheses are planned to be controlled, so that there should be only one variable at a time. In routine, mostly it is not possible to construct an experiment with absolute certainty in which only one parameter is variable.

Unrecognized variables do influence the experimental results. Many different experiments needs to be conducted on a particular issue or a question to find the truthfulness or falseness of a hypothesis, as many different predictions are tested over the course of time. If a hypothesis leads to a prediction that is shown experimentally to be false, the hypothesis has to be modified or changed all together. So hypotheses are altered over the course of time as number of experiments is conducted to test their rationality.

After modification or alteration, hypotheses (scientific guess) get closer to the reality. Because of this self-testing and experimental in nature, scientific knowledge is always keep transforming. There is no surety that yesterday's truth will remain the same as tomorrows. A hypothesis which has been extensively tested, and which generates a large number of true predictions, is often called a theory. Even the words 'hypothesis' and 'theory' are regularly used synonymously, but a scientific hypothesis may not the same as a scientific theory. The scientific knowledge exists at a particular time present the best explanation of natural phenomena which has been formulated up to that time. It is not possible to know how close to the "real truth" scientific knowledge at any particular time. The history of science has educated us to be skeptical about everything whatsoever we think is true.

In forensic analysis a results or conclusions made are 'scientific,' public has become accustomed to believe that the results must be right.

Since the fourteenth century, scientific evidences have posed strong challenges for the law and legal system of that time and always had an uneasy association. These challenges arise because of the fundamental differences between the scientific and legal processes. The legal system embraces the adversary process to attain "truth," for the ultimate purpose of attaining an authoritative, final, just, and socially acceptable resolution of disputes. Thus law has fixed standards and seeks to define how public and private relations should function.

In contrast to law's vision of truth, however, science embraces empirical analysis to discover truth as found in certifiable facts. Thus Science does not define how the universe should be, but rather describes how it actually is making it more descriptive.

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### **LAW AND FORENSIC SCIENCE**

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During any crime investigation, the evidences are collected from a crime scene, analyzed scientifically in the Forensic Science laboratory according to the requirements of the investigation and reports are presented in court of law. Each crime scene is unique, and each case presents varied challenges. In

the laboratory complex cases may sometime involve multiple forensic experts having different backgrounds in biology, physics, chemistry, and other disciplines. These forensic scientists analyze evidence separately in a particular case. For example, one of the forensic examiners might be developing a latent finger prints, forensic biologist might be comparing hair and other fibers by using a microscope, and another might be identifying a white powder that was collected from the scene. The investigators will then combine all of the examiners' objective results to interpret and build a case.

Now, it is the duty of both the scientist and the court to ensure that maximum effort should be made to ensure that the jury must understands the competences and limitations of any science presented to them. The scientists must be able to convey the complex subject in as much simpler way as possible. Only then the lawyers and judges can appreciate the value of scientifically analyzed physical evidences and reach to a secure and informed decision.

### **Law and Legal Systems**

When we have to live together and interested in the formation of society, disputes are inevitable. To resolve these issues, perhaps we need to have a framework of code of conduct to maintain our relationships with others. Even from primitive times, history has been logged with accounts of different codes, or laws. In the history of law in many societies, we can discern the same evolution taking place moreover in the same sequence (Table-1).

For conducting human affairs, people were regulating their affairs and relationships in a rather systematic manner by designing laws and legal system, which were based either on their religion or purely secular.

**Table-1 Showing Sequences of Historical Evolution of law in various societies**

<b>First</b>	<b>People Live In Family Units With Rule By <i>The Patriarch</i></b>
<b>Second</b>	<b><i>A Patriarchal Sovereign</i> Who is usually Heroic issues Rulings in individual Cases after The Fact</b>
<b>Third</b>	<b><i>Customary Law</i> grows up from the Sovereign's rulings.</b>
<b>Fourth</b>	<b><i>A Legal Code</i> is Created. This Code bears on Relationships between Families or between the Patriarchs of the families.</b>
<b>Fifth</b>	<b>The Code Begins to bear on <i>Individuals</i> rather than Families.</b>
<b>Sixth</b>	<b><i>More Relationships</i> are defined by <i>Contracts</i> i.e. A Movement from Status to Contract.</b>

Presently, we are accustomed to a legal system with voluminous codes and well defined procedures for contracts, many of these mentioned in table-1 don't sound like much of a legal system to normal ears. But for most of the existence of humans, the following are the systems they lived under:

### **The Patriarch**

In the historical evolution of law, according to earliest ever records available and in the observations of more primitive cultures, the earliest stage of development is characterized by people living in small groups. These groups were based on kinship and ruled by the eldest male. Mostly the ruler was chosen by very strict customs of descent through the eldest sons from the 'original' ancestor. Often this rule was quite complete and always includes property, earnings, and contract. This was entirely at the caprice of the patriarch, with the ruled having none of what we would think of as rights. But it is a



customary responsibility of the patriarch to provide for his family. The males after attaining the age of majority could free themselves from the rule of their father and even start their own patriarchy.

### **The Sovereign**

Later on they develop a sovereign ruling over a grouping of families. This rule is in the same style as of the patriarch; he issues rulings after the fact and without reference to any established rules. Primitive man at this stage supposed that the gods (Themis to the Greeks) dictated to the king what to award. The name of the awards was Themistes. Here important to note that these are not laws but pronounced judgments. The pattern of themistes helps in creating a custom (which is opposite to the theory that the laws embody the customs of a previous era).

### **Customary Law**

The early kings were heroic, but often feebler monarchs followed. Often an oligarchy would grow up around the monarch. These aristocrats mostly became the depository and administrators of the law. This was the epoch of customary law. English common law pretends to be of this type (at one time, the judges relied on rules, principles, but lawyers and the public were not fully aware about these distinctions), but today all this is based on written precedents.

### **A Legal Code**

Finally a legal code has been written down. This happened just after the invention of writing. Quite often the initial code were mixed of civil, religious, and moral issues. But at last we have arrived at a stage where the legal system becomes recognizable. In most of the cases the flavor of the initial code in the form of earlier patriarchal era and primarily deals with relationships between the families or between the patriarchs of the families was retained.

It was only after 800 in English history, when King Alfred the Great declared that the law would be present in a written so that people could know about the law related to the fact (I date the beginning of the Libertarian revolution from this point.)

### **Individuals**

In the next stage, the legal code started dealing with individuals rather than just the patriarch. It even begins to regulate the relations within the family.

### **Contractual Relationships**

Finally, the relationships within a legal system begin to be determined more and more by contracts than by the status of the actors. The most obvious is employment, which becomes a matter of contract between two parties rather than master and slave. This process can be observed in historical times and is still continuing today.

Now let's discuss about the present Legal systems which operate under many different forms of governments. The laws in effect at any given time are usually influenced by or derived from those of earlier periods.

The relationships activities among people of a society are also subject to certain rules and regulations. Government keeps regulating those activities which influence our daily life most closely. The other activities, regarded as contrary to the collective interest or to generally accept moral code and conduct, has to be barred by laws. The existing govt. /authority then provides sanctions against those who engage in the forbidden activities. In a general way, criminal codes governs activities in which society as a whole has an interest, and civil codes tend to manage relations among the individuals or groups.

The distinction between criminal and civil matters depends upon the social and historical context of the society who has created these laws. Legal structures prevalent at particular times are always reflections of particular type of societies. Without any exception, however, the dynamics of their

investigator work side-by-side in the laboratory with the lab analysts. The fire investigator may further need to perform his or her own analysis of the lab data beyond what the laboratory is in a position to do. This usually requires that the fire investigator utilize specialized software and databases to perform this analysis of the lab data.

### Case Application

Proper QA/QC is useful in the assessment of laboratory results to rule in or rule out various potential chemical processes. It may be necessary to assess all these alternative potential chemical processes to discover what actually happened. Without proper QA/QC, valuable lines of investigation can be easily overlooked. Generally it is the investigator, not the laboratory, who identifies appropriate additional analyses based on the assessment of laboratory results at each step in the process.

QA/QC is beneficially used in the court in a number of ways. Inadequate QA/QC is often the foundation for attacking the opposing expert's evidence. This tactic can be pursued based on holes in the laboratory QA/QC, or even on the basis of a complete lack of QA/QC. An experienced investigator can assist in the development of deposition strategies, including the identification of specific lines of questioning. In some circumstances, it is possible to "turn" the opposing expert based on either insufficient QA/QC or on well qualified data that points in another direction. An experienced investigator also helps ensure that the case for their client has solid and impeccable QA/QC. It should not be unexpected that the final outcome of the case is often rooted in the credibility of the chemical analysis and interpretation of the evidence.

### FORENSIC SCIENCE UNIT – I MCQs

1. Dying declaration is to be preferably recorded by

- (A) Doctor
- (B) Police
- (C) Magistrate
- (D) Jury Member

Answer: (C)

2. Murder cases are tried in the following courts

- (A) Chief Judicial Magistrate's Court
- (B) 1st Class Metropolitan Magistrate's Court
- (C) Sessions Court
- (D) High Court

Answer: (C)

3. Police inquest is conducted under section

- (A) 174 CrPC
- (B) 174 IPC
- (C) 176 CrPC
- (D) 176 IPC

Answer: (A)

4. Assertion (A): Blood stains on cloth should be collected after drying in shade under room heater.

Reason (R): It causes disintegration of blood stains.

- (A) Both (A) and (R) are correct.
- (B) Both (A) and (R) are incorrect.
- (C) (A) is correct, but (R) is incorrect.
- (D) (A) is incorrect, but (R) is correct.

Answer: (D)

5. Preservation of footprint on snow can be done by

- (B) Plaster of Paris Cast
- (C) Sulphur Casting
- (D) Tracing
- (E) Wax Casting

Answer: (B)

6. Light that has all its waves pulsating in unison is called

- (A) Maser
- (B) Laser
- (C) Monochromatic light
- (D) Polychromatic light

Answer: (B)

7. Hollow Cathode Lamp (HCL) is used in the following:

- (A) Atomic Absorption Spectrometer
- (B) Atomic Emission Spectrometer
- (C) Infra Red Spectrometer
- (D) X-ray Fluorescence Spectrometer

Answer: (A)

8. Deviations from Beer's Law fall into which categories?

- (A) Real

- (B) Instrumental
- (C) Chemical
- (D) All of the above

Answer: (D)

9. One of the following is not the component of Kastle-Meyer Test

- (A) Phenolphthalein
- (B) Glacial Acetic Acid
- (C) Zinc dust
- (D) Potassium Hydroxide

Answer: (B)

10. Confirmation of menstrual blood stain is done by the following method:

- (A) Isoenzyme marker
- (B) Fibrin Degradation Product (FDP)
- (C) Protein Marker
- (D) Restriction enzymes

Answer: (B)

11. The complementary base pairs among four nucleotides (A, T, G, C) are as

- (A) A = G and T = C
- (B) A = C and G = T
- (C) A = T and G = C
- (D) All of the above

Answer: (C)

12. Seminal fluid is a gelatinous material produced in males by seminal vesicles, prostate and

- (A) Adrenal gland
- (B) Pituitary gland
- (C) Cowper's gland
- (D) Thyroid gland

Answer: (C)

13. The level of toxicity of Datura plant on the basis of increasing level is

- (A) Root, Seeds, Fruit, Leaf
- (B) Leaf, Root, Fruit, Seeds
- (C) Fruit, Root, Seeds, Leaf
- (D) Seeds, Leaf, Root, Fruit

Answer: (B)

14. Sodium and Potassium hydroxides are strongly corrosive due to

- (A) Their solvent action on protein material
- (B) Their saponifying action on the lipids
- (C) Their ability to extract water from the tissues
- (D) All of above

Answer: (D)

15. In case of carbon monoxide poisoning which preservative is recommended for the preservation of blood samples

- (A) Sodium Chloride
- (B) Sodium Fluoride
- (C) Sodium Carbonate
- (D) No preservative

Answer: (D)

16. Free sulphuric acid is rarely found in stomach contents in acid poisoning cases because

- (A) It may be vomited out
- (B) May be neutralised by alkalies given as antidotes
- (C) May combine chemically with the tissue with which it comes in contact
- (D) All the above

Answer: (D)

17. Arrange the following firearms in the proper chronological order:

- (i) Flint lock
- (ii) Wheel lock
- (iii) Match lock
- (iv) Percussion cap lock

Codes:

- (A) (ii) (iii) (iv) (i)
- (B) (iii) (i) (ii) (iv)
- (C) (iv) (ii) (i) (iii)
- (D) (i) (iv) (iii) (ii)

Answer: (B)

18. The Indian Arms Act was enforced in

- (A) 1955
- (B) 1959
- (C) 1961
- (D) 1964

Answer: (B)

19. The bore of the 12 bore gun is

- (A) 0.723"
- (B) 0.727"
- (C) 0.729"
- (D) 0.731"

Answer: (C)

21. The diameter of SG pellet in 12 bore gun cartridge is

- (A) 8.43 mm
- (B) 7.77 mm
- (C) 6.83 mm
- (D) 5.16 mm

Answer: (A)

22. Faeces stains are identified from odour, presence of indigested matter, vegetable fibres and

- (A) Stercobilin
- (B) Dark brown crystals of choline iodide
- (C) Rhombic crystals
- (D) All of the above

Answer: (A)

23. Assertion (A): Hair has paramount importance to establish the link between suspect and victim and linking both with the scene of occurrence.

Reason (R): As per Locards principle of exchange.

Codes:

- (A) (A) is correct, but (R) is incorrect.
- (B) Both (A) and (R) are incorrect.
- (C) (R) is correct, but (A) is incorrect.
- (D) Both (A) and (R) are correct.

Answer: (D)

24. The direction of a wound can be ascertained from which of the following injuries:

- (A) Chop wound
- (B) Contusion
- (C) Incised wound
- (D) Laceration

Answer: (C)

25. Lanugo hairs are

- (A) Pigmented
- (B) Thin and soft
- (C) Medullated
- (D) Scale pattern is complex

Answer: (B)

26. The following method is used for determining the age of an ink by tracking the degradation of certain dyes.

- (A) Thin layer chromatography (TLC)
- (B) Gas chromatography (GC)
- (C) Laser desorption mass spectrometry (LDMS)
- (D) High Performance Liquid Chromatography (HPLC)

Answer: (C)

27. LSD is derived from which of the following plant?

- (A) Cannabis sp.
- (B) Papaver Somniferum
- (C) Erthroxylum sp.
- (D) Clavicepspurpurea

Answer: (D)

28. Linseed, Safflower and Cottonseed are used for

- (A) Paint pigments

(B) Paint oils

(C) Paint solvents

(D) Paint binders

Answer: (B)

29. Assertion (A): As the rhodamine 'B' in ballpoint pen ink degrades, it loses the ethyl groups.

Reason (R): The ethyl groups are replaced by hydrogen atoms.

Codes:

- (A) Both (A) and (R) are true.
- (B) Both (A) and (R) are false.
- (C) (A) is true, but (R) is false.
- (D) (A) is false, but (R) is true.

Answer: (A)

30. For hardening of plaster of paris cast of foot prints, following substance is added:

- (A) Sodium chloride
- (B) Sodium carbonate
- (C) Talcum powder
- (D) Sodium sulphate

Answer: (B)

31. When the temperature of a liquid is raised

- (A) Its refractive index increases
- (B) Its refractive index decreases
- (C) Its refractive index disappears
- (D) Its refractive index doesn't change

Answer: (B)

32. The skid marks on the road in a vehicular accident may give an indication of

- (A) Make of the vehicle
- (B) Speed of the vehicle
- (C) Weight of the vehicle
- (D) Height of the vehicle

Answer: (B)

33. Chemical etching is a method for restoration of erased marks on

- (A) Wood
- (B) Leather
- (C) Metal
- (D) Plastic

Answer: (C)

34. Scales are found on the following fibre:

- (A) Wool
- (B) Cotton
- (C) Linen
- (D) Silk

Answer: (A)

35. Fractures due to heat are

- (A) Radial
- (B) Spiral
- (C) Hackle marks
- (D) Wavy

Answer: (D)

36. Which of the following type of abrasions are associated in sexual assault over the thigh of a woman?

- (A) Graze
- (B) Imprint
- (C) Pressure
- (D) Scratch

Answer: (D)

37. The chromosome pattern in Turner's syndrome is

- (A) XXY
- (B) XO
- (C) XXX
- (D) XYY

Answer: (B)

38. Cusp of Carabelli is found on

- (A) Incisors Central
- (B) Canines
- (C) Bicuspid
- (D) Tricuspid

Answer: (D)

39. ABO grouping is based on

- (A) Red cell surface antigen
- (B) Plasma proteins
- (C) Red cell enzyme
- (D) Nuclear chromatin

Answer: (A)

40. The total number of bones in the human skeleton in an adult is

- (A) 208
- (B) 308
- (C) 408
- (D) 508

Answer: (A)

41. In handwriting comparison the crossing in 't' and the dots in 'i' and 'j' are known as

- (A) Connective signs
- (B) Diacritic signs
- (C) Loop signs
- (D) Shoulder signs

Answer: (D)

42. Which of the following disease affects handwriting?

- (A) Chronic Leukaemia
- (B) Chronic Malaria
- (C) Parkinsonism
- (D) Thalassemia

Answer: (C)

43. The following is a sign of forgery:

- (A) Retouching
- (B) Connecting strokes
- (C) Pen lifts
- (D) All of the above

Answer: (D)

44. The most suitable solvent system for thin layer chromatography/paper chromatography of inks is

- (A) N-butanol: pyridine: water (3: 1: 1.5)
- (B) Amyl alcohol: acetic acid: chloroform (6: 1: 2)
- (C) Ethanol: Acetone: acetic acid (4: 1: 5)
- (D) Amyl alcohol: acetic acid: pyridine (6: 1: 2)

Answer: (A)

45. The following is the most acceptable method for revealing indented writing:

- (A) Oblique lighting
- (B) Intense lighting
- (C) Rubbing with a pencil lead
- (D) ESDA

Answer: (D)

46. Cadaveric spasm indicates

- (A) Suicide
- (B) Homicide
- (C) Natural death
- (D) Last act of a person before death

Answer: (D)

47. The sequence of post-mortem changes in a cadaver includes

- (A) Rigor mortis, primary flaccidity, secondary flaccidity, marbling
- (B) Primary flaccidity, secondary flaccidity, rigor mortis, marbling
- (C) Marbling, secondary flaccidity, primary flaccidity, rigor mortis
- (D) Primary flaccidity, rigor mortis, secondary flaccidity, marbling

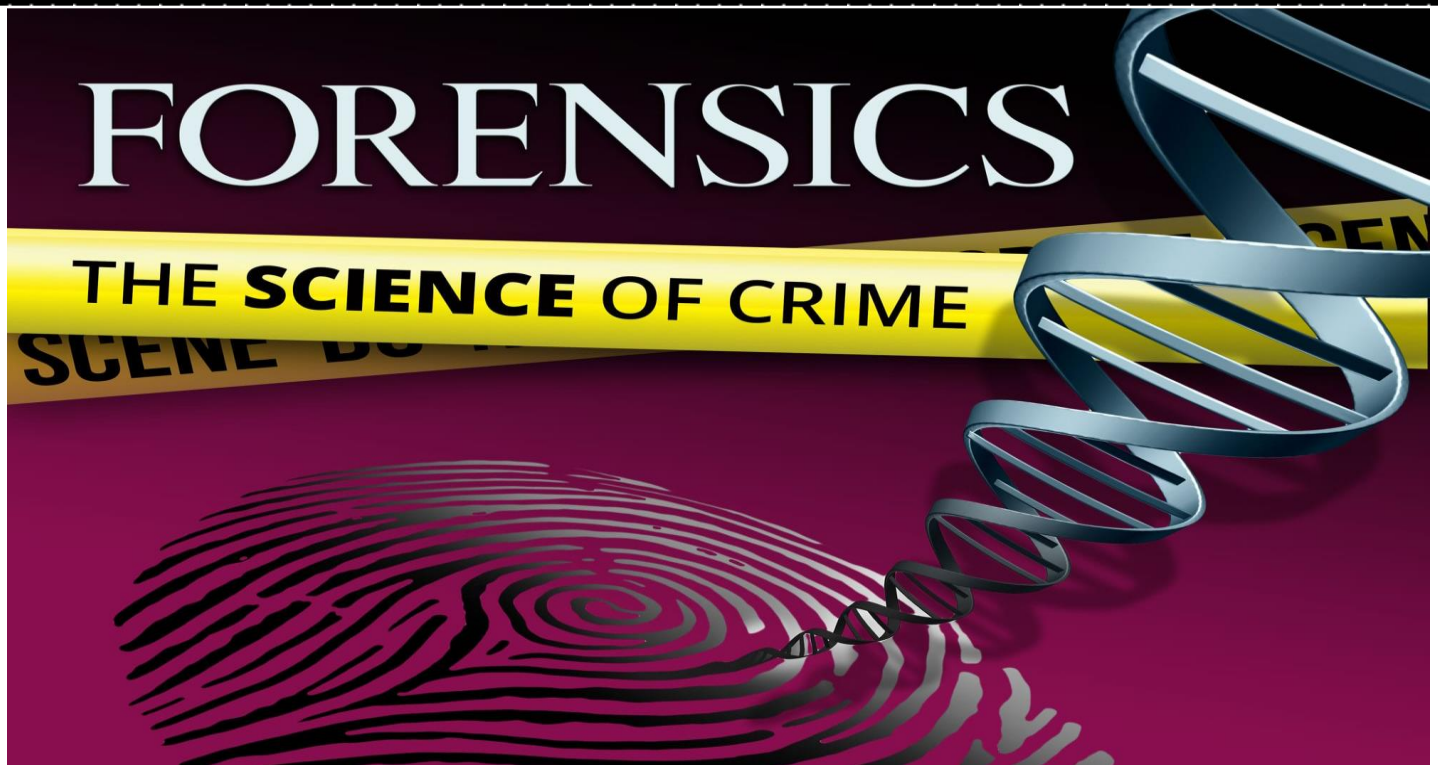
Answer: (B)

48. "Taches Noire Scleroitiques" is a post-mortem feature seen in

- (A) Eyes
- (B) Nostrils
- (C) Ears



# FORENSIC SCIENCE UNIT – II



*As Per Updated Syllabus*

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## MICROSCOPY

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A microscope is an optical instrument that is used for magnifying objects too small to be seen by naked eye.

Investigations or studies of cell architecture by means of the microscope are called microscopy, and the person who pursuing the study is called microscopist.

- Microscopes work on the physical principle of magnification where the image of an object is magnified so that it can be visible.
- The substances that can only be seen with a microscope are called microscopic substances.
- Microscopes are imperative in areas like microbiology that deals with the structure and function of microscopic living beings.
- Microscopy is further divided into three branches; optical microscopy, electron microscopy, and X-ray microscopy.
- X-ray microscopy is a fairly new technology that is responsible for detailed imaging of subcellular organelles like the nucleus and chromosomes.
- Microscopy, importantly optical microscopy, began with the discovery of the first microscope by Anton Von Leeuwenhoek.
- The complexity of microscopy since then has increased rapidly with new and advanced microscopes with higher magnification and resolution.
- In an optical microscope, the rays of light are passed through a series of glass lenses to produce a magnified image on the observer's eyes. Compound microscopes are the most common type of microscope, mostly used for research and teaching purposes.
- In the case of an electron and X-ray microscope, an electron beam is created which produced a digital magnified image of an object.
- Electron microscopes have very high magnification and resolution which produces clear enlarged images of objects as small as an atom.
- Depending on the nature of the sample, different types of microscopes, including bright field microscope, fluorescence microscope, phase contrast, and darkfield microscopes, are also available.
- The magnification of these microscopes depends on the type of lens used in the system which produces images of different magnitude and resolution so that they can be viewed.
- Microscopy is important in different areas of science like histology, cytology, and bacteriology. Microscopic examination of the morphology and structure of cells has been used as an essential technique for the identification of microorganisms.

### History of Microscopy

**1590:** The two Janssen brothers of Holland, Francis Janssen and Zacharias Janssen, who were spectacle makers built the first operational light microscope.

**1611:** Kepler built the first compound microscope.

**1665:** Robert Hooke developed the first laboratory microscope which has a magnification of 14-42 X. He observed small pores in sections of cork that he called cells.

**1674:** Leeuwenhoek discovered protozoa by his self built microscope with magnification of 270 X. He discovered bacteria for the first time 9 years later.

**1905:** Zsigmondy invented dark-field microscopy.

### Properties of Microscope

A microscope has dual property i.e. magnification and resolution. The usefulness of a microscope depends not so much on the degree of magnification but rather on the resolution. Resolution has nothing to do with the magnification.

**(a) Magnification:** Magnification or magnifying power of a microscope is the degree of increase in size of optical image over the actual size of object being viewed.

Magnification = Size of retinal image seen with microscope/Size of retinal image with naked eye

Magnification of microscope is calculated by multiplying the magnification of the objective lens with that of the eye piece (ocular lens). For example, the magnification of eye piece is 10X and the magnification of objective lens is 40X, then the microscope magnifies the object by  $10 \times 40 = 400$  times i.e. magnification is 400X.

The human eye has no power of magnification, so magnifying glasses maybe used to magnify images up to about 10 times. A light microscope in which combination of lens used has a magnification of 100-2000 X. For higher magnification over 400X, oil immersion lens can be used in which cedar wood oil placed between objective and the coverslip increase the light gathering properties of the lens.

### **Units of Measurement used in Microscopy**

$$1 \text{ metre (m)} = 10^2 \text{ cm} = 10^3 \text{ mm} = 10^6 \text{ } \mu\text{m} = 10^9 \text{ nm} = 10^{10} \text{ } \text{\AA}$$

$$1 \text{ centimeter (cm)} = 1/100 \text{ metre (m)} = 0.4 \text{ inch}$$

$$1 \text{ millimetre (mm)} = 1/1000 \text{ metre} = 0.001 \text{ m} = 10^{-3} \text{ m} = 10^{-3} \text{ mm} = 10^6 \text{ nm} = 10^7 \text{ } \text{\AA}$$

$$1 \text{ micrometre (mm)}^{**} = 1/1000 \text{ mm} = 0.001 \text{ mm} = 10^{-3} \text{ mm} = 10^{-6} \text{ m} = 10^3 \text{ nm} = 10^4 \text{ } \text{\AA}$$

$$1 \text{ nanometer (nm)} = 1/1000 \text{ mm} = 0.001 \text{ mm} = 10^{-3} \text{ mm} = 10^{-6} \text{ mm} = 10^{-9} \text{ m} = 10 \text{ } \text{\AA}$$

$$1 \text{ angstrom (A)}^+ = 1/10 \text{ nm} = 0.1 \text{ nm} = 10^{-1} \text{ nm} = 10^{-7} \text{ mm} = 10^{-10} \text{ m}$$

Micrometers were formerly known as microns ( $\mu$ ), and nanometers as millimicrons (m $\mu$ ).

The Angstrom is not an accepted measurement in the International system of Units. It is included here, however, because it was widely used in microscopy in the past.

**(b) Resolution or Resolving power:** Resolution (= resolving power or resolving limit) of an optical device (eye or microscope) is its ability to distinguish between two very closely placed objects as separate objects. The resolving power of a microscope depends on (i) Wavelength of light ( $\lambda$ ) and (ii) numerical-aperture (NA) of the lens system used. Resolution of a microscope can be calculated by Abbe Equation, after the name of German Physicist Ernst Abbe in 1876.

$$\text{Resolution} = 0.61\lambda/\text{NA} = 0.61\lambda/N \sin\theta$$

$$0.61 = \text{a trigonometric constant}$$

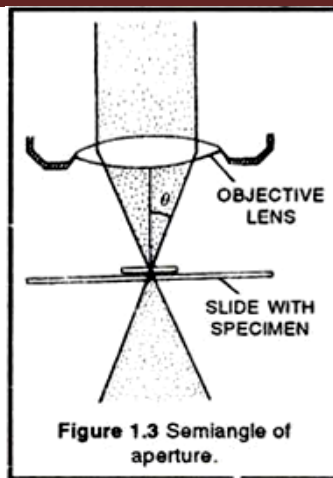
$\lambda$  = Wavelength of light used; 450-750 nm for visible light used in compound microscope, Blue light has shortest wavelength ( $\lambda = 450\text{nm}$ ) gives maximum resolution. Therefore, blue filter blue light commonly used in microscopy.

NA –  $N \sin \theta$ ; where N is the refractive index of the medium (usually air or oil) between the specimen and objective lens. For air  $N = 1.0$  and for immersion oil  $N = 1.5$ .

$\theta$  or  $\alpha$ - half angle of the cone of light entering the objective lens from the specimen. The maximum value of  $\theta$  for the best objective lens is  $70^\circ$  ( $\sin 70^\circ = 0.94$ ). The resolution of light microscope, using air and blue light, will be

$$L_m = 0.61 \times 450\text{nm} / 1.0 \times 0.94 = 292\text{nm} \text{ or } 0.3 \mu\text{m}$$

$$\text{If oil and blue light used, then } L_m = 0.61 \times 450\text{nm} / 1.5 \times 0.94 = 194\text{nm} \text{ or } 0.2 \mu\text{m}$$



Thus, light microscope can never resolve two closer particles less than about 0.2 nm apart, no matter how many times the image is magnified. The resolution of electron microscope is about 0.0005  $\mu\text{m}$  whereas the human eye is about 100  $\mu\text{m}$ . It should be noted that lower the value of  $L_m$  Higher will be resolution, which can be done by changing  $A$ ,  $N$  or  $\theta$ . Resolution will increase with a decrease in  $A$  and with an increase in  $NA$ ; i.e.  $L_m$  is inversely proportional to  $A$  and  $L_m$  is proportional to  $NA$ . The numerical aperture ( $NA$ ) is the light collecting ability of lens.

### PRINCIPLES

Microscopy is necessary to evaluate the integrity of samples and to correlate structure with function. Microscopy serves two independent functions of enlargement (magnification) and improved resolution (rendering of two objects as separate entities).

Light microscopes employ optical lenses to sequentially focus the image of objects, whereas electron microscope uses electromagnetic lenses.

Light and electron microscopes work either in transmission or scanning mode depending on whether the light or electron beam either passes through the specimen and is diffracted or deflected by specimen surface. Polarized light microscopes detect optically active substances in cells; for example, particles of silica or asbestos in lung tissue.

Phase contrast microscopes are often used to improve image contrast of unstained material which is caused either by diffraction by the specimen or even by differences in thickness of the specimen. At their point of focus, the converging light rays shows interference, resulting in either increase or decrease in the amplitude of the resultant wave (constructive or destructive interference, respectively), which the eye detects as differences in brightness.



Fig 1.2: Transmission electron microscope



Confocal microscopy is an imaging technique used to increase micrograph contrast and/or to reconstruct three-dimensional images by using a spatial pinhole to eliminate out-of-focus light or flare in specimens that are thicker than the focal plane. This technique has been gaining popularity in the scientific and industrial communities.

Typical applications include life sciences and semiconductor inspection. In a conventional (i.e., wide-field) fluorescence microscope, the entire specimen is flooded in light from a light source. Due to the conservation of light intensity transportation, all parts of specimen throughout the optical path will be excited and the fluorescence will be detected by a photo detector or a camera.



Fig 1.3: Fluorescent Microscope



Fig 1.4: Confocal Microscope

In contrast, a confocal microscope uses point illumination and a pinhole in an optically conjugate plane in front of the detector to eliminate out-of-focus information. Only the light within the focal plane can be detected, so the image quality is much better than that of wide-field images.

As only one point is illuminated at a time in confocal microscopy, 2D or 3D imaging requires scanning over a regular raster (i.e., a rectangular pattern of parallel scanning lines) in the specimen. The thickness of the focal plane is defined mostly by the square of the numerical aperture of the objective lens, and also by the optical properties of the specimen and the ambient index of refraction.



Fig 1.5: Transmission electron micrograph of silver nanoparticles

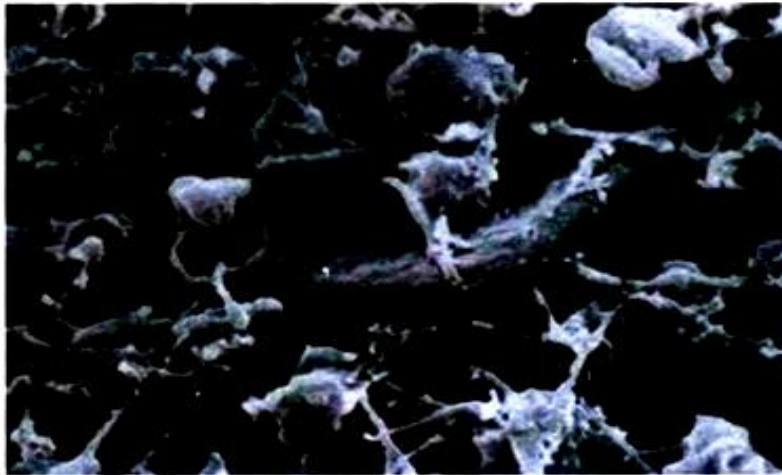


Fig 1.6: Scanning electron micrograph of aggregated platelets and RBC

Microscopes using visible light will magnify approximately 1500 times and have a resolution limit of about 0.2 mm whereas a transmission electron microscope is capable of magnifying approximately 2,00,000 times and has a resolution limit for biological specimens of about 1 nm. This capability of TEM is largely a function of the very short wavelength of electrons accelerated under the influence of an applied electric field. (An accelerating voltage of 100 kV produces a wavelength of  $4 \times 10^{-3}$  nm.)

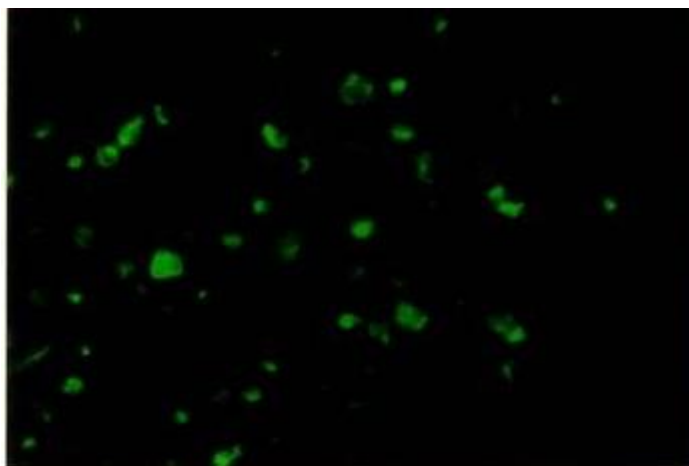
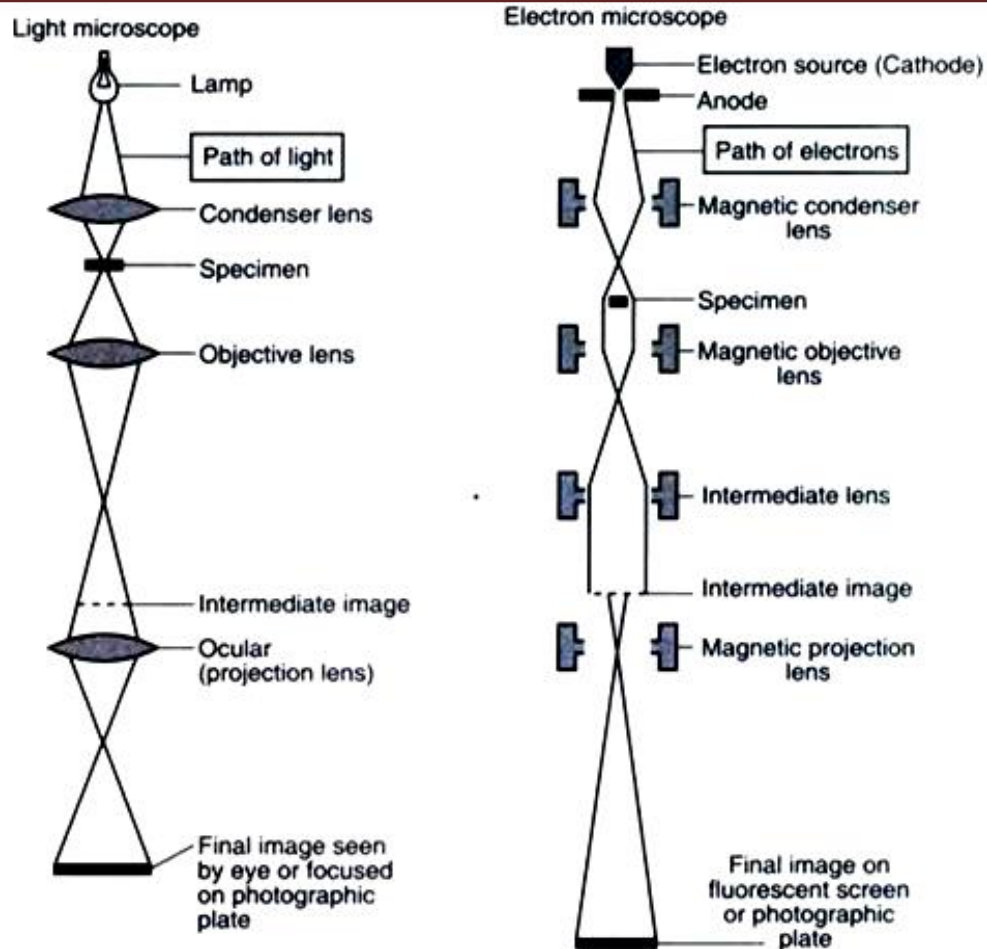
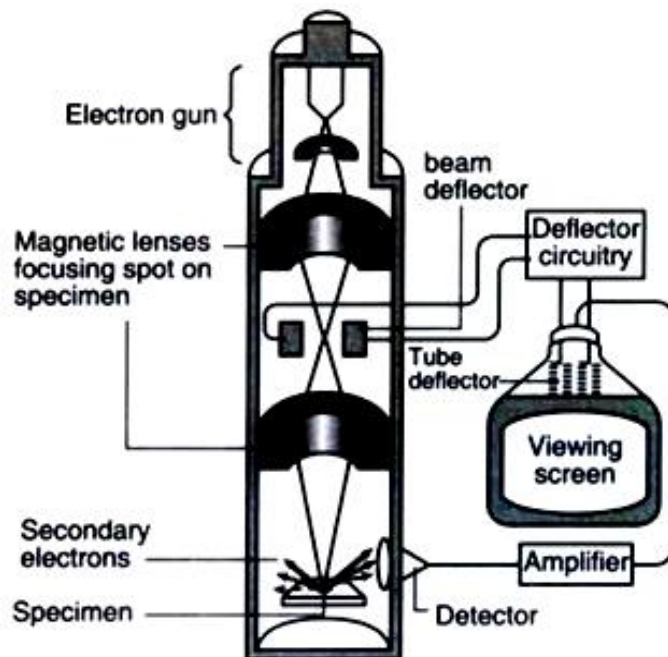


Fig 1.7: Fluorescence microscopic image of activated platelets tagged with FITC conjugated Phalloidin



**Fig. 1.8:** Schematic diagrams of Light Microscope and Electron Microscope



**Fig. 1.9:** Schematic diagram of Scanning Electron Microscope

In addition, smartphone-based immunoassay formats, multiplex bead-based assays, lateral flow immunoassay formats are among the latest developments that have facilitated monitoring and management of health-related complications in real-time.

**FORENSIC SCIENCE UNIT- II MCQs**

1. Which of the following is used in electron microscope?

- a) electron beams
- b) magnetic fields
- c) light waves
- d) electron beams and magnetic fields

Answer: d

Explanation: Electron Microscope uses electron beams and magnetic fields to produce the image, whereas the light microscope uses light waves and glass lenses. In electron microscopy, a much higher resolution is obtained with extremely short wavelength of the electron beam.

2. Electron Microscope can give a magnification up to \_\_\_\_\_

- a) 400,000X
- b) 100,000X
- c) 15000X
- d) 100X

Answer: a

Explanation: The resolving power of the electron microscope is more than 100 times that of the light microscope, and it produces useful magnification up to 400,000X. It is possible to resolve objects as small as 10 Angstrom.

3. Which of the following are true for electron microscopy?

- a) specimen should be thin and dry
- b) image is obtained on a phosphorescent screen
- c) electron beam must pass through evacuated chamber
- d) specimen should be thin and dry, image is obtained on a phosphorescent screen and electron beam must pass through evacuated chamber

Answer: d

Explanation: Since electrons can travel only in high vacuum, the entire electron path through

the instrument must be evacuated; specimens must be completely dehydrated prior to examination. Only very thin specimens can be observed in the conventional electron microscope since the penetrating power of electrons through matter is weak. The magnified image may be viewed on a phosphorescent or fluorescent screen.

4. Degree of scattering in transmission electron microscope is a function of \_\_\_\_\_

- a) wavelength of electron beam used
- b) number of atoms that lie in the electron path
- c) number and mass of atoms that lie in the electron path
- d) mass of atoms that lie in the electron path

Answer: c

Explanation: In a transmission electron microscope, contrast results from the differential scattering of electrons by the specimen, the degree of scattering being a function of the number and mass of atoms that lie in the electron path.

5. Negative Staining is used for examining \_\_\_\_\_

- a) virus particles
- b) protein molecules
- c) bacterial flagella
- d) virus particles, protein molecules and bacterial flagella

Answer: d

Explanation: In negative-staining the electron opacity of the surrounding field is increased by using an electron-dense material such as phosphotungstic acid as a stain. Negative staining is particularly valuable for the examination of very small structures such as virus particles, protein molecules and bacterial flagella.

6. Which among the following helps us in getting a three-dimensional picture of the

specimen?

- a) Transmission Electron Microscope
- b) Scanning Electron Microscope
- c) Compound Microscope
- d) Simple Microscope

Answer: b

Explanation: The scanning electron microscope lacks the resolving power obtainable with the transmission electron microscope but has the advantage of revealing a striking three-dimensional picture. The surface topography of a specimen can be revealed with clarity and depth of field not possible by any other method.

7. The secondary electrons radiated back in scanning microscope is collected by?

- a) specimen
- b) anode
- c) vacuum chamber
- d) cathode

Answer: b

Explanation: In scanning electron microscope (SEM), the surface of the specimen is irradiated with a very narrow beam of electrons. Such irradiations causes low energy (secondary) electrons to be ejected from the specimen which can then be collected on a positively-charged plate or anode thereby generating an electric signal.

8. On what factors do the intensity of secondary electrons depend upon?

- a) shape of the irradiated object
- b) chemical composition of the irradiated object
- c) number of electrons ejected
- d) size and chemical composition of the irradiated object, number of electrons ejected and on the number of electrons reabsorbed by surrounding

Answer: d

Explanation: The irradiations in SEM causes secondary electrons to be ejected from the specimen thereby generating a signal that is proportional to the number of electrons

striking the anode. The intensity or the number of secondary electrons depends on the shape and the chemical composition of the irradiated object and also on the number of electrons ejected and the number of electrons reabsorbed by surrounding.

9. Where do we obtain the magnified image of the specimen in SEM?

- a) cathode ray tube
- b) phosphorescent screen
- c) anode
- d) scanning generator

Answer: a

Explanation: In TEM, the image is obtained on a phosphorescent screen but in SEM the magnified image of the surface topography of the specimen is obtained on the cathode ray tube. The electronic signals generated scan the specimen in a raster pattern in the manner of a television system to produce an image on a cathode ray tube.

10. Which of the following techniques are used in Transmission Electron Microscopy (TEM) for examining cellular structure?

- a) Negative-Staining
- b) Shadow Casting
- c) Ultrathin Sectioning
- d) Negative-Staining, Shadow Casting, Ultrathin Sectioning, Freeze-Etching

Answer: d

Explanation: Numerous techniques are available for use with electron microscopy which extends its usefulness in characterizing cellular structure. Some of them are Negative-Staining (which increases the electron opacity of surrounding), Shadow Casting (helps in producing three-dimensional structure of the object), Ultrathin Sectioning and Freeze-Etching.

11. Which part of the compound microscope helps in gathering and focusing light rays on the specimen to be viewed?

- a) Eyepiece lens
- b) Objective lens



- c) Condenser lens
- d) Magnifying lens

Answer: c

Explanation: Compound microscope contains three separate lens systems. The condenser lens is placed between the light source and the specimen and it gathers and focuses the light rays in the plane of the microscopic field to view the specimen.

12. What is the minimum distance for the eye to focus any object?

- a) 11 cm
- b) 25 cm
- c) 32 cm
- d) 42 cm

Answer: b

Explanation: The eye cannot focus on objects brought closer to it less than 25 cm; this is, accordingly the distance of maximal effective magnification. An object must also subtend an angle at the eye of 1 degree or greater.

13. Resolving power of a microscope is a function of \_\_\_\_\_

- a) Wavelength of light used
- b) Numerical aperture of lens system
- c) Refractive index
- d) Wavelength of light used and numerical aperture of lens system

Answer: d

Explanation: The ability of a microscope to distinguish two adjacent points as distinct and separate is known as resolving power.

Resolving power is a function of wavelength of light used and the numerical aperture (NA) of the lens system. NA refers to the refractive index of the medium multiplied with the sine value of the half-aperture angle.

14. The greatest resolution in light microscopy can be obtained with \_\_\_\_\_

- a) Longest wavelength of visible light used
- b) An objective with minimum numerical aperture
- c) Shortest wavelength of visible light used
- d) Shortest wavelength of visible light used and

an objective with the maximum numerical aperture

Answer: d

Explanation: The relationship between numerical aperture (NA) and resolution is:-

Resolution (d) = wavelength / 2(NA)

Thus maximum resolution is obtained with the shortest wavelength of visible light and an objective with the maximum NA.

15. Oil immersion objective lens has an NA value of \_\_\_\_\_

- a) 0.65
- b) 0.85
- c) 1.33
- d) 1.00

Answer: c

Explanation: NA = refractive index \* sine (half-aperture angle).

The maximum NA for a dry objective is less than 1.0 as the refractive index of air is 1. The values of NA for oil immersion lens is slightly greater than 1.0 in the range of (1.2 to 1.4) as the refractive index of oil is 1.56.

16. In fluorescence microscopy, which of the following performs the function of removing all light except the blue light?

- a) Exciter filter
- b) Barrier filter
- c) Dichroic mirror
- d) Mercury arc lamp

Answer: a

Explanation: In fluorescence microscopy, the function of the exciter filter is to remove all but the blue light; the barrier filter blocks out blue light and allows any other light emitted by the fluorescing specimen to pass through and reach the eye.

17. Total Magnification is obtained by \_\_\_\_\_

- a) Magnifying power of the objective lens
- b) Magnifying power of eyepiece
- c) Magnifying power of condenser lens
- d) Magnifying power of both the objective lens and eyepiece

Answer: d

Explanation: The total magnification is determined by multiplying the magnifying power of the objective by that of the eyepiece. Generally, an eyepiece having a magnification of 10X is used although eyepieces of higher or lower magnifications are available.

18. In light microscopy, which of the following is used as fixatives prior to staining technique?

- a) Osmic acid
- b) Glutaraldehyde
- c) Heat
- d) Osmic acid, glutaraldehyde, heat

Answer: c

Explanation: Most staining techniques kill cells and so preliminary to staining, the cells are sometimes fixed. Commonly used chemical fixatives include osmic acid and mainly glutaraldehyde. But for light microscopy heat is the most commonly used fixative.

19. In Phase contrast microscopy, the rate at which light enters through objects is \_\_\_\_\_

- a) Constant
- b) Inversely proportional to their refractive indices
- c) Directly proportional to their refractive indices
- d) Exponentially related to their refractive indices

Answer: b

Explanation: Phase contrast microscopy is based on the fact that the rate at which light travels through objects is inversely related to their refractive indices. Since the frequency of light waves is independent of the medium through which they travel, the phase of a light ray passing through an object of the higher refractive index than the surrounding medium will be relatively retarded.

20. Which part of the light microscope controls the intensity of light entering the viewing area?

- a) Coarse adjustment screw
- b) Fine adjustment screw

c) Diaphragm

d) Condenser lens

Answer: c

Explanation: On the condenser is mounted a shutter like an apparatus called the diaphragm which opens and closes to permit more or less light into the viewing area. Condenser lens just helps in condensing the light rays. Coarse and fine adjustment screws are used for focusing under different power lens.

21. Which of the following component of the light microscope illuminates the specimen by gathering diffuse rays from the light microscope?

- a) light source
- b) eyepiece
- c) condenser lens
- d) screws

Answer: c

Explanation: The substage condenser lens gathers the diffuse rays from light source and illuminates the specimen by formation of a small cone of bright light. With the help of condenser lens very small parts of the specimen can be seen after magnification.

22. Which of the following magnification of the ocular will occupy maximum retinal surface?

- a) 1X
- b) 5X
- c) 20X
- d) 50X

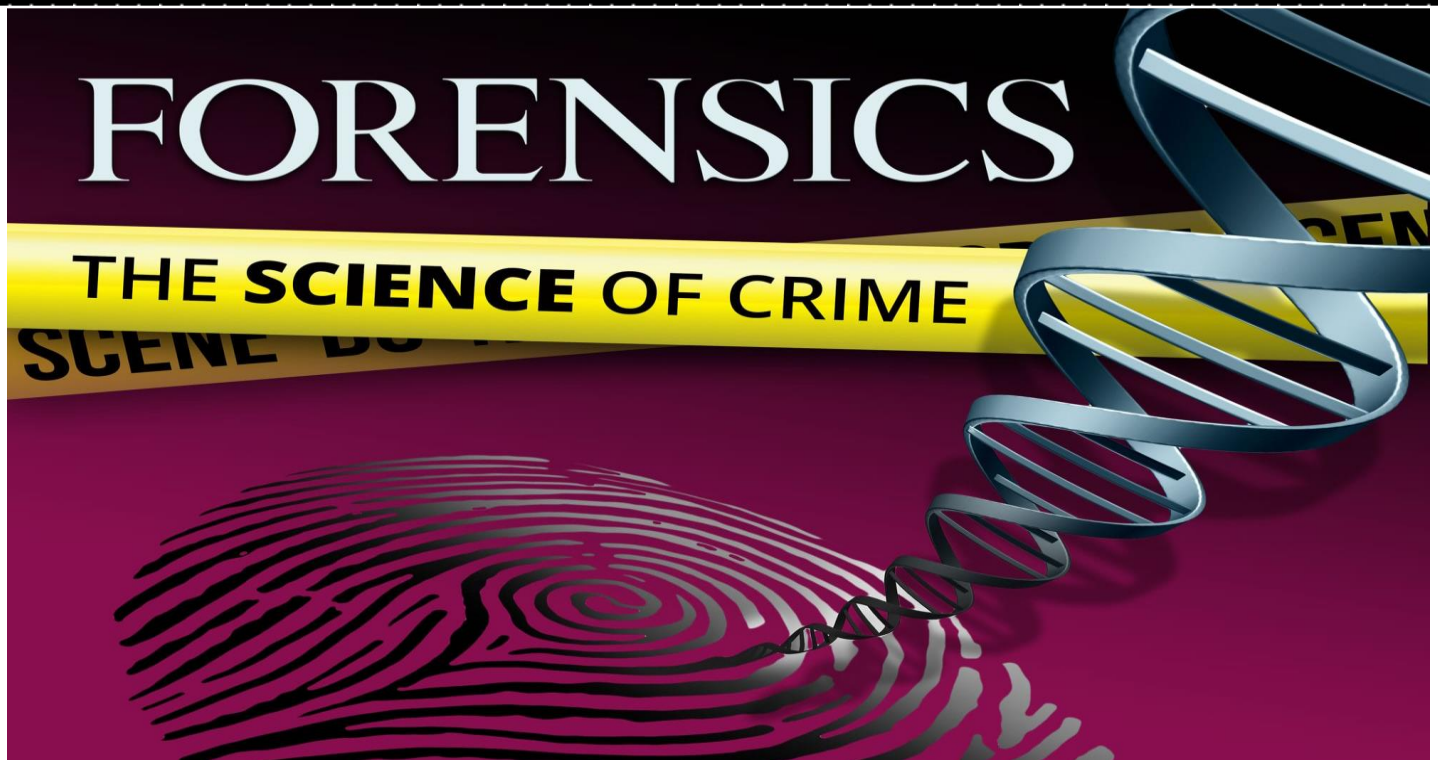
Answer: d

Explanation: The more the magnification of the ocular, the more retinal surface it covers. However, beyond a certain magnification, the image is not clear (termed empty magnification) even if it occupies a greater retinal surface.

23. The resolution attained by a microscope is limited by \_\_\_\_\_

- a) diffraction
- b) refraction
- c) reflection
- d) retraction

# FORENSIC SCIENCE UNIT – III



*As Per Updated Syllabus*

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<b>D.</b>	<b>Detection of Seminal and other body fluids and their Blood Grouping, Red cells Enzymes, Serum Proteins of forensic significance</b>	<b>61-102</b>
<b>E.</b>	<b>DNA: Structure, DNA as genetic marker, DNA Extraction and Profiling Techniques</b>	<b>102-123</b>
<b>F.</b>	<b>DNA Phenotyping and RNA Profiling &amp; their applications</b>	<b>124-143</b>
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## BLOOD

Blood is a complex fluid tissue composed of a liquid portion, plasma, and cellular components. It delivers necessary substances such as nutrients and oxygen to the cells and transports metabolic waste products away from those same cells. Plasma is a mixture of dissolved proteins, salts and other chemicals.

### Composition of Blood

The blood cells are of three main types: red blood cells (erythrocytes), white blood cells (leukocytes), and platelets (thrombocytes).

Erythrocytes are manufactured in the bone marrow. In mammalian blood biconcave disc shaped cells are found because the cells do not retain their nucleus before entering the circulatory system. The usual average count of RBCs is 4.5-5.4 million cells/ mL. The diameter and thickness of each human red cell is about 7.5  $\mu\text{m}$  and 2  $\mu\text{m}$  respectively. Antigens present in the human red cell are also known as agglutinogens. RBCs function to transport  $\text{O}_2$  and  $\text{CO}_2$  and are packed with the complex compound hemoglobin. The heme molecule present in hemoglobin imparts red color to the erythrocytes. The presence of the heme molecule in a stain determines whether blood is present in the sample of unknown origin or not.

The WBC count of a healthy human adult is about 4000-10,000 white blood cells/ mL. WBCs are of various types and they function as body's defensive mechanism. The common type that is present in abundance is the granulocytes or the polymorphonuclear leukocytes. Many granulocytes comprise neutrophilic granules (neutrophils); a small number of comprise granules which stain with acid dye (eosinophils), and several granules stain with basic dyes (basophils). Other type of WBCs found are the cells having large round nuclei and little cytoplasm or lymphocytes, and monocytes, cells with abundant cytoplasm and kidney-shaped nuclei. Genetic marker analysis of blood is probable as DNA can be taken out from these nucleated leukocytes.

Small, granulated bodies also known as platelets are also present in blood. The human blood contains about 300000 platelets/mL having a diameter of 2-4 $\mu\text{m}$ . These cells are involved in the blood clotting mechanism.

The blood, which constitute around 1/13<sup>th</sup> of the body weight consist of medium plasma and suspended cells like-

1. **RBC (Erythrocytes)**- Red blood corpuscles containing hemoglobin are responsible for carrying oxygen from the lungs to various parts of the body. They are formed in the bone marrow.
2. **WBC (Leukocytes)**- White blood corpuscles containing antibodies that fight foreign bodies, which cause infections and disturbs the immune system.
3. **Platelets (Thrombocytes)**- Platelets are blood cells that help in blood clotting.
4. **Plasma**- Plasma is the yellowish, liquid portion of the blood that contains electrolytes, nutrients, proteins and vitamins.

### Major Functions of blood are-

- Transport
- Maintain body temperature
- Control pH (acid base balance)
- Removal of toxins from the body (Excretion)

## BLOOD DETECTION BY CHEMICAL METHODS

Forensic scientists are often asked to determine, both in the field and in the laboratory, whether a particular stain is or is not blood. This is a surprisingly difficult question to answer with certainty.



The discussion is confined to chemical methods and therefore does not consider biological methods such as antigen - antibody reactions. The biological methods are generally slower than chemical methods but more specific. One of the requirements of forensic science is for methods which can be used in the field, and generally only chemical methods possess sufficient speed for this.

### Chemical Methods Used to Detect Blood

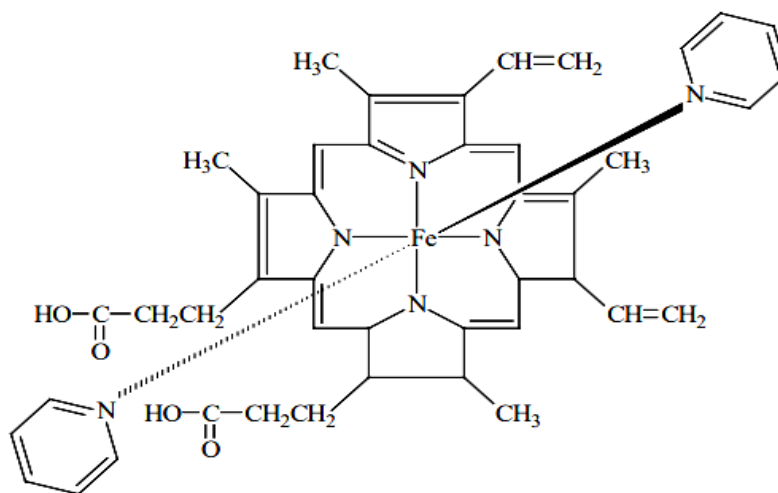
Chemical methods can be divided into 3 categories:

- crystal tests
- catalytic tests
- instrumental methods

All of the methods are in some way dependent on the presence of haemoglobin, and will therefore give positive results for both animal and human blood.

### Crystal Tests

The crystal tests, which are now rarely used, are all based on the formation of haemoglobin derivative crystals such as haematin, haemin and haemochromogen. The test is carried out on a microscope slide, with the reagents being added to the stain under a cover slip, and crystal formation observed microscopically. Probably the best known of the crystal tests is that developed by Takayama about 80 years ago. An alkaline solution of pyridine is added to the stain and, if blood is present, pink crystals of a complex between pyridine and haem form as the slide is warmed. The structure of the complex is shown in Figure 1. As well as pyridine, a number of other nitrogenous bases, including nicotine, methylamine, histidine and glycine have been used in variations of this test.



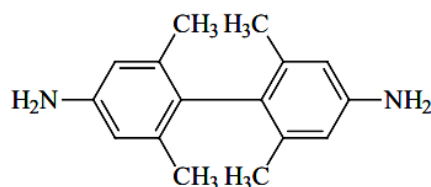
**Figure 1 - Pyridine ferroprotoporphyrin  
(the complex formed in the Takayama test)**

It is generally accepted with the crystal tests that a positive result confirms the presence of blood. The sensitivity is about 0.001 mL of blood or 0.1 mg of haemoglobin. A negative result does not necessarily show that blood is absent - it may, for example, indicate faulty technique - and a positive control should always be run for comparison. Bloodstains up to 20 years old have given positive results in crystal tests.

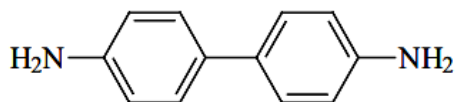
### Catalytic Tests

These methods depend on the fact that the haem group of haemoglobin possesses a peroxidase-like activity which catalyses the breakdown of hydrogen peroxide. The oxidising species formed in this reaction can then react with a variety of substrates to produce a visible colour change. Among substrates in common use are benzidine and various substituted benzidines, ortho-tolidine, leucomalachite green, leucocrystal violet and phenolphthalein - the last of these being known as the

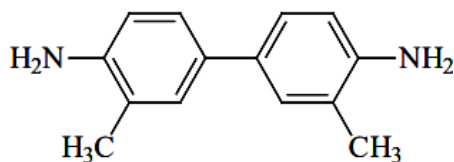
Kastle-Meyer test. The reaction with 3-aminophthalhydrazide (luminol) to form a luminescent rather than a coloured product is also a catalytic test. A derivative of ortho-tolidine is used in the "Sangur" test sticks manufactured by Boehringer Mannheim. These are intended for the detection of blood in urine in clinical situations but are equally useful as a screening test for dried bloodstains.



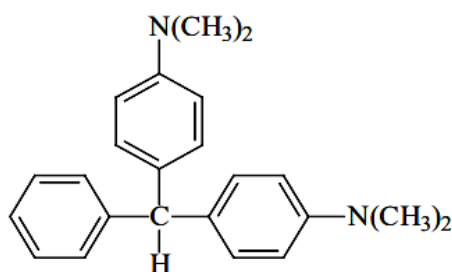
Tetramethyl benzidine



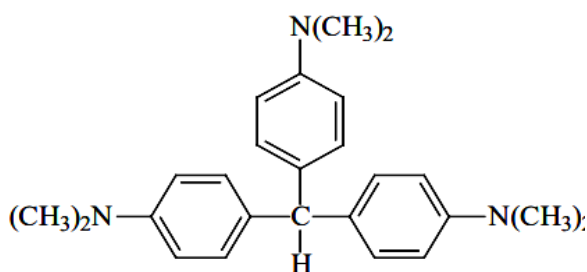
Benzidine



Ortho-tolidine



Leucomalachite green



Leucocrystal violet

The catalytic tests are extremely sensitive (blood can be detected to dilutions of about 1 in 100,000), but are subject to a number of interferences and are therefore not totally specific for blood. Substances which can interfere include enzymes such as catalase and peroxidases (which can occur in both plant and animal materials), oxidising chemicals and metals - in particular copper and iron. There has to be an awareness of this when results are interpreted, particularly when testing outdoors, where many types of plant material can be present, or testing in vehicles, where metal surfaces can interfere. The general principle is that if the test is negative, blood is absent, but that if the test is positive, blood is probably, not definitely present. For this reason the tests are often described as "presumptive" tests.

An interesting example of possible interferences occurred with the testing of the car belonging to Michael and Lindy Chamberlain after the disappearance of their daughter Azaria at Ayer's Rock, Australia, in 1980. The Chamberlains lived in Mt Isa, a copper mining town, with a high concentration of copper-containing dust in the atmosphere. The car was later tested for the presence of blood with ortho-tolidine. Some positive results found were subsequently attributed to the presence of copper.

Instrumental methods High performance liquid chromatography (HPLC) can be used to confirm the identity of blood using the absorbance of haemoglobin for detection. This method can also be used to identify the species of origin from variations in the globin chains, to distinguish foetal haemoglobin from adult haemoglobin, and to give an estimate of the age of a bloodstain.

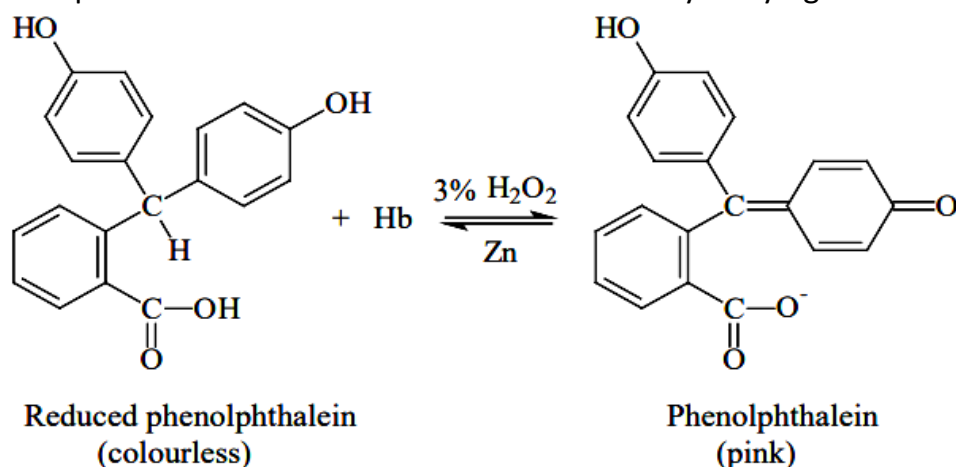
## Applications

### Confirmation that visible stains are (probably) blood

This is largely carried out using either the "Sangur" sticks mentioned earlier, or using the Kastle-Meyer test. The Sangur sticks, in which the detecting reagent is in an immobilised form, require only to be

rubbed gently on the stain and moistened. An immediate change in colour from pale yellow to an intense greenish blue indicates the probable presence of blood. The test is very sensitive but because of the way it is set up is not easily modified to check for possible interferences.

In the Kastle-Meyer test the reduced phenolphthalein is kept in alkaline solution in the presence of zinc. This solution is colourless. Oxidation with haemoglobin and peroxide causes an instant colour change to the well known bright pink. Figure 2 shows the reaction. The test was originally used in one step, but many of the potential interferences can be eliminated by carrying it out in two steps.



**Figure 2 - Oxidation of reduced phenolphthalein by haemoglobin and peroxide**

In the original form, a small amount of the Kastle-Meyer reagent as prepared is mixed with equal volumes of 95% ethanol and 10% hydrogen peroxide solution. The suspect stain is rubbed gently with a small piece of filter paper and a drop of the mixed reagent added to the paper. The development of a pink colour is indicative of the presence of haemoglobin, which has catalysed the breakdown of hydrogen peroxide to an oxidising species. However, used in this form, the test will give an apparently positive result with other oxidising materials.

In the 2-step version of the test, the Kastle-Meyer reagent is mixed only with an equal volume of 95% ethanol. This solution is first added to the stain on the filter paper. If a pink or red colour develops at this point, that is without the addition of hydrogen peroxide, the stain in question is not blood. If there is no reaction at this point, a drop of hydrogen peroxide solution is added, and the presence of a pink colour indicates the likely presence of blood.

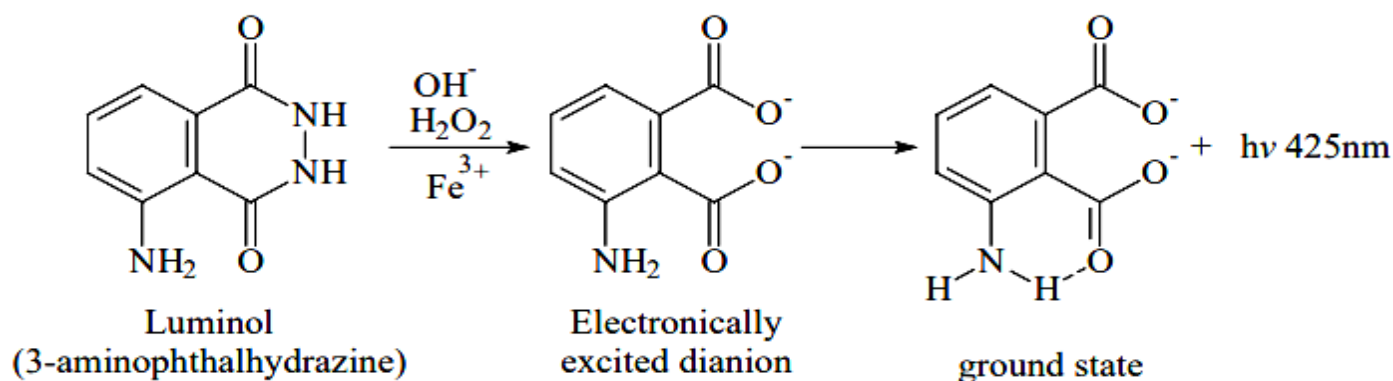
A sample giving a positive result in both the Sangur and Kastle-Meyer tests would be reported as probable blood. Unless a positive result was subsequently obtained with a biological test known to be human-specific, the presence of blood could not be confirmed. Tests which could be used for confirmation would include antigen-antibody reactions such as Ouchterlony double diffusion, the presence of an enzyme such as alpha-2-HS-glycoprotein known to be human specific, or the presence of a DNA sequence specific to humans.

### Detection of non-visible bloodstains

This has traditionally been carried out using luminol. Luminol's major application is in areas where blood may be present but is difficult to see, such as outdoors among vegetation, or where attempts have been made to clean up blood and traces are still present. A positive reaction can also sometimes be given by bloodstained clothing which has been washed.

Luminol is made up in alkaline solution (pH 10.4-10.8) using sodium carbonate, and sodium perborate (NaBO<sub>3</sub>·H<sub>2</sub>O) rather than hydrogen peroxide is used as the source of the oxidising species. Hydrogen peroxide can be used but yields a shorter-lived luminescence than sodium perborate. The solution is applied as a spray and the presence of blood produces a bluish luminescence which persists for about 45 seconds. The luminescence can be restored by additional spraying but this needs to be done

carefully as the stain will lose definition if too much liquid is added to it. The luminescence can be photographed in either black and white or colour but requires some specialised techniques. Figure 3 (adapted from reference 8) outlines the chemistry of the reaction.



**Figure 3 - The reaction of luminol**

As with other catalytic tests, luminol is not specific for blood and can also give a positive reaction with some plant enzymes, oxidising agents and metals. An experienced user of luminol can distinguish these reactions from those given by blood by the colour of the luminescence, how long it persists for, and in the degree of "sparkle" of the luminescent product. Blood tends not to sparkle, but produces a steady luminescence, whereas some metals tend to give a definite sparkling luminescence.

Luminol needs to be used with care as there are uncertainties relating to its safety. Although it is described in some literature as non-mutagenic, its structure suggests that nonmutagenicity or non-carcinogenicity cannot be assumed. In addition, some users find the other contents of the solution irritating if the spray is inhaled during use.

### Enhancement of blood stains

A further area in which blood detection reagents have proved useful has been in the enhancement of existing bloodstains. In cases of partial shoeprints or fingerprints in blood there is often more of the print present than can be seen, and treatment of the print with a chemical which reacts with blood can often produce a much more detailed print. This can then be photographed and subsequently compared with a suspect's shoe or fingerprint. Luminol has been used for this purpose but, as noted earlier, detail can be lost by excessive spraying of the stain, and photography is often difficult.

Leucocrystal violet is now being used extensively for shoeprint enhancement with considerable success. Its major disadvantage is that the stains are indelible, so it cannot be used in situations where the surface is required to be left clean. It is extremely easy to use, as a light spray with the reagent solution produces a purple stain almost immediately. This is very easily photographed. There are a number of other reagents such as amido black which can also be used for shoeprint enhancement. Some of these are specific for protein rather than for blood.

### Subsequent reactions of stains treated with blood detecting reagents

Once it is determined that a stain is probably blood, the next question one asks is "whose?" It is therefore necessary to ensure that the presumptive tests for blood do not interfere with subsequent tests used to "type" or "group" the blood. The Sangur or Kastle-Meyer tests use only a small part of the stain and the major part remains for further testing. Problems can arise when an entire stain is treated with a reagent which can affect subsequent tests. It is well known, for example, that the use of amido black on bloodstains removes any possibility of subsequent blood grouping, while stains treated with leucocrystal violet can still be typed in some systems. If typing of the stain is likely to be required then enhancement reagents known not to interfere must be used. Several compilations of such results have been published. Forensic biology is moving towards almost exclusive use of DNA

polymerase chain reaction (PCR) methods for individualisation of blood. Because these methods can work with degraded DNA, it is likely that most current detection reagents can continue in use.

In a recent case, a pair of apparently washed jeans with no visible blood was treated with luminol and showed the probable presence of blood on both knees. The stain from one knee gave a positive human antigen-antibody test (this is unusual with an invisible stain) and subsequently a positive DNA PCR result in one system. This result identified the stain as not being excluded as the blood of an assault victim but excluded it from coming from the owner of the jeans.

## BLOODSTAIN PATTERN ANALYSIS

**When violent crimes are committed, it is not unusual for the participants to be injured.** If these injuries are accompanied by blood flow, distinctive bloodstain patterns may result that can be used to provide investigative information about the activities which occurred during the commission of the crime. These distinctive bloodstain patterns occur because of the physical properties of the blood and how it reacts when acted upon by physical forces. The analysis of these patterns can provide the investigator with information about the direction of travel of the blood, the level of force used to put the blood in flight, the location of the blood source which was acted upon to create the pattern, movements during bloodshed, movements after bloodshed, and activities during bloodshed. Bloodstain pattern analysis studies how different forces and activities influence the creation and appearance of the bloodstain patterns, so that they can be interpreted as part of the crime scene investigation.

### Bloodstain Characteristics

**Many factors affect the size and shape of the blood drops.** When blood passively drips off of a surface and falls on to a smooth hard horizontal surface, the resulting bloodstain will be round. As the blood falls through the air, it takes the shape of an oscillating sphere. The blood droplets will not break up in the air as they fall due to gravitational force alone. Additional forces, however, can break the drops apart. The diameter of the bloodstain is dependent upon the distance the drop falls to the horizontal surface as well as the volume of the blood drop. As the distance of the fall increases, the diameter of the bloodstain will increase until it reaches a maximum diameter. The maximum diameter for the bloodstain occurs after the drop has fallen approximately 1.8 m (6 ft). The volume of the drop of blood also affects the diameter of the bloodstain. As volume increases, so will the diameter of the resulting bloodstain. Early research identified the average volume of a drop of blood as 0.05 ml. Subsequent research determined that the average volume of a drop of blood varies depending on the surface characteristics of the object that the blood drips from. Blood drops fall off of a surface because its volume increases to the level where the pull of the Earth's gravity overcomes the viscosity of the blood and allows the surface tension to break. The shape and finish of the surface the blood falls from affects the blood volume that is needed for the surface tension of the blood to break and allow it to fall.

**In addition to droplet volume, distance of fall and the blood source surface characteristics,** other factors affect the size, shape and appearance of the bloodstains. Droplet size is a factor of how much force was imparted on the blood source to put it in flight. The viscosity, specific gravity and surface tension of blood make it resistant to being broken up into drops. When an external force is imparted on static blood, it will cause some of the blood to react and be put in flight. The distance the drops of blood fly through the air is dependent upon how much force was used to create the drops, the size of the blood drops, and air currents. In low force events (sometimes referred to as low velocity), the number of blood drops put in flight is low and the size of the droplets tend to be large. The majority



## FORENSIC SCIENCE UNIT – III MCQs

1. Assertion (A): In shotgun, the dispersion of pellets is more in the true cylinder gun than choked gun.

Reason (R): Choking of barrel is not related to dispersion of pellet.

Codes:

- (A) Both (A) and (R) are correct
- (B) (R) is correct, but (A) is incorrect
- (C) (A) is correct, but (R) is incorrect
- (D) Both (A) and (R) are incorrect

Answer: (C)

2. What is the temperature which is maintained by the Freezers in the pathology labs?

- a) 10°C – 15°C
- b) 0°C – 10°C
- c) -10°C – -60°C
- d) -100°C

Answer: c

Explanation: When pathological samples are collected, they may have to be stored for further study or for future use. They are stored at a temperature of -10°C – -60°C. At this temperature, the solution used for preserving freezes without causing damage to the samples.

3. What solution is used to maintain sterility in labs?

- a) Sodium Chloride
- b) Sodium Hypochlorite
- c) Sodium Cyanide
- d) Sodium Sulphate

Answer: b

Explanation: Sodium Hypochlorite acts like a bleaching agent. When it is dissolved in water and used to clean the labs, it gives off nascent oxygen. This oxygen is highly reactive and dangerous to microbes. It destroys the microbes that may have grown in the lab. It also helps remove stains due to its reactive nature and thus reduces/destroys the environment for microbes to breed.

4. An electrophoresis machine is used to

- a) separate DNA
- b) separate blood components
- c) separate the bone components
- d) separate the muscle fibers

Answer: a

Explanation: An electrophoresis machine uses the charge present on the substance and the weight of the substance to separate the various components of the substance. The DNA strand is taken and broken at various places which is detected by markers. These strands all possess individual charges and weights. When a potential difference is developed across the two ends, the strands start moving according to their weight and charges. Thus, the strands of the DNA get separated.

5. Which of the following devices are used to maintain a sterile environment while working with microorganisms?

- a) Laminar Airflow
- b) Microwave oven
- c) Water Bath
- d) Incubator

Answer: a

Explanation: A laminar air flow is like a sterile work station. It has a UV lamp and the whole chamber is aerated with sterile air to destroy any unwanted microorganisms. When any work is being done with microorganisms, they are placed in a petridish and placed in the laminar air flow. This allows sufficient aeration to the organisms without posing danger to the scientists. The scientists can work on the organisms by using gloves and accessing the chamber via special small holes which are wide enough for only the arms to pass through. Thus, the body stays out while the work is being done. This allows for isolation and maintains sterility.

6. Which device is used to separate the components of blood?

- a) auto analyzer
- b) centrifuge
- c) hematocrit
- d) magnetic stirrer

Answer: b

Explanation: The various components of blood precipitate at various speeds. The centrifuge rotates at high speed and the components depending upon their density separate out. The high speed causes them to clump together and then they either float up or sink down.

7. How are the blood cells and plasma separated?

- a) hematocrit

- b) hot plate
- c) centrifuge
- d) water bath

Answer: a

Explanation: The blood is collected, heparin is added to it and then the blood is centrifugated at 10,000 RPM. Then the blood is left to settle down. The cells due to higher weight settle down and the plasma floats up. The RBCs have the highest density so they settle down at the bottom. Then comes a layer of WBCs and then platelets. The plasma is collected above the platelets.

8. The cells related to blood are collectively called as

- a) oestoblast cells
- b) condcrocyte cells
- c) hematopoietic cells
- d) megakaryocytes

Answer: c

Explanation: Hemato is a term that related to blood. All the cellular components of the blood originate from the hematopoietic cells. They are found in the bone marrow of the long bones and are self renewing in nature. These are also considered as stem cells as they can give rise to different types of cells.

9. On what basis is the blood type classified?

- a) Antigen
- b) Antibody
- c) Rhesus Factor
- d) Oxygen Content

Answer: c

Explanation: Rhesus factor, also known as antigen D is used to classify blood into positive and negative. If the RH factor is present, it is called +ve blood type. If the RH factor is absent, it is called as -ve blood type.

10. On what basis is the blood group classified?

- a) Antigen and antibody
- b) Haemoglobin content
- c) Rhesus Factor
- d) Oxygen Content

Answer: a

Explanation: The blood groups have antigens which produce antibodies for the opposite blood group. Thus, blood group A has antigen A and it produces antibody B against the blood group B which contains antigen B. When a blood test is done, the

blood of group A will clump up when antigen B is added to the taken blood. In the same way, the blood of group B will clump when antigen A is added. For blood group AB, no clumping happens and for group O, clumping happens for both Antigen A and B.

11. How many blood types exist including the positive and negative factors?

- a) 3
- b) 4
- c) 8
- d) 6

Answer: c

Explanation: Blood is classified into two factors, antigen-antibody and rhesus factor. There are two types of antigens A and B. The cells having antigen A have antibody B while those having antigen B have antibody A. This makes the blood group A and B. AB blood group have both antigens A and B and no antibodies. Blood group has no antigens but antibodies for both. This makes four blood groups A, B, AB, O. The presence and absence of Rh factor make these four blood groups into 8. A+, A-, B+, B-, AB+, AB-, O+ and O-.

12. If the blood of two different groups is mixed together, what problem is observed?

- a) Coagulation
- b) Agglutination
- c) Thrombus formation
- d) Embolism

Answer: b

Explanation: The blood group is determined by the presence of antigen on the surface of the blood cells. When this antigen exists for one group, an antibody for the other group will exist as well. Thus, when the blood of the opposing group enters the body, the antibody attacks the antigen and they form a clump. This is called as agglutination.

13. Other than transfusion, when is it necessary to take the Rh factor into consideration?

- a) Cathertization
- b) Spleen Rupture
- c) Pregnancy
- d) Blood Donation

Answer: c

Explanation: Erythroblastosis foetalis is a condition that may happen to a woman when she is pregnant. When a woman with RH- blood type conceives a baby with RH+ blood type, the body

perceives it as a threat and starts producing antibodies. This can cause the baby to die in the womb and so RH factor must be monitored especially if the woman is RH-. Certain medications and treatments can help reverse this condition.

14. Which blood type is a universal donor?

- a) O –
- b) O +
- c) AB –
- d) AB +

Answer: a

Explanation: In O- the antigens for A and B are both absent and so is the antigen D (more commonly known as the Rhesus factor). Thus, O- can donate blood to all types of blood groups quite safely.

15. If a person has AB- blood, who all can donate blood to him?

- a) A+ , B-, O -, AB+
- b) A- , B -, O+, AB+
- c) A+, B+, O+, AB-
- d) A-, B-, O-, AB-

Answer: d

Explanation: A person with AB- blood has antigens for A and B and the antibodies for them are absent. Since the blood group is negative, it means that antigen D or the Rhesus factor is also absent in the blood. Thus, all blood groups with negative rhesus factors can donate blood.

16. Extraction of a small section of diseased tissue to study and diagnose is called \_\_\_\_\_

- a) autopsy
- b) biopsy
- c) necrosis
- d) endoscopy

Answer: b

Explanation: A cell is the smallest individually functioning unit of a body. Thus, when a body is diseased, all the cells of the diseases tissues shows a similar kind of response and reactions. Thus, a small part of the diseased tissue is extracted which is used to study and analyze the problem. This is called a biopsy.

17. On collecting blood, what solution is added to it?

- a) sodium citrate
- b) potassium citrate
- c) sodium phosphate
- d) potassium phosphate

Answer: a

Explanation: Sodium Citrate is an anticoagulant. Adding it ensures that the blood does not coagulate in the blood bags. If the blood is left static, i.e. if it does not keep moving, it starts coagulating. Once even if a small amount of blood has coagulated, even if it has a few cells, the coagulation spreads. The coagulation of blood can begin within a few minutes after extraction of blood and can finish within a few hours. To avoid this situation, anticoagulants are used.

18. Which of the following tests can be performed on the extracted blood?

- i) HIV ii) Diabetes iii) Hepatitis B surface antigen iv) Malaria v) Antibody to Hepatitis C vi) Serological test for Syphilis vii) Dengue viii) Creatinine
- a) i, iii, v, vi
- b) i, ii, iii, iv, v, vi, viii, viii
- c) ii, iv, vii, viii
- d) i, v, viii

Answer: a

Explanation: Once the blood has been donated, before storing it, it undergoes some basic tests to ensure that it can be used for transfusing into a patient in times of need. The tests include all those pathological problems that can be transmitted via blood. This blood test serves two purposes, one is to check if the donated blood is appropriate for transfusion, the second is that if any abnormality is detected, the person can be called back for further tests.

19. How much blood does the body have in reserve and where is it stored?

- a) 150 ml stored in liver
- b) 10 ml stored in gall bladder
- c) 100 ml stored in the spleen
- d) 15 ml stored in the heart

Answer: c

Explanation: Spleen stores almost three months of reserves of blood. When the body suffers from blood loss due to injury or trauma, the spleen releases blood to act as a buffer and prevent any critical situations from happening. Even after blood donation, the spleen releases blood to cover up for the loss. It takes almost three months for the spleen to recover its lost blood.

20. What is the optimum temperature to store blood right after collecting it?

- a) 25°C

- b) 22°C
- c) 20°C
- d) 15°C

Answer: b

Explanation: Once the blood is collected, it is stored at 22°C. At this temperature, the blood proteins are safe from degeneration, the cells function properly and the presence of anticoagulants ensures that the blood is not clotting.

21. How is dengue detected in blood?

- a) Low level of RBCs
- b) Low level of WBCs
- c) Low level of Platelets
- d) Low level of fibrin

Answer: c

Explanation: While dengue expresses itself in the form of fever, the sure test for dengue is the reduction in the number of platelets. The loss of platelets reduces the blood clotting capacity. When a person suffers from external injury the bleeding does not stop easily. When there is a severe reduction of platelets, the person may suffer from internal bleeding and die.

22. What machine is used to test the blood?

- a) Auto analyzer
- b) Hemodialyzer
- c) Diathermy machine
- d) Ventilator

Answer: a

Explanation: An auto analyzer has various tests and processes of doing those tests fed into it. It can do a test for over hundred samples using minimum amounts of blood within a span of few hours. Thus, an auto analyzer is currently the most favorite machine to do the various tests regarding blood.

23. What is the normal haemoglobin content of the RBCs?

- a) 0.3pgm
- b) 3.0pgm
- c) 30pgm
- d) 300pgm

Answer: c

Explanation: Haemoglobin is the protein that is responsible for the transport of oxygen and carbon-dioxide from the lungs to the cells in the body. Haemoglobin is carried by the RBCs and every RBC has 30pgm of haemoglobin.

24. Anaemia is caused due to deficiency of \_\_\_\_\_

- a) Haemoglobin
- b) Fibrin
- c) Thrombin
- d) Neutrophils

Answer: a

Explanation: An important component of haemoglobin is iron. When the quantity of iron goes down in body, it causes the haemoglobin content of the body to go down. Anemia symptoms include fatigue, dizziness, shortness of breath etc.

25. Hemophilia is more dominant in \_\_\_\_\_

- a) Males
- b) Females
- c) Young children
- d) Transvestite

Answer: a

Explanation: Haemophilia is a genetic problem in which the X chromosome is the carrier of the mutated gene. If a person is suffering from hemophilia, their blood has lost the ability to clot or the blood clotting ability has gone down. Since men have one X and one Y chromosome, they are more susceptible to get hemophilia.

26. A virus that destroys the immunity of the body and can spread through blood is \_\_\_\_\_

- a) HIV
- b) Gonorrhea
- c) Anemia
- d) Hemophilia

Answer: a

Explanation: HIV stands for Human Immunodeficiency Virus. HIV attacks the immune system of the body and causes AIDS (acquired immunodeficiency syndrome). A common method for HIV to propagate is through the transfusion of blood. It can stay hidden for long periods of time and can be discovered much later after it has attacked.

27. In a normal adult male, what is the ration of RBCs: WBCs: Platelets per ml of blood?

- a) 5000:11:300
- b) 50:11:300
- c) 5000:11:3
- d) 50:11:3

Answer: a

Explanation: A normal adult male is 5000000 RBCs,

# FORENSIC SCIENCE UNIT – IV

## Forensic Science



*As Per Updated Syllabus*



**FORENSIC SCIENCE UNIT – IV**

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## ANALYSIS OF ETHYL ALCOHOL IN BEVERAGES, LIQUORS, BIOLOGICAL FLUIDS AND BREATH; ANALYSIS OF METHANOL AND DENATURANTS;

### BEVERAGES

The term 'Beverage' is originated from a Latin word 'bever' which literally means 'rest after work'. A beverage or drink is a type of liquid which is specially prepared for the consumption of humans. There are vast varieties of drinks which can be classified as non-alcoholic drinks, alcoholic drinks, vegetable or fruit juices, soft drinks, etc. In addition to fulfilling a basic need, beverages have become an intermingled part of the culture of human society.



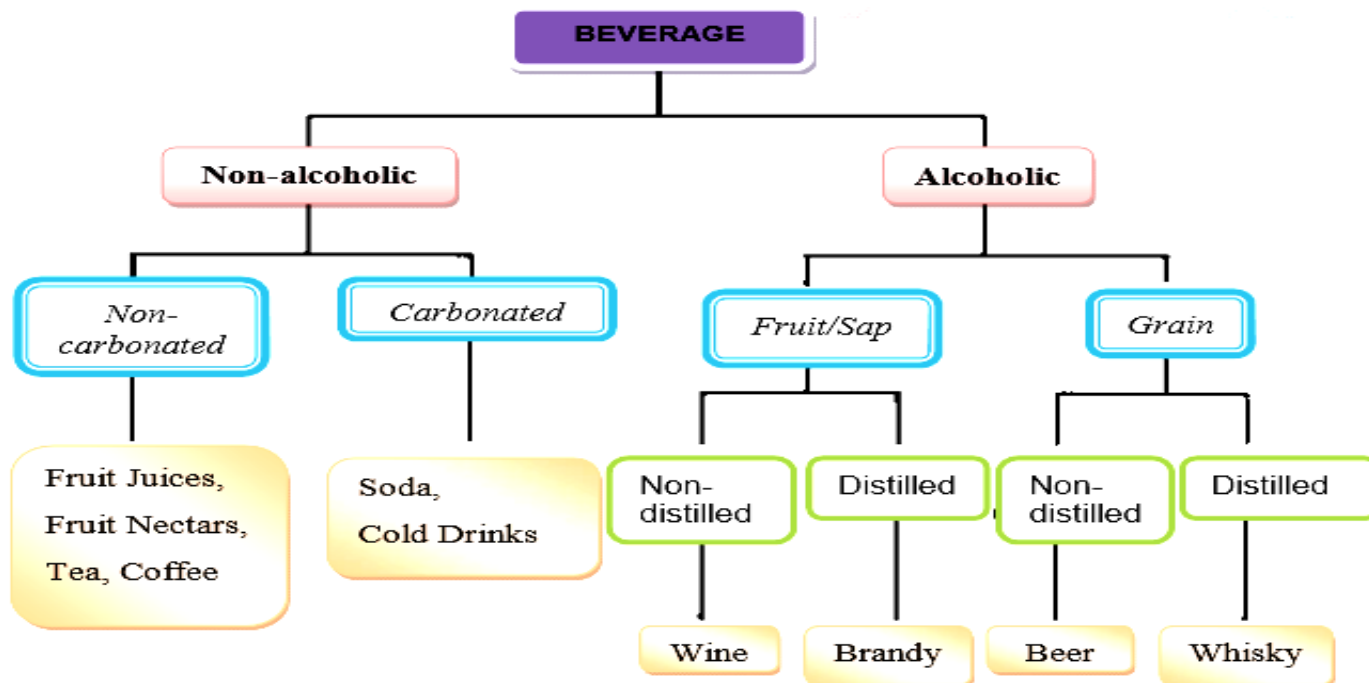
Figure1. Beverages

### Classification of Beverages

The term beverage is very vast as it incorporates a number of drinks in it, each differing in its contents and manufacturing processes. Beverages are chiefly classified into following two categories, based on the alcohol percentage present in them:

- Non-alcoholic Beverage
- Alcoholic Beverage

Schematic representation of Classification of Beverages



The term non-alcoholic doesn't necessarily signifies that the beverage should be absolutely free from alcohol. However, a non-alcoholic beverage is a type of beverage that contains less than 0.5% alcohol by volume. On the other hand, as the name itself implies, an alcoholic beverage contains a much higher percentage of alcohol as compared to a non-alcoholic one.

**Non-Alcoholic Beverages** As discussed earlier, a non-alcoholic beverage (also known as a virgin drink) is a type of beverage that contains less than 0.5% alcohol by volume. Non-alcoholic versions of some common and popular alcoholic beverages such as non-alcoholic beer ("near beer") and cocktails ("mocktails") are widely available in the market.

Some drinks like Sodas, juices, etc. contain no alcohol. But non-alcoholic wines and beer undergo a process in which alcohol is removed which may still leave alcohol in traces. That's why, some states have legally restricted non-alcoholic beer and wine. On the other hand, certain beverages contain stimulant drugs, prominently caffeine, which is marketed as physical and mental stimulant. Such drinks are commonly known as Energy Drinks in the market.

A non-alcoholic beverage could further be classified into non-carbonated and carbonated groups. Non-carbonated beverages are such drinks that are not added with carbon dioxide. A list of noncarbonated drinks would include tea, water, coffee, juices, milk, sports drinks, etc.



On the other hand, a carbonated beverage which is popularly called soft drink, is a beverage that typically contains water, including a flavouring agent and a sweetener.

Soft drinks are called "soft" in contrast to the alcoholic beverages which are known as "hard drinks". Even soft drink may contain small amounts of alcohol, but it should be less than 0.5% of the total volume. Soft drinks may also contain colourings, caffeine, preservatives along with other ingredients.

### **1. Carbonation**

Carbon dioxide ( $\text{CO}_2$ ) is a compound which is made up of one carbon and two oxygen atoms. Carbonation is the process of adding  $\text{CO}_2$  in a liquid under high pressure, most often to improve the texture, taste or both. Carbonation, in some cases may occur naturally like in underground water which absorb  $\text{CO}_2$  underground or artificially by application of pressure. Beer is another naturally carbonated beverage, as the fermentation also generates carbon dioxide as a by-product.

In artificially carbonated beverages when the pressure is released by opening up the bottle,  $\text{CO}_2$  escapes out in the form of small bubbles, which causes effervescence or "fizz" in the solution as shown in the Figure 2 given below. Addition of carbon dioxide in water to form carbonated water is an example of carbonation.

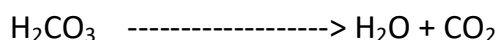


Figure2. Carbonation

A simple trick to check out whether the drink is carbonated or not, is that the drink makes bubbles on shaking or not. A carbonated beverage shows the generation of bubbles.

## 2. Chemistry

As carbon dioxide is very sparingly water soluble, it has a tendency to escape out from the liquid. The process of CO<sub>2</sub> escaping out of a solution is represented by the following chemical reaction. This shows aqueous carbonic acid converts to carbon dioxide and water:

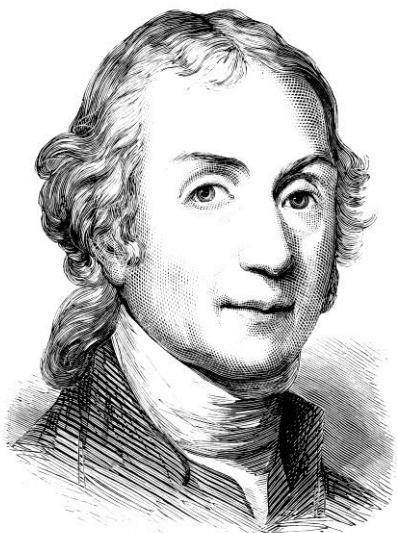


## 3. Why Some Beverages are Carbonated?

There are a number of reasons for which the beverages are carbonated. One of them is that the fizzy sensation and the slight different taste that it produce is liked by various people. In early times, carbonated spring water- a natural carbonated beverage, was used to cure the stomach upsets.

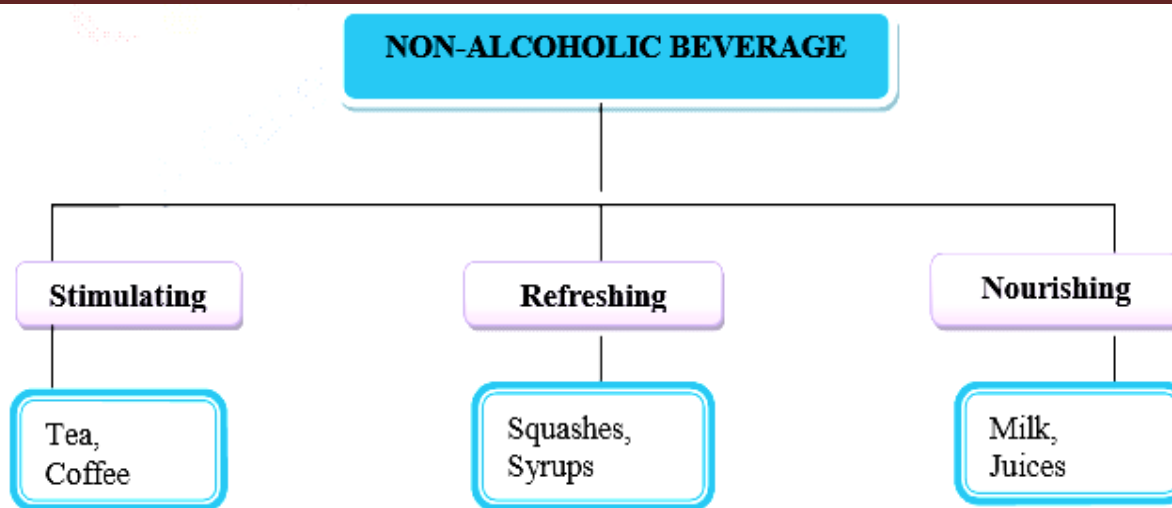
## 4. History

Sparkling wine and beer are naturally carbonated during the process of fermentation and their usage was reported even in the 17th century. English chemist Joseph Priestly is credited with the invention of first artificial carbonated beverage who previously invented a way to infuse CO<sub>2</sub> in water in 1767.



Joseph Priestley

A non-alcoholic beverage could also be classified on the basis of effects that they produce-



### Alcoholic Beverages

An alcoholic beverage is a drink and psychoactive drug containing ethyl alcohol which is commonly referred to as ethanol. Alcohol is a colourless liquid diluted with water belonging to the group of inebriants which are generally characterized by a set of two symptoms namely, excitement and narcosis.

Ethanol is a small molecule having a backbone made up of 2-carbon (C) with 5 hydrogens (H) and a hydroxyl (OH) group at one end, attached to it. The alcohol is characterized by this terminal hydroxyl group as shown in Figure 3.

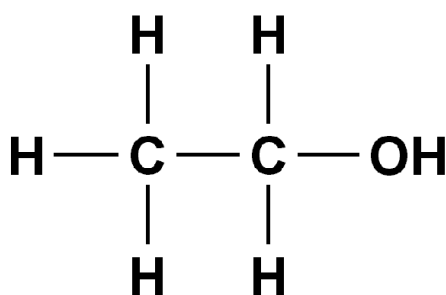
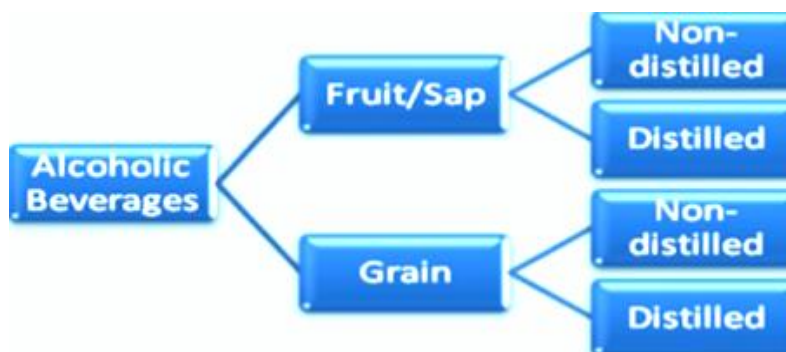


Figure 3 Chemical Structure of Ethanol

Ethanol is formed by a natural conversion process known as fermentation where yeast, a fungus, converts sugar into alcohol and carbon dioxide. The natural sugars found in fruit, berries and malted grains are fermented to produce the alcoholic beverages like beer and wine. Liquors, however, are produced through the process of distillation.

The classification of an alcoholic beverage can be made on the basis of their basic ingredient i.e. fruit/sap or grain. Each category is further classified into non-distilled and distilled alcoholic beverages.





A distilled liquor or beverage is an alcohol containing beverage produced by distilling alcohol that is produced by fermenting fruits and grains. Distilled and unsweetened alcoholic beverages that have an alcohol concentration a minimum of 20% ABV(alcohol by volume) are called spirits. The process of distillation not only concentrates the alcohol but also removes some of the congeners present in it. This excludes non-distilled fermented beverages such as wine, beer and cider. Types of distilled beverages include gin, vodka, tequila, whisky, brandy, rum, etc.

In North America the term hard liquor is used for distilled beverages which are stronger in alcohol concentration as compared to the non-distilled ones.

- Neutral grain spirit is a flammable, clear and colorless liquid having high alcohol concentration are distilled from a grain mash. The term neutral actually signifies to those spirits that lack any flavor. In some cases, other kinds of spirits like whiskey are distilled to a much lower alcohol concentration for preserving the mash flavor.
- "Rectified spirit" is a neutral alcohol which is further purified by the process of "rectification" (i.e., repeated distillation). Rectified spirits must have at least 95% ABV. Generally it is employed in medicinal purposes but can also be used in making homemade liquors.

Alcoholic beverages also act as a source of energy. One gram alcohol provides 7.1 kcal, and each milliliter provides 5.6 kcal.

### **1. History**

Evidences have been found from the Neolithic era of the use of alcoholic beverages. The earliest evidence of alcohol use was found in Jiahu, dated back from 7000–6600 BC. The practice of alcohol production and consumption is present in almost every culture of the world, right from hunter-gatherer peoples ranging to nation-states.

### **2. Reasons for using Alcoholic Beverages**

Since ancient times, people around the whole world have been drinking alcoholic beverages. Reasons for drinking alcoholic beverages vary and include:

- Being part of a standard diet
- Medical purposes
- Relaxant effects
- Euphoric effects
- Recreational purposes
- Artistic inspiration
- Happiness

### **Forensic Aspects of Beverages**

The study of drugs in drinks and foodstuff can be an important aspect in forensic cases. Generally beverages and food stuffs do not have any drug as their natural constituent except gammahydroxybutyric acid (GHB) which occurs naturally in wines and juices, especially which are produced by fermented red grapes. The presence of a drug in a drink can raise suspicion as to the nature of the case that may often needs forensic investigation. A drink can be spiked to fulfill a malicious intention ranging from abortion, miscarriage, sexual assault and even to attempted murder. Such suspected drinks are carefully collected from the scene of crime and send to forensic laboratory for analysis of any toxicological material. So, the basic knowledge about the type of beverage will facilitate the analysis.

### **FATE OF ALCOHOL IN BODY**

## 1. Absorption of Alcohol

Alcohol appears in blood within a few minutes after consuming an alcoholic beverage. It is absorbed through all the regions of gastrointestinal tract by simply diffusing out into the blood stream. However, due to its very large area that is available for alcohol absorption, small intestine is a way ahead in its efficiency to absorb alcohol as compared to any other region of the gastrointestinal tract.

That's why, when a person consumes alcohol, only about 20% of the absorption of alcohol takes place in stomach and remaining 80% is carried out in small intestine and its level in blood stream starts rising. After absorption, the alcohol gets dissolved into the water content of the bloodstream. Alcohol is carried out throughout the body via blood. From blood, the alcohol is carried to all parts of the body, then enters and gets dissolved in the water that is present inside each and every body tissue except fat tissues, as alcohol is insoluble in fat. The alcohol is evenly distributed throughout the watery content of the body. Once alcohol absorption is complete, equilibrium occurs so that blood at points in all the system contains approximately the similar concentration of alcohol. Those parts which are low in water content like bones, fat, hair, etc. contain a very little amount of alcohol as compared to the absorbed quantity. That's why if blood is not available, in some autopsy situations as the case may be, the medical examiner should look for those organs which are rich in water so as to determine the body alcohol content with a reasonable degree of accuracy.

When all the alcohol is absorbed, the blood alcohol concentration reaches to a maximum and the alcohol starts exerting its effects on the body. The effects so developed are in proportion to the BAC, which in turn is dependent on the amount of the consumed alcohol. Effect of alcohol continues till the ingested alcohol remains in the body.

After this initial stage of absorption, a post-absorption stage is followed in which the level of alcohol in blood stream starts decreasing until a level of zero is reached again.

A blood alcohol curve is generated by the combined effects of absorption, distribution, & finally elimination as shown in Figure 1 given below. This curve represents the following 3 phases:

- Absorption Phase
- Distribution Phase
- Elimination Phase

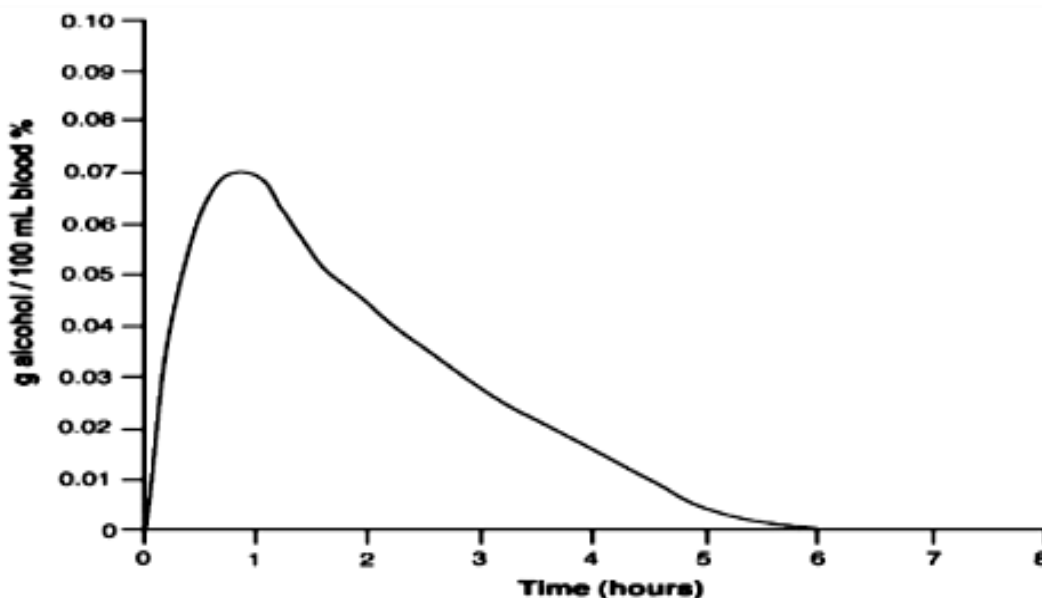


Figure 1. Blood Alcohol Concentration over time

## 2. Metabolism of Alcohol

autopsy and with information gathered by police, and other investigators. Conversely, inferences and suspicions derived from autopsy and investigation must be refined and confirmed through toxicological studies. Only then can reliable opinions be formulated regarding the role of drugs or poisons in a death investigation.

**FORENSIC SCIENCE UNIT – IV MCQs**

1: Low alcohol content is present in

- (A) Wine
- (B) Arrack
- (C) Brandy
- (D) Rum

Answer: (A)

2: Fatty liver syndrome is due to

- (A) Infection by a virus
- (B) Intake of excessive fat
- (C) Intake of excessive alcohol
- (D) Intake of tobacco through chewing

Answer: (C)

3: Alcohol produces

- (A) Cirrhosis of liver
- (B) Cancer of pancreas
- (C) Drug dependence
- (D) Gall bladder stones

Answer: (A)

4: Tunnel vision is caused by

- (A) Lack of vitamin A
- (B) Alcohol
- (C) Smoking
- (D) Barbiturates

Answer: (B)

5: A drunken person should not drive a vehicle because alcohol

- (A) Increases reaction time
- (B) Affects coordination of body parts, alertness and judgement
- (C) Causes rashness and carelessness
- (D) All the above

Answer: (D)

6: Alcohol addiction

- (A) Increases blood sugar
- (B) Reduces blood sugar
- (C) Leads to increased use of barbiturates

(D) Both B & C

Answer: (B)

7: The drug which does not have sedative effect but alongwith alcohol produces marked drowsiness is

- (A) Barbiturate
- (B) Valium
- (C) Antihistamine
- (D) Marijuana

Answer: (C)

8: A useful drug that damages gastric mucosa if taken with alcohol is

- (A) Valium
- (B) Antihistamine
- (C) Aspirin
- (D) Morphine

Answer: (C)

9: Alcoholic beverages contain

- (A) Methyl alcohol
- (B) Ethyl alcohol
- (C) Propyl alcohol
- (D) A mixture of all the above

Answer: (B)

10: High alcohol content of a beverage is achieved through

- (A) Prolonged fermentation
- (B) Distillation
- (C) Fortification
- (D) Both B and C.

Answer: (D)

11: Effervescence of champagne is due to

- (A) Soda bicarb
- (B) Sugar
- (C) CO<sub>2</sub>
- (D) O<sub>2</sub>

Answer: (C)

12: Alcoholic beverage Vodka is

(A) British

(B) French

(C) Indian

(D) Russian

Answer: (D)

13: Vodka is prepared by fermenting

(A) Potato

(B) Fruit juice

(C) Molasses

(D) Cashewnut thalamus

Answer: (A)

14: An Indian alcoholic drink from Goa is

(A) Perry

(B) Cider

(C) Fenny

(D) Bourbon

Answer: (C)

15: Toddy is prepared from sweet juice tapped from

(A) Inflorescence

(B) Stem

(C) Root

(D) All the above.

Answer: (A)

16: Arrack is alcoholic beverage prepared from

(A) Potato

(B) Molasses

(C) Toddy

(D) Pearl Millet.

Answer: (C)

17: Beer is prepared in India from

(A) Maize

(B) Pearl Millet

(C) Barley

(D) Rye

Answer: (B)

18: Whisky is distilled from fermented product of

(A) Grains

(B) Fruit juice

(C) Molasses

(D) Potato

Answer: (A)

19: Which one is alcoholic beverage from molasses

(A) Brandy

(B) Rum

(C) Perry

(D) Gin

Answer: (B)

20: Sake is alcoholic beverage of

(A) Japan

(B) France

(C) Holland

(D) England

Answer: (A)

21: Sake is prepared from

(A) Maize

(B) Rye

(C) Rice

(D) Oat

Answer: (C)

22: Alcohol is

(A) Hallucinogen

(B) Tranquilliser

(C) Stimulant

(D) Depressant.

Answer: (D)

23: A part of alcohol is excreted through

(A) Breath

(B) Urine

(C) Sweat

(D) All the above.

Answer: (D)

24: Percentage of alcohol metabolised in mitochondria is

(A) 5%

(B) 7.5%

(C) 10%

(D) 15%

Answer: (C)

25: Alcohol not metabolised is changed into

(A) Butanol

(B) Butyric acid

(C) Acetic acid

(D) Acetaldehyde.

Answer: (D)

26: Enzyme required to oxidise acetaldehyde is

- (A) Ethanol oxidase
- (B) Acetaldehyde dehydrogenase
- (C) Alcohol hydrogenase
- (D) Glyceraldehyde dehydrogenase

Answer: (B)

27: Enzyme present in limited quantity in Asians for metabolism of alcohol is

- (A) Alcohol dehydrogenase
- (B) Succinate thiokinase
- (C) Acetaldehyde dehydrogenase
- (D) Both A and B.

Answer: (C)

28: Hangover is

- (A) Increased desire to take alcohol
- (B) Feeling of giddiness and nausea in the morning after having taken alcohol
- (C) Development of gastritis and peptic ulcers due to alcohol addiction
- (D) Feeling of flushing after taking alcohol

Answer: (B)

29: Hangover is due to accumulation of

- (A) Ethanol in liver
- (B) Ethanol in lungs
- (C) Acetaldehyde in body
- (D) Formation of formaldehyde from acetaldehyde

Answer: (C)

30: Alcoholics have reduced number of

- (A) Erythrocytes
- (B) Leucocytes
- (C) Blood platelets
- (D) All the above

Answer: (D)

31: Energy content of alcohol is 7.1 kcal/gm. It is more than carbohydrates and slightly less than fats. Intake of alcohol provides

- (A) Enough energy to body for its working
- (B) Releases heat energy
- (C) Uses energy of body for dissipation of alcohol produced heat
- (D) Both B and C.

Answer: (D)

32: Alcohol is

- (A) Appetiser
- (B) Gastric irritant
- (C) Stimulates gastric secretions
- (D) Speeds up digestion

Answer: (B)

33: Bone part which undergoes necrosis in alcoholics is

- (A) Head of femur
- (B) Shaft of femur
- (C) Vertebrae
- (D) Carpals.

Answer: (A)

34: An alcoholic woman has

- (A) Reduced fertility
- (B) Little menstruation
- (C) Spontaneous abortion
- (D) All the above.

Answer: (D)

35: An alcoholic male develops

- (A) Impotence
- (B) Early sexual maturity
- (C) Increased appetite
- (D) Both B and C.

Answer: (A)

36: Alcohol is absorbed in

- (A) Mouth
- (B) Mouth and oesophagus
- (C) Stomach and proximal intestine
- (D) Throughout alimentary canal

Answer: (C)

37: Alcohol is mostly metabolised in

- (A) Liver
- (B) Kidneys
- (C) All body cells
- (D) Connective tissue

Answer: (A)

38: Alcoholism induces fat deposition in

- (A) Fat bodies
- (B) Dermis of skin
- (C) Around heart
- (D) Liver



Answer: (D)

39: Vitamin which is most likely to become deficient in alcoholics is

- (A) Ascorbic acid
- (B) Thiamine
- (C) Niacin
- (D) Riboflavin

Answer: (B)

40: In alcoholics, liver cells come to accumulate fat. Fat formation is stimulated by

- (A) Alcohol
- (B) Acetaldehyde
- (C) Thiamine deficiency
- (D) Folic acid deficiency

Answer: (B)

41: In alcoholics, urine is

- (A) Hyperosmotic
- (B) Isoosmotic
- (C) Hypoosmotic
- (D) Alkaline

Answer: (A)

42: Thiamine deficiency of alcohol addicts produces Wernicke's syndrome which is characterised by

- (A) Double vision
- (B) Reduced muscular coordination
- (C) Decreased mental function
- (D) All the above

Answer: (D)

43: Alcohol addicts suffer from loss of memory. The phenomenon is called

- (A) Amnesia
- (B) Neuritis
- (C) Dementia
- (D) Insomnia

Answer: (A)

44: Syndrome found in alcohol addicts which is characterised by loss of recent and past memory without affecting normal intelligence is

- (A) Wernicke's syndrome
- (B) Korsakoff's syndrome
- (C) Laenec's syndrome
- (D) Foetal alcohol syndrome

Answer: (B)

45: Alcohol taken along with barbiturate will cause

- (A) Enhanced excitement
- (B) Marked depressant effect
- (C) Hallucination
- (D) Both B and C

Answer: (B)

46: Alcohol

- (A) Increases reaction time
- (B) Decreases reaction time
- (C) Does not affect reaction but diminishes decision making
- (D) Both B and C

Answer: (A)

47: Drinking

- (A) Stimulates muscle activity
- (B) Speeds up impulse transfer
- (C) Impairs coordination of different body parts
- (D) Makes the driver extra careful

Answer: (C)

48: Disulfiram antagonises

- (A) Alcohol dehydrogenase
- (B) Acetaldehyde dehydrogenase
- (C) Impulse transfer
- (D) Excretion of alcohol

Answer: (B)

49: Antabuse is used in deaddiction process for alcohol because

- (A) It causes aversion reaction
- (B) It indicates the amount and time of recent drinking
- (C) It satisfies the craze for alcohol
- (D) It immediately informs AA

Answer: (A)

50: A bottle of alcoholic drink has a print line indicating 125% proof. The alcohol content of the drink is

- (A) 12.5%
- (B) 25%
- (C) 49.29%
- (D) 61.6%

Answer: (D)