Model Answer for Question Paper Code: AU6214
Model answer
LZT 104: Histology and Biostatistics
M. Sc. First Semester
Department of Zoology, Guru Ghasidas Vishwavidyalaya

Q 1: Multiple Choice Questions (Tick the appropriate answer) (2X10=20)
All questions are compulsory.

1. c) Median and Mode
2. c) 1.7
3. b) 3/28
4. b) 52, 10
5. c) 50
6. b) Alcohol
7. a) free aldehyde group of carbohydrate
8. c) Pinkish Purple colour
9. d) bound water molecule from proteins only
10. d) None of the above

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Q 2: Descriptive type (Answer any 4 questions within 200 words) (4X10=40)

Question no 1: Define dehydrating agent? Why it is necessary for histology?

Answer 1:
Dehydration:
The aim of tissue processing is to remove water from tissues and replace with a medium that solidifies to allow thin sections to be cut. It is the first step in tissue processing by which the intercellular and extracellular water from the tissue are removed after fixation and prior to wax infiltration**.**

Dehydrating Agents:
Solvents utilized in the removal of water, (e.g. graded strengths of alcohol).

Characteristics of An Ideal Dehydrating Agent:

- Dehydrates rapidly, producing noconsiderable shrinkage or distortion of tissues.
- Does not evaporate very fast.
- Ability to dehydrate fatty tissues.
- Does not harden tissues excessively.
- Does not remove stains.
- Non-toxic and not a fire hazard.

Commonly Used Dehydrating Agents

1) Alcohol (most common)
2) Acetone
3) Dioxane 4 – cellosolve
4) Triethyl phosphate
5) Tetrahydrofuran
6) Cellosolve

Alcohol

A. Ethyl alcohol (ethanol)

It is recommended for routine dehydration of tissues and considered to be the best dehydrating agent. It is clear, colorless and flammable fluid that is fast-acting, mixes with water and many inorganic solvents, penetrates tissueeasily, not poisonous and expensive.

B. Methyl alcohol (methanol)

It is primarily used for blood and tissue films, andfor smear preparations. It is toxic to the body

C. Butyl alcohol

It is utilized in plant and animal micro-techniques. It is a slow dehydrating agent producing less shrinkage and hardening than ethanol. It is a slow dehydrating agent thus, it is not suitable for rapid tissue processing.

The strength of initial alcohol required in each concentration will depend upon the size, and nature of the tissue and fixative used. Smaller and more delicate tissues require lower conc. and shorter intervals between succeeding ascending grades of alcohol. Concentrated alcohols produce shrinkage and make the tissue hard, brittle and difficult to cut. Do not penetrate the deeper parts. The tissue may be stored in70-80% alcohol (not for longer periods of time).
Prolonged storage in lower concentrations (below 70%) tends to macerate the tissue. While conc ranging from 70-80% alcohol at very long periods of time might interfere with the staining properties of the specimen.

Acetone

It is clear, colorless fluid that mixes with water, ethanoland most organic solvent. It is cheap, rapid-acting dehydrating agent which itdehydrate in ½ to 2 hours. It is more miscible when epoxy resins than alcohol. It is highly flammable, penetrates tissues poorly,causes brittleness (tissues placed for prolongedperiod of time). Most lipids are removed from tissues.
Because of considerable tissues shrinkageproduced, acetone is not recommended forroutine dehydration purposed.

Dioxane (Diethylene Dioxide)

It is an excellent dehydrating and clearing agent that readily miscible in water, melted paraffin, alcohol and xylol. It produces less tissues shrinkage. Tissues can be left in this reagent for long period of time withoutaffecting the consistency or staining properties of the specimen. It is

expensive, extremely dangerous, tissues sections tend to ribbon poorly. Its vapor produces a cumulative and highly toxic thus it should not be used routinely.

Cellosolve

It is Ethylene glycol monoethyl ether. Tissue can be stored in it for months without producing hardening or distortion. It is toxic and combustible at 110-120 degree F.

Triethyl phosphate

Removes water very readily and produces very little distortion and hardening.
Used to dehydrate sections and smears.
Produce minimum shrinkage.

Tetrahydrofuran

Both dehydrates and clears tissues Dissolve many substances including fats
Miscible with lower alcohols, ether, chloroforms, acetone, benzene
May be used for remixing, clearing and dehydrating paraffin section.
Most staining procedures give improved results with tetrahydofuran.

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Question no 2: What is Alum hematoxylin? Describe composition and preparation with suitable example.

Answer 2:

This group comprises most of those that are used routinely in the hematoxylin and eosin stain, and produce good nuclear staining. The mordant is aluminum, usually in the form of ' potash alum' or 'ammonium alum'. All stain the nuclei a red color, which is converted to the familiar blue black when the section is washed in a weak alkali solution. Tap water is usually alkaline enough to produce this color change but occasionally alkaline solution such as saturated lithium carbonate, 0.05% ammonia in distilled water are necessary. This procedure is known as 'bluing'. The alum hematoxylin can be used regressively meaning that the section is over stained and then differentiated in acid alcohol.

There are several types of alum hematoxylin like Ehrlich's hematoxylin (1886), Delafield's hematoxylin (1885), Mayer's hematoxylin (1903), Harris's hematoxylin (1900) and others.

Ehrlich's hematoxylin

This is a naturally ripening alum hematoxylin which takes about two months to ripen; the ripening time can be shortened somewhat by placing the unstoppered bottle in a warm sunny place. Once satisfactorily ripened this hematoxylin solution will last in bulk of years, and retains its staining ability in a caplin jar for some months. Ehrlich's hematoxylin, as well as being an excellent nuclear stain, also stains mucins including the mucopolysaccharides of cartilage.

Preparation of solution

Ingredient	Amount
Hematoxylin	2g
Absolute alcohol	100ml
Glycerin	100ml
Distilled water	100ml
Glacial acetic acid	10ml
Potassium alum	15g

The hematoxylin is dissolved in the alcohol, and the other chemicals are added. Glycerin is added to slow the oxidation process and prolong the hematoxylin self life. Ehrlich's hematoxylin, being a strong hematoxylin solution, stains nuclei intensely and crisply,and stained sections fade much more slowly than those stained with other alum hematoxylin. Ehrlich's hematoxylin is not ideal foer frozen sections.

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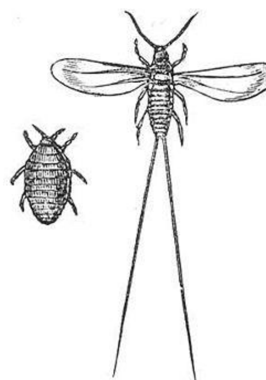
Question no 3: Explain the following: Animal Dye, Microtome

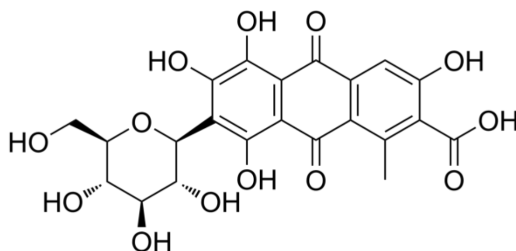
Answer 3:

Animal Dye

Carminic acid ($C_{22}H_{20}O_{13}$) is a red glucosidal hydroxy anthrapurin that occurs naturally in some scale insects, such as the cochineal and the Polish cochineal. The insects produce the acid as a deterrent to predators. Carminic acid is the colouring agent in carmine
The chemical structure of carminic acid consists of a core anthraquinone structure linked to a glucose sugar unit. Carminic acid was first synthesized by organic chemists in 1991.

Order:	Hemiptera
Superfamily:	Coccoidea
Family:	Dactylopiidae
Genus:	*Dactylopius*
Species:	*D. coccus*





Structure of Carminic acid

Carminic acid, typically 17–24% of dried insects' weight, can be extracted from the body and eggs, then mixed with aluminium or calcium salts to make carmine dye, also known as cochineal. Carmine is today primarily used as a food coloring and for cosmetics, especially as a lipstick coloring.

The carmine dye was used in Central America in the 15th century for coloring fabrics and became an important export good during the colonial period. Cochineal insects are soft-bodied, flat, oval-shaped scale insects. The females, wingless and about 5 mm (0.20 in) long, cluster on cactus pads. They penetrate the cactus with their beak-like mouthparts and feed on its juices, remaining immobile unless alarmed. The nymphs secrete a waxy white substance over their bodies for protection from water loss and excessive sun. This substance makes the cochineal insect appear white or grey from the outside, though the body of the insect and its nymphs produces the red pigment, which makes the insides of the insect look dark purple. Carminic acid is extracted from the female cochineal insects and is treated to produce carmine, which can yield shades of red such as crimsonand scarlet. The body of the insect is 19–22% carminic acid. The insects are processed by immersion in hot water or exposure to sunlight, steam, or the heat of an oven. Each method produces a different colour that results in the varied appearance of commercial cochineal. The insects must be dried to about 30% of their original body weight before they can be stored without decaying. It takes about 80,000 to 100,000 insects to make one kilogram of cochineal dye.

Microtome

Microtomy is the means by which tissue is sectioned and attached to surface for further microscopic examination. The basic instrument used in microtomy is **microtome.** The specimen moves vertically past the cutting surface and a tissue section is produced. There are several types of microtome, each with a specific purpose, as following;

Rotary microtome: This type of microtome is often referred to as the 'Minot' after its inventor. The basic mechanism requires the rotation of a fine advance hand-wheel by 360^0 degrees, moving the specimen vertically past the cutting surface and returning it to the starting position.

Base sledge microtome: With this microtome, the specimen is held stationary and the knife slides across the top of the specimen during sectioning. It is used primarily for the large blocks, hard tissues or whole mounts and is especially useful in neuropathology and ophthalmic pathology.

Rotary rocking microtome: This type of microtome is commonly used in cryostats. Here the retracting action moves the tissue block away from the knife on the upstroke, producing a flat face to the tissue block.

Sliding microtome: In this type, the knife is stationary and the specimen slides under it during sectioning.

Ultramicrotome: This type of microtome is used exclusively for electron microscopy**.**
The basic pattern for the carrying out the microtomy process is as;

- Setup of the microtome,
- Trimming the tissue blocks,
- Cutting sections,
- Floating out sections and then
- Drying the sections.

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Question no 4: Define the preparation and mechanism of Periodic acid schiff technique?

Answer:

The PAS technique is the most versatile and widely used technique for the demonstration of carbohydrates or glycoconjugates. The first Histochemical use of this technique was by McManus (1946) for the demonstration of mucin.

Preparation of Schiff reagent:
Schiff reagent is prepared from basic fuchsin. A number of methods for the synthesis of Schiff reagent have been described since Schiff's original report in 1866. All of these methods, however share a common theme in the production of an aqueous solution of sulfurous acid. The sulfurous acid may be generated from the reaction of sodium metabisulfite ($Na_2S_2O_5$) react with a mineral acid such as hydrochloride acid (HCl) or by the reaction of thionyl chloride ($SOCl_2$) with water.
The reaction of sulfur dioxide with basic fuchsin results in the addition of a sulfonic acid group to the central carbon of the triarylmethane molecule.

PAS technique (modified McManus 1946):

Periodic acid solution

Periodic acid	1g
Deionized or distilled water	100ml

Preparation of Schiff reagent

Dissolve 1g of basic fuchsin and 1.9g of sodium metabisulfite ($Na_2S_2O_5$) in 100ml of 0.15N hydrochloric acid (HCl). Shake the solution for 2 hours. The solution should be clear and yellow to light brown in color. Add 500mg of activated charcoal and shake for 1 to 2 minutes. Filter the solution. The filtered solution should be clear and colorless. If the solution is yellow repeat the

charcoal decolorization using a fresh lot of activated charcoal. Store at 4°c. solution is stable for several months.

Mechanism of the PAS technique:

The PAS technique is based upon the relativity of free aldehyde groups within carbohydrates with the Schiff reagent to form a bright magenta end product. The initial step in the PAS technique is the oxidation of hydroxyl groups attached to adjacent carbon atoms within the carbohydrate. The result is the formation of two free aldehyde groups and the cleavage of the adjoining carbon –to-carbon bond. The oxidation of the 1,2 glycols to form adjacent aldehydes is produced by treatment of the sections with a dilute solution of periodic acid(HIO_4). A 0.5-1.0% solution of periodic acid is used for 5-10 minutes. Other oxidants such as chromic acid and potassium permanganate have been used in variations of the technique. These oxidants, however tend further to oxidize the aldehyde groups to carboxylic groups which are not reactive with Schiff reagent.

The intensity of the color that develops following reaction with Schiff reagent is dependent upon the tissue concentration of reactive glycol structures. Monosaccharides that lack 1,2 glycol bond or containing hydroxyl groups that are involved in an ester or glycosidic linkage are not susceptible to periodic acid oxidation and hence cannot be detected with the PAS technique. Reactive monosaccharides include most of the neutral sugars such as mannose, fucose, galactose and glucose.

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Question no 5: What are the basic principles of immunohistochemistry? How alkaline phosphatase are localized through histochemistry?

Answer:

Azo dye is employed for alkaline phosphatase. The diazonium salt is prepared in two stages. In the first, pararosanilin hydrochloride is dissolved in distilled water and acidified with concentrated hydrochloric acid. The solution is filtered and stored at room temperature where it is stable for several months. Diazotization is achieved by the addition of 4% solution of sodium nitrite in distilled water.

Alkaline phosphatase: Azo Dye coupling method (simultaneous coupling)

Reagents required

1. 0.1M Tris buffer stock solution.
2. Sodium α-naphthyl phoaphate.
3. Diazonium salt (Fast red TR).
4.

Preparation of incubating medium

1. Sodium α-naphthyl phoaphate 10 mg
2. 0.1M Tris buffer stock solution. 10 ml
3. Diazonium salt (Fast red TR). 10 mg
4.

The final pH of the incubating medium should be between 9.0-9.4. Sodium α-naphthyl phoaphate is dissolved in the buffer, the diazonium salt is added and the solution well mixed. The solution is then filtered and used immediately.

Sections

1. Cryostat post fixed.
2. Cryostat pre fixed.
3. Freeze dryed.
4. Carefully processed paraffin sections.
5. Frozen sections.

Incubating method

1. After fixation, bring sections to water, incubate at room temperature for 10-60 minutes.
2. Wash in distilled water.
3. Counterstain in 2% Methyl Green (Chloroform extracted).
4. Wash in running water.
5. Mount in glycerin jelly.

Result

Alkaline phosphatase activity: reddish brown

Nuclei: green.

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Question No 6: What do you understand by mean? Discuss its relative merits and demerits as measures of central tendency.

Answer:

Mean is measure which condenses a huge unwieldy set of numerical data into single numerical values which are representative of the entire distribution. These are the values around which other items of the distribution congregate.

Formula:

$$\bar{X} = \frac{\sum X}{N}$$

$$\bar{X} = \frac{\sum fX}{N}$$

$$\text{Mean} = A + h.\ \frac{\text{Sum of Fd}}{N}$$

Merits:

1. It is rigidly defined
2. It is easy to calculate and understand
3. It is based on all the observations
4. It is suitable for further mathematical treatment.
5. It is affected least by fluctuations of sampling

Demerits:

1. It is very much affected by extreme observations
2. Arithmetic mean cannot be used in the case of open end classes
3. It cannot be determined by inspection neither can it be located graphically
4. Cannot be determined in case of qualitative characteristics which cannot be measured quantitatively.
5. It cannot be obtained if a single observation is missing or lost.
6. In asymmetrical distribution, usually arithmetic mean is not representative of the distribution and hence is not a suitable measure of location.
7. It may lead to wrong conclusions if the details of the data from which it is obtained are not available

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Question No 7: Find the mean deviation from the mean for the following data:

Class interval	0-10	10-20	20-30	30-40	40-50	50-60	60-70
Frequency	8	12	10	8	2	3	7

Answer:

Class interval	Frequency	X	D (A=35)	Fd	X-Mean	$F(X - \text{Mean})$
0-10	8	5	-3	-24	-24.2	193.6
10-20	12	15	-2	-24	-14.2	170.4
20-30	10	25	-1	-10	-4.2	42
30-40	8	35	0	0	5.8	46.4
40-50	2	45	+1	+2	15.8	31.6
50-60	3	55	+2	+6	25.8	77.4
60-70	7	65	+3	+21	35.8	250.6
	N=7.14			$\sum Fd =$ -29		$\sum f.(X - \text{Mean}) = 812$

$\text{Mean} = A + \frac{h\sum fd}{N}$

= 35 +10(-29)⁄50
=35 - 5.8
=29.2

Md about Mean = $\frac{\sum f.(X-X)}{N}$
=812⁄50
=16.24

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Question No 8: Obtain the equation of line of two lines of regression for the data given below:

X	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Y	**9**	**8**	**10**	**12**	**11**	**13**	**14**	**16**	**15**

Answer:

X	Y	dx	dy	dx^2	dy^2	dx.dy
1	9	-4	-2	16	9	12
2	8	-3	-3	9	16	12
3	10	-2	-1	4	4	4
4	12	-1	1	1	0	0
5	11	0	0	0	1	0
6	13	1	2	1	1	1
7	14	2	3	4	4	4
8	16	3	5	9	16	12
9	15	4	4	16	9	12
Mean of X = 45⁄9=5	Mean of Y = 108/9=12			$\sum dx^2$ = 60	$\sum dy^2$ = 60	$\sum$dx.dy = 57

Coefficient of Regression are bxy and byx.

$$bxy = \frac{\sum dx.dy}{\sum dy2} = 57/60 = \mathbf{0.95}$$

$$byx = \frac{\sum dx.dy}{\sum dx2} = 57/60 = \mathbf{0.95}$$

Line of regression of X on Y is : **X-X= bxy (Y-Y)**

(X-5)= 0.95- (Y-12)
X-5= 0.95Y-11.4
X=0.95Y-11.4+5
X=0.95Y-6.4

Line of regression of Y on X is : **Y-Y= byx (X-X)**

Or, (Y-12)= 0.95(X-5)
Y-12= 0.95X- 4.75

$Y= 0.95X+12-4.75$

$\mathbf{Y=0.95X+7.25}$

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