AS - 2199

M. Sc. First semester, 2013

LZT 104: Histology, Histochemistry & Biostat

Section A

(1) (b) Range

(2) $b_{xy} = \frac{r\sigma_x}{\sigma_y}$; $\sigma_x = \frac{b_{xy} \cdot \sigma_y}{r}$

$= \frac{0.5 \times 4}{0.8} = 2.5$

(a) 2.5

(3) (d) 09

(4) $P(A) = \frac{\text{Favourable no of cases}}{\text{Exhaustive no of cases}} = \frac{1}{6}$

(d) None of the above

(5) Physical fixation includes — All (d)

(6) Rotary microtome is used to cut sections embedded in — All (d)

(7) Feulgen nuclear reaction gives —
(a) green color to cytoplasm

(8) Methyl green pyronin is for localization of — DNA (b)

(9) Millon reaction for tyrosine was given by — (b) Baker 1956

(10) Calcium lipase method for triglycerides gives — none of the above

(2) Importance of fixation - For various histological and cytological study of a biological material, if a piece of tissue is cut out of a living or recently dead organism and no special care is taken to keep it alive or maintain its structure, it will soon undergo marked changes. When it will in the air or in fluid, it may be shrinken or swollen or it may be attacked by bacteria and moulds. Besides this, tissue will gradually fall to piece by self digestion or autolysis. For these reasons, it is usual to treat a piece of a tissue with a fluid called as fixative.

To preserve a piece of tissue one requires a fluid that will not shrink or swell or dissolve or distort, will kill bacteria and moulds and will render the autolytic enzymes inactive, such fluid is a preservative. A fixative must do everything that a preservative does, but in addition, it must modify the tissue in such a way that they become capable of resisting subsequent treatment of various chemicals. Of these chemicals / treatments, the ones that are most likely to cause damage are embedding, sectioning and mounting. Fixation usually makes many tissue constituents readily colorable by suitable dye. Essential function of fixation is stabilization of protein part of the framework of the cell.

Characteristics of fixatives – For proper fixation of tissue, fixatives must have following characteristics –

1> Non proteolytic in nature – The first requisement of a fixative is that it should not be proteolytic. Any substance that breaks the peptide links and set free soluble amino-acids is the opposite of a fixative.

2> Quick penetration – Different primary fixatives penetrate into tissues at different rates. It is desirable that a fixative should penetrate quickly so that the tissue may be stabilized in structure before autolysis has damaged it

3> Unalterability to material – Ideally a fixative would leave the volume of a piece of a tissue and of its constituent cells unaltered, and resistant to alteration by fluid in which it was subsequently placed.

4> Salinity – Addition of 'indifferent' or non fixative salts to fixatives often improves the results obtained. A saline solution may be prepared having about same osmotic pressure as the body fluid of the organism from which tissue is to be taken and the fixative substance should be dissolved in this instead of distilled water

(3) Any four kinds of microtomes -

There are several types of microtomes and each one is designed for specific purpose. However, several microtomes have multifunctional roles -

(i) Rotary microtome - The rotary microtome is often referred to as the "Minot" after its inventor. The basic mechanisms require the rotation of a fine advance hand wheel by 360°, moving specimen vertically past the cutting surface and returning it to starting position. Rotary microtome may be manual, semi automated or fully automated. The mechanism for block advancement may be retracting or non-retracting. It has ability to cut thin(2-3 μm) sections and its easy adaptation to all type of tissues (hard, fragile and fatty) sectionings. Technological advances in automation of microtomy have improved section quality, increased productivity and improved occupational safety for the technologists

(ii) Base sledge microtomes - With the sledge microtomes, specimen is held stationary and the knife slides across the top of specimen during sectioning. Used primarily for large blocks, hard tissues, or whole mounts, it is especially useful in neuropathology and opthalmic pathology. Three

micron sections are difficult to produce

(iii) Rotary rocking microtome - It is commonly used in cryostate. Retracting action moves the tissue block away from the knife on upstroke, producing a flat face to tissue block. Modern version of this microtome are small & lightweighted instrument, which are very suitable for classwork and teaching of the microtomy. Because of the lightness of the machine and rather jerky, pulling action required to operate the feed mechanism, microtome may tend to move about on the bench during operation. This may be prevented by placing microtome on a mat of non slippery material such as sponge rubber.

(iv) Sliding microtome - In this microtome, knife or blade is stationary and specimen slides under it during sectioning. This is generally used for cutting nitro-cellulose embedded tissue or celloidin-embedded tissue blocks. It is not good for cutting large and hard materials such as cartilage, bones, entire eyes etc.

④ Standard alcian blue technique — Alcian blue is a large conjugated dye molecule that initially was used for the dyeing of textile fibres. It is composed of a central copper containing phthalocyanine ring linked to four isothiouronium groups via thioether bonds. Iso thiouronium groups are moderately strong bases and account for cationic nature of alcian blue.

Preparation of alcian blue —

Alcian blue solution —

Alcian blue 8GX — 1g
3% acetic acid solution — 100ml
} mix well

Nuclear fast red —

Aluminium sulphate $(Al_2SO_4)_3.18H_2O$ — 5g
Distilled water — 100ml
Nuclear fast red — 0.1g

Dissolve aluminum sulphate in water with heat. Add nuclear fast red to water while still hot and filter it.

Protocol to stain biological material —

1- Dewax slide preparation in xylene and rehydrate it through graded series of ethyl alcohol to distilled water.
2- Dip the slide in alcian blue solution for 30 minutes.
3- Rinse in running tap water for 5 minutes.
4- Counterstain in nuclear fast red for 10 minutes.

5- Wash in running tap water for 1 minute
6. Dehydrate in graded ethyl alcohol.
7. Clear in xylene and mount in a miscible medium i.e. DPX mountant.

Observations -

→ Acid mucins, proteoglycans and hyaluronic acid appear in blue color.

→ Nuclei appear in red color.

(5) Methyl green pyronin –

Methyl green is an impure dye containing methyl violet. By removing methyl group with chloroform, pure methyl green appears to be specific for DNA.

The rationale of technique is that both dyes are cationic when used in combination. Methyl green binds preferentially, and specifically to DNA leaving pyronin to bind to RNA. Methyl green specific reactivity is attributed to the spatial alignment of the $-NH_2$ group of dye to phosphate radicals on DNA double helix. On the other hand, pyronin staining does not show this spatial affinity and any negatively charged tissue constituent will stain red. This means that in practice, as well as RNA, acid mucins and cartilages also will stain.

Methodology –

Fixation — Carnoy preferred but formalin acceptable.

Solutions –

Methyl green pyronin Y –

2% methyl green in dH_2O (chloroform washed) → 9 ml

2% pyronin Y in dH_2O → 4 ml

Acetate buffer pH 4.8 → 23 ml.

Glycerol → 14 ml

50 ml

Mix well before use

Protocol –

1- take sections down to water.
2- Rinse in acetate buffer pH 4.8
3- Place in methyl green pyronin Y solution for 25 minutes.
4- Rinse in buffer
5- Blot dry
6- Rinse in 95% ethyl alcohol
7- Rinse in Absolute alcohol
8- Rinse in xylene and mount

Observations –

DNA ——— Green blue color
RNA ——— Red color

(6) Basic principle of fluorescence staining —

Fluorescence is a term that refers to emit light of visible region after absorbance of radiation of near UV region. Any dye that emits fluorescence, plays important role in fluorescence staining in histology. Fluorescent dye may be attached directly to cellular components or may be attached indirectly via antigen or antibodies to biological material. Immunofluorescence is a laboratory technique whereby antigens are detected by specific antibodies that are conjugated directly to a readily identifiable fluorescent label. This method has been well established in the identification and localization of antigen deposits and cells within tissue sections.

A method called direct immunofluorescence, whose specific primary antibody is conjugated directly with the fluorochrome and viewed under a fluorescent microscope. On the other hand, in indirect immunofluorescent method, specific primary antibody is unlabeled and is detected in a second stage by use of fluorochrome labeled antispecies specific ~~ant~~ immunoglobulin antiserum.

Immunofluorescent methods can be used to detect antigens such as double stranded DNA antibodies as well as cell surface receptors on lymphocytes for example. The advantage of

tissue immunofluorescence method over other immunological-methods like ELISA, is the ability to not only identify the presence of antigen but also identify the deposition site within the tissue section. Success in immunofluorescent staining techniques depends upon many factors including —

(a) Preservation of substrate antigens and quality of tissue sections.

(b) Affinity and specificity of antibodies and conjugates

(c) Detection method

(d) Microscope and

(e) Quality control of staining procedure.

(7) Calculate mean for the following distribution

Marks obtained	No of Student	x	$d = \frac{x-A}{h}$, A = 55.5	fd
11 – 20	4	15.5	−4	−16
21 – 30	12	25.5	−3	−36
31 – 40	40	35.5	−2	−80
41 – 50	41	45.5	−1	−41
51 – 60	27	55.5	0	0
61 – 70	13	65.5	1	13
71 – 80	9	75.5	2	18
81 – 90	4	85.5	3	12
	N = 150			Σfd = −130

$$\bar{X} = A + \frac{h \, \Sigma fd}{N}$$

$$= 55.5 + 10 \times \frac{-130}{150}, = 55.5 - 8.6$$

Ans = 46.83

(13)

8→ Find mean deviation from the median for the following data -

Class Interval	Frequency	Less than CF	X	X − Md	F |X−Md|
0 − 10	8	8	5	20	160
10 − 20	12	20	15	10	120
20 − 30	10	30 (Median class)	25	0	0
30 − 40	8	38	35	10	80
40 − 50	3	41	45	20	60
50 − 60	2	43	55	30	60
60 − 70	7	50	65	40	280
	Σf or N = 50				Σ F|X−Md| = 760

(1) $N/2 = 25$

$$\text{Median} = l + \frac{h}{f}\left(\frac{N}{2} - C\right)$$

$$= 20 + \frac{10}{10}(25-20), = 20 + 5$$

Median = 25

$$\text{Mean deviation about Median} = \frac{\Sigma F|X-Md|}{N} = \frac{760}{50}$$

Ans = 15.2

(9) Calculate the coefficient of regression of Y on X –

Marks in Theory (X)	Marks in Practical (Y)	$X-\bar{X}$	$Y-\bar{Y}$	$(X-\bar{X})^2$	$(X-\bar{X}).(Y-\bar{Y})$
25	43	-7	5	49	-35
28	46	-4	8	16	-32
35	49	3	11	9	33
32	41	0	3	0	0
31	36	-1	-2	1	2
36	32	4	-6	16	-24
29	31	-3	-7	9	21
38	30	6	-8	36	-48
34	33	2	-5	4	-10
32	39	0	1	0	0
$\Sigma X = 320$	$\Sigma Y = 380$			$\Sigma(X-\bar{X})^2 = 140$	$\Sigma(X-\bar{X}).(Y-\bar{Y}) = -93$

$$\bar{X} = \frac{\Sigma X}{n} = \frac{320}{10} = 32$$

$$\bar{Y} = \frac{\Sigma Y}{n} = \frac{380}{10} = 38$$

Coefficient of regression of y on x –

$$b_{yx} = \frac{\Sigma |(X-\bar{X}).|Y-\bar{Y}|}{\Sigma (X-\bar{X})^2} = \frac{-93}{140}$$

Ans = −0.664