



SRI LAKSHMI NARAYANA INSTITUTE OF MEDICAL SCIENCES
OSSUDU AGARAM VILLAGE; KUDAPAKKAM POST, PONDICHERRY - 605003

Date 4.1.2021

From
Dr. Pammy sinha ,
HOD
Pathology
SriLakshmiNarayanaInstituteofMedicalSciences,Puducherry
Bharath Institute of Higher Education and Research,
Chennai.

To
The Dean,
SriLakshmiNarayanaInstituteofMedicalSciences,Puducherry
Bharath Institute of Higher Education and Research,
Chennai.

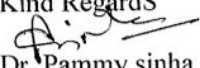
Sub: Permission to conduct value-added course: Hematoxylin and Eosin staining

Dear Sir,

With reference to the subject mentioned above, the department proposes to conduct a value-added course titled: Hematoxylin and Eosin staining

On Feb - April 2021. We solicit your kind permission for the same.

Kind RegardS


Dr. Pammy sinha

FOR THE USE OF DEANS OFFICE

Names of Committee members for evaluating the course:

The Dean: Dr. Jayakumar

The HOD: Dr. PAMMY SINHA

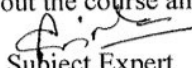
The Expert: Dr. PAMMY SINHA

The committee has discussed about the course and is approved.

Dean

(Sign & Seal)

DEAN

Subject Expert

(Sign & Seal)

(Sign & Seal)

HOD

(Sign & Seal)

(Sign & Seal)

SRI LAKSHMI NARAYANA INSTITUTE OF MEDICAL SCIENCES
OSSUDU, AGARAM VILLAGE,
KODAPAKKAM POST,
PUDUCHERRY - 605 502

PROFESSOR & HEAD, DEPT. OF PATHOLOGY
SRI LAKSHMI NARAYAN INSTITUTE OF
MEDICAL SCIENCES,
PUDUCHERRY - 605 502.

PROFESSOR & HEAD, DEPT. OF PATHOLOGY
SRI LAKSHMI NARAYAN INSTITUTE OF
MEDICAL SCIENCES,
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SRI LAKSHMI NARAYANA INSTITUTE OF MEDICAL SCIENCES
OSSUDU AGARAM VILLAGE; KUDAPAKKAM POST, PONDICHERRY - 605003

11.1.2021

Circular

Sub: Organising Value-added Course: Hematoxylin and Eosin staining

With reference to the above mentioned subject, it is to bring to your notice that **SRI LAKSHMI NARAYANA INSTITUTE OF MEDICAL SCIENCES Bharath Institute of Higher Education and Research**, is organising "Hematoxylin and Eosin staining" from **Feb 2021**. The course content is enclosed below."

The application must reach the institution along with all the necessary documents as mentioned. The hard copy of the application should be sent to the institution by registered/ speed post only so as to reach on or before 30.1.2021. Applications received after the mentioned date shall not be entertained under any circumstances.

Encl: Copy of Course content


Dean

DEAN
SRI LAKSHMI NARAYANA INSTITUTE OF MEDICAL SCIENCES
OSUDU, AGARAM VILLAGE,
KUDAPAKKAM POST,
PUDUCHERRY - 605 502

Course Proposal

Course Title: Hematoxylin and Eosin Staining

Course Objective:

1. To define the staining properties and characteristics
2. Should know about the principles of hematoxylin and eosin staining.
3. Should be able to perform the hematoxylin and eosin staining and should also know about the trouble shooting

Course Outcome: Should know about the hematoxylin and eosin staining in detail

Course Coordinator: Dr.Pammy sinha

Course Faculties with Qualification and Designation:

1. Dr.Pammy sinha, Professor & HOD

2. Dr.Sivaganesh@Porko, Assistant Professor

Course Curriculum/Topics with schedule

SINo	Date	Topic	Faculty	Time	Hours
1.	4.02.2021	Hematoxylin properties and classification	Dr.Pammy Sinha	1.30-4 pm	2.5 hrs
2.	11.02.2021	Types of Alum hematoxylin	Dr.Sivaganesh @porko	1.30-4 pm	2.5 hrs
3.	18.02.2021	Iron hematoxylin	Dr.Pammy Sinha	1.30-4 pm	2.5 hrs
4.	25.02.2021	Tungsten hematoxylin	Dr.Sivaganesh @porko	1.30-4 pm	2.5 hrs
5.	4.03.2021	Lead hematoxylin	Dr.Pammy Sinha	1.30-4 pm	2.5 hrs
6.	11.03.2021	Molbdenum hematoxylin	Dr.Sivaganesh @porko	1.30-4 pm	2.5 hrs
7.	18.03.2021	Eosin Characteristics and Types	Dr.Pammy Sinha	1.30-4 pm	2.5 hrs
8.	25.03.2021	Automation	Dr.Sivaganesh @porko	1.30-4 pm	2.5 hrs
		Practical Class			

9.	1.04.2021	Hands on training of Hematoxylin and Eosin staining	Dr.Pammy Sinha	1.30-4 pm	2.5 hrs	
10.	8.04.2021	Drying and Mounting	Dr.Sivaganesh @porko	1.30-4 pm	2.5 hrs	
11.	15.04.2021	Labelling	Dr.Pammy Sinha	1.30-4 pm	2.5 hrs	
12	22.04.2021	Assessment and giving feedback	Dr.Sivaganesh @porko	1.30-4 pm	2.5 hrs	
			Total			30 hrs

REFERENCE BOOKS:

1 Theory and Practice of Histological Techniques

Book by John D Bancroft

2 Culling histotechniques

VALUE ADDED COURSE

1. Name of the programme & Code

Hematoxylin and Eosin Staining and PA02

2. Duration & Period

30 hrs & Feb – April 2021

3. Information Brochure and Course Content of Value Added Courses

Enclosed as Annexure- I

4. List of students enrolled

Enclosed as Annexure- II

5. Assessment procedures:

Multiple choice questions- *Enclosed as Annexure- III*

6. Certificate model

Enclosed as Annexure- IV

7. No. of times offered during the same year:

8. Year of discontinuation: 2022

9. Summary report of each program year-wise

Value Added Course- Feb – April 2021					
Sl. No	Course Code	Course Name	Resource Persons	Target Students	Strength & Year
1	PA02	Hematoxylin & Eosin Staining	Dr. Pammy Sinha	2 nd MBBS	Feb - April 2021

10. Course Feed Back

Enclosed as Annexure- V

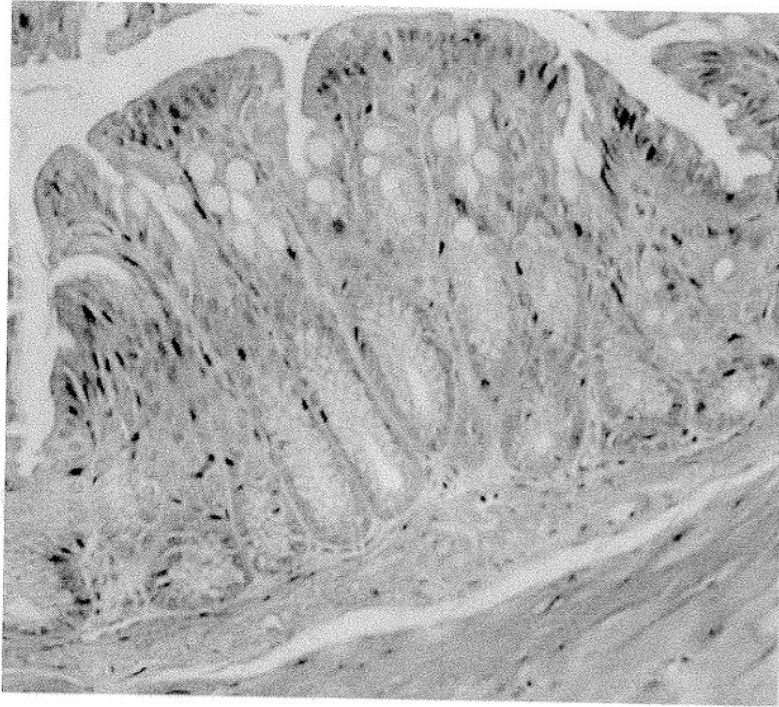

RESOURCE PERSON

PROFESSOR & HEAD, DEPT. OF PATHOLOGY
SRI LAKSHMI NARAYAN INSTITUTE OF
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COORDINATOR

PROFESSOR & HEAD, DEPT. OF PATHOLOGY
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Hematoxylin & Eosin Staining



PARTICIPANT HAND BOOK

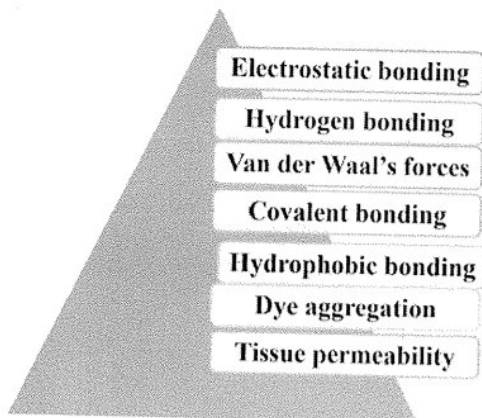
COURSE DETAILS

Particulars	Description
Course Title	Hematoxylin & Eosin Staining
Course Code	PA02
Objective	<ul style="list-style-type: none"> ⊖ Hematoxylin <ul style="list-style-type: none"> • Principles of hematoxylin • Oxidation • Mordant ⊖ Classification of hematoxylin ⊖ Alum hematoxylin <ul style="list-style-type: none"> • Method of use of alum hematoxylin • Bluing • Differentiation • Deterioration of the hematoxylin • Types of alum hematoxylin • Staining time with alum hematoxylin • Disadvantages of alum hematoxylin ⊖ Eosin <ul style="list-style-type: none"> • Introduction • Types of eosin commercially available • Substitutes for eosin • Differentiation • Difficulties encountered
Further learning opportunities	Automated H&E Staining
Key Competencies	On successful completion of the course the students will have skill in performing H&E staining
Target Student	2 nd MBBS Students
Duration	30hrs Feb-April 2021
Theory Session	20hrs
Practical Session	10hrs
Assessment Procedure	Short Notes

STAINING

It is the process of coloring cells, cellular constituent & tissue fibers to facilitate optical differentiation by microscopic examination. It is the union between a colored dye & a tissue substrate which resists simple washing. It involves visual labeling of some entity by attaching, or depositing in its vicinity a marker of characteristic color or shape. Stain is the marker or reagent used to generate the marker.

STAINING MECHANISMS



INTRODUCTION

Hematoxylin & eosin stain is the most widely used histologic stain. Hematoxylin component stains \diamond cell nuclei blue/black with good intranuclear detail. Eosin stains cell cytoplasm & most connective tissue fibers in varying shades & intensities of pink, orange & red. Hematoxlin - Greek word Haimato(blood) and Xylon(wood), referring to its dark red color in natural state and to its origin(wood). It is extracted from the core of the tree HAEMATOXYLON CAMPECHIANUM. The hematoxylin is extracted from logwood with hot water and then precipitated out from the aqueous solution using urea.

NATURALLY RIPENED HEMATOXYLINS	CHEMICALLY RIPENED HEMATOXYLINS
Ripening by exposure to light & air	Ripening by exposure to chemical oxidizing agents.
Slow process (3-4 months)	Ripening instantaneous, ready to use immediately after preparation
Long shelf life, retain stability for a long time	Shorter shelf life (because of continuing oxidation process in air & light eventually destroys much of the hematein converting it into a colourless compound)
Example <ul style="list-style-type: none"> • Ehrlich's hematoxylin • Delafield's hematoxylin. 	Example <ul style="list-style-type: none"> • Sodium iodate in Mayer's hematoxylin (SIM) • Mercuric chloride in Harris's hematoxylin (MCh)

MORDANTS

Biological staining – substance intermediate between dye and tissue. Hematin is anionic. Tissue is also anionic. Therefore, hematin has poor affinity for tissue Making hematin inadequate as a nuclear stain without the presence of a 3rd element (mordant). Mordant forms a link between the “tissue and the stain”

MOLECULAR PROPERTY OF MORDANTS

Mordants are always di-valent and tri-valent salts or hydroxides of metals. • They combine as hydroxides with the dye by displacing a hydrogen atom from the dye. • The remaining valences of the mordant serves to attach/bind the dye-mordant complex to the tissue components (phosphate groups of the nucleic acid) . Although simple salts such as sulfates and chlorides will do, generally double sulfates or alums are used. The double sulfates have

- A. An active usually trivalent metal such as iron, aluminium or chromium,
- B. Together with potassium or ammonium as a second cation. Most Commonly Used
 - 1) Salts of aluminium in the form of potash alum or ammonium alum.
 - 2) Salts of iron

3) Salts of Tungsten.

CLASSIFICATION OF HEMATOXYLIN

I. Based on the Oxidation Procedure

1. Natural oxidation – Ehrlich's and Delafield's
2. Chemical Oxidation - Mayer's and Harris

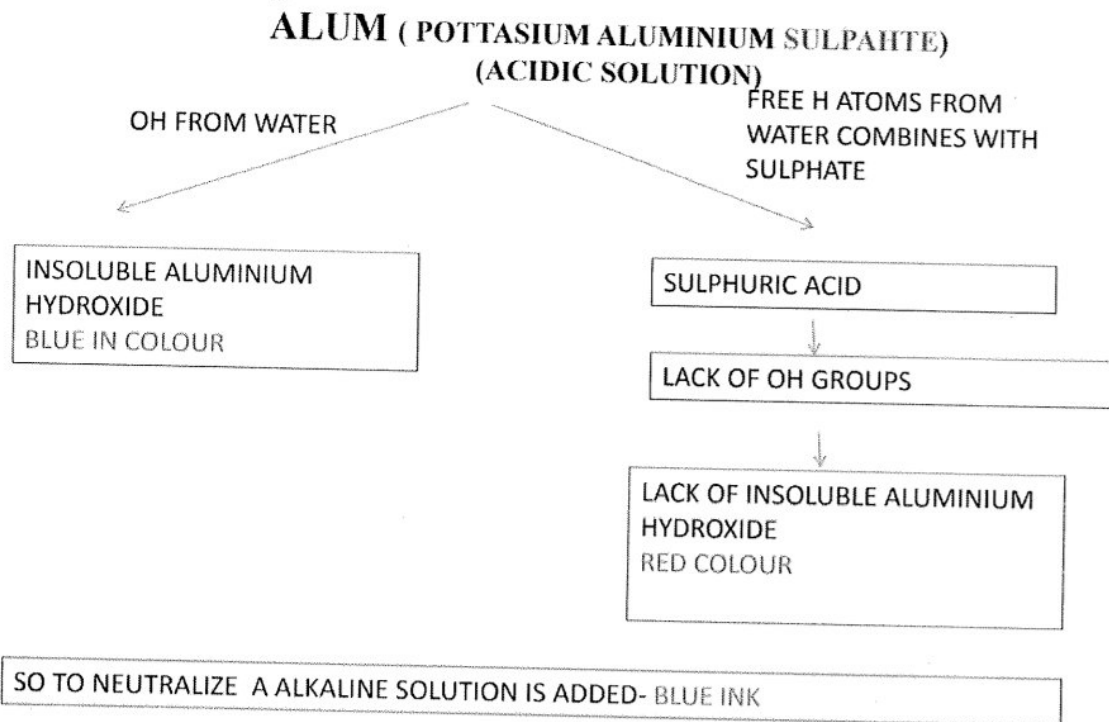
II. Based on the Mordant Used

1. Alum hematoxylin
2. Iron hematoxylin
3. Tungsten hematoxylin
4. Lead hematoxylin
5. Molybdenum hematoxylin
6. Hematoxylin without mordant

ALUM HEMATOXYLIN: Routinely used. Mordant - "potash alum" (aluminium potassium sulfate) or "ammonium alum" (aluminium ammonium sulfate). Alum hematoxylin can be used in 2 ways
Regressively - the section is over stained & then differentiated in acid alcohol, followed by "bluing".
Progressively – stained for a pre-determined time so as to adequately stain the nuclei but leave the background tissue relatively unstained.

BLUING: After differentiating the hematoxylin with acid alcohol, the nuclei in tissue are red colour. This red colour is converted to blue black when section is washed in weak alkali solution.

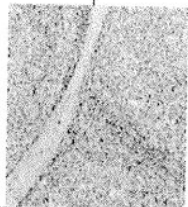
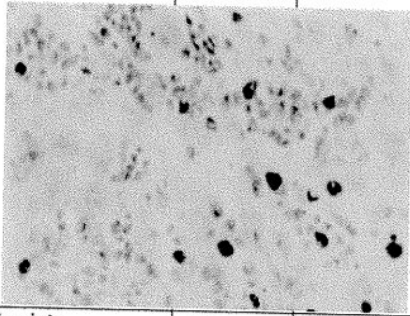
Principle of bluing



TYPES OF ALUM HEMATOXYLIN

1. Ehrlich's hematoxylin (Ehrlich 1886)
2. Delafield's hematoxylin (Delafield 1885)
3. Mayer's hematoxylin (Mayer 1903)
4. Harris hematoxylin (Harris 1900)
5. Cole's hematoxylin (Cole 1943)
6. Carazzi's hematoxylin (Carazzi 1991)
7. Gill's hematoxylin (Gill et al 1974)

Delafield's	Alum	Natural	Hematoxylin (4g) 15g/100ml Saturated aqueous Ammonium alum (400ml) 95% alcohol (125g) Glycerin (100ml)	Nuclear stain	Lasts long
Mayer's Progressive + regressive	Alum	Sodium iodate	Hematoxylin Potassium/ammonium alum Sodium iodate (mordant) Distilled water Citric acid(acidifying agents) Chloral hydrate(preservative)	Nuclear stain. Nuclear counterstain - during demonstration of glycogen.	Glycogen demonstration - Stain is applied for short time (5 - 10 mins) until the nuclei is stained and is then "blued" without any differentiation
Harris's Progressive (cytology) + regressive	Alum	Mercuric chloride	Hematoxylin Potassium alum Mercuric oxide or Sodium iodate Distilled water Absolute alcohol Glacial acetic acid	General purpose hematoxylin. Nuclear stain	Differentiation is required. Mercuric chloride - highly toxic, environmental unfriendly, corrosive on automated staining machines
Cole's	Alum	Alcoholic Iodine	Hematoxylin Potash alum 1% iodine in 95% alcohol	Nuclear staining with eosin	



Hematoxylin	Mordant	Oxidizing / ripening agent	Composition	Application	Other features
Ehrlich's Progressive	Alum	Natural	Hematoxylin Potassium alum Distilled water Glacial acetic acid Absolute alcohol Glycerin	<u>Nuclear stain.</u> <u>Mucins</u> MPS of cartilage. Staining of bone.	Takes 2 months to ripen Emergency - <i>sodium iodate</i> Glycerin - slow the oxidation Lasts long suitable for tissues that have been subjected to acid decalcification or, more valuably, tissues that have been stored for a long period in formalin fixatives which have gradually become acidic over the storage period not ideal for frozen sections

STEPS IN STAINING PROCEDURE FOR ALUM HEMATOXYLIN

- 1) Dewaxing the sections (hot plate and then into xylene)
- 2) Hydrating the sections (through graded alcohols 100%, 90%, 80%)
- 3) Bring the sections to water
- 4) Nuclear stain (Hematoxylin – harris – 5 – 10 mins)
- 5) Differentiation (1% acid alcohol = 1% HCl in 99ml 70% alcohol) – 5-10s
- 6) Wash well in tap water until sections are 'blue'(10-15 minutes)
- 7) Bluing - Blue by dipping in an alkaline solution (eg.ammonia water), followed by 5 min tap water wash.
- 8) Stain in 1% Eosin Y for 10 min
- 9) Dehydration
- 10) Clearing
- 11) Mounting

EOSIN: Most suitable stain to combine with alum hematoxylin. It has the ability for proper differentiation to distinguish between the cytoplasm of different types of cells & between the different types of connective tissue fibers and matrices, by staining them different shades of red and pink. Eosins are xanthine dyes (tetrabromofluorescein)

TYPES OF EOSIN - commercially available

⊗ Eosin Y

⊗ Ethyl eosin

⊗ Eosin B

SUBSTITUTE FOR EOSIN: Phloxine, Bierbrich scarlet – gives a more intense red color to the tissues. They are rarely as amenable to subtle differentiation as eosin and are generally less valuable

DIFFERENTIATION OF EOSIN: Occurs in the subsequent tap water wash. Further differentiation occurs during the dehydration through the alcohols.



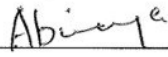
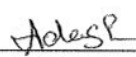



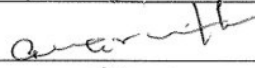
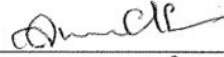
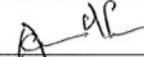

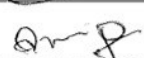
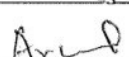
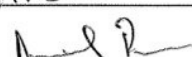
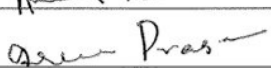

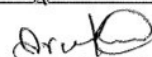
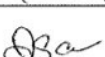
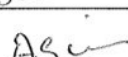
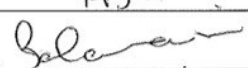
DIFFICULTIES ENCOUNTERED Under circumstances Eosin staining is intense and difficulty may be experienced in obtaining adequate differentiation (this may occur after mercuric fixation). Over differentiation of the eosin may be continued until only red blood cells and granules of eosinophil polymorph are stained red. This is occasionally used to facilitate the location and identification of eosinophils.

RESULTS	
Nuclei, RNA rich cytoplasm	Blue/black
Cytoplasm	Varying shades of pink
Muscle fibers	Deep pink/red
RBCs	Orange/red
Fibrin	Deep pink
Fungal hyphae	Faintly <u>hematoxyphilic</u>
Calcium deposits	Deep blue-black
Keratin	Bright red

VALUE ADDED COURSE

Hematoxylin & Eosin Staining and PA 102

List of Students Enrolled Feb - April 2021

2ND Year MBBS Student			
Sl. No	Roll NO	Name of the Student	Signature
1	U15MB250	AARTHI .H	
2	U15MB251	ABARNA. M	
3	U15MB252	ABINAYA.J	
4	U15MB253	ADARSH .S	
5	U15MB254	AGILAN .A	
6	U15MB255	AKSHAYA .S	
7	U15MB256	AKSSHAYA .M.R	
8	U15MB257	AMARNATH. S	
9	U15MB258	AMUDHESAR .K.M	
10	U15MB259	ANANDH.S	
11	U15MB260	ARCHITH VIGNESH .B	
12	U15MB261	ARJUNBALAJI .A	
13	U15MB262	ARUL NIVETHINI V.A	
14	U15MB263	ARUL PRINCE. E	
15	U15MB265	ARUN PRASAD. K	
16	U15MB266	ARUNA .S	
17	U15MB264	ARUNKUMAR .S	
18	U15MB267	ASAN THASTHAGIR. M	
19	U15MB268	ASWIN. B	
20	U15MB269	BALAMANI KANDAN. S	


RESOURCE PERSON

PROFESSOR & HEAD, DEPT. OF PATHOLOGY
SRI LAKSHMI NARAYAN INSTITUTE OF
MEDICAL SCIENCES,
PUDUCHERRY - 605 002.


COORDINATOR

PROFESSOR & HEAD, DEPT. OF PATHOLOGY
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PUDUCHERRY - 605 002.



**SRI LAKSHMI NARAYANA INSTITUTE OF HIGHER EDUCATION
AND RESEARCH**

HEMATOXYLIN & EOSIN STAINING

SHORT NOTES

Course Code: PA02

I. ANSWER ALL THE QUESTIONS

- 1. WRITE SHORT NOTES ON DIFFERENT TYPES OF HEMATOXYLIN?**
- 2. STAINING PROCEDURE FOR HEMATOXYLIN & EOSIN STAINING?**
- 3. WRITE SHORT NOTES ON PRINCIPLE OF STAINING?**
- 4. EXPLAIN THE TYPES OF EOSIN AND ITS SOURCE?**
- 5. PRINCIPLE OF BLUING?**

Hematoxylin & Eosin Staining

21
30

1 Different types of Hematoxylin

- Alum
- Iron
- Tungsten
- Molybdenum
- Lead
- Hematoxylin with mordants

2 Staining procedure

- Dewax
- Hydrate with graded alcohols
- Harris Hematoxylin - 5-10 min
- Differentiation with 1% acid alcohol
- Blueing for 10-15 min
- Stain with 1% Eosin - 10 min

hematoxylin and eosin staining.

1. Write short notes on different types of hematoxylin?

22
30

There are 6 different types of hematoxylin: β

- (1) Alum haematoxylin ✓
- (2) Iron haematoxylin
- (3) Tungsten haematoxylin
- (4) Molybdenum haematoxylin
- (5) Lead haematoxylin
- (6) Haematoxylin without mordants

(1) Alum haematoxylin - Mordant used are aluminium salts, either aluminium potassium sulphate or aluminium ammonium sulphate.

(2) Iron haematoxylin - Iron salts are used as mordant, these are also oxidizing agents.

Types:

- Weigert's haematoxylin
- Heidenhain's haematoxylin
- Verhoeff's haematoxylin
- Loyez haematoxylin.

(3) Tungsten haematoxylin - Malloxy phosphotungstic acid haematoxylin (PTAH) and phosphotungstic acid is used as mordant.



Sri Lakshmi Narayana Institute of Medical Sciences


Affiliated to Bharath Institute of Higher Education & Research
(Deemed to be University under section 3 of the UGC Act 1956)



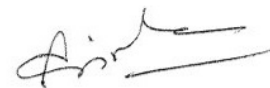
CERTIFICATE OF MERIT

This is to certify that ABARNA.J has

actively participated in the Value Added Course on *HEMATOXYLIN & EOSIN STAINING*
held during Feb - April 2021 Organized by Sri Lakshmi Narayana Institute of Medical
Sciences, Pondicherry- 605 502, India.


Dr. PAMMY SINHA
RESOURCE PERSON

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PUDUCHERRY - 605 502.


Dr. PAMMY SINHA
COORDINATOR

PROFESSOR & HEAD, DEPT. OF PATHOLOGY
SRI LAKSHMI NARAYAN INSTITUTE OF
MEDICAL SCIENCES,
PUDUCHERRY - 605 502.



Sri Lakshmi Narayana Institute of Medical Sciences

Affiliated to Bharath Institute of Higher Education & Research
(Deemed to be University under section 3 of the UGC Act 1956)



CERTIFICATE OF MERIT

This is to certify that ANANDH.S has

actively participated in the Value Added Course on *HEMATOXYLIN & EOSIN STAINING*
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Sciences, Pondicherry- 605 502, India.


Dr. PAMMY SINHA

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Dr. PAMMY SINHA

COORDINATOR

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MEDICAL SCIENCES,
PUDUCHERRY - 605 502.

Student Feedback Form

Course Name: HEMATOXYLIN & EOSIN STAINING

Subject Code: PA02

Name of Student: AKSHAYA S Roll No.: U15HB255

We are constantly looking to improve our classes and deliver the best training to you. Your evaluations, comments and suggestions will help us to improve our performance

Sl. NO	Particulars	1	2	3	4	5
1	Objective of the course is clear				✓	
2	Course contents met with your expectations					✓
3	Lecturer sequence was well planned				✓	
4	Lectures were clear and easy to understand					✓
5	Teaching aids were effective					✓
6	Instructors encourage interaction and were helpful					✓
7	The level of the course					✓
8	Overall rating of the course	1	2	3	4	5

** Rating: 5 - Outstanding; 4 - Excellent; 3 - Good; 2 - Satisfactory; 1 - Not-Satisfactory*

Suggestions if any:

Date:

Signature

Student Feedback Form

Course Name: HEMATOXYLIN & EOSIN STAINING

Subject Code: PA02

Name of Student: ASWIN B Roll No.: U15HB268

We are constantly looking to improve our classes and deliver the best training to you. Your evaluations, comments and suggestions will help us to improve our performance

Sl. NO	Particulars	1	2	3	4	5
1	Objective of the course is clear				✓	
2	Course contents met with your expectations					✓
3	Lecturer sequence was well planned				✓	
4	Lectures were clear and easy to understand				✓	
5	Teaching aids were effective				✓	
6	Instructors encourage interaction and were helpful				✓	
7	The level of the course					✓
8	Overall rating of the course	1	2	3	4	5

* Rating: 5 - Outstanding; 4 - Excellent; 3 - Good; 2 - Satisfactory; 1 - Not-Satisfactory

Suggestions if any:

Date:

Signature

Date: 22.04.2021

From

Dr.Pammy sinha
Professor and Head,
Department of pathology
Sri Lakshmi Narayana Institute of Medical Sciences
Bharath Institute of Higher Education and Research,
Chennai.

Through Proper Channel

To

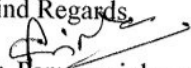
The Dean,
Sri Lakshmi Narayana Institute of Medical Sciences
Bharath Institute of Higher Education and Research,
Chennai.

Sub: Completion of value-added course: Hematoxylin and Eosin Staining

Dear Sir,

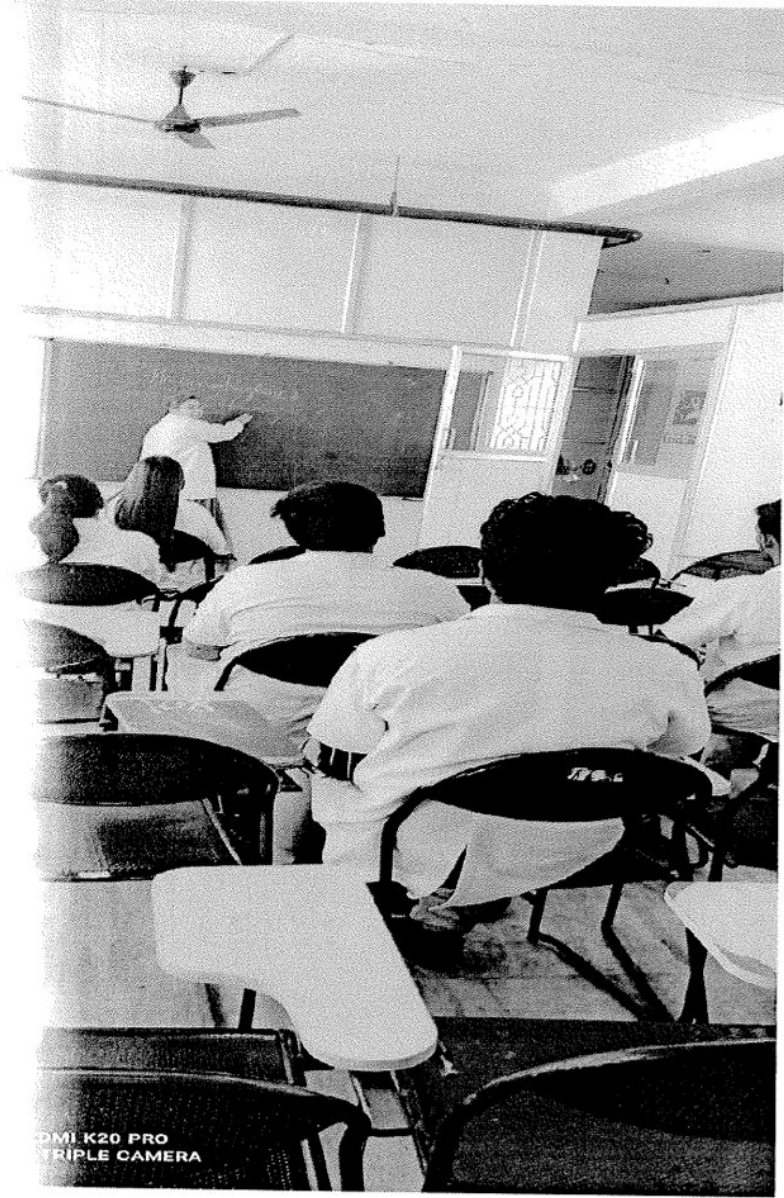
With reference to the subject mentioned above, the department has conducted the value-added course titled: **Hematoxylin and Eosin Staining** in IInd MBBS ,FEB- APRIL 2021 for 20 students . We solicit your kind action to send certificates for the participants, that is attached with this letter. Also, I am attaching the photographs captured during the conduct of the course.

Kind Regards,


Dr. Pammy sinha
PROFESSOR & HEAD, DEPT. OF PATHOLOGY,
SRI LAKSHMI NARAYAN INSTITUTE OF
MEDICAL SCIENCES,
PUDUCHERRY 605 502.

Encl: Certificates

Photographs



DMI K20 PRO
TRIPLE CAMERA

