وزارة التعليم العالي والبحث العلمي
الجامعة التقنية الشمالية
المعهد التقني - الدور

الحقيبة التعليمية

القسم العلمي:	تقنيات المختبرات الطبية
اسم المقرر:	شرائح نسيجية
المستوى:	المستوى الاول
الفصل الدراسي:	الاول
السنة الدراسية:	٢٠٢٥ - ٢٠٢٦

اسم المقرر:	**شرائح نسيجية**						
القسم:	تقنيات المختبرات الطبية						
المعهد:	التقني الدور						
المستوى	الاول						
الفصل الدراسي:	الاول						
عدد الساعات الاسبوعية:	نظري	١	عملي	٢			
عدد الوحدات الدراسية:	٣						
الرمز:							
نوع المادة	نظري		عملي		كلاهما	✓	
هل يتوفر نظير للمقرر في الاقسام الاخرى	كلا						
اسم المقرر النظير							
القسم							
رمز المقرر النظير							

معلومات تدريسي المادة

اسم مدرس (مدرسي) المقرر:	**مصطفى طالب خلف**
اللقب العلمي:	
سنة الحصول على اللقب	
الشهادة:	**دبلوم عالٍ / تحليلات مرضية**
سنة الحصول على الشهادة	**٢٠١٩**
عدد سنوات الخبرة (تدريس)	٨

الوصف العام للمقرر

يهتم المقرر بالتعريف بطرق الحصول على شرائح نسيجية من مختلف الأعضاء بغية تشخيص الامراض المختلفة التي تصيبها

الاهداف العامة

- يتمكن الطالب من معرفة تراكيب جسم الانسان على المستوى التشريحي والنسجي
- يتمكن الطالب من إدارة المختبر ومعرفة الآليات مختلفة لاستلام العينات وتسجيلها
- يتمكن الطالب من التفريق بين العينات المختلفة وطرق التعامل معها حسب نوعها
- يتمكن الطالب من معرفة أنواع مختلفة من المواد والسوائل الكيميائية المستخدمة في هذا المقرر وتطبيقاتها والمحاذير المهمة اثناء استخدامها

الأهداف الخاصة

- المعرفة الكاملة بأنواع المواد الحافظة واستخداماتها لحفظ مختلف أنواع العينات.
- المعرفة الكاملة بأنواع العينات النسيجية وكيفية التعامل مع كل نوع.
- التعرف على أنواع الأجهزة المستخدمة في تحضير الشرائح النسيجية وخصوصاً جهاز المشراح الدوار
- تعلم أساليب تقطيع العينات النسيجية وتوجيهها بالاتجاه المناسب لغرض الحصول على شريحة مناسبة للفحص
- تعلم أساليب تلوين الشرائح النسيجية لأغراض مختلفة تعتمد على تحديد أنواع مختلفة من الامراض

الأهداف السلوكية او نواتج التعلم

- فهم النظريات والمفاهيم الأساسية لكل خطوة من خطوات التحضيرات النسيجية
- القدرة على تحليل وتفسير البيانات المتعلقة بتركيب النسيج
- القدرة على استخدام الأدوات والمعدات المختبرية الخاصة بتحضير الشرائح النسيجية
- تطبيق المعرفة عملياً والقدرة على حل المشاكل التي ممكن ان تواجههم في المختبر
- التعامل الأخلاقي والمهني مع العينات الواردة الى المختبر

بعد الانتهاء من الدرس (المحاضرة) سيكون الطالب قادرا على ان:

- يعرف الغاية المهمة من مقرر الشرائح النسيجية
- يميز بين أنواع المواد والسوائل المستخدمة في تحضير الشرائح النسيجية
- يميز بين أنواع الانسجة المختلفة وطرق التعامل مع كل نوع
- يقيم مستوى خطورة المواد الكيمياوية المستخدمة في المختبر وكيفية تلافي المخاطر الممكن حدوثها
- تحضير شرائح نسيجية جيدة ومناسبة للفحص

المتطلبات السابقة

- معرفة تركيب جسم الانسان
- وظائف أجهزة الجسم

أساليب التدريس (حدد مجموعة متنوعة من أساليب التدريس لتناسب احتياجات الطلاب ومحتوى المقرر)

الاسلوب او الطريقة	مبررات الاختيار
١. التدريس المباشر الذي يعتمد على المحاضرات وتقديم المعلومات بصورة مباشرة	نقل معلومات بقدر كبير وبشكل فعال وسريع مما يجعل للمدرس مساحة كافية في التحكم في المحتوى والتوقيت
٢. التعليم التعاوني الذي يعتمد على انشاء مجموعات عمل صغيرة مهمتها انجاز مهام او حل لمشكلات معينة	يعمل هذا الأسلوب على تعزيز التعاون العلمي والعملي بين الطلبة فضلاً عن زيادة في مهارات التواصل والعمل الجماعي
٣. الأسلوب المعتمد على المشاريع من خلال عمل مشاريع طويلة الأمد تشمل المعرفية النظرية والتطبيق العملي	يعزز هذا الأسلوب الفهم العميق للمعلومات النظرية من خلال التطبيق العملي فضلاً عن تطوير مهارات التفكير
٤. التعليم المدمج الذي يضم التعليم التقليدي والتعليم الالكتروني	يسمح هذا الأسلوب للطالب بالوصول الى المعلومات من من خلال موارد تعليمية مختلفة مما يعزز فرص التعليم الفردي
٥. التعليم التجريبي الذي يعتمد على تطبيق أنشطة وتجارب عملية تجعل الطالب على تماس مباشر مع المواد المختبرية	يعزز هذا الأسلوب من قدرة الطالب على ربط المعلومات النظرية بالتطبيق العملي مما يزيد الفهم من خلال التجارب العملية

الكورس الاول							
عنوان الفصل	الوقت		عنوان المحاضرة		طريقة التدريس	التقنيات	طرق القياس
التوزيع الزمني	النظري	العملي					
الأسبوع الاول	١ ساعة	٢ ساعة	تعريف بعض المصطلحات التي تتعامل مع علم الأنسجة وعلم الخلية	التقنيات الدقيقة	تدريس مباشر	تقديم محاضرة بالاعتماد على شاشة عرض	أسئلة مباشرة بعد المحاضرة
				التشريح	تدريس مباشر	تقديم محاضرة بالاعتماد على شاشة عرض – تطبيق عملي (تشريح حيوان تجارب)	أسئلة مباشرة بعد المحاضرة
				الانسجة	تدريس مباشر	تقديم محاضرة بالاعتماد على شاشة عرض	أسئلة مباشرة بعد المحاضرة
				الخلية	تدريس مباشر	تقديم محاضرة بالاعتماد على شاشة عرض	أسئلة مباشرة بعد المحاضرة
				المسؤوليات التقنية	تدريس مباشر	تقديم محاضرة بالاعتماد على شاشة عرض	أسئلة مباشرة بعد المحاضرة
الأسبوع الثاني	١ ساعة	٢ ساعة	طرق جمع العينات	أنواع الخُزع	تدريس مباشر	تقديم محاضرة بالاعتماد على شاشة عرض	أسئلة مباشرة بعد المحاضرة
				طرق اخذ الخُزع	تدريس مباشر	تقديم محاضرة بالاعتماد على شاشة عرض – تطبيق عملي (تشريح حيوان تجارب واخذ خزعات من الأعضاء المختلفة)	أسئلة مباشرة بعد المحاضرة

الكورس الاول						
عنوان الفصل	الوقت		عنوان المحاضرة		طريقة التدريس	التقنيات	طرق القياس
التوزيع الزمني	النظري	العملي					
الأسبوع الثالث	١ ساعة	٢ ساعة	خطوات تحضير الانسجة لغرض الدراسة، التثبيت والمثبتات	التثبيت	تدريس مباشر	تقديم محاضرة بالاعتماد على شاشة عرض	أسئلة مباشرة بعد المحاضرة
				أنواع المثبتات	تدريس مباشر	تقديم محاضرة بالاعتماد على شاشة عرض	أسئلة مباشرة بعد المحاضرة
				طرق التثبيت	تدريس مباشر	تقديم محاضرة بالاعتماد على شاشة عرض	أسئلة مباشرة بعد المحاضرة
				تحضير المثبت	تدريس مباشر	تقديم محاضرة بالاعتماد على شاشة عرض – تطبيق عملي	أسئلة مباشرة بعد المحاضرة
الأسبوع الرابع	١ ساعة	٢ ساعة	المثبتات الروتينية والخاصة وخصائصها	Formalin	تدريس مباشر	تقديم محاضرة بالاعتماد على شاشة عرض – تطبيق عملي	أسئلة مباشرة بعد المحاضرة
				Picric acid	تدريس مباشر	تقديم محاضرة بالاعتماد على شاشة عرض – تطبيق عملي	أسئلة مباشرة بعد المحاضرة
				Acetone	تدريس مباشر	تقديم محاضرة بالاعتماد على شاشة عرض – تطبيق عملي	أسئلة مباشرة بعد المحاضرة
				Zenker’s solution	تدريس مباشر	تقديم محاضرة بالاعتماد على شاشة عرض – تطبيق عملي	أسئلة مباشرة بعد المحاضرة
				Bouin’s solution	تدريس مباشر	تقديم محاضرة بالاعتماد على شاشة عرض – تطبيق عملي	أسئلة مباشرة بعد المحاضرة

الكورس الاول						
عنوان الفصل	الوقت		عنوان المحاضرة		طريقة التدريس	التقنيات	طرق القياس
التوزيع الزمني	النظري	العملي					
الأسبوع الخامس	١ ساعة	٢ ساعة	محاليل غسل العينات	الماء	تدريس مباشر	تقديم محاضرة بالاعتماد على شاشة عرض – تطبيق عملي	أسئلة مباشرة بعد المحاضرة
				الكحول	تدريس مباشر	تقديم محاضرة بالاعتماد على شاشة عرض – تطبيق عملي	أسئلة مباشرة بعد المحاضرة
الأسبوع السادس	١ ساعة	٢ ساعة	التجفيف وانواع المجففات	Ethyl alcohol	تدريس مباشر	تقديم محاضرة بالاعتماد على شاشة عرض – تطبيق عملي	أسئلة مباشرة بعد المحاضرة
				Methyl alcohol	تدريس مباشر	تقديم محاضرة بالاعتماد على شاشة عرض – تطبيق عملي	أسئلة مباشرة بعد المحاضرة
				Butyl alcohol	تدريس مباشر	تقديم محاضرة بالاعتماد على شاشة عرض – تطبيق عملي	أسئلة مباشرة بعد المحاضرة
				Isopropyl alcohol	تدريس مباشر	تقديم محاضرة بالاعتماد على شاشة عرض – تطبيق عملي	أسئلة مباشرة بعد المحاضرة
الأسبوع السابع	١ ساعة	٢ ساعة	الترويق وانواع المروقات	xylol	تدريس مباشر	تقديم محاضرة بالاعتماد على شاشة عرض – تطبيق عملي	أسئلة مباشرة بعد المحاضرة
				Chloroform	تدريس مباشر	تقديم محاضرة بالاعتماد على شاشة عرض – تطبيق عملي	أسئلة مباشرة بعد المحاضرة
				Toluene	تدريس مباشر	تقديم محاضرة بالاعتماد على شاشة عرض – تطبيق عملي	أسئلة مباشرة بعد المحاضرة
				Benzene	تدريس مباشر	تقديم محاضرة بالاعتماد على شاشة عرض – تطبيق عملي	أسئلة مباشرة بعد المحاضرة
				Cedar wood oil	تدريس مباشر	تقديم محاضرة بالاعتماد على شاشة عرض – تطبيق عملي	أسئلة مباشرة بعد المحاضرة

الكورس الاول							
عنوان الفصل	الوقت		عنوان المحاضرة		طريقة التدريس	التقنيات	طرق القياس
التوزيع الزمني	النظري	العملي					
الأسبوع الثامن	١ ساعة	٢ ساعة	الارتشاح وانواع اوساط الطمر	Paraffin wax	تدريس مباشر	تقديم محاضرة بالاعتماد على شاشة عرض – تطبيق عملي	أسئلة مباشرة بعد المحاضرة
				Resin	تدريس مباشر	تقديم محاضرة بالاعتماد على شاشة عرض	أسئلة مباشرة بعد المحاضرة
				Celloidin	تدريس مباشر	تقديم محاضرة بالاعتماد على شاشة عرض	أسئلة مباشرة بعد المحاضرة
الأسبوع التاسع	١ ساعة	٢ ساعة	صب القوالب ونحتها	أنواع القوالب	تدريس مباشر	تقديم محاضرة بالاعتماد على شاشة عرض – تطبيق عملي	أسئلة مباشرة بعد المحاضرة
				طرق صب العينات	تدريس مباشر	تقديم محاضرة بالاعتماد على شاشة عرض – تطبيق عملي	أسئلة مباشرة بعد المحاضرة
				نحت القوالب	تدريس مباشر	تقديم محاضرة بالاعتماد على شاشة عرض – تطبيق عملي	أسئلة مباشرة بعد المحاضرة
الأسبوع العاشر	١ ساعة	٢ ساعة	التقطيع	تقنيات التقطيع	تدريس مباشر	تقديم محاضرة بالاعتماد على شاشة عرض – تطبيق عملي	أسئلة مباشرة بعد المحاضرة
				أنواع أجهزة التقطيع	تدريس مباشر	تقديم محاضرة بالاعتماد على شاشة عرض	أسئلة مباشرة بعد المحاضرة
الأسبوع الحادي عشر	١ ساعة	٢ ساعة	جهاز المشراح وانواعه	تشغيل وصيانة جهاز المشراح	تدريس مباشر	تقديم محاضرة بالاعتماد على شاشة عرض – تطبيق عملي	أسئلة مباشرة بعد المحاضرة
				الاليات الخاصة باستخدام جهاز المشراح	تدريس مباشر	تقديم محاضرة بالاعتماد على شاشة عرض – تطبيق عملي	أسئلة مباشرة بعد المحاضرة
الأسبوع الثاني عشر	١ ساعة	٢ ساعة	تلوين الشرائح النسجية	خصائص الملونات	تدريس مباشر	تقديم محاضرة بالاعتماد على شاشة عرض – تطبيق عملي	أسئلة مباشرة بعد المحاضرة

الكورس الاول						
عنوان الفصل	الوقت		عنوان المحاضرة		طريقة التدريس	التقنيات	طرق القياس
التوزيع الزمني	النظري	العملي					
				خصائص الخلايا	تدريس مباشر	تقديم محاضرة بالاعتماد على شاشة عرض – تطبيق عملي	أسئلة مباشرة بعد المحاضرة
الأسبوع الثالث عشر	١ ساعة	٢ ساعة	الملونات الروتينية للشرائح النسجية	صبغة الهيماتوكسيلين ومكوناتها	تدريس مباشر	تقديم محاضرة بالاعتماد على شاشة عرض – تطبيق عملي	أسئلة مباشرة بعد المحاضرة
				صبغة الايوسين "أنواعها ومكوناتها"	تدريس مباشر	تقديم محاضرة بالاعتماد على شاشة عرض – تطبيق عملي	أسئلة مباشرة بعد المحاضرة
الأسبوع الرابع عشر	١ ساعة	٢ ساعة	الملونات الخاصة للشرائح النسجية	Van gieson method	تدريس مباشر	تقديم محاضرة بالاعتماد على شاشة عرض – تطبيق عملي	أسئلة مباشرة بعد المحاضرة
				Gomori's method for iron	تدريس مباشر	تقديم محاضرة بالاعتماد على شاشة عرض – تطبيق عملي	أسئلة مباشرة بعد المحاضرة
				Mcmanus for glycogen (PAS)	تدريس مباشر	تقديم محاضرة بالاعتماد على شاشة عرض – تطبيق عملي	أسئلة مباشرة بعد المحاضرة
الأسبوع الخامس عشر	١ ساعة	٢ ساعة	الفحص المجهري	طرق استخدام المجهر في فحص الشرائح النسيجية	تدريس مباشر	تقديم محاضرة بالاعتماد على شاشة عرض – تطبيق عملي	أسئلة مباشرة بعد المحاضرة

رقم المحاضرة	**1**
عنوان المحاضرة:	تعريف بعض المصطلحات التي تتعامل مع علم الأنسجة وعلم الخلية
اسـم المدرس:	مصطفى طالب خلف
الفئة المستهدفة:	المستوى الاول
الهدف العام من المحاضرة:	معرفة الطالب تراكيب جسم الانسان على المستوى التشريحي والنسجي
الأهداف السلوكية او مخرجات التعلم:	١. القدرة على تحليل وتفسير البيانات المتعلقة بتركيب النسيج ٢. قدرة الطالب على التفريق بين العينات المختلفة وطرق التعامل معها حسب نوعها
استراتيجيات التيسير المستخدمة	١. التعليم التعاوني ٢. استخدام طريقة التعليم المدمج ٣. التغذية الراجعة ٤. العصف الذهني ٥. التعليم التجريبي
المهارات المكتسبة	١. معرفة تشريح أعضاء جسم الانسان ومواقعها وارتباطها مع بعضها البعض ٢. معرفة اغلب المصطلحات المتعلقة بتحضير الشرائح النسيجية
طرق القياس المعتمدة	أسئلة مباشرة بعد المحاضرة شفوياً او تحريرياً

الأسبوع الأول

الأسئلة القبلية

س١ / مما يتكون جسم الانسان؟

س٢ / ما هي وحدة بناء جسم الانسان؟

Histopathology- it is a branch of pathology which deals with the study of disease in a tissue section.

The tissue undergoes a series of steps before it reaches the examiners desk to be thoroughly examined microscopically to arrive at a particular diagnosis. To achieve this, it is important that the tissue must be prepared in such a manner that it is sufficiently thick or thin to be examined microscopically and all the structures in a tissue may be differentiated. The objective of the subsequent discussions will be to acquaint the staff with their responsibility, the basic details of tissue handling, processing and staining.

Anatomy- a field in the biological sciences concerned with the identification and description of the body structures of living bodies.

Histology- branch of biology concerned with the composition and structure of plant and animal tissues in relation to their specialized functions.

The term **histochemistry** means study of chemical nature of the tissue components by histological methods.

The **Cell** is the single structural unit of all tissues.

The study of cell is called **Cytology**.

A **Tissue** is a group of cells specialized and differentiated to perform a specialized function. Collection of different type of cells forms an **Organ**.

Responsibility of a technician

The technician is responsible for:

1. Specimen preservation.

2. Specimen labeling, logging and identification.

3. Preparation of the specimen to facilitate their gross and microscopy.

4. Record keeping.

To obtain these aims the following point need consideration.

1. As soon as the specimen is received in the laboratory, check if the specimen is properly labeled with the name, age, Hospital Registration No. and the nature of tissue to be examined and the requisition form is also duly filled.

2. Also check if the specimen is in proper fixative. Fixative should be fifteen to twenty times the volume of the specimen add fixative if not present in sufficient amount.

3. Check if the financial matters have been taken care off.

4. Make the entries in biopsy register and give the specimen a pathology number called the accession number. Note this number carefully on the requisition form as well as the container. This number will accompany the specimen everywhere.

5. If the specimen is large inform the pathologist who will make cut in the specimen so that proper fixation is done. Container should be appropriate to hold the specimen without distorting it.

6. Blocks of tissues taken for processing should be left in 10% formalin at 60°C till processing. These would be fixed in 2 hours.

7. Slides should be released for recording after consultation with the pathologist.

8. Specimens should be kept in their marked container and discarded after checking with pathologist.

9. Block must be stored at their proper number the same day. Note the blocks have to be kept preserved for life long. Slides should be stored in their proper number after 3 days. It gives time for the slides to be properly dried.

الأسئلة البعدية

س١ / ما العلم الذي يهتم بدراسة نوع المرض على مستوى النسيج؟

س٢ / ما هي مسؤوليات التقني في مختبر الشرائح النسيجية؟

رقم المحاضرة	**2**
عنوان المحاضرة:	طرق جمع العينات
اسـم المدرس:	مصطفى طالب خلف
الفئة المستهدفة :	المستوى الاول
الهدف العام من المحاضرة :	معرفة الطالب بأنواع العينات الواردة الى مختبر الشرائح النسيجية وطرق الحصول عليها بشكل صحيح وآمن للأغراض التشخيصية والبحثية
الأهداف السلوكية او مخرجات التعلم:	١. التعريف بالمفاهيم الأساسية المتعلقة بجمع العينات ٢. قدرة الطالب على التفريق بين العينات المختلفة وطرق التعامل معها حسب نوعها ٣. التعرف على الطرق المناسبة لجمع العينات
استراتيجيات التيسير المستخدمة	١. التعليم التعاوني ٢. استخدام طريقة التعليم المدمج ٣. التغذية الراجعة ٤. العصف الذهني ٥. التعليم التجريبي
المهارات المكتسبة	٣. تعلم الخطوات الصحيحة لجمع العينات ٤. معرفة كيفية الحفاظ على العينات من التلف ٥. القدرة على تحديد المخاطر المحتملة اثناء جمع العينات
طرق القياس المعتمدة	أسئلة مباشرة بعد المحاضرة شفوياً او تحريرياً

الأسبوع الثاني

الأسئلة القبلية

س١ / ما المقصود بالخُزعة؟

س٢ / ما هي أنواع العينات الواردة الى مختبر الشرائح النسيجية؟

Type of material obtained in laboratory

The human tissue comes from the surgery and the autopsy room from surgery two types of tissue are obtained.

1. As biopsy- A small piece of lesions or tumor which in sent for diagnosis before final removal of the lesion or the tumor (Incisional biopsy).

2. If the whole of the tumor or lesion is sent for examination and diagnosis by the pathologist, it is called excisional biopsy.

3. Tissues from the autopsy are sent for the study of disease and its course.

Types of Histological preparation

The histological specimen can be prepared as

1. Whole mount

2. Sections

3. Smears.

1. **Whole mounts**- These are preparation entire animal eg. fungus, parasite. These preparations should be no more than 0.2-0.5 mm in thickness.

2. **Sections**- The majority of the preparations in histology are sections. The tissue is cut in about 3-5 mm thick pieces processed and 5 microns thick sections are cut on a microtome. These are then stained and permanently mounted. Microtomes are special instruments which have automatic mechanism for cutting very thin sections. To cut the sections on the microtome. the tissue must be made hard enough to not get crushed. There are 2 methods of hardening the tissues. One is by freezing them and the other is by embedding them in a hard material such at paraffin wax or gelatin.

3. **Smears**- Smears are made from blood, bone marrow or any fluid such as pleural or ascitic fluid. These are immediately fixed in alcohol to presence the cellular structures are then stained. Smears are also made by crushing soft tissue between two slides or an impression smear in made by pressing a clean slide in contact with the moist surface of a tissue. By doing this, the cells are imprinted on the slide and these may be stained for cytological examination.

الأسئلة البعدية

س١ / عدد مصادر العينات الواردة الى مختبر الشرائح النسيجية؟

س٢ / ما الفرق بين Biopsy و Autopsy؟

رقم المحاضرة	٣ – ٤ - ٥
عنوان المحاضرة:	خطوات تحضير الانسجة لغرض الدراسة، التثبيت والمثبتات
اسـم المدرس:	مصطفى طالب خلف
الفئة المستهدفة :	المستوى الاول
الهدف العام من المحاضرة :	توضح عملية تحضير الانسجة بدءاً من تثبيت العينات فضلاً عن التعرف على الأنواع المختلفة للمثبتات وكيفية اختيار المثبت المناسب
الأهداف السلوكية او مخرجات التعلم:	١. تعلم الخطوات العملية والقدرة على وصفها ٢. القدرة على تطبيق تقنيات التثبيت المختلفة واختيار المناسب ٣. قدرة الطالب على التفريق بين العينات المختلفة وطرق التعامل معها حسب نوعها ٤. الالتزام بمعايير السلامة عند التعامل مع المثبتات
استراتيجيات التيسير المستخدمة	١. التعليم التعاوني ٢. استخدام طريقة التعليم المدمج ٣. التغذية الراجعة ٤. العصف الذهني ٥. التعليم التجريبي
المهارات المكتسبة	١. مهارات فنية عالية في تنفيذ خطوات تحضير الشرائح ٢. المعرفة العلمية بالمواد الكيميائية المتعلقة بعملية التثبيت ٣. تقييم جودة الشرائح واختيار المثبت المناسب حسب نوع النسيج
طرق القياس المعتمدة	أسئلة مباشرة بعد المحاضرة شفوياً او تحريرياً

الأسبوع الثالث – الرابع - الخامس

الأسئلة القبلية

س١ / ما المقصود بالتثبيت؟

س٢ / ما هي طرق التثبيت؟

FIXATION- It is a complex series of chemical events which brings about changes in the various chemical constituents of cell like hardening, however the cell morphology and structural detail is preserved. Unless a tissue is fixed soon after the removal from the body it will undergo degenerative changes due to autolysis and putrefaction so that the morphology of the individual cell will be lost.

Principle of fixation- The fixative brings about crosslinking of proteins which produces denaturation or coagulation of proteins so that the semifluid state is converted into semisolid state; so that it maintains everything in vivo in relation to each other. Thus, semisolid state facilitate easy manipulation of tissue.

Aims and Effects of fixation

If a fresh tissue in kept as such at room, temperature it will become liquefied with a foul odour mainly due to action of bacteria i.e. putrefaction and autolysis so the first and fore most aim of fixation is:

1. To preserve the tissue in as lf like manner as possible.

2. To prevent postmortem changes like autolysis and putrefaction.

Autolysis is the lysis or dissolution of cells by enzymatic action probably as a result of rupture of lysosomes.

Putrefaction The breakdown of tissue by bacterial action often with formation of gas.

3. Preservation of chemical compounds and microanatomic constituents so that further histochemistry is possible.

4. **Hardening**: the hardening effect of fixatives allows easy manipulation of soft tissue like brain, intestines etc.

5. **Solidification**: Converts the normal semifluid consistency of cells (gel) to an irreversible semisolid consistency (solid).

6. Optical differentiation - it alters to varying degrees the refractive indices of the various components of cells and tissues so that unstained components are more easily visualized than when unfixed.

7. Effects of staining - certain fixatives like formaldehyde intensifies the staining character of tissue especially with hematoxylin.

Properties of fixatives

1. Coagulation and precipitation as described above.

2. Penetration Fixation is done by immersing the tissue in fluid containing the fixative. Faster a fixative can penetrate the tissue better it is penetration power depends upon the molecular weight e.g.

formalin fixes faster than osmic acid.

3. Solubility of fixatives - All fixatives should be soluble in a suitable solvent, preferably in water so that adequate concentrations can be prepared.

4. Concentration - It is important that the concentration of fixative is isotonic or hypotonic.

5. Reaction - Most fixatives are acidic. It may help in fixation but can affect staining so has to be neutralized e.g. formalin is neutralized by adding of calcium carbonate.

Amount of fixative

The fixative should be at least 15-20 times the bulk of tissue. For museum specimens the volume of fixative is > 50 times.

Note: If the specimen is large then see that the sections are made to make slices which have a thickness of 1.5 cm so that fixative can penetrate the tissue easily.

Reagents employed as fixatives (simple fixatives)

1. **Formaldehyde** - Formaldehyde is a gas but is soluble in water to the extent of 37-40% w/v. This solution of formaldehyde in water is called formalin or full-strength formalin. Formalin is one of the commonly used fixatives in all laboratories since it is cheap penetrates rapidly and does not over harden the tissues.

a. It preserves the proteins by forming cross-linkage with them and the tissue component.
b. It denatures the proteins.
c. Glycogen is partially preserved hence formalin is not a fixative choice for carbohydrates.
d. Some enzymes can be demonstrated in formalin fixed tissues.
e. It neither preserves nor destroys fat. Complex lipids are fixed but has no effect on neutral fat. After formalin fixation fat may be demonstrated in frozen section. Pure formalin is not a satisfactory fixative as it overhardens the tissue. A 10% dilution in water (tap or distilled) is satisfactory.

Since it oxidizes to formic acid if kept standing for long period so it should be neutralized by phosphates or calcium carbonate otherwise it tends to form artifact; a brown pigment in tissues. To remove this pigment picric alcohol or saturated alcoholic sodium hydroxide may be used. Concentrated formalin should never be neutralized as there is a great danger of explosion. The commercial formalin becomes cloudy on standing especially when stored in a cool place due to formation of precipitate of paraformaldehyde which can be filtered. Formalin on prolonged exposure can cause either dermatitis its vapour may damage the nasal mucosa and cause sinusitis.

Time required for fixation.

At room temperature - 12 hours

For small biopsies - 4-6 hours

At 65°C fixation occurs in - 2 hours

Mercuric Chloride (HgCl2)

2. **Mercuric chloride** is a very good salt employed in fixing but is rarely used alone because it causes shrinkage of the tissue.

a. It brings about precipitation of the proteins which are required to be removed before staining by using potassium iodide in which they are soluble.
b. The size (thickness) of the tissue to be fixed in mercuric chloride is important, since if the tissue is more than 4 mm, then it hardens the tissue at the periphery whereas the center remains soft & under fixed.
c. It penetrates rapidly without destroying lipids.
d. It neither fixes nor destroys carbohydrates. Treatment of the tissue with mercuric chloride brings out more brilliant staining with most of the dyes.
e. Tissues fixed with mercuric chloride containing fixatives contain black precipitates of mercury which are removed by treating with 0.5% iodide solution in 70% ethanol for 5-10 minutes, sections are rinsed in water, decolorized for 5 minutes in 5% sodium thiosulphate and washed in running water.

3. Picric acid - It produces marked cells shrinkage hence it is not used alone.

It has to be stored in a damp place because of its explosive nature it is preferably stored under a layer of water.

Advantage: It penetrates well and fixes rapidly.

It precipitates proteins and combines with them to form picrates some of the picrates are water-soluble so must be treated with alcohol before further processing where the tissue comes into contact with water.

Note: All the tissues fixed in picric acid containing fixatives should be thoroughly washed to remove the yellow discoloration to ensure proper staining of tissue sections.

If the fixative is not removed by washing thoroughly with time even the embedded tissue loses its staining quality.

4. Acetone: Cold acetone is sometimes used as a fixative for the histochemical demonstration of some tissue enzymes like phosphatases and lipases.

Its mode of action as fixative is similar to that of alcohol.

5. Zenker's fluid

(a) Mercuric chloride 5gm

(b) Potassium dichromate 2.5 gm

(c) Sodium sulphate 1.0 gm

(d) Distilled water to 100 ml

(e) Add immediately before use: Glacial acetic acid : 5 ml

• Specific features

- Good routine fixative

- Give fairly rapid and even penetration

- It is not stable after the addition of acetic acid hence acetic acid (or formalin) should be added just before use Washing of tissue in running water is necessary to remove excess dichromate.

6. Bouin's fluid

(a) Saturated aqueous picric acid 75ml

(b) Formalin 25ml

(c) Glacial acetic acid 5 ml

• Specific features

- Penetrates rapidly and evenly and causes little shrinkage

- Excellent fixative for testicular and intestinal biopsies because it gives very good nuclear details, in testes is used for oligospermia and infertility studies

- Good fixative for glycogen

- It is necessary to remove excess picric acid by alcohol treatment.

Washing out- After the use of certain fixative it in urgent that the tissues be thoroughly washed in running water to remove the fixative entirely.

Tissues treated with potassium dichromate, osimium tetraoxide and picric acid particularly need to be washed thoroughly with water prior to treatment with alcohol (for dehydration).

الأسئلة البعدية

س١ / ما هو أساس خطوة التثبيت؟

س٢ / عدد أنواع المثبتات واذكر الأشهر او الأكثر استخداماً؟

رقم المحاضرة	6
عنوان المحاضرة:	التجفيف وانواع المجففات
اسـم المدرس:	مصطفى طالب خلف
الفئة المستهدفة :	المستوى الاول
الهدف العام من المحاضرة :	فهم أهمية التجفيف في الحفاظ على التركيب الخلوي للعينة
الأهداف السلوكية او مخرجات التعلم:	١. تحديد المعايير المطلوبة لاختيار نوع المجفف ٢. استخدام تقنيات مختلفة لضمان الحصول على دقة التجفيف ٣. قدرة الطالب على تحليل وتقييم الشرائح النسيجية المجففة بطرق مختلفة ومقارنتها مع بعضها
استراتيجيات التيسير المستخدمة	١. التعليم التعاوني ٢. استخدام طريقة التعليم المدمج ٣. التغذية الراجعة ٤. العصف الذهني ٥. التعليم التجريبي
المهارات المكتسبة	١. مهارات التحضير المختبري ٢. العمل الجماعي الذي يعزز مهارات التواصل واجراء التجارب ومناقشة النتائج ٣. القدرة على إدارة الوقت اثناء عملية التجفيف
طرق القياس المعتمدة	أسئلة مباشرة بعد المحاضرة شفوياً او تحريرياً

الأسبوع السادس

الأسئلة القبلية

س١ / ما المقصود بالتجفيف؟

س٢ / ما هي طرق التجفيف؟

Dehydration - After fixation in aqueous solvent the delicate tissue needs to be dehydrated slowly starting in 50% ethyl alcohol. The other routine tissue specimen may be put in 70% alcohol. A higher concentration of alcohol initially is in inadvisable because this may cause very rapid removal of water may produce cell shrinkage. An exception to this is in case of Heidenhain's Susa fixed tissue where it may be placed directly in 95% alcohol. Tissue transferred from alcoholic based fixative like Carnoy's fixative may be placed in higher grades of alcohol or even in absolute alcohol.

For routine biopsy and postmortem tissue of 4-7 mm thickness 70%, 90% and absolute alcohol (2-3 changes for 2-4 hours each) are sufficient to give reasonably satisfactory result.

Use of solid dehydrants

Anhydrous copper sulphate is used in higher grade of dehydrating alcohols. A layer 1-2.5 cm thick is placed at the bottom of a dehydrating vessel or beaker and is covered with 2 or 3 filter papers to prevent contamination of the tissues. Anhydrous copper sulphate is white, it removes water from alcohol which in turn has been diluted upon absorption of water from the tissues. The change of colour of copper sulphate from white to blue indicates that both alcohol and water should be changed. Use of copper sulphate enhances the process of dehydration and also prolongs the life of alcohol.

Other dehydrating agents

1. Acetone - It is clear, colourless volatile inflammable fluid.

• It has a rapid action in dehydrating the tissue but produces shrinkage and distortion and subsequent brittleness to the tissue.

• Low cost is also an advantage.

Acetone usually dehydrates within 20-30 minutes, but four changes of acetone should be used, it is preferable to use acetone after low strength of alcohol so that distortion of the tissue is less.

2. Dioxane - It dehydrates and clears at the same time. It is miscible with paraffin and with water and alcohol, tissue from dioxane can be transferred straight to paraffin.

• There is less shrinkage of tissues

• Tissues can be left in dioxane without danger of hardening for longer period of time.

Disadvantage: It is more expensive than alcohol.

* It is toxic to man

3. Isopropyl alcohol

• It is miscible with water and other organic solvents

• It does not harden the tissue like alcohol

• It is expensive

الأسئلة البعدية

س١ / عدد أنواع المجففات؟

س٢ / ما هو المجفف الأكثر استخداماً في تحضير الشرائح النسيجية؟

رقم المحاضرة	٧
عنوان المحاضرة:	الترويق وانواع المروقات
اسـم المدرس:	مصطفى طالب خلف
الفئة المستهدفة :	المستوى الاول
الهدف العام من المحاضرة :	فهم أهمية الترويق في الحفاظ على التركيب الخلوي للعينة
الأهداف السلوكية او مخرجات التعلم:	٤. تحديد المعايير المطلوبة لاختيار نوع المروق ٥. استخدام تقنيات مختلفة لضمان الحصول على دقة الترويق ٦. قدرة الطالب على تحليل وتقييم الشرائح النسيجية المروقة بطرق مختلفة ومقارنتها مع بعضها
استراتيجيات التيسير المستخدمة	٦. التعليم التعاوني ٧. استخدام طريقة التعليم المدمج ٨. التغذية الراجعة ٩. العصف الذهني ١٠. التعليم التجريبي
المهارات المكتسبة	٤. مهارات التحضير المختبري ٥. العمل الجماعي الذي يعزز مهارات التواصل واجراء التجارب ومناقشة النتائج ٦. القدرة على إدارة الوقت اثناء عملية الترويق
طرق القياس المعتمدة	أسئلة مباشرة بعد المحاضرة شفوياً او تحريرياً

الأسبوع السابع

الأسئلة القبلية

س١ / ما المقصود الترويق؟

س٢ / ما هي طرق التجفيف؟

Clearing- means appearance of tissue after it has been treated by the fluid chosen to remove the dehydrating agent. Most of these tissues have similar refractive index to that of protein therefore, the tissue is left translucent.

Clearing agent is required when the dehydrating agent is not miscible with the impregnating medium. It is essential for a clearing agent to be miscible both in dehydrating agent as well as embedding agent.

Commonly used clearing agents are as follows:

1. Xylene - It has a rapid action. Biopsy specimens of 3-4 mm thickness are cleared in 2-4 hours.

Immersion time must not be prolonged otherwise the tissue become brittle.

2. Toluene and Benzene are similar in properties to xylene but are less damaging to the tissues on prolonged exposure.

3. Chloroform - It is slower in action, but it causes less brittleness therefore, tissue can be left in it overnight.

• It does not affect the refractive index of the tissue is not rendered translucent.

• It is expensive.

• It is inflammable.

4. Carbon tetrachloride - It has similar properties to chloroform but is cheaper.

5. Cedar wood oil (Histological): It is good for treatment of delicate tissues as it has the least hardening effect.

• It is very slow in action.

• It is very expensive.

Care should be taken not to confuse it with cedar wood oil (microscopic) used with oil immersion lens.

Techniques of clearing: If the tissue is being cleared in chloroform or carbon tetrachloride it may be left overnight. In automatic tissue processor three changes of one hour each are usually satisfactory.

In Xylene, benzene or toluene one change after 30-60 minutes is satisfactory to give a clear translucent appearance to the tissue.

الأسئلة البعدية

س١ / عدد أنواع المروقات؟

س٢ / اذكر آلية تطبيق عملية الترويق؟

رقم المحاضرة	٨ - ٩
عنوان المحاضرة:	الارتشاح وانواع اوساط الطمر - صب القوالب ونحتها
اسـم المدرس:	مصطفى طالب خلف
الفئة المستهدفة :	المستوى الاول
الهدف العام من المحاضرة :	فهم أهمية الارتشاح وطرق صب قوالب العينات
الأهداف السلوكية او مخرجات التعلم:	١. تحديد المعايير المطلوبة لاختيار نوع وسط الارتشاح والطمر ٢. استخدام تقنيات مختلفة لضمان الحصول على دقة الارتشاح ٣. قدرة الطالب على تحليل وتقييم الشرائح النسيجية المرشحة بطرق مختلفة ومقارنتها مع بعضها
استراتيجيات التيسير المستخدمة	١. التعليم التعاوني ٢. استخدام طريقة التعليم المدمج ٣. التغذية الراجعة ٤. العصف الذهني ٥. التعليم التجريبي
المهارات المكتسبة	١. مهارات التحضير المختبري ٢. العمل الجماعي الذي يعزز مهارات التواصل واجراء التجارب ومناقشة النتائج ٣. القدرة على إدارة الوقت اثناء عملية الارتشاح
طرق القياس المعتمدة	أسئلة مباشرة بعد المحاضرة شفوياً او تحريرياً

الأسبوع الثامن ـ التاسع

الأسئلة القبلية

س١ / ما المقصود بالارتشاح؟

س٢ / ما معنى صب القوالب؟

Impregnation- It is the complete removal of clearing reagents by substitution of paraffin or any such similar media.

Impregnation with paraffin wax takes place in an oven heated to 56-60°C depending upon the melting point of the wax in use. Frequent check of the temperature of paraffin baths is required since temperature 5°C above the melting point of the paraffin will cause tissue shrinkage and hardening.

Properties of paraffin wax

1. Easy to prepare large number of tissue blocks in comparatively short time.
2. Minimum supervision is required
3. It is cheaper than other impregnating media
4. During staining there is very little difficulty than other media.

Points to be remembered during use of paraffin wax

1. It should be free from dust, grit and other foreign matter.
2. It should not contain water, which causes it to crystallize and turn it white.
3. The wax has to be filtered before use by use of ordinary filter paper.
4. Higher melting point waxes are hard to ribbon.

For impregnation the wax oven has to be kept at high temperature, making the tissue hard, too low melting point wax may not be hard enough to support the tissue during cutting. If the wax is overheated and remains in that state for a long time, it tends to crystallize and become useless.

Impregnation with Paraplast: This is mixture of highly purified paraffin and several plastic polymers. It has greater elasticity than normal paraffin wax, therefore, the results are superior. It ribbons well allowing almost wrinkle free serial sections to be cut with ease at 4-micron thickness. It should not be used for thin-walled structures as it prevents complete expansion of the specimen.

Impregnation with Bioloid - Good embedding medium in which thin-walled structures can be sectioned satisfactorily.

Technique of impregnation:

The tissue is transferred from clearing agent to molten paraffin wax. The amount of wax should be 25-50 times the volume of tissue. The tissue must be submitted to 3 changes in wax. The temperature of the wax bath should be 2-3°C above the melting point of wax.

Time of impregnation

Depends on the following 3 factors

1. The size and type of tissue

2. The clearing agent employed

3. The use of vacuum embedding oven.

1. Size and type of tissue:

The thicker the tissue the longer will be the time required for wax to penetrate to the centre in addition a thick tissue has more of clearing agent so, more changes of wax are necessary to remove it. If even small amounts of clearing agents remain with the wax this will cause crystallization and produce crumbling of the sections during cutting. The type of tissue is also important since bone, skin, CNS needs twice as long as soft tissue like liver or kindly. Tissue like muscle and fibrous tissue tends to overharden and become brittle in wax bath so the time for impregnation must be kept to a minimum. The reduction of time can be achieved by using vacuum embedding medium.

2. Clearing agent employed

Some clearing agents are more rapidly and easily cleared than other e.g. Xylene, benzene and toluene are easiest to remove, and one change of wax is normally sufficient; whereas for chloroform and carbon tetrachloride 2-3 changes are needed.

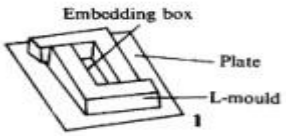
3. Use of vacuum embedding oven

With the use of normal paraffin oven, 2 changes of paraffin wax for a period of 4 hours are needed but by using vacuum embedding oven this time may be halved.

Embedding - It is the orientation of tissue in melted paraffin which when solidified provides a firm medium for keeping intact all parts of the tissue when sections are cut.

Types of molds

a) Leuckhart's L pieces - These are two 'L' which are resting metal usually brass, which are resting on a flat metal or glass plate.



b) Compound embedding units - consists of square shaped brass or metal plates in a series of interlocking plates.

c) Others like plastic embedding blocks (tissue Tek system).

Techniques of casting (embedding)

1. Molten paraffin wax which is heated at a temperature 2-3° above the melting point is poured into the mold to an adequate depth so as to cover the thickest tissue block.

2. The wax touching the mold will quickly form a thin semi solid layer, Now introduce the tissue with a prewarmed forceps to prevent the wax to stick to it. The tissue is pressed in this semisolid wax to orient it at the bottom of mold in a correct plane.

3. Fix the label in position by pressing one edge against solidifying wax usually, sides of the mold are preferred.

4. As soon as a film of solid wax is formed on the surface, the whole block with mold is submerged in cold water at 20°C. If this is not done there will be crystallization of wax, using ice water to do initial cooling will also cause the block to crack.

5. When blocks are set hard, they are removed from mold. The tissue surface towards the mold base is from where the sections are to be cut this surface should be trimmed lightly with a scalpel so as to expose the tissue.

Following points must be taken care off during casting.

1. Paraffin should not be allowed to cool around the tissue to be blocked for this before introducing the tissue in the mold it should be kept in heated wax or in cassette placed over thermostatic hot plate.

2. To prevent excess of wax solidifying on the bottom of the block during winter prewarmed molds may be used.

3. The cutting surface of the tissue should be facing at the bottom of the mold.

4. If two or more tissues have to be casted remember to keep them both at the same depth.

5. If small biopsy fragments have to be casted, the largest piece should be first blocked, and other pieces should be as near it as possible.

6. All four corners of the block should be in one horizontal plane.

7. The tissue should have at least 2 mm wax around its edges.

8. Smear mineral or machine oil on the inner surface of the mold for facilitating easy removal of block.

9. Whitish areas around tissue in block denotes crystallization which may be due to moisture or due to incomplete removal of clearing agent.

Most tissue sections are cut from the largest area, but some tissue needs special mention.

1. Tissue of tubular nature are cut transversely so should be embedded vertically.

2. Skin is cut in a plane at right angles to the surface so should be embedded at right angles to the bottom.

3. Muscle biopsy should be sectioned in both transverse and longitudinal planes.

الأسئلة القبلية

س١ / اذكر العوامل المؤثرة على عملية الارتشاح؟

س٢ / اذكر أنواع قوالب الصب؟

رقم المحاضرة	١٠ - ١١
عنوان المحاضرة:	التقطيع – جهاز المشراح وانواعه
اسـم المدرس:	مصطفى طالب خلف
الفئة المستهدفة :	المستوى الاول
الهدف العام من المحاضرة :	معرفة الطلبة بمهارات التقطيع بشكل دقيق لتحضير شرائح نسيجية عالية الجودة وجاهزة للفحص
الأهداف السلوكية او مخرجات التعلم:	١. التعرف على الأدوات والأجهزة المرافقة لعملية التقطيع ٢. اتقان عملية التقطيع بشكل جيد باستخدام جهاز المشراح الدوار ٣. تعلم كيفية التعامل مع مختلف أنواع الانسجة وطرق معالجتها
استراتيجيات التيسير المستخدمة	١. التعليم التعاوني ٢. استخدام طريقة التعليم المدمج ٣. التغذية الراجعة ٤. العصف الذهني ٥. التعليم التجريبي
المهارات المكتسبة	١. مهارات التحضير المختبري ٢. العمل الجماعي الذي يعزز مهارات التواصل واجراء التجارب ومناقشة النتائج ٣. اتقان كيفية ضبط واعداد جهاز المشراح ٤. التعامل الجيد مع الانسجة الحساسة دون اتلافها ٥. مهارات الصيانة والتنظيف لجهاز التقطيع والمعدات الاخرى
طرق القياس المعتمدة	أسئلة مباشرة بعد المحاضرة شفوياً او تحريرياً

الأسبوع العاشر – الحادي عشر

الأسئلة القبلية

س١ / ما الجهاز المستخدم في عملية تقطيع الانسجة؟

س٢ / ما عدد الشرائح النسيجية التي تعتقد ان تؤخذ من النسيج لغرض الفحص؟

Microtomes- These are mechanical devices for cutting uniform sections of tissue of appropriate thickness. All microtomes other than those used for producing ultra-thin sections for election microscopy depend upon the motion of a screw thread in order to advance the tissue block on knife at a regulated number of microns.

Motion of screws can be direct or through system of gears or levers to magnify the movement.

Types of microtomes

1. Hand microtomes – limited for use in botanical sections
2. Rocking microtome
3. Rotary microtome
4. Freezing microtome
5. Base sledge microtome
6. Vibrating knife microtome

Size of Knives

110 mm Knife - Frozen sections

120 & 185 mm - Routine paraffin block

Paraffin section cutting

Equipment required

1. Microtome
2. Water bath preferably thermostatically controlled.
3. Hot plate or drying oven thermostatically controlled.
4. Fine pointed forceps.
5. Small hair brush.
6. Seeker
7. Scalpel
8. Clear cloth or paper towel.
9. Slide rack
10. Clean glass slides
11. Section adhesive
12. Fluff less blotting paper
13. Ice cubes
14. Diamond marker pencil

Water bath- thermostatically controlled for paraffin wax of melting point 56ºC, a water temperature of 45ºC is sufficient ordinary distilled water is satisfactory; addition of a trace of detergent to water is beneficial in flattening of sections.

Hot plate or drying oven- Drying of sections at around the melting point of wax is satisfactory

Brush, seeker, forceps – needed to remove folds and creases in sections after floating out.

Slides - Majority of sections fit comfortably on a 76 x 25 x 1.2 mm slide.

Diamond pencil – needed to write the identification details like name or specific number.

Section adhesives- An adhesive is a substance which can be smeared on to the slides so that the sections stick well to the slides.

Most of the tissue sections which are adequately thin and thoroughly dried without any air bubble trapped under them do not require an adhesive, as in case of routine H and E staining, but for histochemical methods requiring alkaline solutions eg ammonia tend to remove sections from slide for such cases adhesive is required. Also, adhesive is required for tissues like brain, spinal cord, blood clot, decalcified tissues which have a tendency to detach themselves from the slide. Tissue impregnated with ester wax also require section adhesive.

Types of adhesives

1. Albumin
2. Gelatin
3. Starch
4. Cellulose
5. Sodium silicate
6. Resin
7. Poly L Lysine

Adhesives are either added to water bath or smeared thinly on slide.

1. Agar – add 50 ml of melted agar to water bath

2. Gelatin – Add 30 ml of melted gelatin to water bath

3. Mayer's glycerol albumin – This is the most popular adhesive for routine use:

1. Fresh egg white 50 ml

2. Glycerol 50 ml

3. Sodium salicylate 1 ml

Mix and agitate the ingredients filter through coarse filter paper smear

fluid over the slide. This fluid may be diluted 1:20 with distilled water and

section floated on the fluid, while manipulating the albuminized slide under

water in the floatation bath to pick up the section, avoid dipping the entire

slide as the albumin may wash off.

Section cutting of paraffin embedded tissue

Fixing of block

1. Fix the block in the block holder on the microtome knife in such as position that it will be clear of the knife when it is in position, block may be fixed directly, or it may be fixed to a metal carrier which in turn is fixed to the microtome.
2. Insert the appropriate knife in the knife holder and screw it tightly in position. Adjust if required. The clearance angle should be set at 3-4 degree and angle of slope should be set permanently at 90 degrees. It is important to tighten the knife clamp screw securely and block clamp screws most also be firm. The exposed ends of the knife must all the times be protected by magnetic or clip on knife guards to avoid any accidents.
3. Trimming of tissue block : Move the block forward so that he wax block is almost touching the knife. To trim away any surplus wax and to expose a suitable area of tissue for sectioning, the section thickness adjusters are set at 15 microns.
4. On exposing a suitable area of tissue the section thickness is set to the appropriate level for routine purposes to 4-6 microns.
5. Apply ice to the surface of the block for a few seconds and wipe the surface of block free of water. This step is optional but makes sections cut easily.
6. Note that the whole surface of the block will move parallel to the edge of the knife in order to ensure a straight ribbon of sections.
7. The microtome is now moved in an easy rhythm with right hand operating the microtome and left hand holding the sections away from the knife. The ribbon is formed due to the slight heat

generated during cutting, which causes the edges of the sections to adhere. If difficulty is experienced in forming the ribbon it is sometimes overcome by rubbing one of the edges of the block with finger.

8. During cutting the paraffin wax embedded sections become slightly compressed and creased. Before being attached to slides the creases must be removed and the section flattened. This is achieved by floating them on warm water. Thermostatically controlled water baths are now available with the inside coated black. These baths are controlled at a temperature 4-6ºC below the melting point of paraffin wax. It is easy to see creases if the inside of water bath is black.
9. The action in floating out must be smooth with the trailing end of ribbon making contact with water first to obtain flat sections with correct orientation, floating out with the shiny surface towards the water is essential. When the ribbon has come to rest on water the remaining wrinkles and folds are removed by teasing apart by using forceps or seeker.
10. Picking up sections – The ribbon of sections floating on water is split Picking up a section on slide is achieved by immersing the slide lightly smeared with adhesive vertically to three fourths of its length bringing the section in contact with the slide. On lifting the slide vertically from the water, the section will flatten on to the slide. The sections are then blotted lightly with moistened blotting paper to remove excess water and to increase contact between section and slide. For delicate tissues or when several ribbons of sections are placed on the slide, omit the blotting instead keep the slide in upright position for several minutes to drain.
11. Drying of section: Sections are then kept in incubator with a temperature 5-6ºC above the melting point of wax i.e. at 60ºC for 20- 60 minutes. It is better to overheat than underheat. If the sections are not well dried, they may come off during staining. The sections should not be allowed to dry without a good contact with the slide, such sections will come off during staining.

SOME USEFUL HINTS IN SECTION CUTTING

Methods of removing bubbles trapped beneath the sections Bubbles may get trapped under a section while in the tissue flotation bath. These need to removed before the section are picked on the slides this may be done by:

1. With the edge of slide.

2. Can be teased out with bent dissecting needle.

3. Place the sections on slide and run 2% alcohol under them. Any foldor bubbles are removed.

To cut a tissue which tends to crumble or fragment while cutting. With the mouth open and sounding a soft long drawn 'H' thus 'h h h h h ' exhale gently on to the section as it leaves the knife and cut very slowly. This also helps to reduce the effect of static electricity. If sections fragment due to large amount of blood in tissue, the block should be coated with celloidin between sections. The surface of the block should be wiped dry, and painted with a camel hairbrush which has been dipped in 1% Celloidin. After allowing few seconds for the Celloidin to dry a section is cut in usual way.

It must be remembered that when floating the sections to remove the creases, the celloidin layer must be uppermost, and the water should be a little hotter than usual to counteract the effect of celloidin. Following drying in usual way, the cellodin is removed with equal parts of ether and alcohol before removing wax with xylol.

Serial sectioning- Serial sectioning may be needed to study the track of some structures

or to find the extent of a lesions. Sections are collected from the very first cut that includes any tissue. Ribbons of ten - 1-10, 11-20, 11-20, 21-30 so on are picked up and mounted on the slides.

Step sections- This is an alternative for serial sections and for the same reason. Sections are taken at periodic level through the block. The request is made for every nth section for a total of 'n' sections.

Cooling block and knife. In general, keep ice cubes ready at hand and cool the surface of block and knife before cutting, always dry and block and knife and block after the application of knife.

الأسئلة البعدية

س١ / اذكر المعدات المستخدمة في عملية التقطيع؟

س٢ / اذكر الفرق بينSerial sections و Step section؟

س٣ / عدد أنواع أجهزة التقطيع؟

رقم المحاضرة	١٢ – ١٣ - ١٤
عنوان المحاضرة:	تلوين الشرائح النسجية
اسـم المدرس:	مصطفى طالب خلف
الفئة المستهدفة :	المستوى الاول
الهدف العام من المحاضرة :	تدريب الطلبة على تلوين المقاطع النسيجية من خلالها يتم الحصول على معلومات دقيقة حول بنية الخلية والانسجة
الأهداف السلوكية او مخرجات التعلم:	١. القدرة على تمييز مكونات الانسجة ٢. تحديد وظائف الخلايا حيث ان بعض الملونات ترتبط بمكونات معينة للخلية ٣. تشخيص الامراض حيث ان الملونات تساعد على كشف التغيرات المرضية التي ممكن ان تحصل في الخلية
استراتيجيات التيسير المستخدمة	١. التعليم التعاوني ٢. استخدام طريقة التعليم المدمج ٣. التغذية الراجعة ٤. العصف الذهني ٥. التعليم التجريبي
المهارات المكتسبة	١. مهارات التحضير المختبري ٢. العمل الجماعي الذي يعزز مهارات التواصل واجراء التجارب ومناقشة النتائج ٣. اتقان كيفية عمل الملونات المختلفة ٤. التعامل الجيد مع الانسجة الحساسة دون اتلافها ٥. مهارات التقييم والتحليل للنتائج التي يتم الحصول عليها بعد عملية التلوين
طرق القياس المعتمدة	أسئلة مباشرة بعد المحاضرة شفوياً او تحريرياً

الأسبوع الثاني عشر ـ الثالث عشر – الرابع عشر

الأسئلة القبلية

س١ / ما الغاية من تلوين الاشياء؟

س٢ / ما الأجزاء الرئيسية في بنية الخلية؟

Staining

The sections, as they are prepared, are colourless and different components cannot be appreciated. Staining them by different colored dyes, having affinities of specific components of tissues, makes identification and study of their morphology possible.

Certain terminologies used in the following account are given below.

1. Basophilic: Substances stained with basic dyes
2. Acidophilic: Substances stained by acid dyes

Vital staining

Staining of structures in living cells, either in the body (in vivo) or in a laboratory preparation (in vitro). e.g. Janus green is taken up by living cells and stains the mitochondria.

Metachromatic staining

There are certain basic dyes belonging to aniline group that will differentiate particular tissue components by staining them a different color to that of original dye. The phenomenon is known as metachromasia. The tissue elements reacting in this manner are said to be exhibiting metachromasia.

The generally accepted explanation of this phenomenon is that change in color is due to polymerization.

Sulfated substances are highly metachromatic e.g. Mast cell granules. These contain Heparin which is highly sulfated. Some of the common metachromatic dyes are :

1. Methylene blue
2. Methyl violent
3. Thionine
4. Crystal violent
5. Toluidine blue

Thionine and toluidine blue dyes are commonly used for quick staining of frozen selection using their metachromatic property to stain nucleus and cytoplasm differently.

Tissue components often demonstrated by metachromatic stains:

1. Amyloid material
2. Mast cell granules
3. Mucin
4. Cartilage

Direct staining

Application of simple dye to stain the tissue in varying shades of colors.

Indirect staining

It means use of mordant of facilitate a particular staining method or the use of accentuator to improve either the selectivity or the intensity of stain.

Progressive staining

Stain applied to the tissue in strict sequence and for specific times. The stain is not washed out or decolorized because there is no overstaining of tissue constituents. Staining is controlled by frequent observation under microscope.

Regressive staining

Tissue is first overstained and then the excess stain is removed from all but the structures to be demonstrated. This process is called differentiation and should always be controlled under microscope.

Decolorization

Partial or complete removal of stain from tissue sections. When the color is removed selectively (usually with microscopic control) it is called differentiation. In case decolourization is to restain the selection with some other stain, acid alcohol treatment is the method of choice.

Differentiation

In regressive staining differentiation is the removal of washing out of the excess stain until the colour is retained only in the tissue components to be studies.

Impregnation

It is the deposition of salts of heavy metals on or around cells, tissue constituents etc. It has followed characteristics:

1. Structures demonstrated are opaque and black

2. The colouring matter is particulate

3. The deposit is on or around but not in the element so demonstrated.

Histochemical staining

Staining which is used to indicate the chemical composition of the tissue or cellular elements.

Counter stains

A counter stain is the application to the original stain, usually nuclear, of one or more dyes that by contrast will bring out difference between the various cells and tissues. A heavy counterstain is to be avoided lest it mask the nuclear stain. It can be done either by using dilute stain or cutting down the staining time. Some counterstains which are acidic may lighten or remove the nuclear stains.

Mordants

Substance that causes certain staining reactions to take place by forming a link between the tissue and the stain. The link is referred as lake. Without it, dye is not capable of binding to and staining the tissue. e.g. Ammonium and Potassium alum for hematoxylin.

Accentuators

These are substances that causes an increase in the selectively or in the staining power of dye. Thus, they lead to more intense staining. e.g. Phenol in Carbol fuchsin, KOH in Mehtylene blue.

Leuco compounds

Conversion of a dye into a colourless compound by the destruction of its chromophore. Prefix leuco is applied to it, e.g. leucofuchsin used in PAS stain.

Dyes used in staining

Dyes are classified in various ways :

1. According to source	2. Affinity to tissues	3. Chemical composition
a. Natural	a. Acidophilic	a. Thiazines
b. Synthetic	b. Basophilic	b. Azo-dyes
		c. Rosailins

Synthetic dyes have greater staining capacity, much greater spectrum of colours.

Natural dyes

These are very few in numbers. They are mainly two in common use:

1. Hematoxylin: This is the most popular dye used as a nuclear stain. It is derived from the log tree mainly found in Mexico. It develops staining property after oxidation. It is a weak dye and to make it give sharp stain a mordant is needed.

2. Carmine: It is a scarlet dye made from the ground bodies of cochineal beetles.

Synthetic dyes

Most of these are in Aniline base and derived from coal tar. These aniline dyes offer wide range of color and action. These aniline dyes offer wide range of colour and action. Chemical composition may be basic, acidic, amphoteric (neutral). According to these characters stain different components of tissue.

Basic dyes

These are cationic dyes and stain nuclei, basophilic granules or bacteria.

Acidic dyes

These are anionic dyes and stain mainly cytoplasm, eosinophilic granules.

Theories of staining

Physical theories:

1. Simple solubility e.g. Fat stains are effective because the stain is more soluble in fat than in 70% alcohol.

2. Adsorption: This is a property by which a large body attracts to itself minute particles from a surrounding medium.

Chemical theories

It is generally true that acid dyes stain basic elements (Cytoplasm) and basic dyes stain acidophilic material (nucleus) however this far from being complete truth, Indeed hematoxylin, which is an acid dye, does not stain the cytoplasm, but (in the presence of mordant) is one of the most widely used nuclear stains.

Staining of paraffin section

The most common method of histological study is to prepare thin sections (3-5 micron) from paraffin embedded tissues. These are then suitably stained and mounted in a medium of proper refractive index for study and storage. Commonest mountants used are resinous substances of refractive index close to that of glass. These are soluble in xylol. Hence sections are dehydrated and cleared in xylol and mounted. Mounting in aqueous mounting media is done directly after staining for sections which cannot be subjected to dehydrating and clearing agents.

The basic steps in staining and mounting paraffin sections are as follows:

1. Deparaffinization

2. Hydration

3. Removal of mercury pigments wherever needed

4. Staining

5. Dehydration and clearing

6. Mounting

1. Deparaffinization

Removal of wax is done with xylol. It is essential to remove the wax completely, otherwise subsequent stages will not be possible. At least 2 to 3 changes in xylol are given for suitable length of time. Sections of this stage should appear clear and transparent. Presence of any patches indicates the presence of wax and sections should be kept longer in the xylol.

2. Hydration

Most of the stains used are aqueous or dilute alcoholic solutions. Hence it is essential to bring the section to water before the stains are applied. The hydration is done with graded alcohols from higher concentration to lower concentration. Alcohol and acetone are miscible with xylol. First change is made to absolute alcohol or acetone followed by 90%, 70% alcohol and finally distilled water. Sections now should appear opaque. Presence of any clear areas are indicative of the presence of xylol. To remove this xylol, sections should be returned to absolute alcohol and rehydrated.

3. Removal of mercury pigments wherever needed

In case mercury containing fixatives e.g. Zenker, Susa etc. are used, mercury pigments are precipitated on the sections. It has to be removed before staining is done. This is brought about by treatment with iodine solutions which changes mercury to an iodine compound. This in turn is converted to tetrathionate by thiosulphate, which is readily soluble in water. The slides are placed in running water to wash out all extraneous chemicals.

4. Staining

Various staining procedures are applied from this hydrated stage. The most common stain applied for histological study is Hematoxylin and Eosin. Various types of haemotoxylin formulations are used. Certain of the stains use strong chemicals e.g. ammonia. Sections tend to float off the slides in such stains. This can be prevented by coating the sections by a thin layers of celloidin. For this sections are returned to absolute alcohol and then dipped in a dilute solution of celloidin and finally hardened in 70% alcohol. Washing and rinsing of tissue sections is a necessary part of most staining techniques. It eliminates carrying over of one dye solution to the next. Excess dye, mordants, or other reagents might react unfavorably or precipitate when placed in the fluid employed in the next step.

5. Dehydration and clearing

Dehydration is done is graded alcohols or acetones from 70% to absolute alcohol or acetone. Dehydrating alcohol and acetones can remove some of the stains. Time has to be suitably modified to minimize fading of stains. Since alcohol and acetone are miscible in xylol, it is used for clearing the sections. Any sections from which water has not been completely removed would give a milky appearance after the first xylol. Such sections should be returned to absolute alcohol and the process repeated. Mounting is done after 2nd or 3rd xylol.

6. Cover slipping and mounting

Make quite sure that the sections are quite clear. Do not let the section go dry before mounting:

1. Hold the slide between the thumb and the forefinger of one hand and wipe with a clean cloth both ends of the slides. Look for the engraved number to make sure the side the sections are present.

2. Clean carefully around the section and lay on a clean blotting paper with section uppermost along with appropriate coverslip which has already been polished.

3. Place a drop of mountant on the slide over coverslip. Amount of mountant should be just enough. Invert the slide over the coverslip and lower it so that it just adheres to the cover slip quickly turn the slide over, then lay it on a flat surface to allow the mountant to spread.

Do not press or push the slide at all. It can damage the section.

4. After the mountant has spread to the edge of the coverslip wipe around it for neatness. If proper care has been taken there should be no air bubbles. If many are present, slide should be returned to the xylol to remove the coverslip. It will slip off and remounting is done. No attempt should be made to pull the coverslip. Slight warming of the slide from below will make the small air bubbles to escape from the slide of the coverslip.

5. Coverslip should be in the center of the slide with neatly written label on one slide.

Mountants

Histological sections which need to be examined for any length of time or to be stored, must be mounted under a coverslip. There are two types of mounting media:

1. Aqueous media - Used for material which is unstained, stained for fat, or metachroamtically stained.

2. Resinous media - For routine staining.

Aqueous media

There are used for mounting sections from distilled water when the stains would be decolorized or removed by alcohol and xylene, as would be the case with most of fat stains (Sudan methods). Some stains, e.g. methyl violent, tend to diffuse into medium after mounting. This can be avoided by using Highman's medium. Aqueous mountants require addition of bacteriostatic agents such as phenol, crystal of thymol or sodium Merthiolate to prevent the growth of fungi.

Permanent seal

After mounting the cover slip can be ringed by clear nail polish for storage. Following are some of the commonly used aqueous mounting media: For formulation see the appendix.

1. Apathy's medium (R.I- 1.52) A very useful medium for mounting sections for fluorescent microscopy.

2. Farrant's medium (R.I. 1.43) Recommended for fat stains.

3. Glycerine jelly (R.I. 1.47) An excellent routine mountant for fat stains.

4. Highman's medium (R.I. 1.52) Recommended with the metachroamtic dyes especially methyl violent.

Resinous mounting media

Natural or synthetic resins dissolved in benzene, toluene or xylene. These are purchased readymade. In case they become too viscous they may have to be diluted with xylene. Following is some of these media.

1. Canada balsam - Natural resin (R.I. - 1.52) It is used as 60% resin by weight in xylene. H.&E-stained slides are fairly well preserved but basic aniline dyes tend to fade and Prussian blue is slowly bleached. Slides take few months to dry.

2. D.P.X. (R.I. 1.52) Polystyrene resin dissolved in xylene as a 20% solution. It is most commonly used.

3. There are many other synthetic resins sold under various trade names e.g. Coverbond (R.I. 1.53), H.S.R. (Harlew synthetic Resin), Histoclad (R.I. - 1.54), Permount (R.I. 1.54), Pro-Texx (R.I. 1.495).

Criteria of acceptable mounting media

1. Refractive index should be as close as possible to that of glass i.e. 1.5.

2. It should not cause stain to diffuse or fade.

3. It should not crack or appear granular on setting.

4. It should be dry to a nonstick consistency and harden relatively quickly.

5. It should not shrink back from edge of cover-glass.

6. It should be free flowing and free from air bubbles.

Cover glasses used in histopathology

Care has to be exercised in selecting cover glasses for mounting, these are available in variable sizes and thickness and are supplied usually in 10 gm packings.

Following sizes are commonly available:

1. 22 x 22 mm
2. 25 x 50mm
3. 22 x 30 mm
4. Circular
5. 22 x 40 mm

Cover glass should preferably be the No. 1 thickness (0.13 - 0.16 mm), but never more than No. 1 ½ thickness (0.16 - 0.19 mm).

Haematoxylin

Haematoxylin as supplied has no staining properties until it has been ripened by oxidation into haematin. This ripening is achieved by two methods:

1. Exposure of prepared solutions to the air for periods upto 6-8 weeks, preferably in sunlight

2. Addition of an oxidizing agent such as sodium iodate, potassium permaganate or mercuric oxide.

In this ripening process Haemtoxylin (C16 HI & O6) loses two hydrogen atoms to become Haematin (C16 H12). Sufficient Haematoxylin should be left unoxidized in solution, so that natural oxidation can continue. It prolongs shelf life of the stain.

Bluing

Alum Haematoxylin stains nuclei and red color, which is converted to blue black color, when the section is washed in weak alkali. Tap water is usually alkaline enough to produce this color change.

Following may be used for rapid bluing of the sections.

1. 1% Lithium carbonate.

2. 2% Ammonia (Ammonia Water).

3. Scott's water

- Sod. or Pot. Carbonate 2 to 3 gm
- Magnesium sulphate 20 gm
- Dist. water 1000 ml

There are many formulations for preparing hematoxylin stains. Use of many is a matter of personal preference of whether progressive or regressive staining is being used. In situations where hematoxylin staining is followed by acidic stains, Iron hematoxylin is preferred as it resists decolorization by these counter stains. Various formulations differ mainly in regards to mordant and the shorter oxidizer used.

Cytoplasmic stains

• EOSIN (AFIP)

Eosin 1% stock

Dissolve 1gm of eosin Y water soluble in 20ml of distilled water and 80ml of

95% alcohol.

Eosin working

Stock Eosin - 1 Part

Alcohol 80% - 3 Parts

Add 0.5ml of acetic acid just before use per 100 ml

• Eosin Phloxine (AFIP)

Eosin B 1% and distilled water

Eosin Phloxine working

Stock eosin 1% in distilled water - 100ml

Stock Phloxine 1% in distilled water - 10ml

Alcohol 95% 780ml

Acetic acid 4 ml

Working solution to changed weekly

• Nuclear fast red (Kernechtrot)

Aluminum sulphate 5 gms

Distilled water 100 ml

Heat and dissolve and cool

nuclear fast red 0.1gm

Dissolved with the aid of heat, cool and filter. Add a crystal of thymol.

Nuclear stains

• Hematoxylin Ehrlich's

2% haematoxylin in alcohol 100 ml

3% ammonium or potassium alum in distilled water 100ml

Mix the two above and add the following in order

Glycerol 100 ml

Acetic aid 10ml

Keep the bottle loosely plugged

Let it ripen for 1-3 months

• Hematoxylin Harris

10% ammonium or potassium alum in distilled water 100 ml

10% alcoholic hematoxylin 10 ml

Bring the alum to boiling point and add the haematoxylin solution carefully till the solution is deep red. Add 0.5 gm of red oxide of mercury, solution becomes become deep purple. Promptly remove the flame and plunge into ice cold water. This is the most important part. Leave over night at room temperature and filter. Add 2 or 4 ml of acetic acid before use per 100 ml in the stain.

Note: In place of red oxide of mercury 0.177 gm of potassium permanganate can be used but should added after cooling the solution and never while boiling.

• Hematoxylin phosphotungstic acid Mallory's

Haematoxylin 1.0gm

Phosphotungstic acid 20gm

Distilled water 1000 ml

Dissolve haematoxylin and phosphotungstic acid separately in distilled water with the aid of heat, when cool, combine the solution and make upto 1 liter with distilled water. Let it stand for 5-6 weeks before use. Staining time - 12-24 hours

Note: Quick ripening may be done by adding 0.177 gm of potassium permanganate. However, the results are not so good.

• Weigert's iron Hematoxylin

A. Haematoxyln 1gm

Alcohol 100 ml

Let it ripen for a week

B. 30% solution of ferric chloride 4ml

Distilled water 100 ml

Hydrochroloric acid (conc.) 1 ml

Immediately before use mix equal parts of A&B add B to A and not vice versa.

Staining time 20-30 minutes

• Mayer's Hematoxylin

Hematoxylin 1gm

Distilled water 1000ml

Heat distilled water to 55 to 60°C and add hematoxylin rotate till dissolved.

Ammonium or potassium alum 50 gm

Sodium iodate 0.20gm (to be weighed exactly)

Add the above in order given

Citric acid 1gm

Chloral hydrate 50.0 gm

Above must added in order given. Allow to stand overnight before use. Solution is stable for 6-8 weeks. Staining time 6-8 minutes to increase after a month.

Some basic rules for staining

1. Keep stains and solutions covered when not in use.

2. After the slides are removed from oven these should be cooled before being put in xylene.

3. Filter stains before use.

4. Once the slides have been put in the xylene to remove paraffin they should not be allowed to dry out. Particular care must be taken not to let the sections dry at the time of mounting as the xylene easily evaporates and if the section dried before mounting preparation would become useless.

5. Care should be taken that level of any solution used during staining is such as to cover the slides.

6. Drain the slides well and blot the bottom on filter paper before putting into the next solution. This is particularly necessary in transferring from 95% to absolute alcohol and absolute alcohol in xylol.

7. Xylol used to remove paraffin should not get mixed up with the clearing xylol. It also should be frequently changed as it tends to get saturated.

8. If for bluing an alkali e.g. ammonia has been used, it should be well washed out. Failure to do that will lead to disagreeably hazy blue color of nuclei.

Haematoxylin and Eosin staining

Procedure

1. Deparaffinize in hot air oven.
2. Hydrate the section.
    - 3 dips in xylene (2 Min. each)
    - 3 dips in acetone / alcohol (2 Min. each)
    - In running tap water for 5 Minutes.
3. Mayer's haemotoxylin for 15 minutes.
4. Wash in running tap water for 20 minutes
5. Counter stain with eosin for 2 minutes
6. Dehydrate the section in 95% and absolute alcohol/ acetone 2 changes (2minutes each).
7. Clear in xylene 3 changes (2 minutes each)
8. Mount in DPX

Results

1. Nucleus - blue
2. Cytoplasm and background - pink

Causes of poor quality of staining

1. Poor or inadequate fixation of tissue.

2. Over or under-ripened Hematoxylin.

3. Overused or worked out Hematoxylin.

4. Over or under differentiation of haematoxylin

5. Insufficient blueing following differentiation.

6. Failure to wash blueing agent out of section before counter staining with eosin (especially when ammonia is used).

7. Insufficient differentiation of eosin during washing or dehydration.

8. Insufficient dehydration and clearing of sections.

9. Contamination of stains.

SPECIAL STAINING

VAN GIESON METHOD

Aim: Staining of the connective tissue

Principle: In the routine staining method collagen, elastic fibers and smooth muscle appear pink or reddish in colour. In the Van Gieson stain, collagen and most reticulin stain selectively with acid aniline dyes (acid fuchsin). Picric acid acts as counter stain for muscle and cytoplasm and form complex with the dyes. This complex has special affinity for collagen.

Reagents

1. Solution A

    (a) Haematoxylin 1.0 gm

    (b) Alcohol 95% 100 ml

2. Solution B

    (a) 29% (w/v) ferric chloride 4 ml

    (b) Conc. Hydrochloric acid 1.0 ml

    (c) Distilled water 95.0 ml

3. Weight's iron heamatoxylin solution: mix equal quantities of solution A and solution B. Color of this reagent should appear violet black.

4. Van Gieson’s solution

    (a) Saturated aqueous picric acid – 10 ml

    (b) 1% (m/v) acid fuchsin – 1.5ml

Procedure

(1) Deparaffinize with xylene

(2) Hydration take sections to water

(3) Stain with Weigert's haematoxylin for 20-40 minutes

(4) Wash in distilled water

(5) Van Gieson stain 1-3 min

(6) Rinse well in distilled water

(7) Dehydrate in absolute alcohol (2 changes)

(8) Clear in xylene (2 changes)

(9) Mount in DPX

Results

1. Collagen Red

2. Muscle and Cornified epithelium Yellow

3. Nuclei Blue to Black

Clinical significance

Several pathological changes are associated with cellular changes in connective tissues. Histological diagnosis of collagen diseases is based on the study of the section of various connective tissues.

McMANUS FOR GLYCOGEN (PAS)

Aim: Staining and identification of the various types of carbohydrates (polysaccharides & mucopolysaccharides).

Principle: Tissue structures like liver & heart, striated muscles are studied by Periodic acid Shiff stain. Periodic acid reacts with aldehyde group of the carbohydrates and afterwards reaction with the schiff's reagent produces a red or purple red colour.

Reagents

1. 0.5% w/v periodic acid solution

2. Schiff's reagent

    (a) Dissolve 1.0 gm of basic fuchsin in ≈100 ml of boiling distilled water cools to about 60 degrees and filter.

    (b) Add 20 ml of 0.1 N hydrochloric acid, cool further and add 1.0 gm of sodium metabisulphite and mix well.

    (c) Keep in the dark for 24-48 hours. When the solution becomes straw colored, add 300 mg of activated charcoal, shake vigorously, filter and store.

3. 1 N Hydrochloric acid

4. 0.1 gm of light green in 100 ml of 0.1% (v/v) acetic acid.

5. Harris hematoxylin stain.

Procedure

1. Deparaffinize and hydrate to distilled water.

2. Oxidize in periodic acid solution for 5 minutes

3. Rinse in distilled water

4. Schiff's regent solution for 15 minutes.

5. Wash in running water for 10 minutes for pink colour to develop.

6. Harris hematoxylin for 6 minutes or light green counter stain for a few seconds.

7. Wash in running water.

8. Differentiate in 1% acid alcohol solution 3-10 quick dips.

9. Wash in running water.

10. Dip in ammonia water to blue the sections

11. Wash in running water for 10 minutes

12. Dehydrate in 95% alcohol, absolute alcohol, clear in xylene two changes each.

13. Mount in DPX.

Results

With hematoxylin counterstain

1. Nuclei – blue
2. Glycogen, mucin, hyaluronic acid, reticulin, colloid droplets, amyloid infiltration, thrombi. – purple red
3. Fungi – Red
4. Background – pale green (with light green counter staining).

Gomori's method for iron

Aim: Staining of section for haemosiderin (a tissue pigment)

Principle: Hemosiderin is a brown granular pigment occurring at the site of previous hemorrhage. It is a product of the breakdown of hemoglobin. It reacts with potassium ferrocyanide in acid medium and yields a Prussian blue color.

Reagents

1. Solution A - 20% hydrocholroc acid solution (stock)

Hydrochloric acid (conc.) 20ml

Distilled water 80ml

Distilled water 80ml

2. Solution B - 10% potassium ferrocyanide solution (stock)

Potassium ferrocyanide 10 gm

Distilled water 100ml

3. Acidified potassium ferrocyanide solution. Prepare fresh by mixing equal part of solution A and solution B and leave for 20 minutes

4. Nuclear fast red stain. Dissolve 5.0 gm of aluminium sulphate is hot distilled water and add 0.1 gm of nuclear fast red mix well and filter.

Add a crystal of thymol as a preservative.

Procedure

1. Deparaffinize and hydrate to distilled water

2. Put the slides in acidified potassium ferrocyanide solution for 30 minutes

3. Rinse in distilled water

4. Counterstain in nuclear fast red solution for 5 minutes.

5. Rinse in distilled water

6. Dehydrate in 95% alcohol, absolute alcohol, and clear in xylene 2 changes each

7. Mount with DPX

Results

1. Iron pigments - bright blue
2. Nuclei - Red
3. Cytoplasm - Light pink.

الأسئلة البعدية

س١ / عدد أنواع الملونات الخاصة؟

س٢ / ما الفرق بين الملونات الخاصة والملونات الروتينية؟

س٣ / اذكر أهمية تلوين الشرائح النسيجية.

خارطة القياس المعتمدة:

المحتوى التعليمي	عنوان الكورس	الأهمية النسبية	الأهداف السلوكية					عدد الفقرات
			المعرفة	الفهم	التطبيق	التحليل	التقييم	
الكورس الاول	شرائح نسيجية	% ١٠٠	٪٥٠	٪١٥	٪٢٥	٪١٠	٪١٠	٥

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روابط مقترحة ذات صلة: