THE CUTTING EDGE

REGION IX NEWSLETTER

SUMMER NEWSLETTER ~ JULY 2010

Greetings from the Region IX Director

Heather Nymeyer

Vancouver may have successfully hosted the 2010 Winter Olympics, but this city must also boast for hosting the 2010 Region IX Education Session. Although the attendance was down from previous years, the quality of lectures and speakers met the standards. The participation of the vendors was outstanding as usual and greatly appreciated by the Region IX Executive. Without the vendor's support Region IX would not be able to continue to provide these sessions.

Along with the thank you to the vendors and speakers, Mark Elliott must be recognized for his hard work on selecting a venue, organizing speakers and many more necessary arrangements for this education session. Ann Lynde must also be recognized for providing the necessary elements for the on-line registration. The overall response for event registration was positive. Thanks to everyone else for all their hard work before, during and after this event.

Who says that Histotechnologists cannot adapt to change? During our recent Education session a speaker's flight was delayed and we were faced with a dilemma of filling this time slot. As true histologists, everyone jumped at the chance to exchange ideas discuss such topics and consolidation, fixation related to IHC and quality issues. The exchange of ideas was absolutely outstanding and the feedback given to us will allow us to expand on lectures and consider having further round table discussions. Thank you to Minnie Downie for moderating this session on such short notice.

The first Histotechnology Professional Day was held on March 3, 2010. At the recent NSH Board meeting the success of the first Histotechnology Professional Day was discussed.

This year a new Region IX director will be elected and this in turn will change the appearance of the Region IX executive. I have enjoyed my time as Director and would like to thank my executive team for all their hard work and support. I look forward to providing support to our next Region IX Board and the continued development of Region IX.

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Congratulations

May Chin's article "Developing an Internal Quality Assurance Program for Immunohistochemistry Interpretation of Her-2/neu has been selected for the cover of the Journal of Histotechnology Volume 33, Number 2, June 2010

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Seattle, WA
September 24 - 29, 2010

Program now posted on the NSH website

www.nsh.org

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Future Dates NSH S/C Dates

- **37th Annual Symposium/Convention**
  September 16-21, 2011 in Cincinnati, OH
- **38th Annual Symposium/Convention**
  September 28 - October 3, 2012 in Vancouver, BC Canada
- **39th Annual Symposium/Convention**
  September 20-26, 2013 in Providence, RI
- **40th Annual Symposium/Convention**
  August 21 – 27, 2014 in Austin, TX

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Education Update

Mark Elliott mark@nshregionix.org
Region IX Education Committee, Chair

It is hard to believe, but another Region IX Education Event has come and gone. The latest version was held in Vancouver, the end of May and from talking to people who attended it, the event was enjoyed by all. We had a wide variety of topics which were well received by those in attendance. We did have a couple of glitches this year, which is to be expected. One of our speakers was held up on the East Coast due to severe weather and could not make his session on the Friday. As Heather alluded to in her message, we quickly managed to pull together a round table discussion at the last minute, under the expert direction of Minnie Downie. This was well received by those present and there were a number of good ideas that came out of the session. Notes were taken and are available. Contact Heather for a copy. There was one other late cancellation by a speaker, but Ted Pearson from DAKO graciously stepped in and agreed to sponsor Trish Wegrynowski from Toronto to come and speak on the Newfoundland Experience. Luckily Trish was able to make it at the last minute. I would like to thank ALL of the vendors who attended. Their presence made the event even that much better and it gave everyone a chance to see what is new in the field. I would particularly like to thank Tina from Somagen for bringing in Bill DeSalvo who gave two talks on standardization/quality control; Leica for sponsoring Mark Rees and Russell Myers; and ESBE for once again brining in Donna Willis to discuss the use of microwaves in the Histo lab. I would also like to thank Garry Davis from Davis Diagnostics for providing bags for all of the attendees.

As Heather mentioned in her comments on the front page, attendance was down this year from past years and a number of possible reason have been proposed, including the fact that CSMLS was on the same weekend, the NSH S/C is being held in Seattle this year so people were saving up to go there instead, cut-backs in amount of spending to send people to educational events and an apparent lack of interest from some of the techs. Most are valid reasons and the Executive is discussing what to do with regards to future events. Next year's Education Event is scheduled to be held in Montreal, but we are just in the very beginning stages of planning, so keep your eyes open for more information to follow. Any ideas as to how to increase attendance would be greatly appreciated. For those who attended this year's event, your CEU credits are available on-line if you are a NSH member. If you are not a member, they should have been mailed to you already. If you haven't received them, contact the NSH Head Office.

As I mentioned this year's S/C is being held in Seattle and should be a great time. They have instituted some shorter, 90 minute, sessions this year which should be good.

Looking forward to seeing a lot of you at the S/C and enjoy the rest of what is left of summer.

NSH 2010 Teleconference
The complete schedule and teleconference application are available on the NSH website

www.nsh.org

August 25, 2010
The Association of Cancer and Infectious Agents
Presented by Sheron C. Lear, HT(ASCP)HTL, QIHC & Alvin W. Martin, MD, CPA Laboratory, Louisville, KY

September 22, 2010
Upper Gastrointestinal Tract Biopsies: Tissue Identification, Diseases and Stains (Special and IHC)
Presented by Mitual Amin, M.D., Beaumont Hospital, Royal Oak, MI

October 27, 2010
Connective Tissue Special Stains
Presented by Kimberly Feaster, BS, HTL(ASCP)QIHC, West Virginia University, Morgantown, WV

November 17, 2010
From LEAN to Green: A Partnership for Making an Environmentally Friendly Histology Laboratory
Presented by Carole Barone, AS, BA, HT (ASCP), Nemours-A.I. Dupont Hospital for Children, Wilmington, DE (Note: 1 week early due to holiday)

To purchase teleconferences that have already past please visit the NSH Live Learning Center on the NSH website, ww.nsh.org.

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Common Special Stains – understanding its rationale, clinical significance and be efficient and cost-effective

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Introduction

In a service histology laboratory, pathologists have to make diagnosis for every specimen that is sent by the clinicians, be they physicians, obstetricians, gynaecologists, surgeons and radiologists. The diagnosis is for the benefit of the patients that they could get an appropriate treatment to relieve their ailment. Pathologists will usually have an understanding of the specimen during gross examination by themselves or through the pathology assistants. They then make a diagnosis with their expertise knowledge when viewing routine H&E stained microscopic slides prepared from relevant tissue blocks. Special stains and IHC may be ordered to reinforce their definitive diagnosis. Though IHC is quite routinely requested nowadays, special stains still remain as the indispensable tool in providing a full picture of the morphology of each slide. Understanding of each special stain in their rationale and clinical significance will surely help histotechnologists to perform their tasks knowledgeably, efficiently and more cost-effectively.
Since his emigration to Toronto, the author has the privilege to work in many laboratories due to the merging and closing of hospitals which he has been employed during the harsh financial time in the last decade of the twentieth century. This allows him to view so many protocols and work accordingly. As his children have matured into their adulthood, the author does have the luxury to spend his spare time after work in a rather long period of time, thus doing some trifle research on special stains, especially on the modification of strength and storage of many working reagents.

H&E Stain

A good H&E stained slide will surely give pathologists a complete picture of what they are viewing under microscope. This is the first slide they are examining, in which case should provide as much information in morphology as well as cellular details. A satisfactory and acceptable H&E slide should demonstrate nucleus with crisp chromatin pattern and various eosin shades in different tissue fibres and cytoplasm. Nucleus abnormality, be the increase or decrease in size and chromasia, is crucial for the pathologist to ponder about any possibility of neoplasm. This is the reason that a well balanced nuclear staining is so important. Appropriate eosin counterstain will not mask the nucleus staining and give a more differentiation of various types of tissue.
Criteria for a satisfactory H&E slide should include a blue nucleus staining with crispy chromatin pattern when comparing various cells with leukocytes which are usually a little bit dense in colour. Calcified area should also stain blue. Eosin colour should at least have three shades, more satisfactorily if we could have more than that, with

(1) muscle, keratin, coarse elastic fibres and fribinoid bright red

(2) collagen, reticulin, myelinated nerve fibres and amyloid pink

(3) red blood cells orange

Good controlling of a satisfactory H&E staining lies on

(1) the strength of the acid alcohol and its time duration in haematoxylin differentiation

(2) the time duration in eosin differentiation in water and its subsequent dehydration in upgrading alcohol as they may lose the intensity in the process

Commercial haematoxylin and eosin usually serve satisfactorily in routine staining. However, we have to monitor the haematoxylin on daily basis if they are over-ripened. In addition, in some cold days in winter, some commercial

haematoxylin may give a pale staining due to their under-ripening as the vendor will try to give some leeway in storage and transportation that they may slow down the oxidation in the manufacturing procedure. This can be rectified by slightly warming up the reagent, thus hastening the ripening.
Sometimes, the eosin may suddenly loss their strength due to diluting out or contamination during the staining process.

Helicobacter pylori Stains

Helicobacter pylori is recognized to be the causative agent of gastritis and duodenal ulcers in human. Patients could suffer gastritis either causing peptic ulcer (due to increased gastric acid production) or prone to developing gastric atrophy such as intestinal metaplasia and gastric adenocarcinoma (due to decreased gastric acid production). Demonstration of the presence of these bacteria in any gastric biopsy could render an administration of antibiotic to a patient for their eradication. Their absence may also be a sign of a successful treatment.
Cresyl violet, Giemsa and its modified protocol, silver impregnation methods (modified Steiner, Warthin & Starry) and IHC are all preferred methods. Cresyl violet and Giemsa, especially its modified protocol, remain to be the most employed methods. These three staining methods all base on the strong affinity of the bacteria to the stain even after vigorous differentiation in water and acetic acid.
Cresy violet remains to be the most simple, effective and economical method of choice. A well-capped bottle of 100 ml working solution could be reused for at least two weeks through pouring in and out of coplin jars with an average of 30 t0 50 slides per day. Of course, we have to pay attention to minimize any dilution effect of the carrying over of water adhered to the slides.
Demonstration of H pylori in Cresyl violet stain is usually found as dark purplish-blue stumpy curved rods on the epithelial surface or in the mucosal glandular folds. A satisfactory stain should stain the nucleus purple with a very pale purple cytoplasm and clear background.
Modified Giemsa method (with no differentiation in acetic acid) should also demonstrate the bacteria as purplish-blue rods similar to that in Cresyl violet. With **traditional Giemsa method** (with differentiation in acetic acid), the blue coloured bacteria stand out more prominently against a pinkish background of mucin and cytoplasm. The working solution for both stains could not be stored and is only good for a day.

Carbohydrates: Mucin and glycogen Stains

PAS & PASD

There are quite a lot of PAS-positive substances in tissue, for example, glycogen, neutral mucin, thyroid colloid, fibrin, basement membranes and fungi. The rationale of Periodic Acid Schiff Technique (PAS) is based on the periodic acid oxidation of any glycol groups in substances to form dialdehydes. These dialdehydes would then combine with Schiff's reagent to form an insoluble magenta compound. Schiff's reagent is actually basic fuchsin with its chromophoric groups broken by sulphuration to form a colourless solution. In the presence of free aldehyde groups, an insoluble magenta coloured compound is formed. Periodic acid is the choice of oxidant as further oxidation of the formed aldehydes to carboxyl groups is avoided, thus eliminating a false negative Schiff's reaction.
Among all suggested protocols, the use of 1% periodic acid for oxidation of 10 minutes and reaction in Schiff's reagent for 30 minutes is recommended as an all-around procedure since basement membrane could be satisfactorily demonstrated. Counterstain in haematoxylin should be light, not to mask over the magenta colour of those PAS positive substances. 100 ml of periodic acid may be kept in capped bottle in room temperature and reuse on a daily basis for at least a month. So is the working Schiff's reagent which should be stored in refrigerator around 5°C. It could also be reused for a month. Its strength could be demonstrated by addition of one drop of formalin to about 5 ml solution. Loss of magenta colour shows loss of strength and should be discarded.
Diastase or amylase digestion of section slides could render the hydrolysis of glycogen to water-soluble maltose which will be washed away by subsequent rinsing in water. The comparison of a digested to a PAS slide could demonstrate any presence of glycogen. α-amylase seems to be a very effective agent in glycogen digestion. It could be used as a 0.5% solution in warm water. It does not need to be distilled water, tap water could serve the purpose. The solution should be freshly prepared. 10 minutes is quite sufficient with no sign of over-digestion which will be shown as breakdown of nucleus and cytolysis. α-amylase keeps well in refrigerator. There is no loss of activity even for a bottle which has been kept for 3 years.
Presence of glycogen in tumour cells may suggest a diagnosis of mesothelioma to that of neutral mucin (digestion resistance) in adenocarcinoma. This is the reason that pathologists sometimes order both a PAS and PASD requests.

However, simply a PASD may be the choice of request by many pathologists for the demonstration of mucins or other non-glycogen substance, giving them a more clear-cut picture.

Southgate's mucicarmine

It is very specific for mucins, even though it only stains strongly for acidic mucins and negative or weakly for neutral mucins. It is especially useful for the demonstration of Cryptococcus neoformans. The rationale of this stain hinges on the bonding of the positively charged aluminium carmine complex with the negatively charged acid mucins.
Nuclei should be well-stained with Harris' haematoxylin with no differentiation in acid alcohol but bluing in Scott's solution or warm water, as the carmine solution will later on take away the blue colour, giving it a just right picture. Some protocols suggest a dilution of the carmine solution at proportion of 1:3 to 1:9 with distilled water. Undiluted solution gives even better results with 30 minutes staining time. Counterstain with metanil yellow is not necessary as it will wash away the red colour. Working undiluted Mucicarmine solution should be stored in small staining vial and kept in refrigerator. It could be reused for at least six months on a weekly usage of one to two days.

Alcian Blue

Alcian Blue is copper phthalocyanin dyes containing positively charged groups capable of salt linkage with certain polyanions. Acid mucins, having these polyanions of sulphate and carboxyl radicals, are stained intensely and permanently with Alcian Blue. It is quite safe, or so as to speak specific, to regard any positive staining by Alcian Blue as mucin. Varying the pH of the solution produces more information concerning the types of acid mucin present. At a pH of 0.2 strongly sulphated mucins are ionized and react, at pH 1 weakly and strongly sulphated mucins react, whilst at pH 2.5 most acid mucins will stain.

Preparation of various pH Alcian Blue solutions

pH 0.2	1 gram Alcian Blue in 100 ml of 10% sulphuric acid stain only the strongly sulphated acid mucins
pH 1	1 gram Alcian Blue in 100 ml of 1.8% hydrochloric acid stain only weakly and strongly sulphated acid mucins
pH 2.5	1 gram Alcian Blue in 100 ml of 3% acetic acid stain most acid mucins

It is a good practice to immerse slides in their respective pH solvents before staining in the stain solution to avoid carry-over of water and thus avoid altering their pH. Staining time of different protocols varies from 5 to 30 minutes. For Alcian Blue at pH 2.5, staining for 30 seconds in a household microwave oven at half the power strength seems to be an efficient and effective choice of procedure. The staining of the nuclei with Alcian Blue may occur as a sign of over-power usage in microwave. A well-capped bottle of 100 ml of pH 2.5 Alcian Blue solution could be reused for a month, even with a daily batch of 30 t0 50 slides. Beware that prolonged staining in neutral red as counterstain will take away the positive blue colour of mucins.

PAS-Alcian Blue

This is a useful technique for distinguishing between acid and neutral mucins, demonstrating most mucins in a single slide preparation. Sometimes pathologists simply request a PASD-Alcian Blue instead for their convenience to rule out glycogen as well. In such cases, enzyme digestion should be performed prior to staining in Alcian Blue and subsequent PAS reaction. The rationale of the method is that acid mucins stain first with Alcian Blue and are unable to react with the subsequent PAS treatment. Neutral mucins and glycogen which do not take up Alcian Blue would then react with PAS and stain red. Cells having mixtures of acid and neutral mucins will show up as purple in colour.

Hale's Colloidal Iron

The use of dialyzed iron-Prussian blue reaction for the demonstration of acid mucins is very sensitive. Even scanty deposits of acid mucins could be shown in this stain. Many dermatopathologists order this request on skin biopsies as

connective tissue mucin production may be increased in a non-specific tissue reaction. The rationale of the method is that at low pH colloidal iron is selectively absorbed on to acidic groups of mucin. The adsorbed iron is subsequently visualized by forming Prussian blue with potassium ferrocyanide.
A duplicate control slide to rule out any haemosiderin pigment will not be performed under normal circumstance except specially requested by the pathologists. Lower concentration of potassium ferrocyanide and hydrochloric acid (both at 2%) and shorter staining time (5 minutes) is preferred to avoid a heavy background staining, when compare to the traditional iron pigment stain.
The working colloidal iron solution is quite stable and could be kept in refrigerator and reused for a month, or longer if the order is not that frequent on daily basis. On the contrary, the working solution of ferrocyanide with hydrochloric acid has to be freshly prepared and discarded even after one use.

Connective tissue Stains

Collagen stains are of particular value in identifying the increase in collagenous tissues that occurs in chronic inflammatory processes. This is particularly the case for gastrointestinal biopsies on which some laboratories simply employ a saffron stain (Haematoxylin Phloxine Saffron HPS) on a routine basis as a special stain.

van Gieson's Stain

Though the stain is very specific for collagen, it is rather used as a counterstain for other method such as Verhoff's for elastic fibre. This is because it could not demonstrate young collagen fibres. The rationale of the method is that the small molecules of picric acid are retained in the close-textured red blood cells and muscle while the larger molecules of the acid fuchsin could displace the picric acid and bind to the larger pores of the collagen fibres.
Weigert's iron haematoxylin is employed to stain the nuclei. If used with the routine alum haematoxylin, van Gieson's solution will take away the blue colour, resulting a very pale nucleus staining. This is because of the picric acid as the ingredient. Rinsing in distilled water rather than tap water before and after the van Gieson's solution gives brighter staining results. Dehydration in alcohol should be quick as picric acid may be extracted during this process.
van Gieson's solution kept in a working bottle could be reused for at least 3 months. Weigert's haematoxylin should be freshly prepared and discarded afterwards.

Verhoff's Elastic stain

Elastic fibres are seen in tissue such as dermis of skin, lung, heart and blood vessel walls. They may be increased due to presence of tumour cells, for example, in breast cancer and elastoma dorsi. Due to hypertension, the elastic fibre may be increased in the elastic laminae of the blood vessel. On the contrary, they will be lost due to degenerative process.
Verhoff's technique seems to be a good choice for their demonstration when compare to other methods such as Orcein and aldehyde fuchsin. Very fine elastic fibres could be demonstrated with Verhoff's haematoxylin, though with skilful technique in its differentiation with ferric chloride solution. Working solution should be freshly prepared, as ferric chloride acts as an oxidizing agent to ripen the haematoxylin and iodine as a mordant. As van Gieson is usually the counterstain which could provide a better picture with the staining of collagen and muscle, the slides should be a little bit under differentiated with ferric chloride that fine elastic fibres could still stand out nicely after the counterstain. It is because the van Gieson's solution contains picric acid and has the effect for further differentiation, thus making the very fine elastic fibre invisible.

Masson's Trichrome

It is very useful to demonstrate young collagen fibres and for differentiating muscle from connective tissue. However, formalin fixed tissues give poor results and they need to be pre-mordant in Helly's or Bouin's fluid. Bouin's fluid is preferred as Helly's contains mercuric chloride. Moreover, the treatment time is shorter. Some laboratories obtain very satisfactory result by treating the slides in Bouin's at 80°C oven even for 15 minutes. In this condition, charged slides are recommended to avoid section falling off. Bouin's fluid should be kept in flammable cabinet. They could be reused for at least 3 months.The rationale of most trichrome methods is based on the relative molecular sizes of ingredients to that of the tissues being demonstrated. Electrostatic forces may also pay a role. The smallest molecules (ponceau 2R or Biebrich scarlet)

enter and firmly attach to any tissue with small intermicellar spaces, e.g. rbc. The larger molecules (acid fuchsin) could not enter those spaces and could only hold to tissue with larger intermicellar spaces such as muscle and collagen. However, the dye acid fuchsin which holds to the collagen is displaced by phosphomolybdic acid and phosphotungstic acid as an act of differentiation since they are not securely bonded. This provides a chance for the largest molecules (light green or aniline blue) to take the vacant space and thus stain the collagen. The success of the stain depends on the degree of differentiation of the ponceau-acid fuchsin by the PMA and PTA. Successful differentiation is achieved as the connective tissue is almost unstained.
Recipes of Biebrich Scarlet and Acid fuchsin for different laboratories range from equal part of both dyes in 1% acetic acid to a proportion of 9:1. This is not that important as they all give satisfactory result. They are quite stable and could be reused for even 6 months. In addition, some laboratories use the combination of PTA and PMA while others just use PMA. PMA alone gives comparable if not better result in differentiation. Discard the PMA afterwards. Apply the green or blue stains onto the slides without any rinsing of them in water will give a strong collagen staining effect. These counterstains could not be reused and should be discarded as they are contaminated with PTA / PMA.
Similar to van Gieson, nuclei should be stained with Weigert's iron haematoxylin as the subsequent treating reagents contain acetic acid as well as PMA.

Gomori One Step Trichrome

This is a very simple method and gives comparable result to that of Masson's Trichrome. For formalin fixed sections, they also need to be treated in Bouin's fluid. The nuclei should be stained with Weigert's iron haematoxylin. This one step trichrome staining solution keeps very well. A bottle of 100 ml could be reused for 3 months.

Reticulin stain (Gordon and Sweet's Technique)

Reticulin fibres form a delicate supporting framework for many highly cellular organs, such as liver and lymph nodes. The loss of this framework in lymph node or bone marrow may be suggesting the diagnosis of malignant lymphoma. So is the importance of a diagnosis of cirrhosis in liver biopsy for any irregularity in the reticulin framework.
The rationale of this technique is based on the argyrophilia of the fibre with the enhancement by iron alum, as other argyrophilic substances such as nerve fibres are suppressed by the treatment of potassium permanganate. Acidified potassium permanganate is not mandatory as normal aqueous solution does achieve the same effect. Staining protocol without toning in gold chloride will give more distinction characteristics between collagen and reticulin (brown and black), though it is not commonly practised here in Toronto.
A good reticulin stain depends on the appropriate preparation of the ammonical silver solution. Over titration of the silver solution with ammonia gives poor results. Some precipitate granules should remain rather than having a clear solution in the first titration. This could be reverted by adding of silver nitrate for any over-titrated clear solution. After the addition of sodium hydroxide, the number of drops of ammonia should be much the same in the second titration. Much more than this could lead to a suspicion of over titration, since excess ammonia may form precipitation granules again with no obvious observation of a clear end-point. This working solution could be kept in refrigerator in a well-capped plastic coplin jar and reused for up to two weeks. Filtering with filter paper is not necessary as the bleach remained in the paper may have an effect and deteriorate the staining property of the filtered solution subsequently, especially after storage. Silver deposit is not observed in good preparation even without filtering. The impregnation time seems to be sufficient for at least one and up to two minutes. For sub-optimal prepared solution (over titrated), a good staining could be achieved by double impregnation. That is a treating of the section in ammonical silver solution once again after reduction by the formalin if staining is pale macroscopically. Rinsing in distilled water is mandatory before the second impregnation. The sections which look pale even after double impregnation could be put back in iron alum, to be re-stained with a better and fresh solution which may lose its strength after being used for a period of week or so. Counterstain should be light not to mask over the collagen if toning is employed.
Potassium permanganate, iron alum, formalin in tap water and gold chloride could be reused for more than 3 months with no loss of strength

Pigments Stains

Pigments can be classified as artefact, endogenous and exogenous. Endogenous pigments are produced normally by the body and are found in pathological conditions.

Iron and haemosiderin

Perl's Prussian Blue

The rationale of this technique is that the acid-split ferric ion from the bound protein will react with ferrocyanide to form blue deposits. Demonstration is best seen with equal portion of 2% potassium ferrocynide and 2% hydrochloric acid with a reaction time of 5 minutes. Working solution should be freshly prepared. Sections should be well washed in water before counterstain with neutral red to avoid heavy dye precipitation. Staining for three to five minutes in 0.2% of filtered neutral red solution serves very good to give an acceptable counterstain without masking nor precipitation. This solution could be kept in room temperature for at least a week.

Calcium

Calcium is usually seen in necrotic tissue as an inflammatory change, such as breast and prostate tissue. Radiographic demonstration is nowadays quite common for their very minute presence. This is more superior to any known histological special stain which could only demonstrate larger deposits of calcium salts.

Von Kossa Stain

This is the widely used method for calcium demonstration even though it is not specific. The rationale is that the calcium in calcium salts is substituted by silver which is then reduced to black metallic silver by light. A higher concentration of 10% silver solution is superior for the demonstration when compared to the usual two to five percent. The solution could be reused for three months when kept in refrigerator. Some laboratory omits the fixing step with hypo treatment which does have some bleaching effect on the dark deposit. However, the slide should be examined as soon as possible since the colour will fade away and could not be kept for a rather long period of time.

Melanin

Identification of melanin as secondary deposits is important in diagnosis of malignant melanoma. Melanin is easily bleached with potassium permanganate, not necessary in acidified solution with sulphuric acid. Bleaching of melanin pigment is required either to confirm its identity, or to more easily study the cell picture in a heavily pigmented lesion. Fifteen minutes of bleaching is more than sufficient for their removal. However, charged slides should be used as otherwise sections may easily fall off.
The rationale of melanin stain is its reducing capabilities with silver (Masson – Fontana) or ferricyanide (Schmorl).

Masson – Fontana Ammonical Silver Technique

This is a mostly employed technique for demonstration of melanin pigment in the hospitals of Greater Toronto Area although it does not show marked differentiation between other brown and dark pigments. A capped coplin jar of well titrated solution could be reused even for up to six months.

Schmorl's Technique

This is a preferred technique as it gives distinction between other brown and dark pigments. It is rather quick when compared to the silver technique. The staining solution should be freshly prepared.

Abnormal protein

Amyloid

Amyloid is abnormal protein which is usually associated with chronic inflammatory diseases such as myeloma, endocrine and thyroid tumour, tuberculosis, colitis, cardiomyopathy and Alzheimer's disease. Amyloids have some carbohydrate content ranging from 1 to 5%, thus the name amyloid indicating starch-like material. Different types of amyloid are associated with particular diseases.
Alkaline Congo Red is a preferred technique. The rationale of the stain is the strong affinity of the amyloid to the dye. Together with the alkaline sodium hydroxide, sodium chloride acts as ionic competitors for the dye, thus eliminating any non-specific background staining. This is a progressive method which does not require any differentiation. In addition, the double-pleated structure of the amyloid fibrils could hold the dye in parallel alignment which allows green birefringence when seen under polarized light. This red-green birefringence is a reliable index for the presence of amyloid. Thick sections from 6 to 8 µm are recommended for the good demonstration of birefringence. It is not quite necessary to cut the control section fresh. Moreover, a well-capped coplin jar of 50 ml of either working staining solution could be reused for two weeks. It could be longer for the working alkaline sodium chloride solution, even for up to three months.

Fibrin

Fibrin will be seen in tissues where damage is due to an acute inflammatory reaction. It is especially useful to aid diagnosis in renal necrosis due to abnormal fibrin formation.

Martius Yellow – Brilliant Crystal Scarlet – Soluble Blue (MSB)

This is the method of choice for fibrin demonstration especially it is capable to differentiate between early, mature and old fibrin. The rationale is similar to that of trichrome stain, based on the relative molecular sizes of ingredients to that of the tissues being demonstrated.
Pre-mordant in Bouin's fluid gives better result, especially good differentiation between old fibrin and muscle. An efficient modification of this treatment could be achieved by treating the slides in 80°C for 15 minutes, rather than using the mercury containing Helly's fluid.
All colour stains could be reused for up to six months. PTA should be used for once as it is a differentiating solution for the red colour.

Microbes Stains

ZN Stain

Tubercle bacilli have a lipid-rich cell wall, consisting of many unsaturated lipids which have carboxyl and hydroxyl groups. The rationale of this stain is that these groups hold up the dye upon differentiation by acid and / or alcohol.
Some laboratories eliminate the addition of phenol in the staining solution. There is not much difference as phenol acts as a surface tension depressant to allow the dye ions to enter the lipid envelope of the bacteria. However, some delicate variants may not be stained in phenol-absent solution, especially under vigorous alcohol differentiation. Moreover, the dye is not well dissolved in water and should be filtered before use. In contrast, filtering of the phenol containing solution may not be necessary if thorough mixing during preparation is enhanced by magnet stirring with mild heat for a period of five to seven hours.
Heating in the staining process is not crucial for phenol containing solution as it gives same result even for delicate variants in minute quantity. This is very efficient as slides could be laid flat on a rack and solution applied separately, thus eliminating cross-contamination of any tubercle bacilli from positive cases.

Some tissue (e.g. keratin) may give non-specific staining with carbol fuchsin. Treatment with potassium permanganate and oxalic acid for a short time (one to two minutes each) could bleach away this non-specific staining. This step does not have any effect on the stained TB bacteria.

It is important not to counterstain the slide too dark as it will mask any presence of the bacteria, not giving them a bright red colour.

Wade Fite Stain

These bacilli causing leprosy are only acid fast, thus alcohol could not be used in differentiation and subsequent dehydration. Moreover, dewaxing of the sections should go through a peanut oil and xylene mixture. This is because their lipid envelope is more easily affected by fat solvents, thus diminishing the staining reaction. To avoid the incomplete clearing of peanut oil in the section during dewaxing as it may give patchy red staining in some area, a quick rinse in xylene alone (one to two dips) before blotting the slides with filter paper gives very satisfactory results. Moreover, quick bleach with potassium permanganate and oxalic acid makes the slides more presentable with no patchy staining. As mentioned in the ZN procedure, heat is not necessary in the staining procedure.

Gram's Stain

The rationale of Gram-positive organisms is that these bacteria take up the dye of crystal violet and form a complex with iodine which is resistant to acetone differentiation as the Gram-negative organisms and other cellular substances lose their dye-fastness in this treatment. Gram-positive organisms will be stained dark blue and Gram-negative organisms could still be seen in red colour against a rather clear background with nuclear staining.

A mixture of equal portion of 100% alcohol and 100% acetone gives very good differentiation effect in one to two dips, not having to worry about over-differentiation of the Gram-positive organisms. This mixture could be reused for many times if slides are well drained before differentiation. No blotting of slides is necessary. Solution should be stored in well-capped coplin jar in flammable cabinet.
Types of iodine solution, be they Gram's, Lugol's or Verhoff's, are not critical as they all give good and consistent results. The trick of a well-prepared solution lies on the addition of iodine crystal to the iodide solution (double ion effect). First dissolve the potassium iodide in very small amount of distilled water. Then add the iodine crystal which could then be easily dissolved. Make up the solution to the required amount. Verhoff's is the most concentrated, followed by Lugol's and Gram's.
Neutral red is the preferred counterstain and gives very good result. For distinctive observation of Gram-negative organisms, a mixture of neutral red and fast green could be used as counterstain (Gram-Twort modification). The proportion of 0.2% neutral red, 0.2% fast green and distilled water is 9:1:30. This could be kept in room temperature and reused for three months.

Grocott's Methenamine-Silver Stain GMS / Ammonical silver technique GAS

The rationale of both techniques is based on the chromic acid-formed aldehydes from the polysaccharides of the fungi and Pneumocystis carinii (previously classified as protozoa) could reduce the silver to black in colour in alkaline pH. Ammonical silver technique (GAS) is preferred as it is simpler because the working solution could be kept in refrigerator for three months to half a year when compared to the freshly prepared solution in the methenamine technique.
Oxidation with the chromic acid for ten minutes is quite sufficient to form any aldehydes from the substance in fungi. Treatment with metabisulphite is not necessary if thorough rinsing in distilled water is followed as any acid residue will be washed away. Filtering of both the solutions is also not mandatory if the slides are thoroughly rinsed in distilled water before the silver treatment. This is especially true if a coplin jar is designated for the use and washing it well after bleaching in 0.1% sodium hypochlorite (about 1 in 20 dilution of the commercial product) every time after use. With well control of power application, the ammonical silver solution gives superior result even undergoing microwave procedure with no precipitate at all. This silver process could be finished in less than five minutes.
A good ammonical silver stain for fungi should show intracellular granules with a distinct cell wall in the hyphae and cysts instead of having them totally black in colour. For any severely blackened slide which is the result of prolonged treatment in the silver solution, this could be rectified by a quick dip in 2% ferric chloride solution, giving back a clear section with well demonstration of any fungi. Some pathologists like to order both GMS and PAS at the same time for any suspicious fungal infection as they could compare any blacken hyphae or cyst-like substances in GMS to that of PAS for a clearer picture.

Some dermatopathologists simply request a diastase digested GMS (GMSD) on skin biopsies which they suspect fungal infection to get rid of the positive staining by glycogen that may hinder the observation of any presence of fungi. Methenamine solution could be used for the demonstration of urates (no pre-treatment with any oxidizing agents such as periodic acid or chromic acid) and basement membranes (Jone's techniques with periodic acid pre-treatment).

Toluidine Blue Stain for mast cells

Mast cells are responsible for IgE immunoglobulin production and they are present in large quantity in any hypersensitivity reaction.
Toluidine blue in diluted solution (0.01%) gives a very good result for the demonstration of mast cells. The staining time is quick, less than a minute. They could then be dehydrated through upgrading alcohol with no loss of metachromasia. Concentrated solution stronger than 0.05% and long staining time gives poor demonstration as all the metachromatic granules are masked, showing no differentiation at all.

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Region IX 2010 S/C House of Delegates

Ann Lynde
Michael Ho
Maureen Bechtold
Vern Hurst
Charles Ho
Michele Shackleton
Heather Nymeyer

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S/C Region IX Annual Meeting

Plan on attending the Region IX Annual Meeting in Seattle. This meeting will be held Saturday September 25, 2010 at 4:35 pm. Check your registration package for complete details as to the meeting room.

This meeting will include voting on changes to Region IX bylaws in addition to the presentation of the 2010 Region IX awards.

Come and meet your newly elected Region IX Director.

THE CUTTING EDGE

is the official newsletter of Region IX of the National Society for Histotechnology. It is distributed to all Region IX members.

The Objectives of THE CUTTING EDGE are to:

- Reflect both Canadian and North American articles pertinent to the practice of Histotechnology
- Publish articles that are beneficial to our discipline.
- Promote communication between Region IX members ad members of the other NSH Regions
-
- Provide a medium for exchange of information among members.

Contributions are *welcome.*
Please send your articles to:

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"Lean Histology word search puzzle compliments of Leica Microsystems, Inc."

STAINING

Q S O N E C A S E F L O W H T W Y Z
S Z T W M K E K T B T P P R S A K C
P N O A U O G L J W K K A Q E L I O
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Y B D I K P W Z Q M V N C I W Y C E
C O N T I N U O U S L O A D I N G R
C S N I A T S E N I T U O R O R F N

Key Word for Staining

Automated
Code Rack Technology
Cytology
Glass Slides
Modular
Once Case Flow
Reagents
Selectech
Special Stains
Transfer Station
Cells
Continuous Loading
Eosin
Hematoxylin
Morphology
Open System
Routine Stains
Slides
Staining Protocols
Walkaway Solutions

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