HISTOGRAPH JULY, 2003.

Contents

Editorial 1
Presidents Message 2
Histo Safety: Fire incidents 3
Thoughts from a senior: ISO Accreditation for Anatomical Pathology 5
Report and Abstracts from our March Meeting at Homebush 7
Abstracts 8
- Research at the Microsearch Foundation of Australia 8
- EDUCATION IN HISTOLOGY 8
- What's In The In Tray? 9
- Candid Camera 9
- Powdered Formalin - A Road Test 10

Quality Control: Xylene Purity Test Procedure from CBG Biotech 11
Review Article: Renal Immunoperoxidase on Paraffin Sections 12
OKAMOTO AND UTAMURA MICROWAVE RHODANINE COPPER STAIN 15
SARS: Recommended Histopathology Procedures 17
Next Issue: 19

Editorial

Welcome to the June issue of Histograph.

In “Thoughts from a Senior”, Sari Barany from SEALS at the Prince of Wales Hospital Randwick describes her laboratory’s experience with ISO 9001:2000 accreditation. Andrew Gayagay presents a synopsis of our recent group meeting at Homebush and you will also find the abstracts of the meeting. What a great get-together it was.

The Histo Safety section describes instances of fire in Histotechnology laboratories, purloined from Histonet. A synopsis of recommended Histopathological procedures in cases of SARS is also included.

There is a procedure for assessing xylene quality (thanks to CBG Biotech) in the Quality Control section and this issue’s review article is on Renal Immunoperoxidase. A stain to try is from SEALS (POW) – a Microwave Rhodanine Copper Stain.

The Chairman’s message leads it all off and I would like to take this opportunity to formally acknowledge the outstanding work and tireless dedication to our group shown by our chairman, Bill Sinai, and our secretary/treasurer Penny Marr. How many years have they been the group’s guiding force? Bill and Penny would probably say “too many”! The rest of us will say “Not enough”!!- Aren’t we greedy!!!. I doubt anyone could fill the shoes of these two professional, knowledgeable, highly competent, approachable scientists. Our group is justly proud of our two office bearers.

This is the first issue of Histograph that I have had the pleasure of editing and I hope it meets your approval. If not then give me a serve! Contributions are definitely welcome and REQUIRED. Easy to do, just email me at anthonyh@chw.edu.au or ring me on (02)9845 3306. Beware I will be calling on you!

Thank you for your indulgence,

Tony Henwood.

Presidents Message

Histo Safety: Fire incidents

From Histonet:

“Once upon a time, we were participating in a tissue harvest with a collaborator. She took muscles and we took bones. We finished the harvest for the day and set up for the following day's harvests. Unbeknownst to my boss or me the collaborator placed her isopentane into our regular old –20^{o}C freezer (rather than the sparkless one in our other lab). Sometime in the morning (the fire chief says about 4:00 am) the compressor in the freezer came on with a spark and ignited the isopentane vapours that had filled the freezer space. The resulting explosion blew the door off the freezer, sailing it ten feet across the lab. The fire totally consumed the freezer interior contents before seeking further fodder in the interior spaces of the lab including the ceiling and all the wiring there. Luckily the fire department response time was short, less than five minutes after the alarm signal, but the damage was total.”

A second incident:

“The explosion was caused by ether being ignited in a fridge which was not spark-proof. The incident was caused when a rat was sacrificed by overdosing with ether vapour. The rat was then placed in the fridge, the explosion occurred and the rat was blasted through the laboratory window and impacted on a parked car. It happened in the lab adjacent to mine and as no one was hurt caused some amusement. It also caused us to be very careful in what we store in ordinary fridges and also to purchase some spark-proof fridges and freezers.”

And a third:

“Years ago, in the days of scary lab practises such as doing cut-up without gloves, I managed to singe off my eyebrows and nose hairs in an unexpected fire. This I achieved by flaming some Ziehl Neelsen slides with a wedge of cotton wool soaked in alcohol and held beneath the slides with forceps. Unbeknownst to me, some acetone had spilled into the sink and the fumes ignited with a whoosh when the flame was lit. I had a bad smell in my nose for weeks. This of course, would not happen today (?) as laboratory discipline and safety controls are much greater, and sadly open bunsen flames are no more.”

A fourth:

“A well regarded scientist was working adjacent to a lab where a colleague was boiling ether, don't ask why just accept boiling ether. When the smell of ether started to waft along the corridors it was decided that an evacuation should take place, "where's Dougie? oh **** he's still in the lab." Yes indeed, there was Dougie, slumped on the floor looking very peaceful and no doubt dreaming of the lovely Kylie Minogue. After being dragged out by the ankles he was revived and although much grayer in the hair still lives to talk of "Scientist gassed by colleague.”

A final word from Gordon Couger (gcouger@provalue.net):

“All the safety controls in the world won't stop accidents. They can only reduce them and reduce their severity. There is no way to stop people from making mistakes or making a dangerous person safe.

I would much rather work in dangerous environment with safe people than a safe environment with a dangerous person. I know a fellow that has managed to saw off all the fingers of one hand, blow up two guns with reloads, burn up two tractors and the only time he had good luck was when he looked back and caught a one inch nut in the teeth that had broken off a piece of machinery he was pulling. Had he been looking forward it would have hit him in the base of the skull and probably killed him or worse. He has also managed to mess up his back rather badly.

Safety is not a program, it’s a way of life. You don't eliminate risks but you minimise useless risks. Don't be afraid of risk just know what they are and why you are taking them. All a safety program can do is educate people and reduce the hazards around you. You are the one responsible for your safety.

Some careless people leave a trial of broken equipment, blood and injuries in their path. I expect most of you know one or two. They also tend to have car accidents, marital problems and difficulties in life in general.

If you ever find yourself working with one of these find a place that limits your exposure to them. If you are their supervisor pass them on to your worst enemy. If you get one like my friend it can infect others."

Thoughts from a senior: ISO Accreditation for Anatomical Pathology

Sari Barany
Laboratory Manager,
Histopathology Department
SEALS,
Prince of Wales Hospital, Randwick.

The Anatomical Pathology Department of SEALS Prince of Wales Hospital was audited in October 2002 and had a reassessment surveillance audit in March 2003.

The department was certified to AS/NZS ISO 9001:2000.

The audit lasted for half a day and present at the assessment were the department head, the laboratory manager, the Anatomical Pathology Quality officer, a senior from the Cytology section and the Quality Manager for SEALS.

The ISO assessment identifies whether the requirements for Quality Management Systems are in place, whilst NATA accreditation is specifically for the evaluation of technical competence of the laboratory. The NATA standards are specifically to assess the laboratory's ability to produce precise and accurate test results.

The issues that ISO looked at in Anatomical Pathology were:

1. Meeting procedures and records
    a. Review of effectiveness of the system
    b. Review of preventive actions to identify potential problems
    c. Actions resulting from management review
2. Assessment of internal Audits
    a. Audit scheduling
    b. Allocation of audits and auditor training
    c. Audit reporting
    d. Corrective actions arising from audits
3. Assessment of records of corrective and preventive actions
    a. Customer complaints
    b. System changes and improvements
    c. Non-conforming products or processes
4. Assessment of Document and Data control procedures
    a. Preparation and approval of documents
    b. Control of documents and distribution
    c. Document change procedures and identification of obsolete documents
    d. Control of computer based documentation and data
5. Assessment of Laboratory Operations
    a. Purchasing procedures and selection of suppliers
    b. Tender and contract review procedures
    c. Laboratory accommodation and environment
    d. Staff training and records of competence
    e. Sampling procedures
    f. Sample receipt and identification
    g. Selection and control of test methods
    h. Testing procedures
    i. Checking and approval of test results
    j. Control of nonconforming testing work
    k. Test reports and test record systems
    l. Storage and disposal of samples

6. Assessment of Test Equipment control procedures
    a. Identification and registration of equipment

b. Calibration methods
c. Calibration records and traceability to National Standards
d. Corrective action taken when equipment is found out of calibration

A new requirement is for every department to have a business plan, which must be reviewed at the yearly departmental management review meeting. The business plan must have objectives and expected outcomes that are measurable. This is a new area for everyone and needs a lot more work.

This is more or less what the auditors looked at in Anatomical Pathology. It is quite a lot to cover in half a day. I hope you find this information useful.

Report and Abstracts from our March Meeting at Homebush

Andrew Gayagay
Histopathology Department
The Children's Hospital at Westmead

Attending the Histotechnology group conference last March was a little daunting for me, since it was my first conference. I wondered if I would encounter a group of people that were exclusive and not receptive to newcomers. Fortunately, none of my fears materialised. I was a stranger for less than a minute. Soon after I walked in, a member struck up a conversation with me and I was soon introduced to more members.

The attendance was very well represented with both senior and junior staff coming from interstate, country NSW and Sydney, private and public laboratories. In addition to this, this seminar was a great opportunity to mingle with sales representatives displaying their latest products. A good way to put names on faces. The atmosphere was great and everyone was very friendly. I was certainly made to feel welcome.

The first session of the meeting was on the pros and cons of microwave processing and this proved to be a lively discussion. Grant Taggart and Tony Henwood gave a compelling presentation of how renal, liver and rectal biopsies can be well processed in a short period of time (depending of course on the size of tissue). This certainly made an impact on those who have never used this method or those considering using it.

Ben Wright from the Microsearch Foundation gave us great insight into replantation of severed body parts and microvascular repair. It is great to know that successful research of rat limb allo-transplantion in Lane Cove, Sydney, served as a stepping stone for the world's first successful hand transplant in France in October 1998. Furthermore, he elaborated on the histological aspects of these experiments that enabled the detection of rejection and provided tools for quantifying nerve growth, which is an essential part of rehabilitation.

Dr. Susan Arbuckle of the Children's Hospital at Westmead gave the last talk of the day on tissue retention. It was a presentation that generated a great deal of interest. She began by presenting the issues from the Alder Hey Children's Hospital in Liverpool. The Alder Hey Inquiry found that collections of children's hearts and other organs had accumulated over several decades for intended research, apparently without parental knowledge and permission. This caused a great deal of public concern about past practices by pathology services. The relatives directly involved expressed deep distress, grief and anger over the discovery that their children's organs had been taken and stored without their consent. All of these events led to comprehensive changes to current practices in many hospitals including more explicit consent forms for post mortem examinations. This includes explanations as to the difference between hospital post mortems and coroners' post mortems, more information for relatives on the purpose of the procedure, more detailed descriptions of which organs and tissues are to be retained and full explanations by clinicians on the purposes of organ and tissue retention.

The day ended with a harbour cruise that was organised by Grant Taggart and Penny Marr. Unfortunately I was unable to attend but from what I have heard those who went had their full moneys' worth.

The following day began with Tony Henwood and Grant Taggart giving a brief presentation on special stains using the microwave oven. This was then followed by discussion with a broad range of issues relating to microwave aided special stains.

The quiz tested our knowledge in all aspects of histology. Then there was the H&E, Tol Blue stain and the longest paraffin section competitions. It was all great fun. With the poster competition, though the number of participants was small, it was definitely high in quality.

To cap it all, this was one truly great experience. I came out learning so much and met so many people at the same time. Many thanks to the committee who put a great deal of effort to ensure that everyone enjoyed themselves.

Abstracts

Research at the Microsearch Foundation of Australia

Ben Wright
Microsearch Foundation of Australia

The Microsearch Foundation of Australia was established in 1978 and is recognised as a training centre of excellence for the replantation of severed body parts and microvascular repair.

The skills and techniques developed over 20 years of research culminated with the world's first successful hand transplant in France, October 1998. That accomplishment was the result of a number of years of rat limb allotransplant research in Lane Cove, Sydney.

Since that time, rat limb allotransplant protocols have evolved to encompass the fields of pharmacology, biomechanical engineering, histotechnology and microsurgery.

Histological aspects of these experiments enable the detection of rejection and provide tools for quantifying peripheral nerve growth, an essential part of rehabilitation.

The ultimate goal for limb allotransplant research is to find suitable short term drug regimes that minimise or eliminate the negative side effects associated with the immunosuppressants.

EDUCATION IN HISTOLOGY

A E Woods
Associate Professor
School of Pharmaceutical, Molecular and Biomedical Sciences
University of South Australia
Adelaide, South Australia, Australia.

There are relatively few institutions in Australia that have the capacity to offer specialised courses in laboratory histopathology. These include a number of Universities, primarily those with AIMS-accredited programs, and some TAFE colleges.

Courses are generally taught in two stages - a universally recognised, fundamental course covering tissue collection, fixation, processing, sectioning and staining and a higher level module dealing with contemporary, advanced techniques. The latter tends to reflect the developments which have occurred in diagnostic pathology and thus we have seen a decreasing emphasis on topics such as electron microscopy, neuromuscular pathology and enzyme histochemistry while immunohistochemistry, imaging procedures and tissue-based molecular pathology (such as in situ hybridisation and gene chip technology) have assumed increasing prominence.

Currently there is an international shortage of qualified and experienced pathology staff both medical and non-medical. The reasons are not entirely clear but as a consequence we are seeing a reassessment of laboratory histopathology particularly in the roles and responsibilities of the personnel involved. The emergence of molecular technologies is also predicted to have a major impact on the diagnostic process and give rise to a different kind of service on offer.

The response of the profession to these developments will have a major influence on the type, level, and extent of education required in the future.

What's In The In Tray?

Debbie Pepperall
Anatomical Pathology, University of Newcastle.

My laboratory is a research facility, which deals with a diversity of tissue samples and employs and develops procedures to improve tissue diagnosis using immunohistochemistry under the supervision of Prof. A. Leong.

We have received aquatic samples from the N.S.W. Fisheries Department, Port Stephens and this has involved research on juvenile Australian snapper (Pagrus auratus) in order to identify borfnchial chloride cells within the gill filaments using immunoperoxidase staining. Also histological procedures to help identify the flat worm (Imagine megitalic) and its affect on the viability of Pearl Farming within Port Stephens Waters and the effect on mussels from blue-green algae contamination.

The sectioning of tadpoles through to the mature bullfrog has been performed for endocrinology research. Histotechnological procedures have also been performed on all the usual four-legged research animals these including the pig.

This diversity of histology can be done when required in our laboratory.

Candid Camera

Brincat JA

Candida Albicans is the most common of the Candida species that can cause fungal infections in humans. It is a commensal organism, and an opportunistic pathogen. Growth is usually inhibited by the normal vacterial flora, however changes in the pH or composition of the normal flora (antibiotic therapy) may allow the fungus to proliferate. Candid-type fungi develop as both yeast cells and pseudohyphae (chains of budding cells). In this case study, the specimen of "tissue from a tube" came from an 83 y.o. male with a history of multiple abdominal operations.

The Gram stain shown used Neutral Red as a counterstain, the PAS was performed on a Medite stainer, and both the chromic acid and silver incubation steps in the Grocott methenamine silver were heated in a microwave oven, a practice which required the operator's undivided attention.

The results showed a fungal entity that was difficult to see in the H&E, but which is PAS, Gram and GMS positive. The budding spores and hyphae demonstrated by these techniques suggest a candida species.

Powdered Formalin - A Road Test

Gayagay, A., Mangan, V., Wilkins, G., Henwood, A.

Histopathology Department
The Children's Hospital at Westmead
Hawkesbury Road
Westmead, NSW, Australia

BBC Biochemical has launched an innovative "Powdered Formalin" that only requires water and 25 minutes mixing time to produce 10% Neutral Buffered Formalin. A sample was received and compared with conventional 10% Neutral Buffered Formalin using kidney, liver and lung tissues.

The pH of the reconstituted powdered formalin was 6.7 with an osmolarity of 1273 mOsM. 48 hours after the inclusion of fresh tissue in the powdered formalin, an average pH decrease of 0.21 was found. The concentration of the formaldehyde was found to be in excess of 4%.

Microscopically, there was no discernible difference between fixatives and special stain (PAS, Reticulin, Orcein-H&E and Masson's Trichrome) results were similar. There was also no difference in antigen localisation using immunoperoxidase (AE1AE3, Vimentin, CD45, Smooth Muscle Actin, Factor VIII and EMA).

Advantages of Powdered formalin include:

1. Easy and Safe to prepare
2. No methanol present
3. Cheaper and safer to transport
4. Less storage requirements
5. Decreased fume exposure during preparation.

Quality Control: Xylene Purity Test Procedure from CBG Biotech

Note: The recommended and most accurate method of determining the purity of the recycled xylene is by doing a GC analysis. The following method can be used to obtain an acceptable confidence level in the purity of the recycled xylene.

1. Establish a 1% calibration mixture. Add 5 ml of Absolute Alcohol to 495 ml of xylene to establish this mixture. Since this procedure has a number of variables the calibration mixture will be used as a standard for the person who has to perform the procedure. By testing this standard mixture the person performing the procedure can see how a 1% contamination level reads and can have confidence that all of the readings that they obtain using this method are accurate. It is recommended that this mixture be tested at the beginning of each day.

Testing Procedure

2. To a clean, dry 100 ml mixing cylinder graduate (KIMAX 20039-100 or equivalent), add sufficient recovered xylene so that the bottom of the meniscus is aligned with the top edge of the 85 ml mark on the graduate.

3. Add water to the graduate until the bottom of the meniscus aligns with the top edge of the 100 ml mark on the graduate. At this point, 15 ml of water will have been added to 85 ml of recovered xylene.

4. Stopper the graduate and invert the mixture. Allow the mixture to settle, making sure that all of the water settles to the bottom of the graduate. No water should remain clinging to the sides of the graduate above the xylene/water separation point. This separation point should be near the 15 ml level of the graduate. **(Note: xylene floats on top of the water.)**

5. Carefully inspect and record the point of separation between the water and xylene using the bottom of the meniscus as the separation point.

6. Subtract 15 ml from the quantity of water indicated in step 5. The remainder plus an additional 0.1 correction factor equals the percentage of recovered xylene impurities.

   EXAMPLE:
   Xylene/Water separation point is indicated to be 15.5 ml.
   (15.5 - 15) + 0.1 = 0.6% impurities. Therefore, the recovered xylene is 99.4% pure.

   If you have questions, please call the CBG Biotech Technical Support Division.

Review Article: Renal Immunoperoxidase on Paraffin Sections

In a questionaire by the UK NEQUAS (Renal Pathology) to 58 laboratories who routinely assess renal biopsies, many respondents stated that they would switch from immunofluorescence to immunoperoxidase if only the method was more reliable (3).

Comparison between IF and IPX (3):

Immunofluorescence	Immunoperoxidase
Reliable (if glomeruli are present)	Unreliable
Need frozen sections	Use paraffin sections - can compare results with other stains
Poor morphology	Good morphology
Technically easy	Technically difficult
Time consuming	Time consuming
Not permanent	Permanent
Rapid	Slow
Need UV microscope	Often misses linear staining in Goodpasture's disease

For successful immunoperoxidase detection of immune complexes, various authors have made the following points:

- Enzyme pre-treatment enables the localisation antibodies to be diluted with a reduction in background staining and improved specificity and consistency (1)
- Standardisation of fixation is required prior to controlled treatment with enzymes in order to ensure consistent results (1).
- The purpose of proteolyic enzyme pre-treatment is two-fold, uncover the antigenic sites in the glomeruli and to reduce background staining especially if the plasma has been fixed in the capillary loops (3).
- For the method to be reproducible on renal biopsies, extensive dilution of the localisation antibody is required (eg for IgG a 1/20000 dilution or 10ul in 200ml of antibody diluent when used in a routine PAP or ABC technique) (5,6).
- Plasma Ig's do not stain in frozen sections since they are not fixed, so they wash out leaving tissue bound immune complexes behind. In paraffin sections the plasma proteins are fixed and must be removed by enzymatic digestion (3)

The following results are obtained with differing enzyme reactions (1):

Enzyme Pre-treatment Status	Microscopic Results
Under-digestion	No Staining Staining of protein within capillaries
Correct Digestion	Detection of immune complexes with anti basement membrane antibodies
Partial over-digestion	Loss of complement components (C3 & C4)
Over-digestion	Linear staining of IgG on GBM and Tubular BM Nuclear and collagen staining

Controlling enzyme pre-treatment

Howie etal (2) controlled the enzyme pre-treatment using phase contrast microscopy or a microscope with a lowered condenser. After 3 minutes, a slide was rinsed in PBS and examined microscopically. The first indication of digestion was granularity appearing in tubules. As digestion proceeded the glomerular basement membrane stood out as plasma was removed giving a sharp contrast with the

almost uniform hyaline appearance of an undigested glomerulus. Sometimes red blood cells were also removed from capillary loops, enhancing the contrast. Debris is also removed from Bowman's Space. Optimum digestion produced no staining of plasma in capillary loops but permitted intense staining of immune deposits.

In-built Controls

- Tubular casts commonly contain IgG and IgA and act as in-built positive controls (1)
- Plasma cells which frequently infiltrate the interstitium in small numbers, should only react with antisera against IgG,A or M and not with antisera to complement proteins (1)
- IgG, IgM and Complement often show granular staining in the cytoplasm of tubule cells. This is believed to be associated with mitochondrial uptake and is a useful internal control (5).

Problems with Complement C3 Staining

Howie et al (2) found that C3 was frequently missed by immunoperoxidase. They found that 16/65 renal biopsies positive for C3 using immunofluorescence but negative using IPX on paraffin sections. All 16 IPX negative cases were positive for C9. C9 was found at the same sites as C3 on frozen sections of renal biopsies.

Recommendations:

1. Develop expertise with the microscopic control of enzyme pre-treatment
2. Howie etal (2) recommend that the inconsistency in the demonstration of complement can be avoided by staining for C9 rather than C3.
3. Thomas & Howart (4) recommend that immunofluorescence should be used for cases of acute renal failure when the demonstration of linear IgG by immunoperoxidase is unreliable.
4. Rinse renal biopsies in Hank's solution for several minutes (60min has been recommended) prior to fixation in formalin to reduce the plasma in capillary loops (4).

C4d Immunoreactivity

Peritubular capillary deposition of C4d has been shown to be associated with both acute humoral and vascular rejection and increased graft loss (7,8). This was found to be independent of histological rejection type (7).

Patients were considered C4d positive if 25% of the peritubular capillaries exhibited circumferential staining (7). Successful staining relies on Microwave antigen retrieval.

Peritubular capillary C4d deposition in acute allograft rejection is a predictor of long term graft failure (7) and it has been suggested that these patients would benefit from intensive therapy, potentially preventing the previously reported high graft failure rate (8).

References:

1. MacIver, A.G., Mepham, B.L., (1982) "Immunoperoxidase techniques in human renal biopsy" Histopathology 6:249-267.
2. Howie, A.J., Gregory J., Thompson, R.A., Adkins, M.A., Niblett, A.J., (1990) "Technical improvements in the immunoperoxidase study of renal biopsy specimens" J Clin Pathol 43:257-259.
3. Furness, P.N., Boyd, S., (1996) "Electron microscopy and immunocytochemistry in the assessment of renal biopsy specimens: actual and optimal practice" J Clin Pathol 49:233-237.
4. Thomas, C.M., Howart, A.J (1996) "Assessment of Renal Biopsy Specimens" J Clin Pathol 49(7):614-5.
5. Griffiths, M.H., Miller, K., (1995) "Peroxidase immunocytochemistry on formalin fixed, routinely processed paraffin-wax embedded renal biopsies: a method for demonstrating immune-complexes in glomerulnephritis" UK NEQAS-ICC 6:2-5.

6. Birchall, I.W., (1996) "Direct antibody method for formalin fixed, paraffin embedded renal biopsies: a comparison with the peroxidase labelled streptavidin/biotin method" J. Histotechnol 19(2): 125-129
7. Herzenberg, A.M., Gill, J.S., Djurdjev, O., Magil, A.B., (2002) "C4d deposition in acute rejection: An Independent Long-term Prognostic factor" J Am Soc Nephrol 13(1):234-41.
8. Nickeleit, V., Zeeiler, M., Gudat, F., Theil, G., Mihatsch, M.J., (2002) "Detection of the complement degradation product C4d in renal allografts: diagnostic and therapeutic implications: J Am Soc Nephrol 13(1):242-51

Try this Stain:

OKAMOTO AND UTAMURA MICROWAVE RHODANINE COPPER STAIN

From SEALS, POW Hospital, Randwick

PRINCIPLE

Copper is a normal constituent of many tissues and is an important structural component of some of the oxidase enzymes, particularly cytochrome oxidase and DOPA oxidase. It is usually present in such small amounts that it cannot be demonstrated histochemically.

There are four main methods available for demonstrating copper:

1. The dye lake method using non-ripened alkaline haematoxylin
2. The DMABR (dimethylaminobenzylidinertiodanine technique
3. The rhodanine technique
4. The rubeanic acid technique.

In the rubeanic acid technique, rubeanic acid forms an insoluble dark greenish-black copper rubeanate precipitate when a section containing excess copper is treated with a dilute alcoholic solution of rubeanic acid. The technique is not sensitive but gives good results.

The rhodanine technique is a reliable method for copper. The positive red colour is considered by many to be more easily discerned than the dark green of the rubeanic acid technique. The rationale is that rhodanine forms chelate bonds with copper.

Note:
Copper may be indirectly demonstrated by the orcein technique for copper-associated protein.

SPECIMEN
A 3um paraffin section mounted onto a clean glass slide.

QUALITY CONTROL SECTION

A 3um rhodanine positive control section is stained together with the test material to ensure staining method is working. This is recorded and checked off in the stains quality control book.

REAGENTS

0.5% Celloidin Stock Solution

Celloidin	2.5 g
Absolute Alcohol	250 ml
Diethyl Ether	250 ml

- Dissolve celloidin in absolute alcohol and ether.
- Store in closed stoppered bottles to prevent evaporation.

Celloidin Working Solution

0.5% Celloidin, stock solution	15 ml
Absolute Alcohol	235 ml

- Pour 0.5% celloidin stock solution into staining dish.
- Add absolute alcohol.

Rhodanine (Stock Solution)

Absolute Alcohol	100 ml
5-(4"dimethylaminobenzylidine) rhodanine	0.5 g

- Keep stock solution in amber bottle in refrigerator. Solution stable for 1 month.

Rhodanine (Working Solution)

Sodium Acetate-trihydrate	1 g
Dístilled Water	50 ml
37% Formaldehyde, concentrated	0.15ml
Rhodanine, stock	3 ml

- Do not shake rhodanine stock solution when preparing working solution.

Modified Lille-Mayer Haematoxylin

Haematoxylin (CI 75290)	5 g
Distilled Water	800 ml
Aluminium Ammonium Sulphate (Aluminium Alum)	50 g
Glycerol	200 ml
Sodium iodate	0.3 g
Acetic Acid	3 ml per 100 ml solution

- Dissolve haematoxylin and ammonium alum in the distilled water using stirrer.
- Add glycerol and sodium iodate and mix well using stirrer.
- Before use add acetic acid. This increases the precision and selectivity of the nuclear stain.
- Filter before use.

0.05% Sodium Tetraborate (Borax)

Sodium Tetraborate (Borax)	0.5 g
Distilled Water	1000ml

PROCEDURE

1. Deparaffinise sections in xylene then rinse in 2 changes of absolute alcohol.
2. Place slides in a film of 0.5% celloidin for 2min.
3. Place slides in 80°C oven until dry for 2min.
4. Place slides in 70% alcohol (to harden celloidin) for 1min.
5. Wash slides in running water. Rinse in distilled water.
6. Place slides in filtered rhodanine in plastic coplin jar.
7. Heat in microwave oven at high power for 20sec.
8. Remove coplin jar from microwave oven, agitate slides and place coplin jar in 54° C convection oven for 30min.
9. Rinse well in distilled water.
10. Stain in Lillie-Mayer's haematoxylin for 20sec.
11. Rinse in distilled water.
12. Rinse in 0.05% sodium tetraborate (borax) to blue for 20sec.
13. Rinse well in distilled water.
14. Blot section dry and take sections directly to xylol.
15. Mount in automatic coverslipper.

RESULTS

Copper - bright red
Nuclei - light blue

REFERENCE

- Lindquist, R.R., Arch of Path 87 (4): 370-379,1969.
- Wenger, J,, Armed Forces Institute of Pathology, Washington, U.SA 1989
- Walsh, B. Department of Anatomical Pathology, The Prince of Wales Hospital NSW. 1989.

SARS: Recommended Histopathology Procedures

Tissue Specimens

From the American Centers for Disease Control and Prevention website (1):

Fixed tissues (formalin fixed or paraffin embedded) from all major organs (eg. lung, trachea, heart, spleen, liver, brain, kidney, adrenals)

Formalin fixed tissue is not considered a biohazard or chemical hazard (1)
Store and ship at room temperature. *DO NOT FREEZE FIXED TISSUES* (1).

Work surfaces and equipment should be decontaminated after specimens are processed. Standard decontamination agents that are effective against lipid-enveloped viruses should be sufficient (2).

Autopsies

The Commonwealth department of Health (3) has posted the following guidelines for the performing of autopsy of confirmed and suspected SARS cases:

Healthcare workers should apply full precautions when handling the body of a deceased SARS infected person as listed below:

- Gloves,
- Long sleeve gown,
- Eye protection (goggles, visor or face shield), and
- Properly fitted P2 (N95) mask (respirator).

The body should be sealed in an impermeable body bag. This may require double or triple bagging and sealing of the zip or other openings with airtight tape. Alternatively the bag may be placed within a large thick plastic outer bag that can be sealed.

"There is less risk of spread of infection to workers involved in the transport and routine preparation of bodies by aerosol from the lungs of dead bodies than from those of live patients. Nevertheless, actions, which could result in the forceful expulsion of air from the lungs, such as dropping a body, should be avoided. Also, when workers are in close contact with the upper airways of the body, such as during lifting, it is wise to cover the body's mouth and nose with some form of cloth or padding."
(source: Infection Control Guidelines for the Funeral Industry Part A 1.10)

Post mortem assessment

All post mortem procedures require adherence to standard precautions with use of appropriate and facilities with appropriate safety features. Mechanical devices used during autopsies can efficiently generate fine aerosols that may contain infectious organisms. Thus, personal protective equipment should include both protective garments and respiratory protection.

Protective garments**:** surgical scrub suit, surgical cap, impervious gown or apron with full sleeve coverage, eye protection (eg., goggles, visor or face shield), shoe covers and double surgical gloves with an interposed layer of cut-proof synthetic mesh gloves.

For respiratory protection: a properly fitted P2 (N95) mask/respirator or powered airpurifying respirator (PAPR) equipped with a high efficiency particulate air (HEPA) filter. PAPR is recommended for any procedures that result in mechanical generation of aerosols, eg. use of oscillating saws. Autopsy personnel who cannot wear P2 masks because of facial hair or other fit-limitations should wear PAPR. However, PAPR's should only be used by individuals who have been trained in their use and disinfection.

For autopsies and postmortem assessment of SARS cases, safety procedures should include:

1. Prevention of percutaneous injury: including never recapping, bending or cutting needles, and ensuring that appropriate sharps containers are available, and
2. Careful handling of protective equipment.

Removal of PPE

Personal protection equipment must be removed in a way that does not allow transmission of SARS coronavirus to the wearer.

Gloves are likely to be heavily contaminated and should be removed first.

The other items of PPE are fomites that are likely to harbour virus, particularly if the patient has been coughing. Touching these items could allow introduction of the virus to the wearer through broken skin or hand contact with mucous membranes. Take care, cover wounds and do not to allow contaminated hands to touch mucous membranes.

The steps in PPE removal are:

1. Remove gloves by rolling back from the wrist, do not touch skin.
2. Remove goggles/visor/shield and wipe with an alcohol wipe.
3. Remove gown and fold carefully with contaminated side in and place in covered linen bin.
4. Remove P2 (N95) mask/respirator by touching the tapes only, not the front of the mask, discard in covered receptacle.
5. Immediately wash hands VERY WELL or use an alcohol rub.

References:

1. http://www.cdc.gov/ncidod/sars/pdf/specimencollection-sars2.pdf
2. http://www.cdc.gov/ncidod/sars/pdf/sarslabguide.pdf
3. http://www.health.gov.au/sars/pdf/2_icg.pdf

Next Issue:

Safety – DPX Dangers
Quality Control – Gram controls
Curiosa – Chicken Antibodies – store the eggs in the fridge
A ZN stain on Steroids