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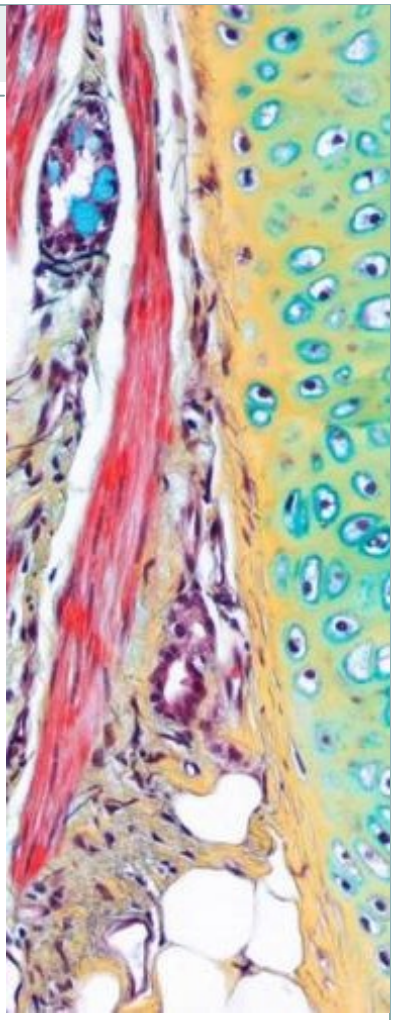
ISSUE 1
April 2021

Newsletter of The Histotechnology Society of NSW

HISTOGRAPH



Editorial

Hi and welcome to the first edition of the Histograph for 2021. Hoping all our members are staying safe and well during this challenging and unprecedented times due to COVID-19 pandemic.

Hope everyone had the time to read the two papers published by Tony Henwood and Dr. Yongfu Wang on the concerns about safely dealing with bodily fluids and tissue from COVID-19 patients which will be ongoing for sometime. The papers were "Coronavirus disinfection in histopathology" and "A review of histology practices in Covid-19 pathology investigation".

Most labs are still continuing to work under biosafety practices when handling SARS-CoV-2 specimens. I have put a list of abstracts together dealing with safety precautions when handling such specimens. Most journals regarding coronavirus safety protocols have open access.

Mark Bromley has written an article on acronyms and the voluntary certification process for Scientist. The medical laboratory science profession in Australia now has its own national professional certification scheme. For more information check out the ACCMLSW website www.ACCMLSW.wildapricot.org to get familiar with the certification process and then decide for yourself if you want to become certified.

Tony Henwood has written an article on Syphilis histochemistry since it is a major public health problem with an estimated 12 million new cases per year worldwide.

Hope you enjoy reading this issue and have some histo fun with our Test and Teach quiz. It is entertaining and highly educational.

Linda Prasad

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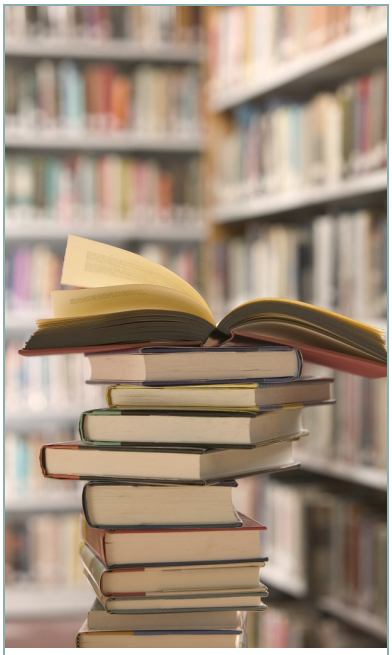

Histotechnology Society of NSW COMMITTEE MEMBERS are volunteers who work tirelessly to promote histotechnology and provide educational opportunities for continuing professional development. Thank you team for the GREAT JOB

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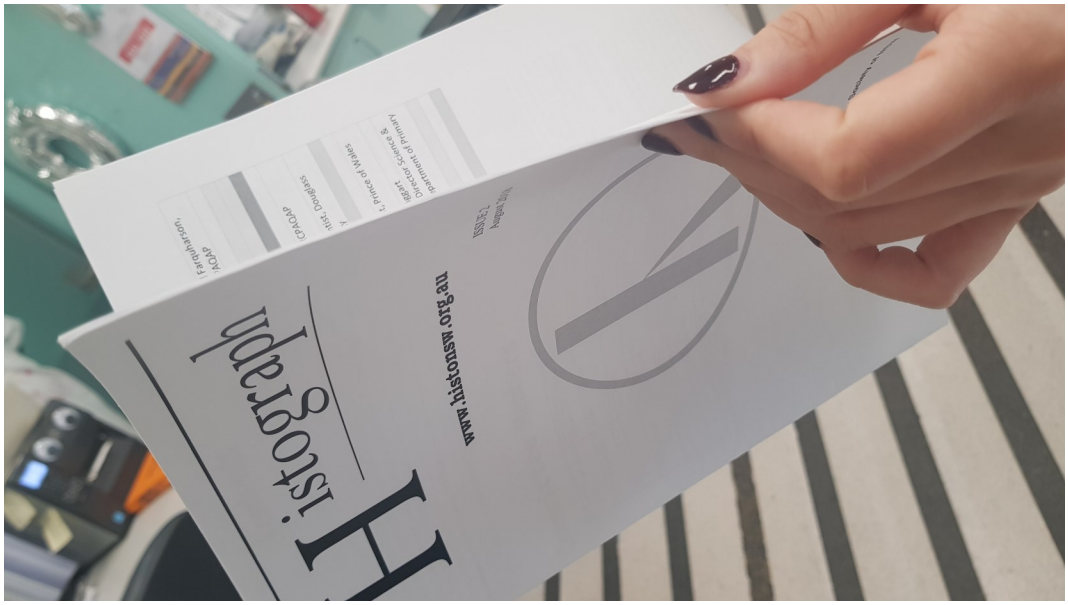

Chairman's Report

On Friday evening the 26th of March 2021, we held our delayed 2020 AGM. Due to continuing COVID-19 issues we held our first virtual AGM by Zoom. There was no guest presentation. All committee positions were filled, some new faces with most of the previous committee renominating. Some constitutional amendments were approved.

Our student committee members prepared a promotional flyer to be distributed to UTS students. They are an important part of our committee and promoting our Society.

We created a new position (Membership Officer). Elana Petrovska has kindly taken on this role which was approved at our AGM. This will enable us to have more contact with our members.

In Conjunction with the Histology Group of Australia (HGA) we are working on holding the next National Conference in Sydney early next year. We are currently exploring venues and locking in a date.

Update from HGA on the "Australian Council for Certification of Medical Laboratory Scientific Workforce (ACCMLSW). HGA is now a member of ACCMLSW with a director on their board and two committee members. We see the accreditation of Histology laboratory staff as an important component of ACCMLSW and encourage our members to register with ACCMLSW noting HGA as their organisation. This can be done through the ACCMLSW website.

Our first Webinar has been held on the 27th of March, on "Embedding" which was well attended.

Please follow the space for other Webinars planned for 2021 on page 12 for details of this years webinars.

Cheers,
Trevor Hinwood, Chairperson
Histology Society of NSW

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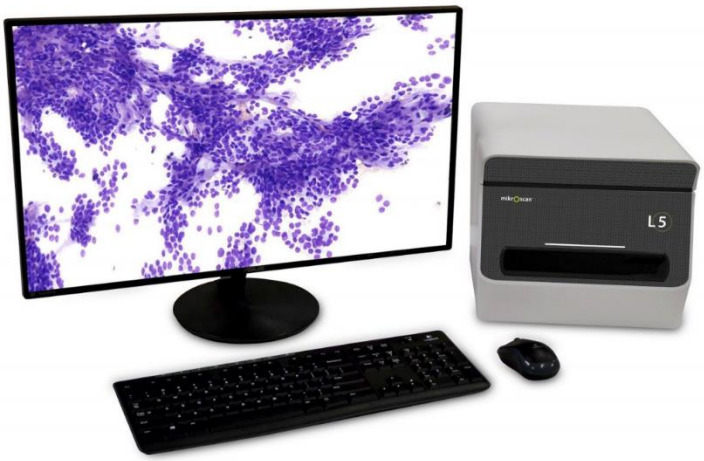

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An Update on Syphilis Histochemistry

Tony Henwood, Histopathology, the Children's Hospital at Westmead, NSW, Australia

The venereal disease known as syphilis has been around since ancient times. In 1906, Levaditi-Manouelian first demonstrated the organisms by impregnating a whole tissue block with silver (1). Syphilis is caused by Treponema pallidum subsp. Pallidum (2).

Treponematoses are infections caused by the spirochetal organisms of the Treponema species. These bacteria are the cause of both syphilis (Treponema pallidum ssp. pallidum) and the so-called nonvenereal or endemic treponematoses consisting of yaws (T. pallidum spp. pertenue), bejel (or endemic syphilis) (T. pallidum spp. endemicum), and pinta (T. carateum). Dilemmas exist in the diagnosis of patients with endemic treponematoses because clinical findings do not always accurately identify patients with the disease, and serologic methods are unable to differentiate these disease entities from venereal syphilis and from each other (3).

Yaws is an infectious disease caused by Treponema pallidum pertenue, a bacterium that closely resembles the causative agent of syphilis and is spread by skin-to-skin contact in humid tropical regions mainly among children. Yaws causes disfiguring, and sometimes painful lesions of the skin and bones. As with syphilis, clinical manifestations can be divided into three stages; however, unlike syphilis, mother-to-child transmission does not occur (3).

Syphilis continues to be a public health problem, with an estimated 12 million new cases per year worldwide. In many developing countries, as much as 10% of the population may be infected (4). The incidence of syphilis is on the rise in the United States. After an all-time low of 2.1 cases of primary and secondary syphilis per 100,000 population in 2000, there has been a steady increase in the number of reported cases of syphilis. In 2013, the rate was 5.3 cases per 100,000 (2, 5). Co-infection with HIV and syphilis has been reported to be as high as 50% in Sydney and the USA (6). Syphilis remains a major, although entirely preventable, cause of death in newborn babies (7).

T. pallidum is a delicate spiral organism with a hard, uniform, tight, and deep helix. The characteristic motion of T. pallidum is a forward and backward movement around the longitudinal axis. Due to the specific spiral-like shape of T. pallidum, morphological tests have been used to screen for primary syphilis using optical microscopy without other special instruments. The sensitivity varies from 71 to 100% depending on the sample (8).

T pallidum cannot be cultured in vitro, rather its isolation and laboratory propagation require laboratory animals, principally the rabbit (4,9). The pathogen is usually identified by dark-field microscopic examination of mucosal or skin lesions and by serology. Classical serology is based on a combination of nontreponemal (rapid plasma reagin or Venereal Disease Research Laboratory) and treponemal tests (T. pallidum hemagglutination assay or T. pallidum particle agglutination) and has proven to be useful for confirmation of the diagnosis of syphilis and for the follow-up of the treatment efficacy. However, these tests could be negative in early stage of syphilis (primary syphilis), and both serological tests and microscopic examinations are limited by low degrees of sensitivity and specificity. The use of direct fluorescent anti-T. pallidum antibodies has improved detection of T. pallidum, showing a sensitivity comparable to that in dark-field examination. Also, PCRs have been developed to detect T. pallidum in serum, cerebrospinal fluid, amniotic fluid, lesion exudate, and fixed tissues (4).

Some gram-negative bacteria including spirochetes (such as Borrelia, Helicobacter, Leptospira, and Treponema) and some small bacilli (including Bartonella, Campylobacter, and Legionella) are colored feebly or not at all in smears or sections stained by the Gram technique. These organisms can be demonstrated with reduced silver techniques like those used to show nerve fibers (10).

The first stage of the “Reduced-silver” procedure involves the formation of submicroscopic clusters (“nuclei”) of silver atoms in the bacteria. In the second step, these nuclei catalyze local reduction of more silver ions by a reducing agent until enough colloidal metal has been deposited to display the organisms as black objects of characteristic form (10). Warthin and Starry developed their Reduced-silver technique for the demonstration of spirochetes in 1920 (11). The Warthin-Starry stain depends on the ability of spirochetes to absorb silver ions from solution, given their distinctive double-layered envelope (diderm) (12).

It needs to be remembered that the reduced silver techniques are not specific for T pallidum. For example, symbiotic Treponema with high similarity to T pallidum and reduced-silver positive, may exist in oral or rectal biopsies (8).

A major problem with the reduced-silver techniques for bacteria is that the technique is prone to significant background staining. It is also known that there are various challenges associated with interpretation, including staining of melanin and reticulin fibers, which can mimic the appearance of spirochetes in tissue (5). Misidentification of fibrils, or extrusions from cells, as spirochetes may produce false positive results. False negative results occur when organisms are sparse or, especially with Treponema pallidum, masked by large amounts of mucoid material (13). It needs to be appreciated that one needs to be wary of the mountant used since some will fade the silver product quite markedly (14).

There has been a plethora of Warthin & Starry variations to improve the sensitivity and specificity of the silver reduction stain for bacteria. Steiner-type modifications use a pre-sensitization step (eg uranyl nitrate) to reduce non-specific nuclear, nerve and connective tissue staining (1). Other variations use differing silver concentrations and incubation temperatures. The Bosma variation of the Warthin & Starry stain technique differs in two ways from the original Steiner method. All preparations are incubated with 1% amylase at 37°C for 90 minutes to remove mucoid host substances surrounding the microorganisms, and the specimens are incubated with 1% silver nitrate solution for 2 hours at 60°C instead of 0.1% silver nitrate solution for 24 hours. Digestion of mucoid substances by amylase appeared to be essential for the demonstration of B. burgdorferi. Hyaluronidase was found to have a similar effect (13).

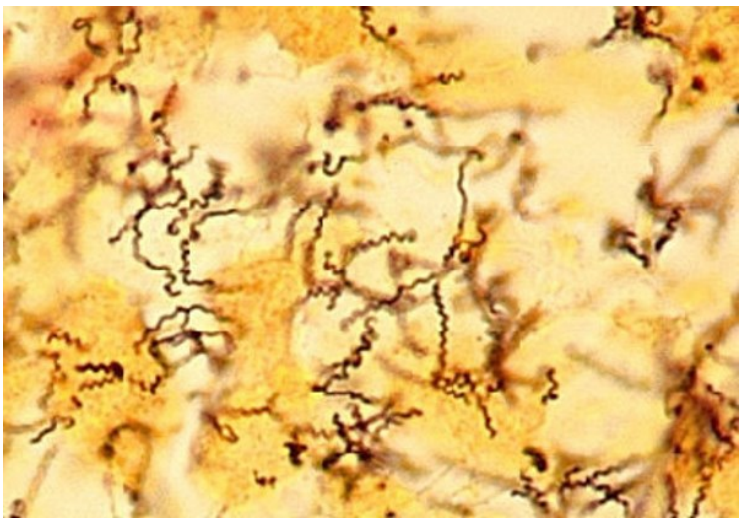

Figure 1: Syphilis Warthin & Starry Stain

It needs to be remembered that the reduced silver techniques were developed to demonstrate all bacteria, especially those that were poorly stained or unstained with existing Giemsa and Gram techniques. As such, these techniques may be quite sensitive, but they lack specificity. There are several commercial antibodies available to demonstrate T pallidum using immunoperoxidase techniques. In fact, one of the seminal papers by Sternberger et al (15) on the introduction of the horseradish peroxidase-anti-horseradish peroxidase, the fore-runner of the ABC and labelled polymer techniques, studied the localization of T pallidum using a polyclonal rabbit antiserum.

Unfortunately, we do not live in a perfect world since there have been several recorded occurrences of cross-reactivity of anti-T pallidum antibody (mainly the Biocare rabbit polyclonal) with other bacteria. Among spirochetes, the immunomarker can be positive for Borrelia burgdorferi and members of the Brachyspira family of spirochetes, which populate the gastrointestinal tract. This cross-reactivity is so strong that the T. pallidum IHC has been used experimentally to identify Brachyspira in intestinal

spirochetosis. One study found that the T. pallidum IHC was positive in 217 of 223 specimens that were known to harbor Brachyspira, suggesting that this cross-reactivity occurs consistently. Reports have also demonstrated cross-reactivity and positivity with non-spirochetal organisms, such as members of the Mycobacteria family (including Mycobacterium leprae). Although the exact mechanism of the cross-reaction is unknown, it is likely that the epitope with which the anti–T. pallidum antibody reacts is common to other spirochetes (16, 17). Sternberger et al (15) did not record cross-reactive staining in their original paper. Later investigators used heat-induced antigen retrieval, and this might have unmasked the cross-reactive antigens. Heat-induced antigen retrieval had not been discovered in 1970.

The potential for cross-reactivity of commercially available antibodies for T pallidum with other spirochete species poses a potential diagnostic pitfall when biopsies are taken from locations wherein spirochetes may be encountered (18).

T pallidum is killed easily by drying, raised temperature and oxygen exposure. The organism multiplies very slowly (once every 30–33 hours), does not survive outside the mammalian host, and cannot be grown in culture (3).

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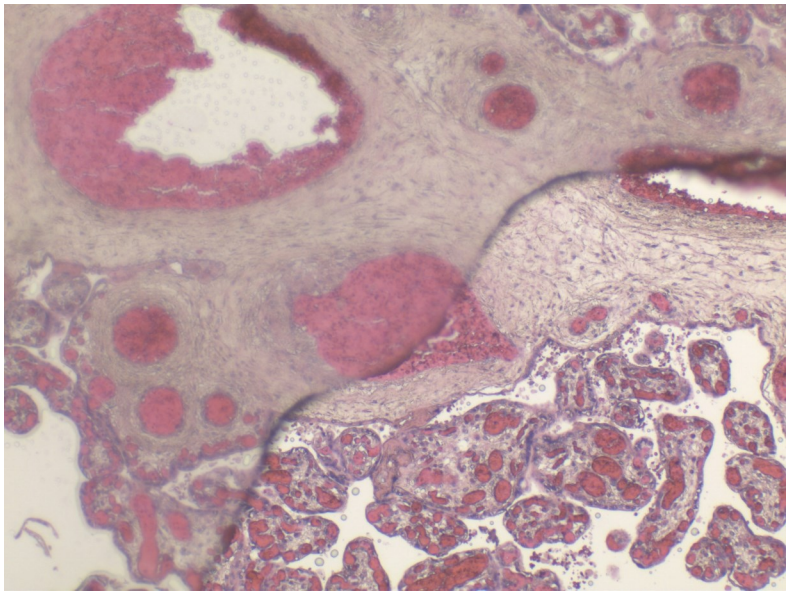
TEST AND TEACH

QUESTIONS

1. What is the tissue?
2. What is the stain?
3. What is the artifact?

Answers will be published in the next issue of the histograph



HISTOTECHNOLOGY SOCIETY OF NSW

2021 Webinars

Mar Sat 27th Embedding

May- 29th, Industry Webinar on Embedding waxes.

Jul Sat 24th Reticuloendothelial System (Thymus, Lymph nodes, Spleen)

Aug-28th, Industry Webinar.

Sep Sat 11th Making Tissue Microarrays (TMA)

Nov Sat 13th Lymphomas- a basic, quick and dirty synopsis

Acronyms are Everywhere!

By Mark Bromley

Acronyms are everywhere! They pervade every aspect of communication, from formal scientific journals through to the TGIF I typed on messenger earlier today. With the explosion of online communication over the last 20 years has come a simultaneous tsunami of acronymization, some are relevant to our daily lives, and others, IMHO, not!

But there are three acronyms that have recently come to the fore. They hold particular relevance for us in Histoland, yet many of us humble citizens of Microtomyville remain blissfully ignorant of them as they sneak up behind us. Two of them are eager to trip us up when we least expect it. And then there is the third, standing knight like between them and you, sword drawn ready to protect you as you have your back turned, unaware of their impending approach. The intent of this article is to shine a torch squarely in their faces of all three, so we can all be aware of them, what they do, and how they can be of use to us.

Firstly, I present to you the longest and frankly the most tongue twisting of them, the ACCMLSW, the imaginatively and romantically named Australian Council for the Certification of the Medical Laboratory Scientific Workforce. Just rolls off the tongue like molasses. So, what on earth is the ACCMLSW?

Certification is defined as the formal recognition of the knowledge, skills and experience of an individual demonstrated by the achievement of standards identified by a profession. *Licensure*, or *registration* as it would be called in Australia for health professionals, is legislated certification.

In most countries around the world medical scientists and technicians are either certified or in many cases registered in an attempt to ensure an appropriate skill level is maintained within the profession. Australia has up until now been an exception to this. The Australian Government has long rejected the need for registration of medical scientists. However, industry bodies have long believed the complete opposite, and in the absence of compulsory registration, deemed that some form of certification was desirable. So, in 2017, a joint AIMS/AACB (shame on you if you don't know the first of those! and you can look the second up yourself, I'm not doing all of the work!) sponsored project was set up to explore potential models for a national professional certification scheme for the medical laboratory scientific and technician workforce within Australia. The outcome of the project was the framework of a voluntary certification scheme, and a company was formed to administer it. That company is the Australian Council for the Certification of the Medical Laboratory Scientific Workforce, or ACCMLSW. Whilst the certification scheme is voluntary, I'm sure employers will increasingly view it favourably in resumes, and I expect eventually only hire certified staff. So first thing on your to-do list is to check out the ACCMLSW website, www.ACCMLSW.wildapricot.org (I kid you not…) get familiar with certification and what it involves, and decide for yourself if you want to become certified (you do!) because it's coming, and you don't want to be left on the shelf.

Part of the ACCMLSW certification process involves continuing education, both doing it and documenting that you have done it. This makes for a nice segue into our next acronym, APACE. For those who haven't heard of APACE, it stands for Australian Professional Acknowledgement of Continuing Education.

Not quite as laryngeally challenging as the ACCMLSW, but it still doesn't quite evoke tingly feelings of gooey joy. Continuing education is something that isn't done well across the majority of Australian histology laboratories, or indeed by the majority of those of us working within them. There is the biennial National Histology conference, and of course the fantastic scientific meetings and presentations put on by state groups, but for most of us that's as far as it goes. But, denizens of Formalinville, I can assure you that it will become increasingly important. NATA (shouldn't have to look that one up unless you're a fresh faced, rosey cheeked newbie graduate, and trust me, you'll get to know what NATA means pretty quickly!) are focusing more and more on the continuing education aspect of ISO 15189 (don't bother with that one unless you suffer with insomnia or have aspirations in Quality Management) and NPAAC regulations (National Pathology Accreditation Advisory Council, the part of the Department of Health that comes up with many of the rules labs have to abide by) both of which mandate Continuing Personal Development (CPD) requirements for laboratory staff. So workplaces are going to look fondly on those who do it and record it. Then there's your certification, which will need it too. So check out APACE, which is the AIMS scheme for recording and certifying continuing personal development activities. I predict we'll all need to utilise some form of CPD registration and validation process at some point in the future, for like certification, it's coming round the corner, not because you **necessarily** want it to, but because what you do want (a job!) will make you want to do it. So https://www.aims.org.au/apace is where to go and become familiar with it. Entirely up to you of course if you want to make use of it, but be aware of it lest it sneaks up and bites you on the derriere at some point in the future. Horses, water & drinking and all that… HWD!!

Finally we get to the warm fuzzy bit, the cosy fluffy acronym that has your back, the one that is keeping a beady eye on the other two, and anything else that tries to sneak up on the innocent and happy residents of the Waxlands, the HGA. I'll say that again. HGA! Just rolls of the tongue! Ok, back on track… The Histology Group of Australia. For many eons there have only been state based histology groups like the HGVT (Victoria), the HGQ (Queensland), the HTSNSW HGSA (Guess!) and the HGWA (YAY! you got it!). Cast your minds back to earlier when we were talking about the ACCMLSW, and how that project was started in 2017 to come up with how it would work. It was actually an organisation called Human Capital Alliance that were tasked with the job, and they asked all of the various national organisations representing medical scientists for their input; AIMS (been there before!), AACB (you were meant to look that one up yourself) NPAAC, HGSA (that's the genetics people, not the Histo Group of SA), ASM (micro), THANZ (vampires) ASC (cyto) bla bla bla.. BUT! There was no national group for histopepes! so we were just forgotten about. It wasn't until the run-up to the excellent National Conference in Adelaide put on by the real HGSA, not those pesky DNA obsessed chromosome heads that stole the acronym for themselves, that word of this movement reached histoears. It was at that conference that representatives from the committees of the five state groups came together and drank wine. Oh! and we formed the HGA too. It was formed with the view of having a national body representing the histology and histotechnology community that could then engage with the ACCMLSW to make sure that your voices are heard moving forward. The HGA constitution was written and it became an incorporated association soon thereafter, and is now an ACCMLSW member association, with representatives on the ACCMLSW committee and its Board of Directors. So go and check out www.NationalHistologyGroup.org.au and see what's there. It's early days yet, the website isn't super flash, mainly because I made it and there is a reason I don't work in IT land! But the fundamentals are in place and the HGA has an exciting future representing you and your interests at the national level. It's got your back! An on that note I'll leave you in peace with one last acronym- TTFN!

Histotechnology Professionals Day

Histotechnology Professionals Day was started by the National Society of Histotechnology (NSH) in the United States in 2010 as a day to spread awareness of the profession of Histotechnology.

Each year since then, Histotechnology Professionals Day has been celebrated on the 10th of March with activities, competitions and events being organised in labs across the US.

Unfortunately the uptake of Histotechnology Professionals Day in Histology labs in NSW is still a little muted, even more so this year due to the pandemic.

But hopefully more labs will take the time to promote this day and use it as an opportunity to acknowledge and appreciate the fantastic work done by Histology professionals every day.

Are these cassette big enough?

"Stains and slides" decorations from the Children's Hospital at Westmead

HPD celebration cake from Dubbo Hospital (Photo: Trisha Lusby)

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Histopathology during the COVID-19 pandemic: resilience through adaptation and innovation

T Bracey, S Arif, AM Ralte, AM Shaaban… - Diagnostic …, 2020 – Elsevier

Histopathology departments have adapted to the challenges posed by the COVID-19 pandemic by a variety of changes including working pattern alterations, technology adoptions and incorporation of techniques. This article summarizes these adaptations and provides references to guide pathologists through the continuing pandemic.

Histopathology laboratory paperwork as a potential risk of COVID-19 transmission among laboratory personnel

A Hasan, K Nafie, O Abbadi - Infection Prevention in Practice, 2020 - Elsevier

Background

Healthcare workers have a higher risk of acquiring coronavirus disease 2019 (COVID-19). The process of requesting pathological investigations is usually handled manually through paper-based forms. This study evaluated the potential for paper-based request forms to transmit severe acute respiratory virus coronavirus-2 (SARS-CoV-2) to laboratory staff in order to make recommendations for dealing with hospital paperwork in a post-COVID-19 world.

Methods

Paper-based forms were tracked from the time of test ordering until the release of the pathology report by calculating the time taken for the forms to reach the laboratory, and the exposure of each staff group to forms received from both high and moderate COVID-19 risk areas.

Results

Four hundred and thirty-two (83%) of 520 forms were received in the laboratory within 24 h. The remaining 88 (17%) forms took ≥24 h to be handled by laboratory personnel. The mean daily exposure time to the paperwork for various laboratory staff was as follows: receptionists, 2.7 min; technicians, 5.5 min; and pathologists, 54.6 min.

Conclusion

More than 80% of the forms were handled by laboratory personnel within 24 h, carrying a high potential risk for viral transmission. It is recommended that paper-based request forms should be replaced by electronic requests that could be printed in the laboratory if required. Another option would be to sterilize received paperwork to ensure the safety of laboratory personnel. More studies are needed to detect the stability of SARS-CoV-2 on different surfaces and determine the potential risk of COVID-19 transmission via paper.

Disinfection of corona virus in histopathology laboratories

Z Luqman, N Iqbal, HM Ali, M Zahid… - Clinical …, 2020 - Wiley Online Library

Severe acute respiratory syndrome (SARS CoV-2/COVID-19) is a highly contagious and deadly disease caused by a virus belonging to the coronaviridae family. Researchers working in histopathology laboratories, dealing with morbid samples, are particularly vulnerable to infection unless they have very strong immunity. Hence, a proper precautionary protocol is required for the safety of the laboratory staff. The current review highlights the biological and physical agents that can be used to inactivate the virus and disinfect the surrounding environment in the laboratory.

Safety considerations in the laboratory testing of specimens suspected or known to contain the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)

PC Iwen, KL Stiles, MA Pentella - American journal of clinical …, 2020 - academic.oup.com

The Ebola virus epidemic of 2014 to 2015 was a wakeup call for the medical community as to the lack of biosafety guidance within the clinical laboratory for the handling of specimens that might contain a highly hazardous pathogen (HHP). Following this epidemic, the Assistant Secretary for Preparedness and Response provided funding to initiate the National Ebola Training and Education Center with a mission "to increase the capacity of the United States public health and health care systems to safely and effectively manage individuals with suspected and confirmed special pathogens," which includes training in laboratory practices (netec.org). The recent emergence of the new pathogen, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), as the cause of coronavirus disease 2019 (COVID-19) has again heightened the need for laboratories to review in-house biosafety practices and to update these practices with the new recommendations that are available. Keep in mind that since the recommendations are often fluid when responding to a new pathogen, strict adherence to the suggested safety practices may not be the best approach and should be based on the needs of the specific facility.

Safe laboratory practices in the light of COVID-19 pandemic: way forward in a resource limited setting

K Odega, E Iyamah, E Ibadin, F Idomeh - 2020 - preprints.org

The 2019 Coronavirus pandemic which was initially referred to as 2019-nCoV, was first identified in Wuhan, China. Early response from the Chinese government included quarantine of infected persons, isolation and total lockdown of Wuhan province to prevent further spread. With the spread of the disease across national borders and declaration of the disease as a global pandemic, there has been a robust response by the international community to contain this deadly virus and prevent its further spread worldwide. Africa is not left out of this rampaging pandemic with documented cases in over 40 countries and still rising. Although extensive studies have been carried out on the novel SARS-CoV-2 on its pathogenesis, mode of infection and virulence but much is still unknown. However, potentially infectious samples are received routinely in the medical laboratory for analysis. This technical note reviews good laboratory practice (GLP) and processes across the different specialities of Medical Laboratory practice that should minimize the risk of infection to laboratory staff especially in resource-limited settings.

COVID-19 and biosafety: A review of biosafety recommendations for cytopathology and histopathology laboratories

I Chakrabarti - IP Archives of Cytology and Histopathology Research, 2020 - achr.co.in

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is the zoonotic coronavirus responsible for the present pandemic of COVID-19. The novel ways of transmission of this virus have eluded and infected the global population, surpassing the confines of the place of its origin in Wuhan, China. The healthcare workers are one of the most susceptible populations and laboratory safety protocols are being devised throughout the world to protect the laboratory personnel, who are the frontline fighters in this war against the virus. The present narrative review is an attempt to encompass the published literature sharing the experience and guidelines of biosafety for those working in histopathology and cytopathology laboratories.