HGV

Org. No. A003523F

PARAFFINALIA NEWSLETTER

VOLUME 23, NUMBER 2
June 2018

The HGV aims to provide a dynamic continuing education program in which all persons with an interest in Histology and Histotechnology are freely invited to participate.

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Committee Page

The members of the Histology Group of Victoria 2018 are:

Name	Institution
Kellie Madigan	Leica Biosystems
Adrian Warmington	Clinicallabs (Ballarat)
Mark Bromley	Sullivan Nicolaides Pathology
Elizabeth Baranyai	Cabrini Health
Samantha Arandelovic	Clinicallabs (Geelong)
Alison Boyd	St. Vincent's Pathology
Kellie Vukovic	Sullivan Nicolaides Pathology
Sue Sturrock	Peter MacCallum Cancer Centre
Meghan Leo	Peter MacCallum Cancer Centre
Darcee Mc Nair	Clinicallabs (Geelong)
Kerrie Scott-Dowell	Dorevitch Pathology/Leica
Emma Pan	Walter & Eliza Hall Institute

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President's Report

Each year the HGV likes to acknowledge the efforts of the State's top performing students in Histology/Anatomical Pathology at each institute with an award and monetary gift.

This year's recipients joined us at the May educational evening to receive their awards and we say congratulations to the following award winners (see photo below)

- Shelley-Anne Jacobs – Gordon TAFE
- Joseph Dawson – Federation University
- Holly Ho – Holmesglen TAFE
- Jessika Thorn – RMIT Degree
- Fanica Bailey – RMIT Diploma

As a Histology community we also congratulate Bron Christiansen who is a Principal Scientist at The Royal Children's Hospital. Bron took on the challenge of taking the AIMS Fellowship in Anatomical Pathology and has just received recognition that she has passed and will be awarded her Fellowship in September. This is quite an achievement for anyone, but it is encouraging that Bron is the first Victorian female to receive her Fellowship in AP within Victoria and is either the first or second female Australia wide. Bron has submitted to us an article she provided to the AIMS journal about her experience and hopes to encourage others to take up the challenge. Bron is a familiar face at our Education evenings and we hope to have her present to us a little more detail about the Fellowship process and her project.

Next month we take a small change with our educational meeting and will instead be heading out South-East on the freeway to visit the Trajan site at Ringwood. Trajan have also offered to bring in an inter-state speaker and recommended Professor Graeme Suthers, who is one of Australia's most respected experts in genetic studies. I encourage everyone to make the trip to Ringwood, this is a unique experience for many of us, and if successful we hope to do similar events on perhaps a once a year frequency.

Kellie Madigan

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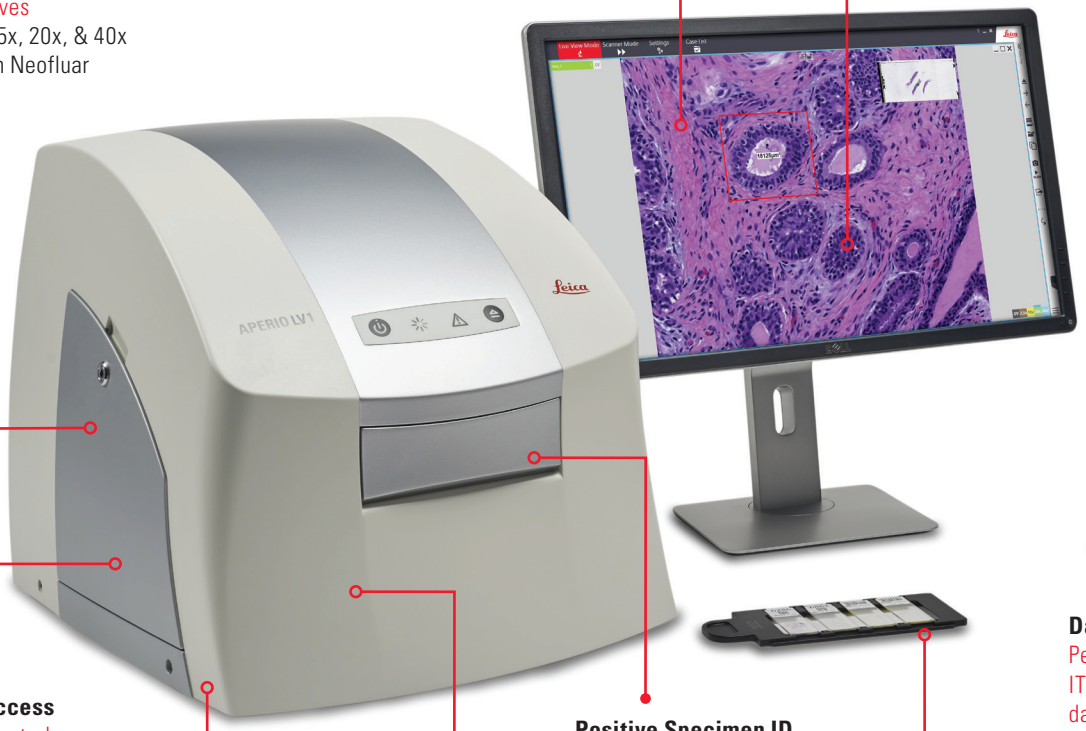

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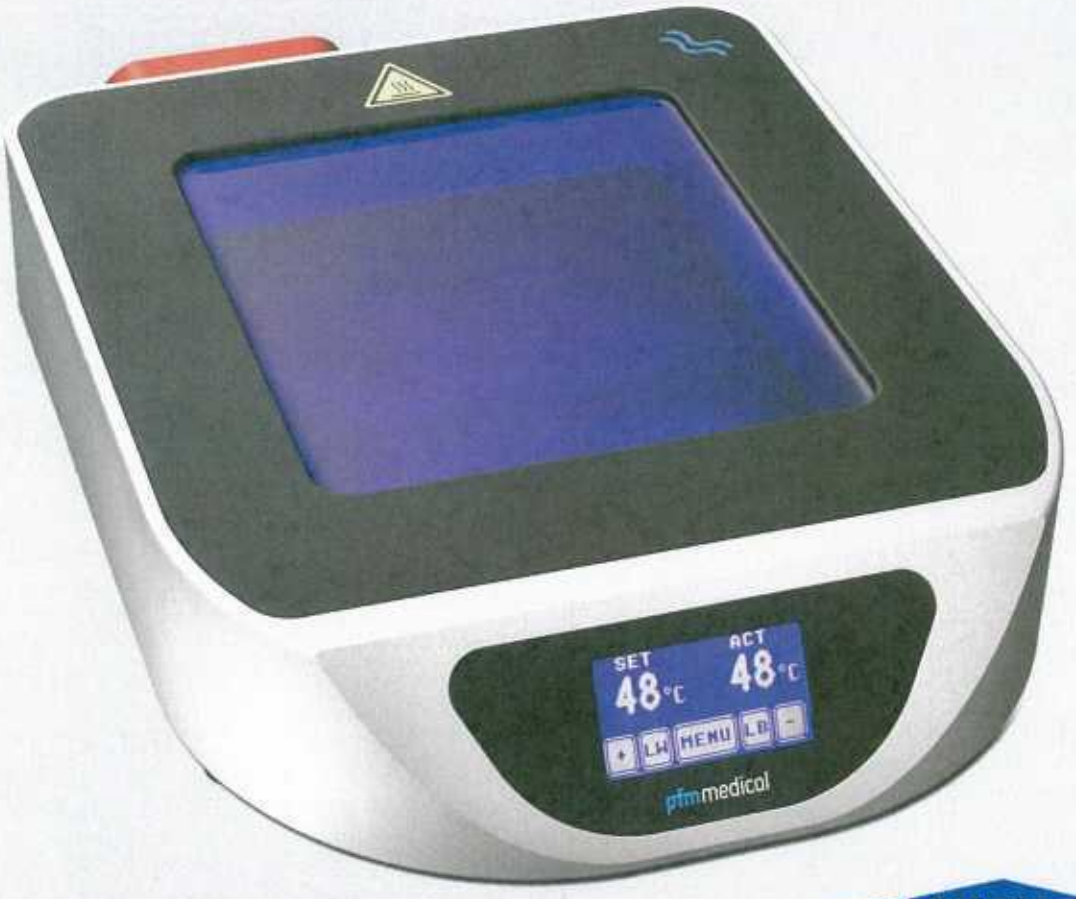

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Under the Microscope with Bron Christiansen

AIMS Anatomical Pathology Fellowship – A reflection

Bron Christiansen

Anatomical Pathology, Royal Children's Hospital, Melbourne.

I decided to begin the Anatomical Pathology Fellowship after a meeting with Piero at the Adelaide National Histology conference a few years ago. At that stage, he had just completed the Fellowship and I was returning to work after my second child. I was inspired to return to study but wondered how I could juggle the demands of family, full-time work and study and still remain sane!

I investigated a large number of courses which would not only assist me professionally but also keep me engaged and keen. Important in the decision making process was also the financial contribution I would need to make to complete additional study. In 2013, I applied to begin the AIMS Fellowship. I specifically chose this course as it was directed towards my specialty subject. I wanted to be confident that advice I gave in the workplace came with firm knowledge and education behind it.

The AIMS Fellowship structure meant I could pace myself with targeted study for each module. It appealed to me that I could concentrate my attention on one area at a time.

And so it began with module 1, Laboratory Histology in 2014. Returning to the well-known books by the masters of Histology; Bancroft and Stevens, Cook and Di Fiore to name a few. I loved revisiting theories and techniques from my undergraduate days but reading with new enthusiasm and appreciation rather than rote learning for an exam. It was punishing for my colleagues with daily "light bulb" moments relayed and discussed!

With my first subject completed I moved on to the next; Specialised Histopathology. This was a challenge! After studying for 6 months re-looking at IHC, special stains and other techniques, I did not pass on the first attempt. I took feedback on board and tried again in 2015 filled with more knowledge about cytokeratins than ever before! I received the eagerly awaited envelope indicating a pass in early 2016.

Now to select some specialist subjects… I elected to choose Electron microscopy as the first one. With John Stirling's book firmly in my grasp, I hounded the EM team with endless questions (thanks to the team for being patient!). I looked at processing, renals, staining and cilia. I looked at old QAP's, negatives and recent images. I studied the electron microscope, watched them load and view. I looked at weird deposits and contaminants on grids in between reading stories to my children. I loved looking at the ultrastructure of our bodies; so many little cogs in a huge machine.

The final elective was Molecular biology. I chose this module as I can see histology becoming increasingly involved with molecular techniques due to our access to archival tissue

samples. With links between DNA genetic codes becoming increasingly linked to the formation of cancer proteins (and treatment), the use of ISH and similar techniques could become more popular in routine testing. With knowledge of processing, probes and PCR now whizzing around in my head, I passed the final module at the end of 2016.

In August of 2017, I sat my VIVA across the table from three of my respected peers. Nervous but full of excitement, I answered their questions (although I cannot remember what any of them were!). At the end of the assessment, Piero ended the quest which he (unknowingly) started – He told me I had passed all of the formal testing. I couldn't believe it. Now the dissertation to complete.

I had followed, with interest, investigations and journal articles by Dr Peter Whitington in relation to Gestational Alloimmune Liver Disease (GALD). Our laboratory had sent a number of samples for his review and we had the preferred antibody on our IHC panel. With a number of diseases causing cross reaction with the marker, I wanted to find a supportive antibody which would help with pathologist confidence in diagnosing this disease. The pilot study indicated that there is potential for a classical complement pathway marker to add sensitivity and specificity to the diagnosis of GALD. In January 2018, I submitted the document for review and assessment.

On Thursday 1st March 2018, I received notification I had passed my Anatomical Pathology Fellowship.

After calling my relatives (and crying a lot) to tell them I had passed, I spoke to my children. When I told them, they asked "Have you been studying mum?" Guess this mum/scientist juggled everything just right….But I could not have done it without the support of my family, friends and work colleagues… Thank you everyone!

This article was published in the AIMS journal and has been supplied to us for sharing with the Victorian Anatomical Pathology community by Bron.

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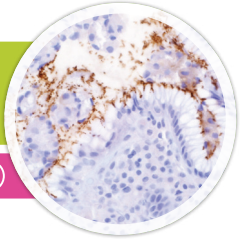

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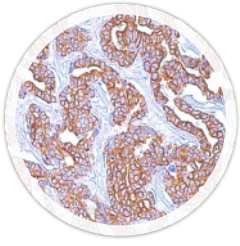

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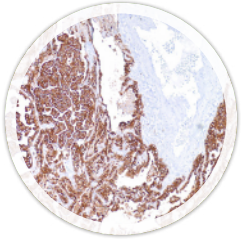

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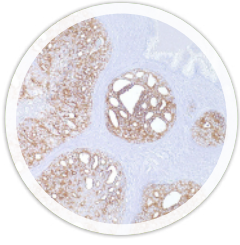

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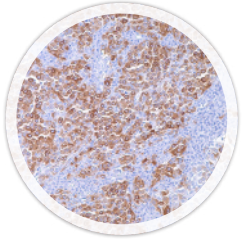

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Review of May Scientific Meeting

by Alison Boyd

Gram stains are one of the quickest stains to perform but are also one of the most poorly done stains. This scientific meeting compared manual and automated staining.

Elizabeth Baranyai was the first presenter and has worked for many years in Histology at Cabrini. Titled Gram Stain – Hints and Tips, Elizabeth talked about the history of the Gram stain, being first developed by Hans Christian Gram in 1884. The principle of the stain involves Gram positive bacteria, with a thick peptidoglycan wall staining purple with crystal violet and Gram negative bacteria with a thinner wall not retaining the purple stain and staining pink with the counterstain safranin. Elizabeth favours the Gram Twort stain, a variation that uses neutral red and light green as the counterstain. Trouble shooting was discussed with helpful tips. Controls are essential for Gram stains and should include positive and negative organisms. Three main ways of obtaining controls were discussed – a good specimen from the lab, which is rare; inoculating fresh tissue with known organisms from the micro lab; or improvising with fresh meat. Elizabeth stumbled across a really good control when feeding her dog Harper. The meat felt greasy and smelt off so Harper was not fed but the meat was kept for a few days then fixed and processed. The control was so good along with the Gram technique that Elizabeth scored 5 for QAP.

Samantha Arandelovic was the second speaker and has also worked in histology for a number of years and is currently based in Geelong. Titled Gram Stain and Automation, Samantha looked at two different platforms for special stains – the DAKO Artisan System and the Ventana Benchmark. Automation has a number of benefits such as precision and consistency but for short stains such as the Gram Stain there can be problems. DAKO is more flexible and allows a shorter run time but Ventana has the problem of the carousel taking four minutes to go around the machine. The Gram Twort is used but sections are overstained to allow longer differentiation with the four minute cycle time. By the time the carousel goes around, the Gram stain could be done manually. Samantha spoke about QAP and getting poor results. The overall comment from QAP was that Gram Twort performed manually gave the best results, followed by the modified Gram done manually, with automation giving the worst results.

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Commonly Used In: Laboratories, nail salons, patient wards, nursing homes and more.

May Meeting Review

Liz Baranyai from Cabrini, shared with us the secrets of a good Gram stain and how to obtain control tissue. The following is her method:

GRAM-TWORT STAIN (TWORT, 1924)

Cationic (Basic Dye), Crystal Violet, totally colours the section. Anionic DNA and ribosomal RNA in bacteria is coloured. Grams Iodine converts the Crystal Violet into salt of low solubility which slows differentiation. Gram positive organisms have a much thicker wall than do Gram negative organisms, allowing differentiation. The counterstain-Neutral Red-is another basic dye which then permeates the Gram negative organisms. In the Gram-Twort, an acid dye (Fast green) is also used in the counterstain to colour the background proteins. (**Note**: I used light green as I didn't have any fast green in the lab and that seemed to work just as well. EB).

A. SOLUTIONS:

1. Crystal Violet Solution
   0.5% Crystal Violet in 25% Alcohol
2. Grams Iodine

Potassium Iodide	2g
Iodine	1g
Deionised water	300ml

Dissolve the Potassium Iodide in 20ml deionised water. Add Iodine and after dissolved, make up to 300ml.

3. Twort Stain
   Mix immediately before use.

1.0% Neutral Red in alcohol	9ml	(0.9ml)
0.2% Light Green in alcohol	5ml	(0.5ml)
Make up to 30ml with deionised water.		(3ml)

4. Acetone
5. Acetic ethanol

Glacial acetic acid	2ml
Absolute ethanol	100ml

B. METHOD:

1. Bring sections to water.
2. Stain in Crystal Violet Solution for 2 minutes.
3. Rinse in running tap water.
4. Stain in Grams Iodine for 5 minutes.
5. Rinse in tap water.
6. Differentiate in acetone until all purple stops bleeding from section. (1 squirt from a wash bottle).
7. Rinse in tap water.
8. Stain in Twort's Stain for 5 minutes.
9. Rinse in acetic ethanol. (1 squirt from a wash bottle).
10. Blot dry.
11. Clear in solvent and mount.

C. RESULTS:

Gram positive organisms	Blue-black
Gram negative organisms	Pink to red
Nuclei	Red
Background	Green
Elastic fibres	Black

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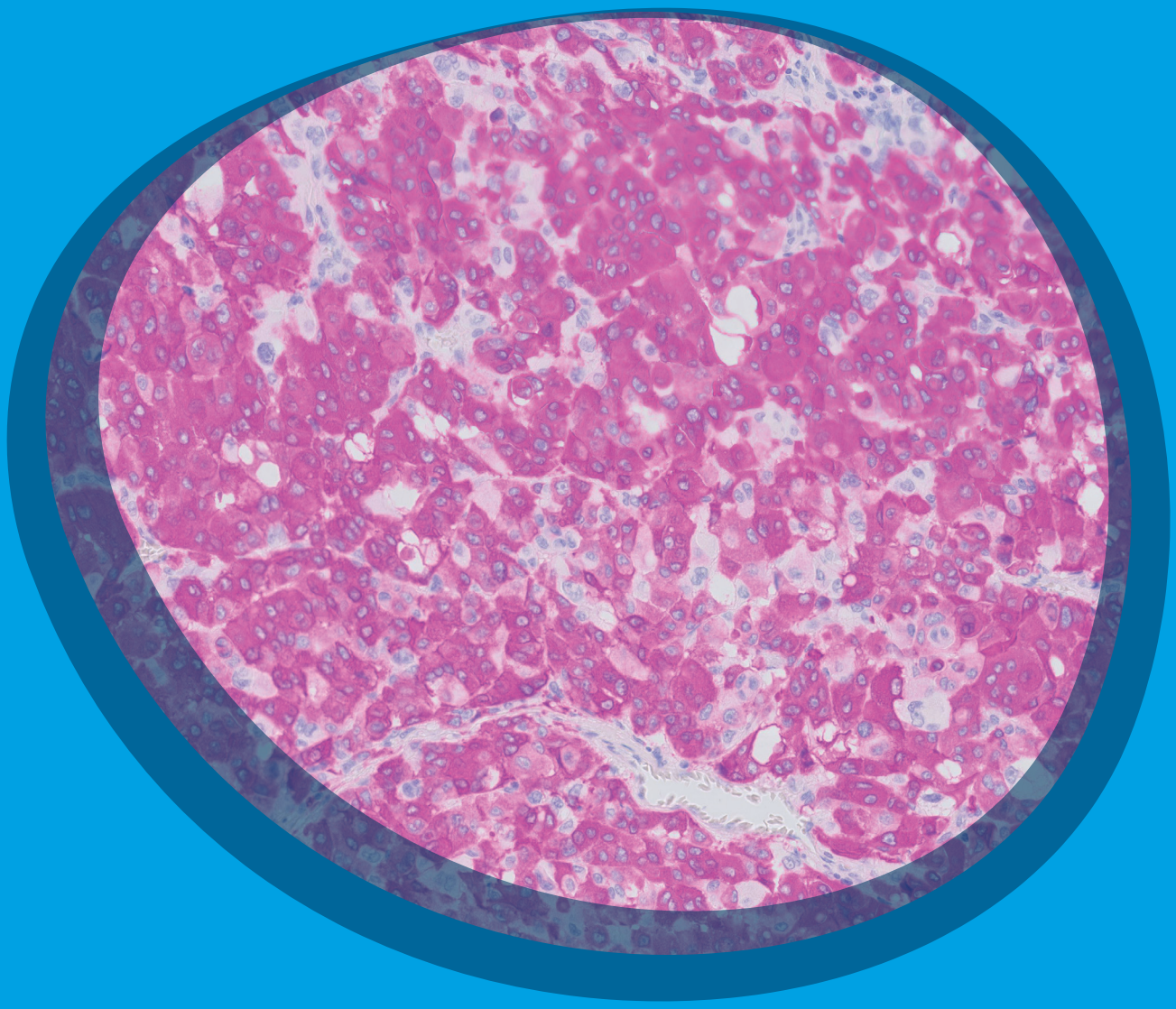

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Cleaved Caspase-3

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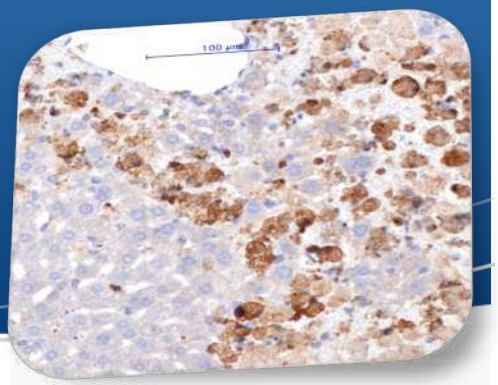
IHC stain of cleaved caspase-3 is popularly used by researchers for studies of cancers, age-related diseases and neurodegeneration diseases. However, the application of this antibody is not yet for clinical use.



Detection of Cleaved Caspase-3 Using Automation

Caspase-3 protein is a member of the family of cytosolic aspartate-specific cysteine proteases. It plays an important role in execution of apoptosis. Commonly, the protein is expressed as latent zymogen. The procaspase is then activated by a cleavage adjacent to the aspartic acid at the P1 position forming p17 and p12 subunits.

Immunohistochemistry stain of cleaved Caspase-3 has rarely been performed on a fully automated platform due to the requirement of prolonged application time of primary antibody on tissue for adequate stain replication. Although the reason behind is yet to be discovered, our lab has developed a methodology to produce desirable results with a much-shortened staining time on the Dako Omnis platform.

Scattered cytoplasmic stain of specific individual cells is usually expected in normal tissue (bottom left). However, certain events can cause elevated expression of this protein, for example, radiation (bottom right). On the other hand, caspase-3 has shown to execute nonapoptotic functions in the central nervous system, which is related to studies of neurodegeneration diseases, such as Alzheimer's and Parkinson's disease.

By Emma Pan

Antibody

R&D	**Active Caspase-3** – Detect the p17 subunit of Caspase-3 in human and mouse tissues.
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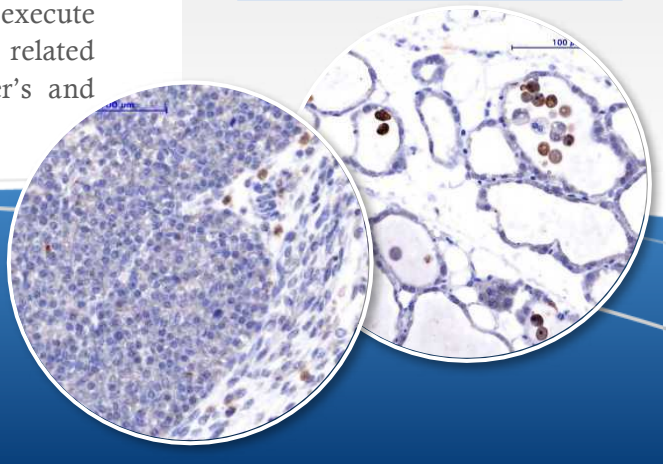
CST	**5A1E** – Detect the larger subunit p17 resulting from cleavage close to Asp175.



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Case Presentation

THE PRESENCE OF OPPORTUNISTIC INFECTIONS IN CONJUNTION WITH HEAVY METAL POISONING IN THE MINING INDUSTRY.

Kerrie Howard

A 47-year-old miner presented to the emergency department with respiratory, neurological and renal symptoms before entering multiple organ failure and dying. The mining industry presents many hazards, heavy metal poisoning shows a great prevalence and with it comes great health risks. Heavy metals such as Mercury can have whole body symptoms. Opportunistic acid fast bacilli often follow, causing great strain on the body. There are various types of acid fast bacilli that are opportunistic infection presenting with little to no symptoms distinguishable from heavy metal poisoning, making the combination extremely hazardous to individuals, often resulting in sudden health decline and death.

Introduction:

A 47-year-old male with an occupation of a miner presents to the emergency department after collapsing at a shopping centre. The patient presented with ataxia, poor movement, reflexes and fatigue which quickly progresses to a cough and he stops urinating. The patient dies 24 hours after admission.

Lungs are essential for gas exchange within the body, where oxygen and carbon dioxide are exchanged to help fuel aerobic respiration within the body and maintain bodily functions. The respiratory system begins from the nose/mouth, following down to the bronchus and bronchioles before reaching the respiratory zone. 'Respiratory zones' allow for gas exchange. The respiratory zones consist of three structures: alveoli, alveolar ducts and capillaries which supply the blood for gas exchange. Within each individual lungs the amount of the respiratory zone structures stays relatively static in number with approximately three million alveoli, fourteen million alveolar ducts and two hundred and eighty capillaries (1). Each alveoli possess two types of cells that make up the structure; type one are involved in gas exchange whereas type two secrete a fluid substance called surfactant, which assists gas exchange by allows for gasses to diffuse across the membranes and into capillaries(2).

Diffuse Alveolar Damage (DAD) is a cellular response of normal lung architecture caused by trauma or infection(3); characterised by oedema, severe inflammation, haemorrhage, alveolar cell damage, hyaline membrane formation and increased membrane permeability. With the presence of all these symptoms, there can be seen to be alveolar cell type one necrosis and type two proliferation ultimately being the cause of these symptoms (4). The condition that this results in as a whole is referred to as Acute Respiratory Distress Syndrome (ARDS)(4).

Mercury is a heavy metal exposed to those in the mining industry, there are three forms of mercury present in the environment, elemental mercury (hazardous as vapour), organic mercury (methyl mercury and ethyl mercury) commonly found in mines and inorganic mercury (mercuric mercury), all three forms exhibit grave health risks. The main forms that is hazardous to individuals in the mining industry is that of organic and elemental mercury as it can often form alloys with many other metals such as gold, silver and tin amongst others;(5) all of which are commonly mined metals.

Mercury affects many body systems in their mechanism of action. It hinders the neurological and musculoskeletal system through affecting ion channels and calcium pumps; it also binds to proteins containing thiol groups which not only helps the mercury move around the body but also can be seen as a protective mechanism as it 'holds' the mercury stopping it from binding to other receptors. Mercury can also be seen to interfere with immune mechanisms ultimately leaving individuals with mercury poisoning susceptible to infection. Absorption of this heavy metal (especially in the form of elemental mercury) through the lungs can lead to 'intoxication' which leads to clinical symptoms such as dyspnoea, cough and hypoxemia. Renal failure is also common as the metal is taken to the kidneys for excretion when attached to proteins; this not only causes proteinuria but also destroys the tubules of the kidney by burdening the cells with high concentrations of the poison(5).

Due to the fact that mercury poisoning causes failed immune responses, there is a high chance

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of opportunistic infections flooding the body. An example of an opportunistic infection that can cause symptoms exhibited by the patient is that of pneumonia. There are many examples of acid fast bacilli causing opportunistic respiratory infections in already immunocompromised patients; atypical pneumonia and other respiratory conditions can be caused by acid fast bacilli such as *Legionella pneumophila,* also known as legionnaires' disease. This disease causes often no fever and cough is only seen in extremely progressed cases, however there is clear respiratory distress with dyspnoea and exhaustion(6). Another type of acid fast bacilli seen in the respiratory tract include *Mycobacterium Tuberculosis(7).*

Method:
Please refer to MEDS129/2113 Histopathology 2/ Advanced Histopathology Laboratory Manual 2018(8) for all staining procedures; stains completed include:
H and E (regressive): Appendix 1, page 12
Ziehl Neelson Kinyoun's method: Appendix 1, page 30
Grocotts Hexamine Silver- Chromic Acid Method: Appendix 1, Page 10
Mercury Pigment removal: Appendix 1, Page 19
Perl's Prussian Blue: Appendix 1, Page 24

Results:
The H and E slide macroscopically presented a single piece of eosinophilic, dense tissue measuring approximately 8x6mm. The dense nature of the tissue is unusual for lung tissue; thus the first sign of disease processes being present in the slide. Microscopically, the slide present a section of lung showing a thickened pleura, pulmonary oedema, some macrophages and a large amount of pigment within the lung. The pigment can be seen both intracytoplasmic and extra-cytoplasmic thus there is questions of a fungi or bacteria intracytoplasmic causing the fatigue and cough. Taking into consideration the occupation of a miner the pigment could also be thought to be a metal or by-product seen commonly within mining environments. Almost all lung architecture is destroyed suggestive of diffused alveolar damage. There is as presence of hyaline membrane formation which is also associated with diffuse alveolar damage.

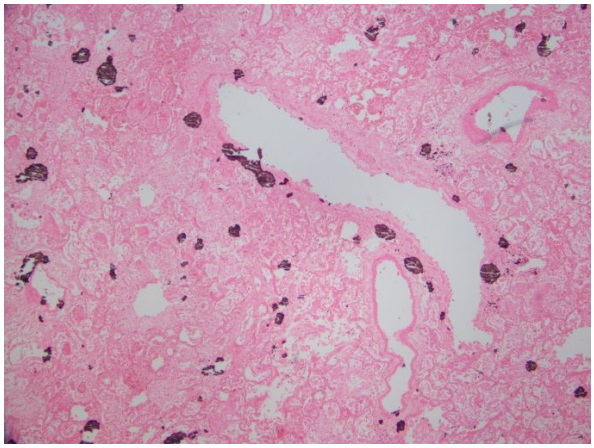

Figure 1: Mercury pigment seen in H and E sections

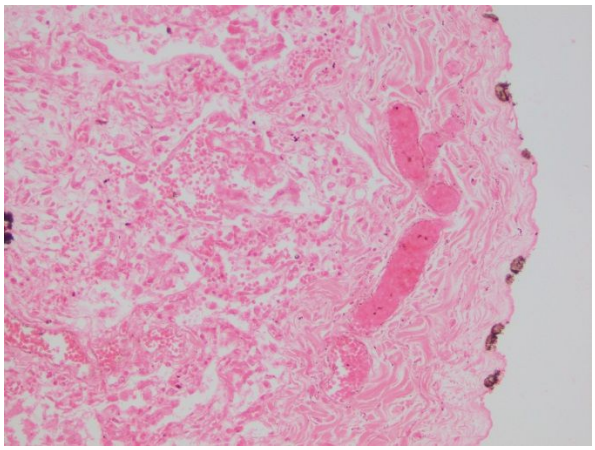

Figure 2: Thickened pleura and haemorrhage caused by DAD

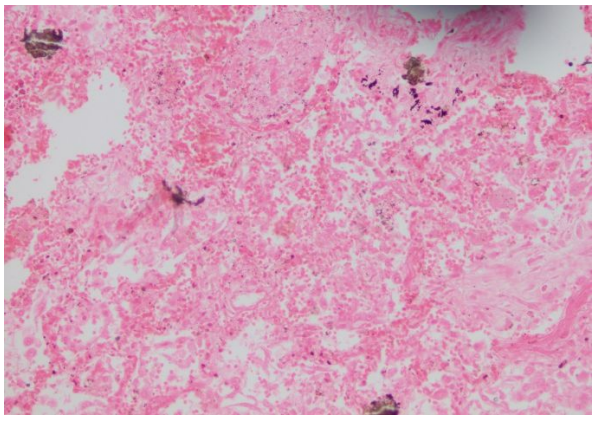

Figure 3: Intracellular pigment (suggested Bacteria or Fungi) and DAD

Further testing was ordered to determine the exact cause of death, the tests ordered include Ziehl Neelson Kinyon (bacteria), Grocotts Hexamine Silver (fungi), Mercury pigment removal (Mercury) and Perl's Prussian Blue (To rule out hemeosiderosis)

Ziehl Neelson (ZN) Kinyon was performed to test for acid fast bacilli, the control tissue showed a strong positive result suggesting the

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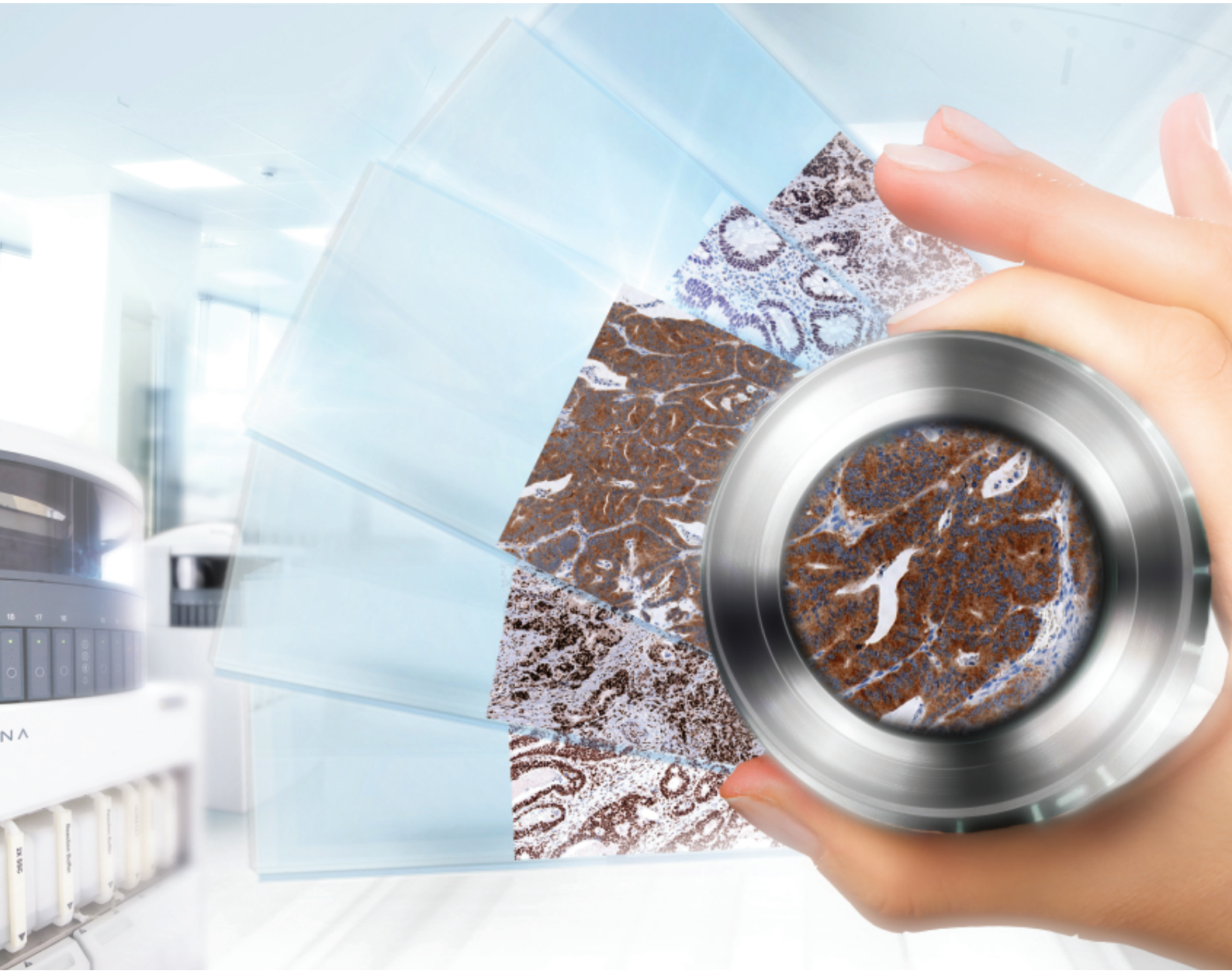

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method was successful. The test section also showed a strong positive reaction by the presence of pink/red acid fast bacilli with a blue background.

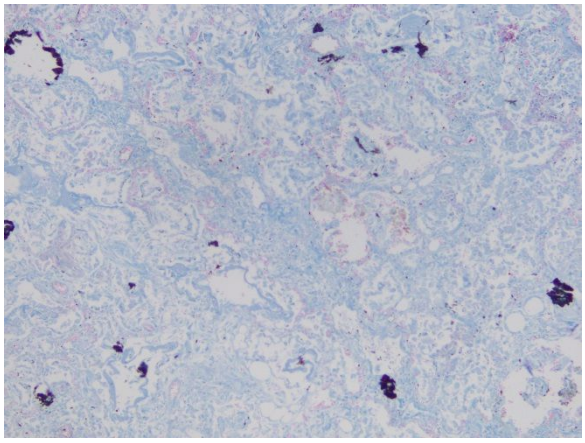

Figure 4: ZN stain showing positive staining of acid fast bacilli seen in pink/red

Mercury pigment removal was completed presenting a positive removal of the large pigments seen throughout the original H and E section. Suggestive of mercury heavy metal present within the lung tissue.

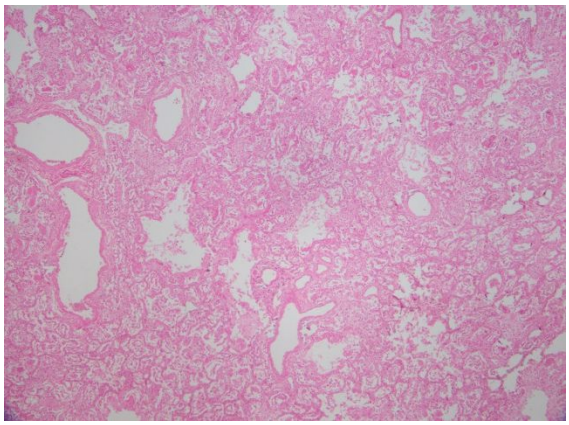

Figure 5: Mercury pigment removal shows success through lack of large extra cellular pigment throughout tissue

Perl's Prussian Blue (PPB) was completed to test for hemosiderin, the control tissue showed a strong positive response suggestive of a successful stain. The test section showed a small positive reaction with blue deposits against a pink background suggestive of some hemosiderin within the lung tissue. Due to the fact that a proportion of tissue floated off the slide, the whole lung section could not be examined.

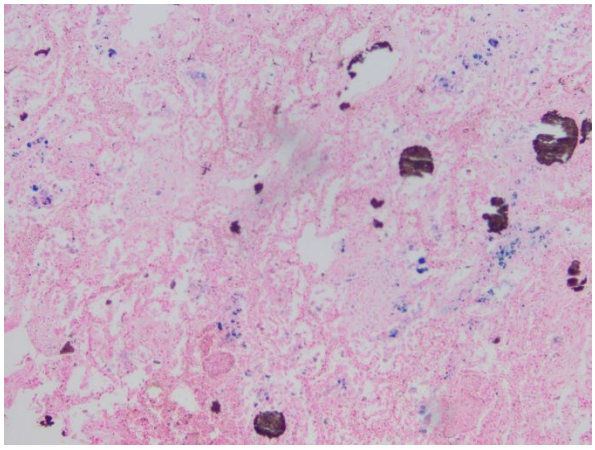

Figure 6: PPB stain shows positive results for hemosiderin through blue pigment

A Grocotts Hexamine Silver- chromic acid method (GMS) stain was completed to investigate the presence of fungi within the tissue. This stain was completed twice due to the fact that the section continually floated. Both controls were positive suggestive of a successful stain. Both sections of test tissue showed a questionable positive result with a black pigment against a blue/green background in what could be seen from the remaining tissue; this suggests that there is a possibility of fungal lung infection. However, due to floating tissue and precipitate being present on slides this cannot be confirmed. Testing would be required to be completed again to gauge a result.

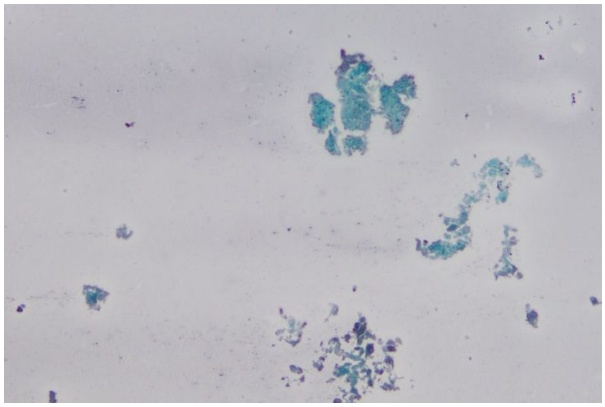

Figure 7: GMS stain attempt one showing lost tissue as well as black pigment suggestive of precipitate.

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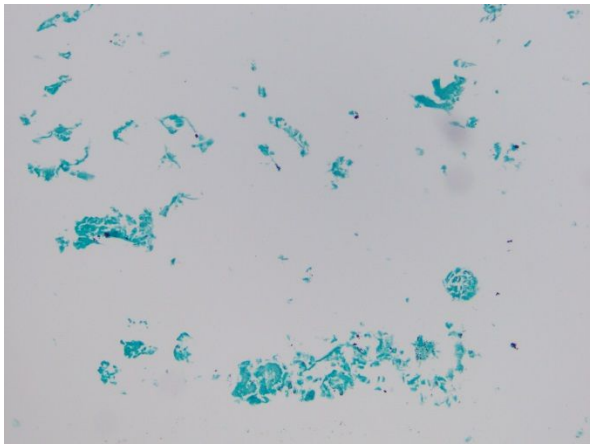

Figure 8: GMS attempt 2 showing less tissue loss and confirming black pigment in tissue suggestive of precipitate.

Discussion:
There were many pathological conditions seen throughout the H and E sections which were further investigated with special stains:

The intracytoplasmic pigment could be attributed to the fact that as a miner he is exposed to various heavy metals which may cause pigmentation, possible pigments include carbon or mercury which also presents with impaired memory function. Diffused Alveolar damage is also seen, this is caused by patients suffering from respiratory trauma such an injury, intubation or infection which often leads to death(9), this causes a surplus of exudate within the alveolar spaces causing a lack of oxygen exchange, ultimately causing asphyxiation.

With the completion of special staining, it can be confirmed that there is a presence of heavy metals, specifically that of mercury, this explains the miners neurological and renal symptoms as well as that of the respiratory symptoms. Mercury poisoning also known as Minamoto disease progresses through the body ultimately leading to multi organ failure and death(5). The section was well stained and provided good visualisation of tissue, there was however two air bubbles seen in the plane of the tissue, one was over the edge of the pleura making a very small section of the pleura unable to be examined microscopically, this did not hinder the diagnosis. The slide is correctly and adequately labelled; the coverslip was well placed, however excess DPX adhesive can be seen towards the edges of the coverslip.

There was also a positive reaction form Ziehl Neelson (ZN) suggestive of acid fast bacilli which may be connected with the differential diagnosis of bacterial infection which also explains the respiratory symptoms. The ZN staining was slightly pale, this could be assisted through ensuring the entire section is adequately covered with stain and possibly leaving stains on for longer if still not satisfied with the overall stain quality. The suspected mercury pigment was also clearly seen, no air bubbles were present in the slides however, there was some artefact seen towards one end of the section. The slide is correctly and adequately labelled; the coverslip was well placed and the use of DPX is in proportion to needed.

A Perl's stain was completed for the differential diagnosis of haemeosiderosis causing some respiratory strain, there was small pockets of hemosiderin seen within the test sample, however this was expected due to the lung trauma causing haemorrhage and the fact that the patients profession left him susceptible to inhalation of many hazardous substances. Part of the section did float resulting in half of the tissue being unable to examined, this could have been rectified through placing the lung sample on positively charged slides as lung tissue is especially friable and lacy causing it to often be difficult to adhere to slides. The section was well stained, with no air bubbles present in the section however some artefact can be seen towards one end of the tissue, this does not hinder the diagnosis. The suspected mercury pigment can also be seen throughout the tissue. The slide is correctly and adequately labelled; the coverslip was well placed, excess DPX can be seen to the upper part of the slide, this does not hinder viewing of the section.

Grocotts Hexamine Silver- Chromic Acid Method was also completed to confirm or deny the presence of fungi within the sample. There was difficulty with this method as the section continually floated over repeated stains, however there was enough tissue left on the second stain to suggest that fungi may be present. The floating sections could have been rectified through using positively charged slides as well as through extra heating to ensure good fixation of the sections to the slides. The first section also showed a great amount of precipitate once removed from the silver solution which can be seen both macroscopically and microscopically in both the test and control sections. There are many reasons why precipitate many have formed during the silver solution incubation such as if metal forceps or other forms of metal came into contact with the solution, if tap water was used

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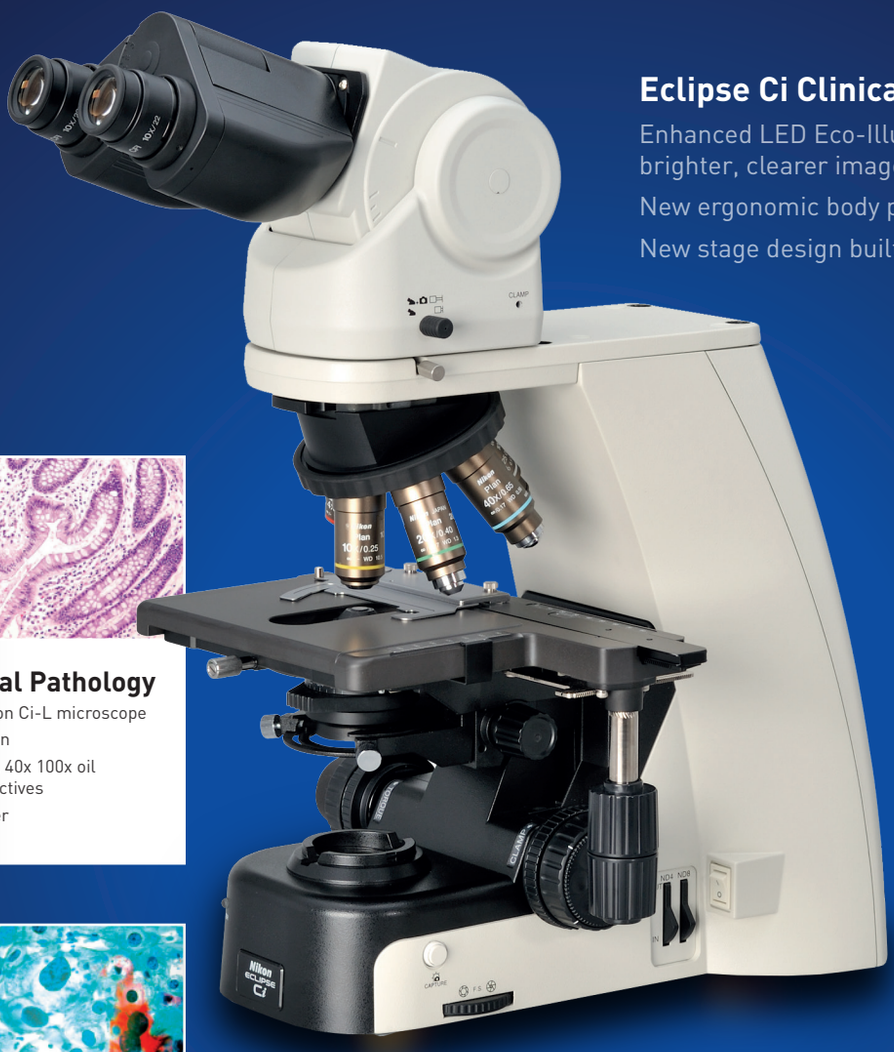

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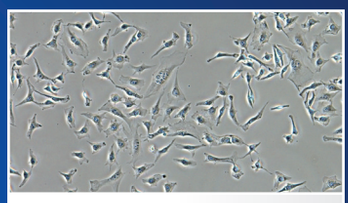

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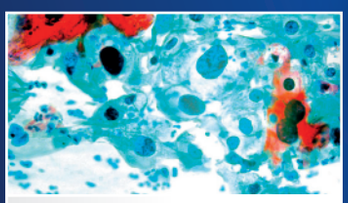

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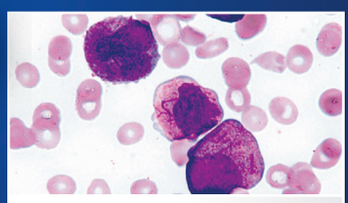

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instead of distilled water precipitate may form with ions in the water, furthermore if there was contamination of water bath water entering the slide mailer containing the silver solution, this could also cause a precipitate to form. The issues seen with the precipitate can be rectified through ensuing that there is complete separation of the water bath and silver solution, distilled water should be used throughout all steps to ensure no contamination of slides and all materials should be thoroughly washed to ensure a successful stain, furthermore the removal of all metal materials such as forceps will prevent accidental use and possibly causing another precipitation reaction(10).

No air bubbles can be seen in the field of the GMS stain tissue over both sections. Section one showed good use of DPX adhesive and the coverslip placed centrally over the tissue. Section two also showed good coverslip positioning however some excess DPX is seen on the left-hand side of the slide; this does not hinder section viewing. Due to the fact that there were copious amounts of precipitate as well as floated tissue it was deemed that the suggested positive results were questionable and required re-staining on positively changed slides.
Haemorrhage was also seen which is suggestive of trauma causing diffuse alveolar damage as the architectural destruction and degraded capillary walls cause a surplus of blood within the tissue and tissue spaces. Diffuse alveolar damage can be caused by trauma or infection, further testing in the case of special stains for fungi and bacteria as well as mercury pigment removal to rule out heavy metal trauma. There was positive mercury pigment removal, suggestive of heavy metal trauma, from the patient's occupation. Hyaline membrane formation is also present within the tissue architecture; this can also be attributed to the presence of diffuse alveolar damage. Hyaline membranes cause lung stiffness through a fibrous layer of dead cells and excess surfactant setting on the alveolar surface causing change in architecture and lung permeability(11).

Alveolar cell damage can clearly be seen throughout the tissue, this is caused by a combination of diffuse alveolar damage and mercury poisoning which causes respiratory distress, furthermore, the presence of acid fast bacilli is suggestive of atypical pneumonia respiratory distress causing bacilli(12) such as *Legionella pneumophila*. There are other acid fast bacilli which also create similar respiratory conditions such as *Mycobacteria tuberculosis.* A thickened pleura was seen which can also be caused trough DAD and infection due to the increased stress on the respiratory system through trauma and further strain on lungs.

Further testing that could be completed to ensure an accurate diagnosis include re completing GMS stating. A tuberculosis skin or serology(13) to determine if *Mycobacteria tuberculosis* is the cause of the infection. *Legionella pneumophila* can be confirmed or denied through testing of urine to see if the antigen is present through an antigen assays such as Bintax(14).

Conclusion:
Through the examination of a routine H and E section as well as special stains there are conclusions that can be drawn including: the patient presents a heavy metal pigment within the lung tissue confirmed to be that of mercury. There is a presence of acid fast bacilli within the lung tissue suggestive of infection such as *Legionella pneumophila* or *Mycobacteria tuberculosis*. A small amount of hemosiderin can be seen within the lung tissue, however whilst not ideal for the patients' health, this is expected in an individual who has been continually exposed to hazardous materials in their line of work. Diffuse alveolar damage is caused by mercury poisoning as well as infection, this is also a side effect of death, consequently, seen in this post mortem specimen.

Differential Diagnosis: Heavy Metal Poisoning, Bacterial infection (pneumonia), Trauma (from collapse), fungal infection or Heavy metal positioning and infection.

Provisional Diagnosis: Mercury poisoning and Bacterial infection.

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