Artifacts, Faults and Failures: a Review

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We have been producing artifacts, faults and failures for very many years (some of us being more productive than others)!

- This is a Cambridge Rocking microtome ("Cambridge Rocker")
- Question: In which year was it first offered for sale?
- Answer: 1885
Introduction

For each of our 70 cases we want you to:

1. Identify the artifact, fault or failure
2. Explain the most likely cause/s
3. Describe how you could overcome the problem and avoid its occurrence in the future

Definitions

- Artifact: a structure that is not normally present in living tissue
- For the Histologist: an artifact is a structure that is not normally present in a well-prepared section
- Fault: a defect or blemish, culpability, offence, misdeed
- Failure: non-performance, not a success, catastrophe, fiasco

Case 1: Lung H&E

Case 1: Lung – inhaled seaweed from drowning (H&E)
Case 1: Example 2 – cellulose in intestinal tumour

Case 2: Liver – formalin fixation

Case 2: Example 2, bone marrow – formalin fixation

Case 2: Example 3, kidney – formalin fixation
Formalin pigment

- Acid formaldehyde hematin
- Formed by reaction of hemoglobin in red cells with formic acid (from oxidation of formaldehyde)
- Forms after prolonged fixation in buffered formalin and more quickly in non-buffered formalin
- Brown to black birefringent pigment
- An atypical form is deposited in fat vacuoles in fatty liver or adipose tissue
- Can be removed from sections with saturated alcoholic picric acid or alkaline ethanol (sodium hydroxide or lithium carbonate)

Case 3: Skin (H&E)

Tattoos

- Tattoo ink consists of pigment in a carrier solvent (alcohol or alternative)
- Pigments may be iron oxides, metal salts or plastics
- Heavy metals include: mercury, lead, cadmium, nickel, zinc, chromium, cobalt, aluminium, iron etc.
- Particles range from 2 – 400 nm are found in phagosomes in keratinocytes, fibroblasts, macrophages and mast cells
- After 3 months pigment found in dermal fibroblasts surrounded by connective tissue that entraps and immobilizes the cell
- Eventually ink appears in regional lymph nodes
Case 3: Example 2, lymph node

- 56 year old male.
- Clinical notes: enlarged lymph gland right groin ? Lymphoma.
- Dense brown to black pigment.
- Perls’ negative.
- Schmorl’s positive.

Schmorl’s X 10

Case 3: Example 2, lymph node

- Areas of normal lymph node architecture.
- S-100 using red detection shows dendritic cells only.
- Conclusion: non-specific reactive hyperplasia.
- Is there a history of tattooing?

S-100 red detection x20

Case 4: Pancreas H&E (same specimen, different blocks)

Ethanol fixed

Formalin fixed

Case 4: Pancreas H&E (same specimen, different blocks)

Ethanol fixed
Case 5: Endoscopic biopsy (H&E)

- Sesame seeds from gut contents

Case 6: Lung (H&E)
Case 6: Lung-mechanical specimen compression (H&E)
- With forceps while tissue is fresh (during removal)
- Local compression (cassette bars)

Case 7: Splenic capsule (H&E)
- Gelfilm® and Gelfoam® are made from absorbable gelatin
- In the form of a thin film or sponge, are used to control bleeding
- Has a characteristic appearance with slightly basophilic gelatin walls
- Usually no tissue reaction
- Fully absorbed in 4 – 6 weeks

Case 7: Splenic capsule - with surgical sponge

Case 8: Example 1, Stomach (H&E)
Case 8: Specimen-to-specimen contamination

- Specimen-to-specimen contamination is very serious
- It can occur when specimen is removed, during fixation or grossing (cutting board, instruments), during processing (migration from cassette to cassette), during embedding (forceps), during flotation or even during staining
- Of particular concern if the same specimen type is involved
- It can result in a compromised or incorrect diagnosis
- Medical literature includes some interesting recent publications

Case 9: Breast (H&E)
Case 9: Breast - heat damage (H&E)

- Seen at margin of surgical biopsies
- Strong acidophilic staining
- Loss of nuclear and cytoplasmic detail
- Coagulation of connective tissue fibres
- Caused by laser-generated heat fixation of tissues (cautery)

Case 10: Fibro-muscular tissue (H&E)

Case 10: Fibro-muscular tissue - delayed fixation (H&E)

Case 11: Oral cyst (H&E)
Case 11: Oral cyst - cholesterol clefths (H&E)

- Caused by tapering needle-like crystals
- In vessel walls in atheroma
- At sites of old haemorrhage
- Crystals dissolve during paraffin processing
- Can be preserved in frozen sections

Case 12: Granuloma (H&E)

- Calcification can occur in long-standing granulomas associated with chronic infections and necrosis
- If noted at grossing decalcification can be used prior to processing
- Surface decalcification is an alternative
- Deposits stain with hematoxylin
- What other artefact is present?

Case 13: Peripheral nerve (H&E)
Case 14: Appendix child – *Enterobius vermicularis* (threadworm or pinworm) (H&E)

Case 15: Cervical smear (Papanicolaou)

Case 15: Cervical smear – *Alternaria* contamination (Papanicolaou)

- An airborne fungus
- Branching mycelium
- Snowshoe-shaped macroconidia
- May settle on smears or sections at any stage of preparation
- Store smears or sections in covered containers

Case 16: Cervical smear (Papanicolaou)
Case 16: Cervical smear – contaminant insect material? (Papanicolaou)

- Two smears collected on the same occasion
- Contain contaminant insect material from an unidentified source

Case 16: Example 2, cardiac muscle – insect contaminant (H&E)

Case 17: Smear from respiratory tract (Papanicolaou)

- Seen in smears from respiratory tract and other sites
- Bizarre but symmetrical shape
- Relatively large size
- Often above the focal plane of the specimen
Case 17: Smear from respiratory tract – pollen grain (Papanicoloau)

- Seen in smears from respiratory tract and other sites
- Bizarre but symmetrical shape
- Relatively large size
- Often above the focal plane of the specimen

Pollen from a variety of common plants
Micrograph (colorized): Dartmouth Electron Microscope Facility (Wikimedia Commons)

Case 18: Breast specimen (H&E)

Case 18: Breast specimen (H&E) – ruptured implant
Case 18: Breast specimen (H&E)

- 43 yo woman had breast augmentation 3 years ago.
- Left breast tissue was lumpy and deformed and it was thought the implant had ruptured. Implant was removed surgically.
- Specimen consisted of prosthetic implant covered by connective tissue capsule with attached firm 2cm nodule.
- Nodule showed silicone in holes and foam cells. Granuloma reaction was seen in nodule.
- Silicone is refractile but not birefringent.

Case 19: Surgical specimen (H&E)

Case 19: Surgical specimen – silk sutures (H&E)

Case 19: Example 2: Cervical smear – silk sutures (Papanicolaou)
Case 22: Surgical specimen (H&E)

In the fresh state some tissues are highly susceptible to damage from crushing with forceps or other surgical instruments (lymphoid and glandular tissue).

Artifact typically seen at periphery of specimen.

In small localized areas.

Can displace cellular antigens in IHC.

Causes irreversible damage.

Case 23: Tonsil (CD20 and H&E)

Case 23: Tonsil (H&E)
Case 23: Tonsil “sulphur grain” Actinomyces

Case 24: Kidney (H&E)
Case 24: Kidney – biopsy-pad artifact (H&E)

- Foam pads used to enclose specimen
- Pads compressed when cassette closed
- If specimen is fresh or only partly fixed a pattern of local pressure effects or moulding can occur
- Becomes permanent as fixation is completed
- Avoided by not using pads until tissue is fixed or using an alternative enclosure

Case 25: Brain (H&E)

- This was a large thick specimen
- Pressure effect during processing caused by structural bars in cassette lid
- Avoided by using a thinner specimen or a larger cassette
Case 26: Pancreas specimen – incomplete fixation & under-processing

- Very short fixation, 2 hour schedule
- Extreme shrinkage occurred after sectioning due to evaporation of residual solvent
- Very poor morphology
- Re-processing is possible
- Several methods are available
- What would you do?

Case 27: Pancreas specimen – retained solvent from under-processing (H&E)

- Gross cracking in central area of block
- Areas where cells are swollen and demonstrate “blue hue”
- Caused by insufficient dehydration and clearing
- Clearing solvent not replaced with wax and is “retained” in the tissue
- Re-processing would allow better sections to be cut
- Morphology will still be compromised
Case 28: Brain processed on a 12 hour ethanol/xylene schedule (post-mortem specimen)

- Schedule should be adequate for this type and size of specimen
- Texture of the block is poor
- It was discovered that the final ethanol was heavily contaminated with water
- Re-processing may improve the block

Case 29: Colon mucosa (H&E)

Case 29: Colon mucosa – “nuclear meltdown” or “blue-hue”
Some causes of “nuclear meltdown” or “blue-hue”

- Allowing a specimen to dry out before fixation
- Using xylene that has been contaminated with water for clearing
- Using wax contaminated with formalin or formalin and ethanol during processing
- Failing to completely replace solvent with wax (retained solvent) – schedule too short, expired reagents, processor fault
- Over-heating the section when drying
- Ineffective de-waxing during staining (“pink disease”)

Avoiding “nuclear meltdown” or “blue-hue”

- Avoid improper handling of fresh specimens
- Fix properly
- Process thoroughly with un-contaminated reagents
- Avoid excessive heat when drying sections
- Make sure de-waxing is complete prior to staining
- Note that re-processing can help, staining may be improved, but morphology generally remains compromised
Example 5: “nuclear meltdown” or “blue-hue” (metastatic melanoma)

Case 30: Spleen H&E (two fields from the same section)

Case 30: Spleen - under-processed

Reprocessing reference: Leica Pathology Leaders (web site)
http://www.leica-microsystems.com/pathologyleaders/
Case 31: Myocardium – separation during flotation

- Retained solvent/incomplete processing can cause fragmentation
- Problem generally avoided in properly processed tissue
- Bath may be too hot
- Sections have been left for too long on bath

Case 32: kidney – blocks from same specimen (H&E)

- Blocks A: 8hr schedule
- Blocks B: 8hr schedule

Thickness:
- Block A: 3 mm thick
- Block B: 4.5 mm thick
Coffee Break?

Case 33: Spleen (H&E)

Case 33: Spleen - fractured during embedding (H&E)

Case 34: Kidney (H&E)
Case 34: Kidney (H&E)

- Uneven thickness produced during microtomy
- Inexperienced microtomist
- Uneven cooling of block face resulted in uneven expansion
- A section re-cut from the same block was uniform in thickness

Case 35: Brain (H&E)

- Cracks occurred during section cutting (block too cold?, cut too fast?)
- Water droplets were trapped when section placed on slide producing "lovely lips"
- Slide dried flat with inadequate draining
- Droplets of very hot water caused local disruption before evaporating
In these cases microtomist was a learner
Forceps were dropped onto blade
Damage to the section was visible in ribbon and during flotation
Section should never have been picked up
Case 37: Uterus – coarse chatter (H&E)

- Occurs with large, dense specimens
- Due to vibration of block or blade during cutting stroke
- Induced by mechanical fault/design fault in microtome usually combined with
- Poor technique (orientation of specimen, poor processing, cutting speed etc.)

Case 38: Rodent liver (reticulin stain)

- Venetian-blind effect/micro-chatter
- Rodent tissue susceptible to over-processing
- Block too cold
- Cutting too fast
- Faulty blade holder
Case 39: Kidney – fine chatter (H&E)

- In this case caused by cutting a cold, brittle block too fast
- Overcome by allowing the block to warm slightly then cutting very slowly
- Other strategies?
- Over-processed blocks tend to be brittle
- Other causes? (microtome or blade?)

Case 37, 38 & 39: Avoiding chatter

- Regular cleaning & maintenance of knife holder is important
- Contaminants between the blade and the clamping plates may allow vibration of the blade during the cutting stroke
- Learn how to disassemble the knife holder

Case 40: Adenocarcinoma (H&E)

- Very cellular organs prone to this problem
- Produced by very coarse rough trimming followed by a failure to properly polish the block face before taking sections
- Usually visible during flotation
- Easily avoided

Case 40: Adenocarcinoma – holes from roughing (H&E)
Case 41: Tongue (H&E)

Case 41: Tongue – dirt & squames (H&E)

A surface decalcifier was used to soften keratin to make sectioning easier.
This damages nuclear staining in epidermis.
Other softening agents are a better choice and should not damage nuclei if used sensibly.

Case 42: Skin (H&E)

Case 42: Skin – poor nuclear morphology (H&E)
Case 43: Deposit (H&E)

Case 43: Deposit – sneeze artifact (H&E)

Ensure the flotation bath is free of bubbles

Case 44: Lymphoid tissue (H&E)

Case 44: Lymphoid tissue – collapsed bubble artifact (H&E)
Case 45: Spleen (H&E)

- Slide dried flat on a hotplate
- Uneven surface temperature produced hot-spots
- Produced cracking and some cell shrinkage

Case 46: Vagina (H&E)

- Contaminant hair (H&E)
Case 47: Mucosa (H&E)

- Only eosinophils and red cells have stained with eosin
- In this case the water wash that follows the blueing alkali was skipped
- Eosin will not bind under alkaline conditions
Case 48: Liver after hematoxylin stain (incomplete H&E)
- Incorrect setting of microscope condenser diaphragm when examining wet section (no cover slip)

Partially closed

Fully open

Case 49: Endoscopic biopsy (H&E)

Case 49: Endoscopic biopsy – residual wax (H&E)

two sections mounted on the same slide

Case 50: Skin (H&E)
Case 50: Skin - over-oxidized hematoxylin (H& E)
- Nuclei lack sharpness & there is an overall brownish tinge
- Precipitated mordant present
- Replace hematoxylin

Case 51: Skin (H& E)

Case 51: Example 2, mucosa (H& E)

Case 51: Skin - over-stained with hematoxylin (H& E)
- Cytoplasm and fibrous elements stained with hematoxylin
- Some nuclei lack chromatin detail
- Requires acid alcohol differentiation
Case 52: Appendix (H&E)

- Crystals present on top of section
- Probably precipitated mordant from hematoxylin
- Crystallization may occur because of solvent evaporation and concentration of salts or dyes in the staining bath
- Evaporation occurs during manual staining
- Filter reagent or replace

Case 52: Example 2, Masson Trichrome

Case 53: Bone marrow (PAS)
Case 53: Bone marrow – residual mucus from saliva (PAS)

- Saliva used as a source of diastase (glycogen – ve control)
- Not properly washed prior to stain
- Use commercial diastase or dilute saliva with water or saline

Case 54: Liver, formalin fixed (PAS)

- Caused by displacement of glycogen by the advancing fixation front
- Can be avoided by using better glycogen fixatives such as Bouin or formal-alcohol

Case 55: Skin (PAS)
Case 55: Skin – under-oxidized (PAS)
- Insufficient oxidation with periodic acid
- Note staining of basement membrane

Case 56: Kidney (H&E)
- Section allowed to partially dry before coverslipping
- Tiny air bubbles are trapped over some nuclei (appear black)
- Prevent drying prior to contact with mountant
- Properly applying another coverslip will overcome the problem
Case 57: Skeletal muscle (cryostat section SDH)

- Caused by slow freezing of tissue allowing the growth of crystals
- Inappropriate quenching method used
- Specimen too large to freeze rapidly
- Some specimens prone to this (muscle)
- How do you avoid this?


Case 58: Salivary gland (H&E)

- Breakdown of poor-quality polystyrene mountant
- Crazing or cracking present
- Spherocrystals present
- Remove cover slip, re-stain and remount
Case 58: Example 2, Small intestine (H&E)

- Coverslip on wrong side
- Slide placed in rack wrong way around for coverslipper
- Slide could be retrieved

Case 59: Tonsil (IHC)

- Residual wax
- Bubble in primary antibody

CD5 CD20
To achieve strong, uniform staining high-quality fixation is essential
- “Zonal fixation” must be avoided
- Specimen dimensions must be appropriate (<5 mm thick)
- As far as possible fixation conditions should be standardized
Case 61: Colon (H&E)

Case 61: Colon - hematoxylin scum (H&E)

Case 62: Submucosa (Masson trichrome)

Case 62: Submucosa (Masson trichrome - sensitization or secondary mordant step omitted)
Case 63 : Gut muscularis externa (H&E)

Case 63 : Gut muscularis externa - bleaching from micro-projector (H&E)

Case 64: Thyroid (H&E)

Case 64: Thyroid - cholesterol crystals in clot (H&E)
Case 65: Cervix (HPV – ISH)

Case 65: Cervix - bubbles formed during pretreatment causing uneven staining (HPV – ISH)

Case 66: Tonsil (CD3 IHC)

Case 66: Tonsil – lifting due to improper flattening and drying (CD3 IHC)
Case 67: Tonsil – CD20 Bond Polymer Refine Detection (H&E for comparison)

- Over-digestion
- HIER 20min with ER1 pH 6 (Citrate)
- Plus 10min with Enzyme 1 (Proteinase K)

Case 67: Tonsil – CD20 Bond Polymer Refine Detection

Optimized retrieval (HIER 10min with ER1)
Unsuitable retrieval (10min with Enzyme 1)

Case 68: Kidney (cryostat section SDH)
Case 68: Kidney - (cryostat section SDH)

- Histochemical method for the mitochondrial enzyme succinate dehydrogenase (should stain mitochondria)
- In acting as a hydrogen acceptor tetrazolium salt (NBT) is reduced to a colored formazan
- Section must be thoroughly rinsed after incubation and before mounting (aqueous) to avoid crystallization of NBT/formazan

Case 69: Submucosa (H&E)

Case 69: Submucosa - microorganisms from contaminated flotation bath (H&E)

Case 70: Pig colon (PAS)
Case 70: Pig colon (PAS)
Unknown material impacted at grossing

Case 70: Example 2, Prostate (H&E)
Wood from dissecting board impacted at grossing

Case 70: Example 3, Breast (H&E)
Grossing problem?
Case 70: Example 3, Breast (H&E)
Whoops!

With special thanks to:
Kerrie Scott-Dowell, Neville Farmer, John Hall and Judy Brincat