RCPAQAP
RCPA Quality Assurance Programs

Perfecting a Gram Stain

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Protecting patients

RCPAQAP

- RCPA Quality Assurance Programs are world leaders in the provision of external quality assurance (EQA) for pathology laboratories.
- RCPA Quality Assurance Programs Pty Ltd was formed in 1988 by the Royal College of Pathologists of Australasia (RCPA).
- The company was formed to provide the following:
  - ❖ External proficiency testing
  - ❖ Quality assessment
  - ❖ Appropriate education programs to public and private medical testing (pathology) laboratories in Australia, New Zealand and other countries.
- The programs have been developed with assistance from a number of professional bodies and with significant input from participating laboratories.

Where is RCPAQAP?

Technical proficiency module

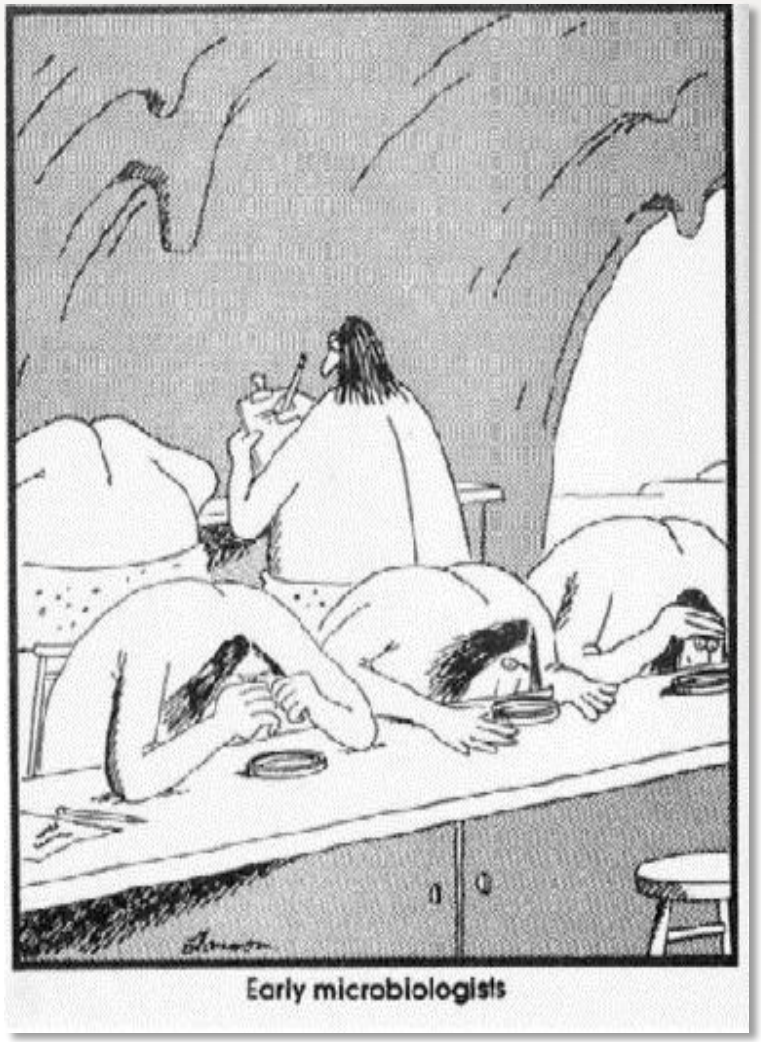
Comprises of 3 surveys per annum

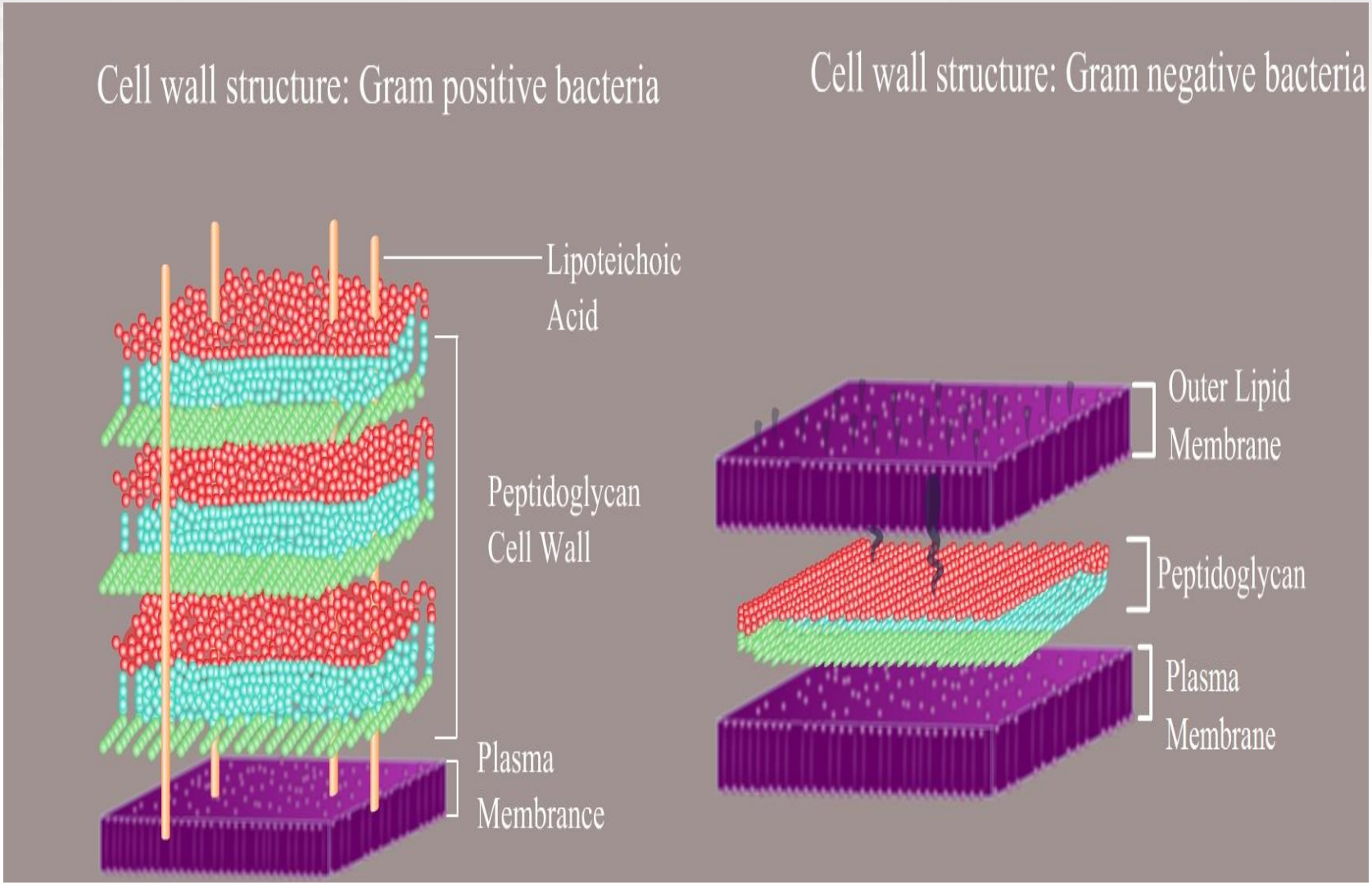
- TM2017-1 (January)
  - Special staining
  - Processing and sectioning exercise.
- TM2017-2 (May)
  - Haematoxylin and Eosin staining exercise.
- TM2017-3
  - Repeat special stain exercise.

- Technical frozen module-TF2017-1. Frozen sectioning and staining exercise.

Gram Stain Background

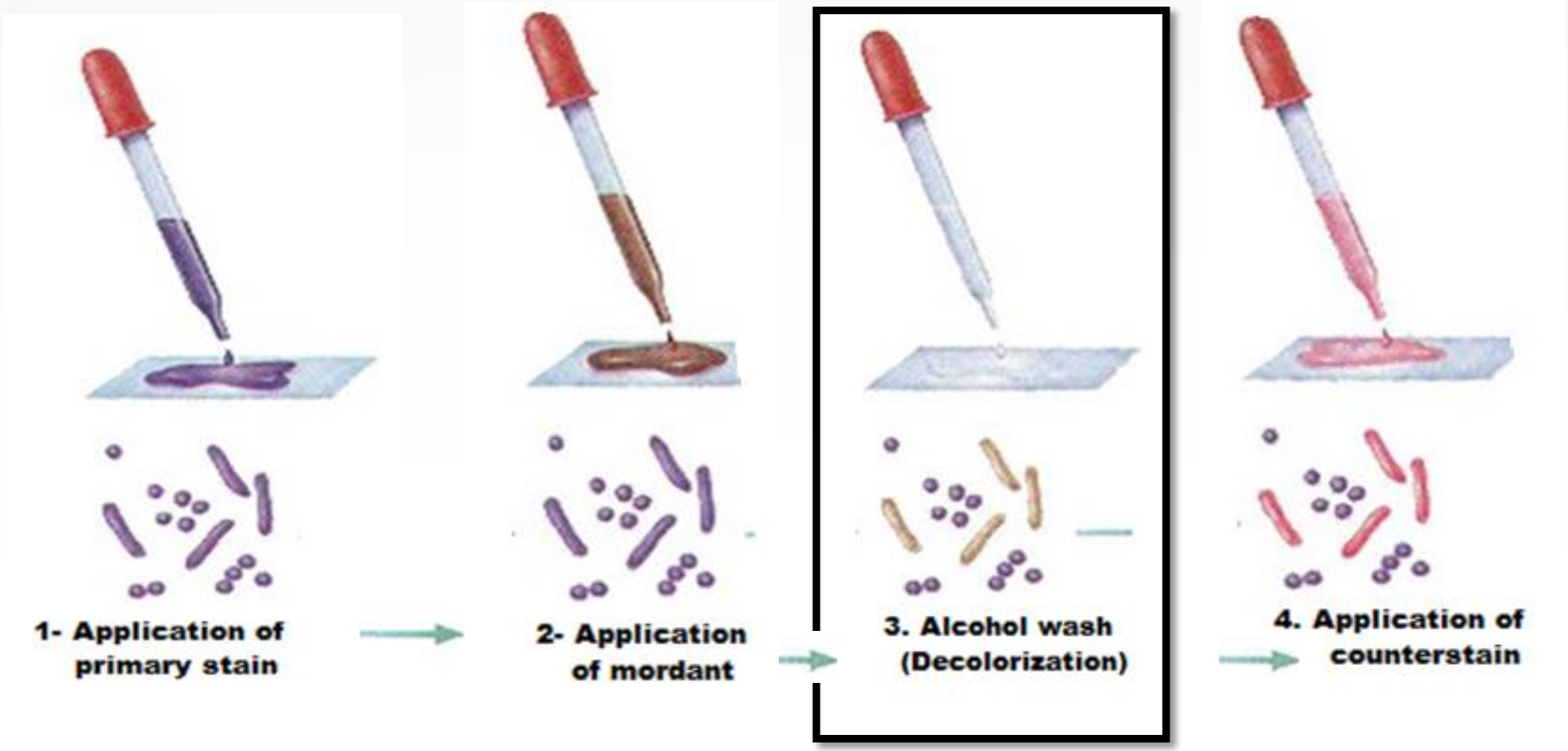
- Discovered in 1882 (published in 1884) -Hans Christian Gram, a Danish bacteriologist.
- Standard procedure performed for identification of bacteria.
- Bacterial cells- preferentially retained certain stains during staining.
- Gram positive organisms have a thick mesh-like cell wall where about 50-90% is made of peptidoglycan which retains the crystal violet iodine during staining.
- Gram-negative bacteria have a thinner layer of peptidoglycan (10% of the cell wall) and lose the crystal violet-iodine complex during decolorization with the alcohol rinse, but retain the counter stain Safranin, thus appearing reddish or pink.





(http://ibabmsc01.blogspot.com.au/2012/03/gram-staining.html)

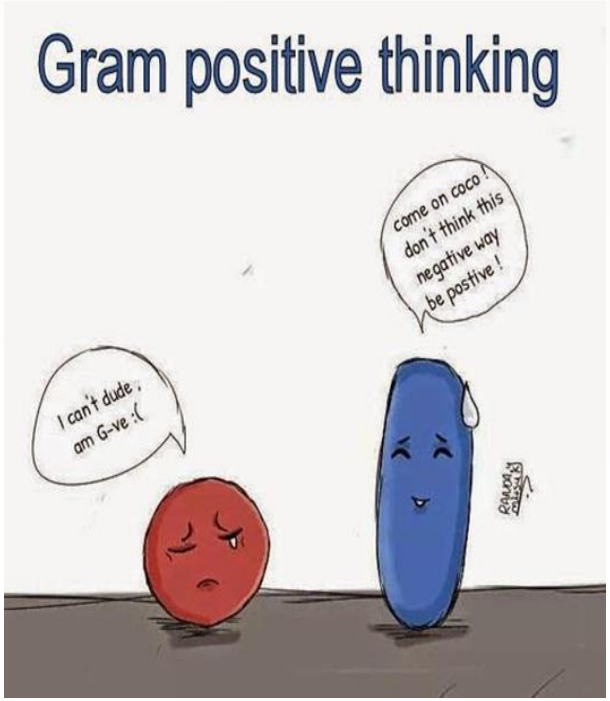
Gram Staining procedure

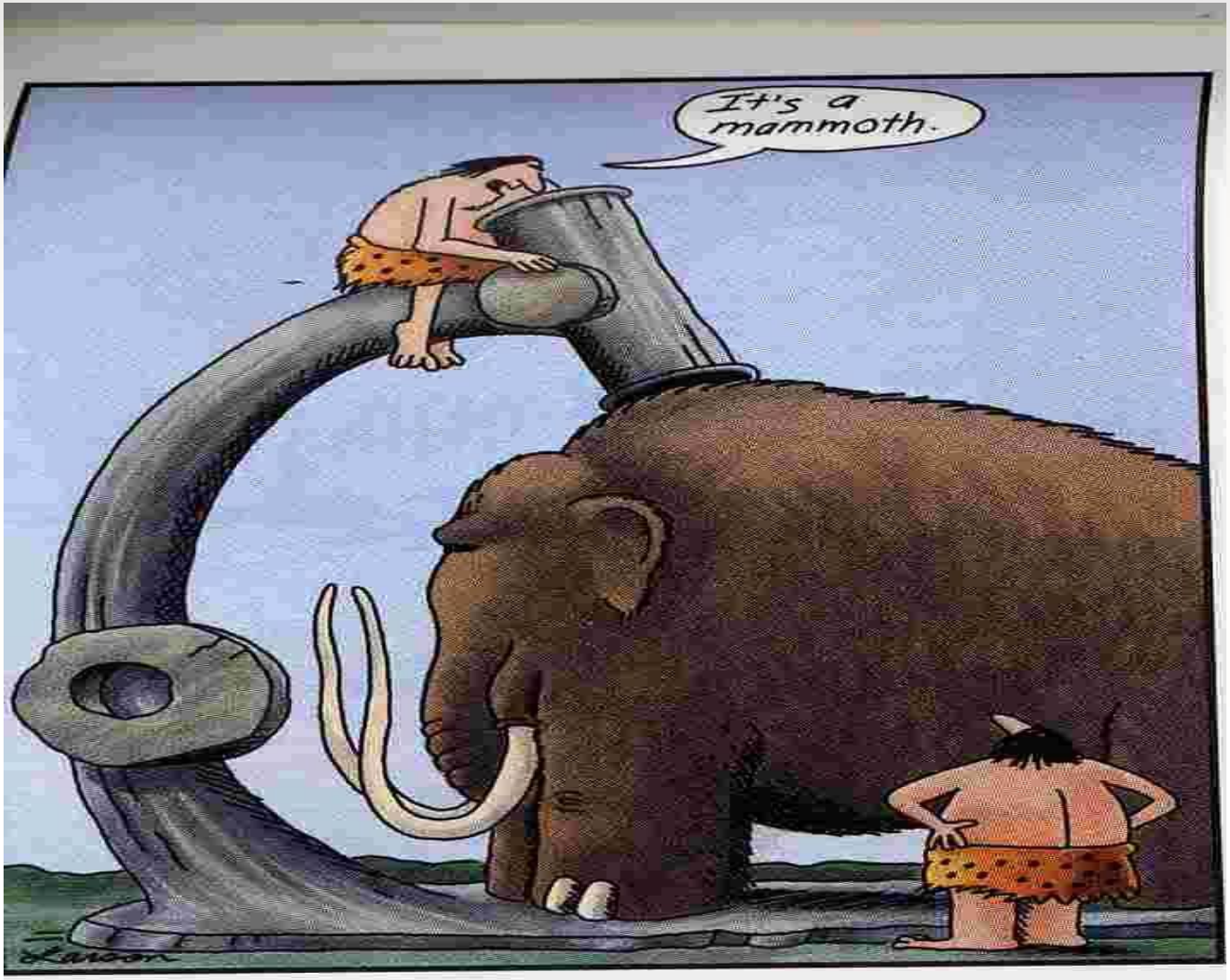


(http://www.biologyjunction.com/bacteria_notes_b1.htm)

Quality control of Gram stain
Why assess Gram staining?

- Staining varies between laboratories.
- Artefacts can interfere with diagnosis.
- The need for consistency of staining to avoid difficult histological interpretation.
- Quality depends on many factors- initial handling, fixation, processing, staining procedure and types of reagents used, etc.



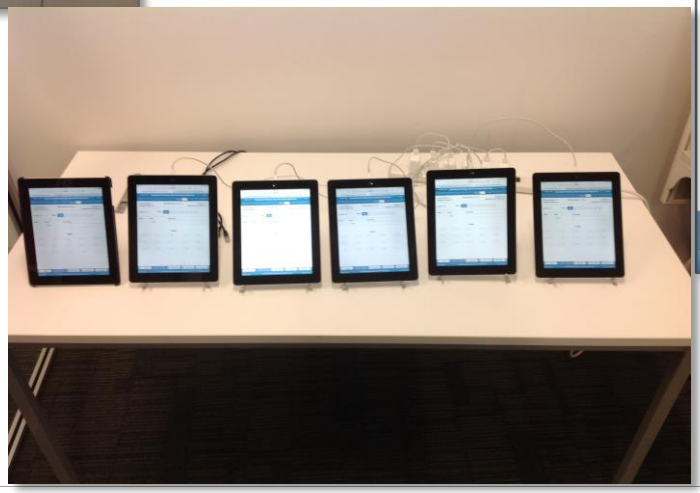


Early microscope

Assessment process

- The assessment of slides lasts for a duration of 3-4 days.
- A panel of four assessors from the technical advisory committee convene to assess the slides.
- Results are recorded using an iPad app which was implemented in 2014. Prior to that, results were recorded in paper booklets.
- Pre-determined assessment criteria, together with comment codes are used as a guideline to determine the quality of the staining.

H&E Assessment – WADE app



Gram stain - Assessment criteria

The submitted material according to the following criteria:

- Clear distinction of microorganisms from the background.
- Correct proportions of Gram positive and Gram negative microorganisms.
- Uniformity of staining of both gram positive and negative microorganisms.
- Intensity of staining sufficient to detect morphology of the microorganisms.
- Counterstain not interfering or masking microorganisms
- Absence of contaminants.
- Absence of artefacts from dehydration, clearing and mounting

Gram stain - Assessment comment codes

Codes	Comments
BA	Blotting artefact
BGS	Background staining present obscuring microorganisms
CBT	The staining of control better than test.
CH	Counterstain heavy, obscuring microorganisms
CMP	Contaminants present
CW	Counterstain weak
FN	False negative staining of microorganisms
GND	Decreased percentage of Gram negative organisms seen.
GNO	Gram negative organisms overstained
GNP	Decreased percentage of Gram positive organisms seen.
GNW	Gram negative organisms weakly stained
GPO	Gram positive organisms overstained
GPW	Gram Positive organisms weakly stained
LD	Lacks differentiation
LIF	Section lifting from the slide
MAP	Mounting artefact
PTY	Patchy uneven staining
SOC	Inappropriate tissue choice for control.

Assessment process

Standardised grading categories:

- Satisfactory: ≥3.0
- Borderline: ≥2.5 and <3.0
- Unsatisfactory: <2.5
- Unable to be assessed (*)

(*) Material submitted late, or with insufficient or illegible identification , or with technical problems preventing assessment by the committee.

Assessment scoring table

Scoring of slides

0: No staining

1: Not diagnosable, very poor staining and none of the assessment criteria met.

2: Unsatisfactory staining - criteria has not been met, diagnosis would be affected

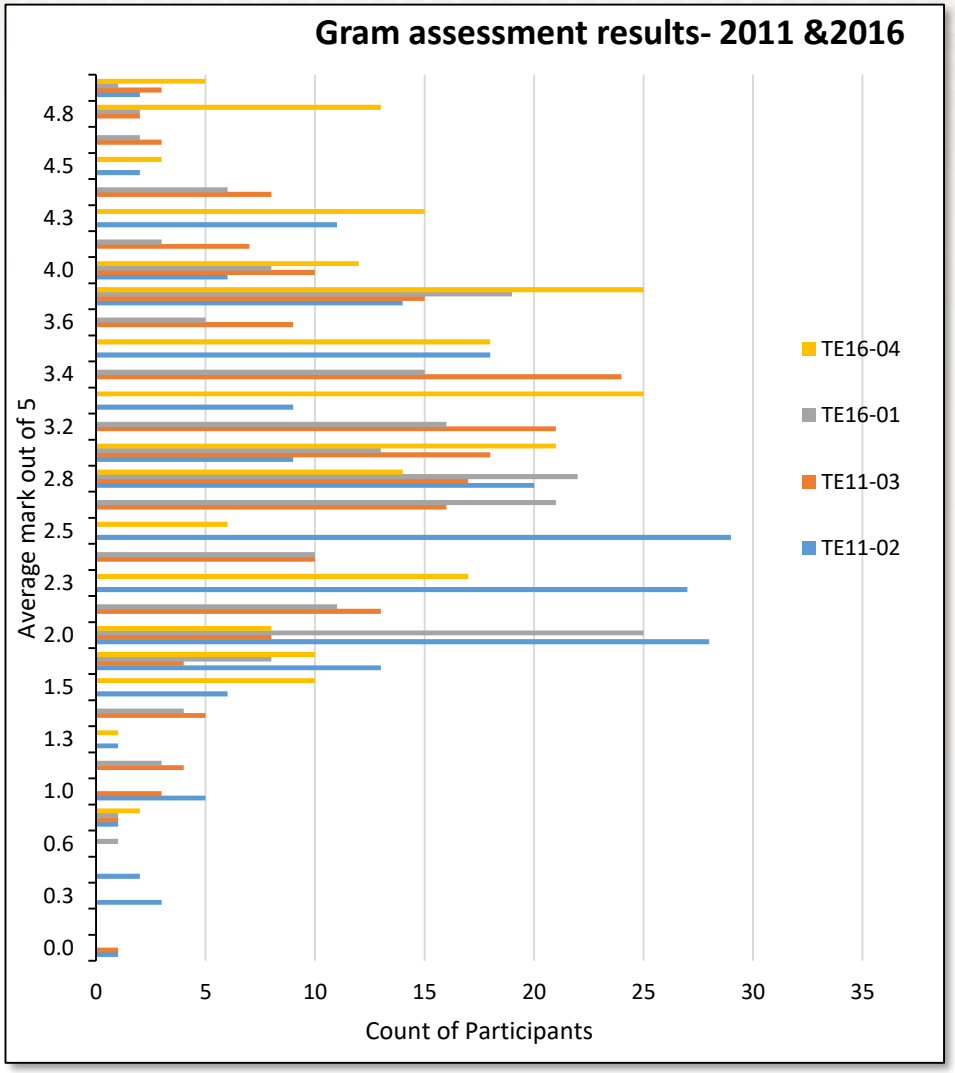
3: Criteria has been met at a basic level

4: Above average

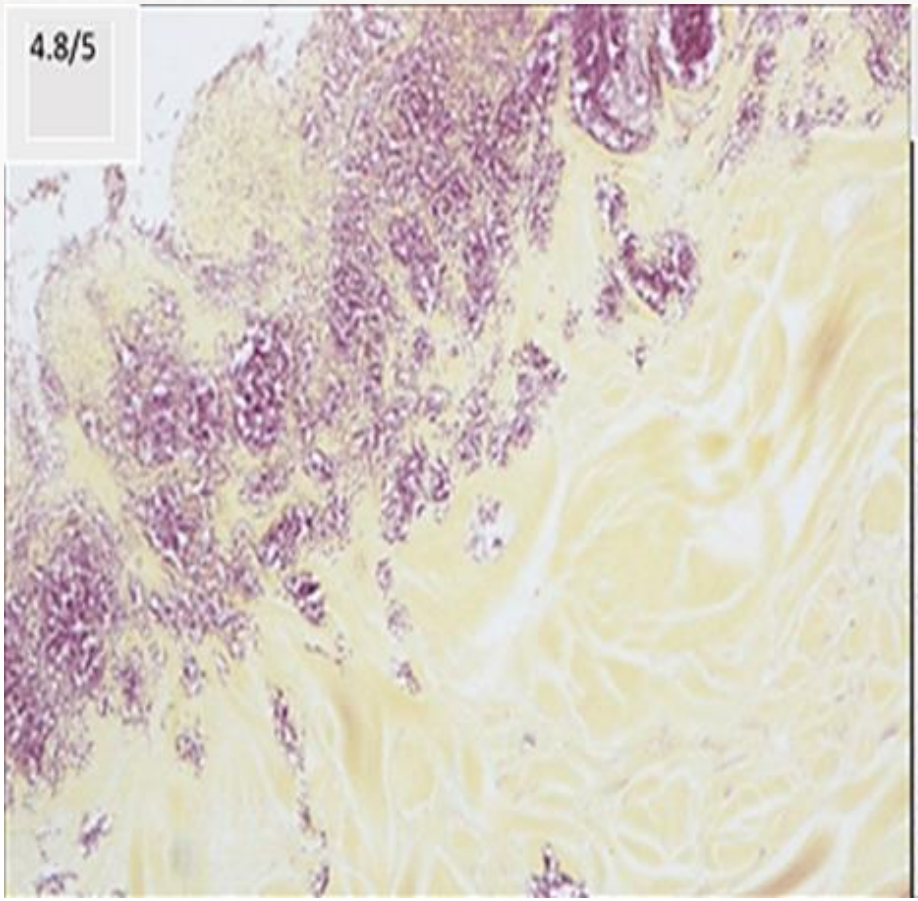
5: Reflects a perfect fulfilment of the criteria

RCPAQAP Gram stain results

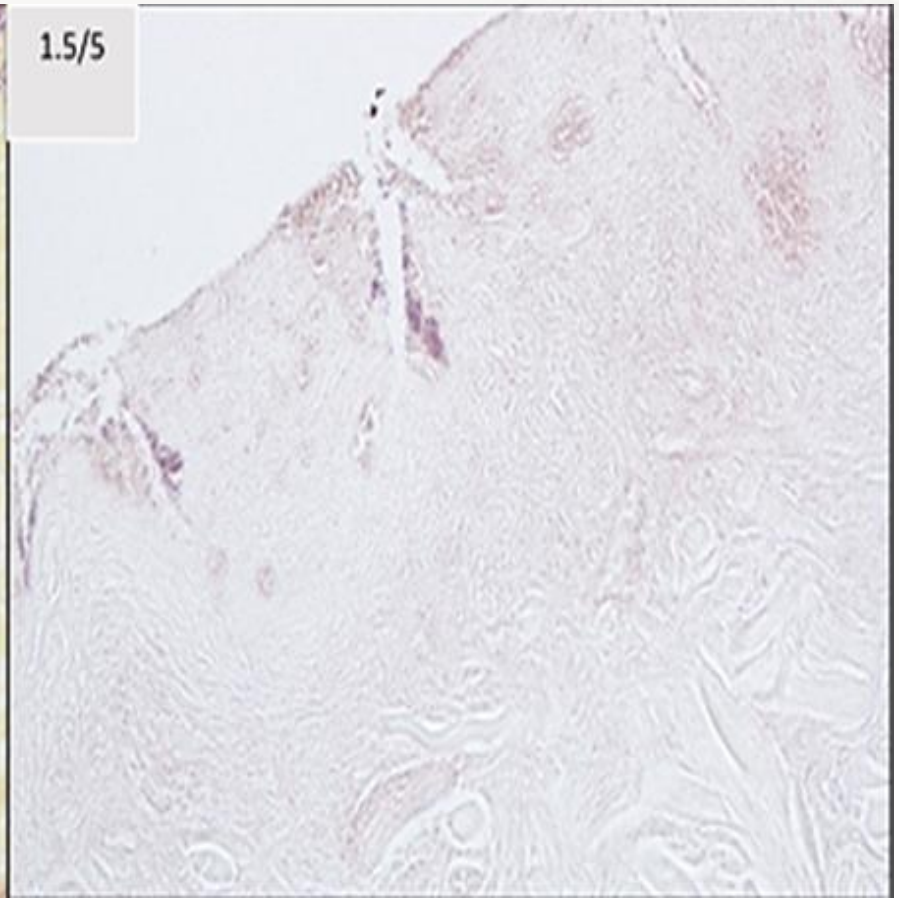
- Improvement in staining quality in 2016.
- More participants achieved satisfactory results in the 2nd survey 2016.
- It was indentified from these exercises that sourcing the right type of controls was an issue for a few participants.



Results from the Gram Assessment

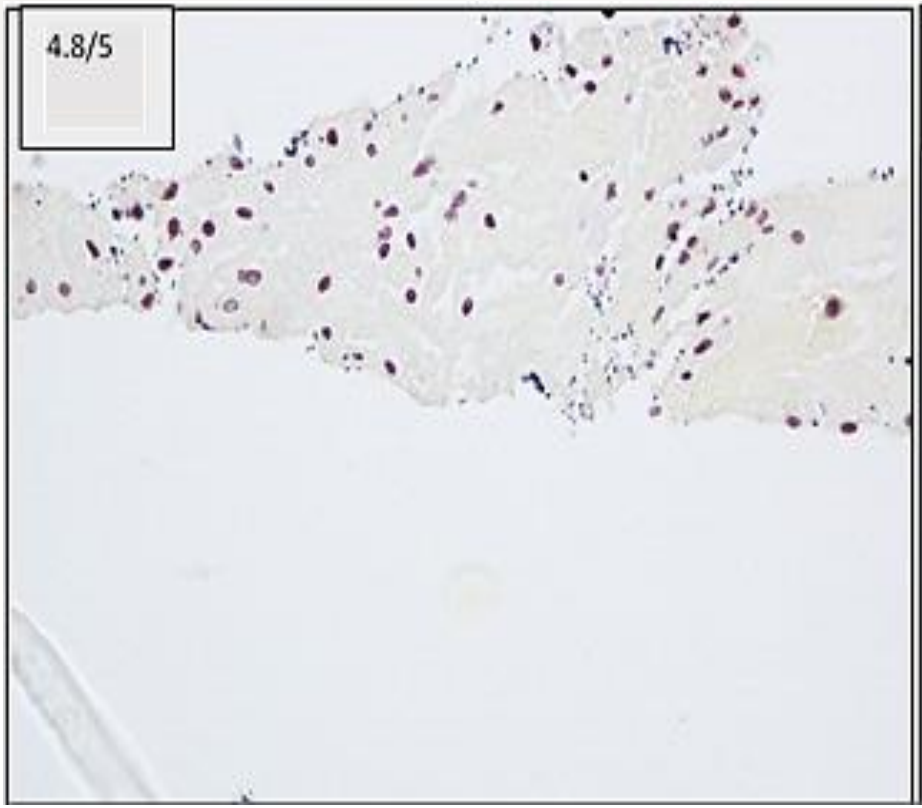


Optimal staining of gram negative organisms. Brown and Hopps method. 40x.

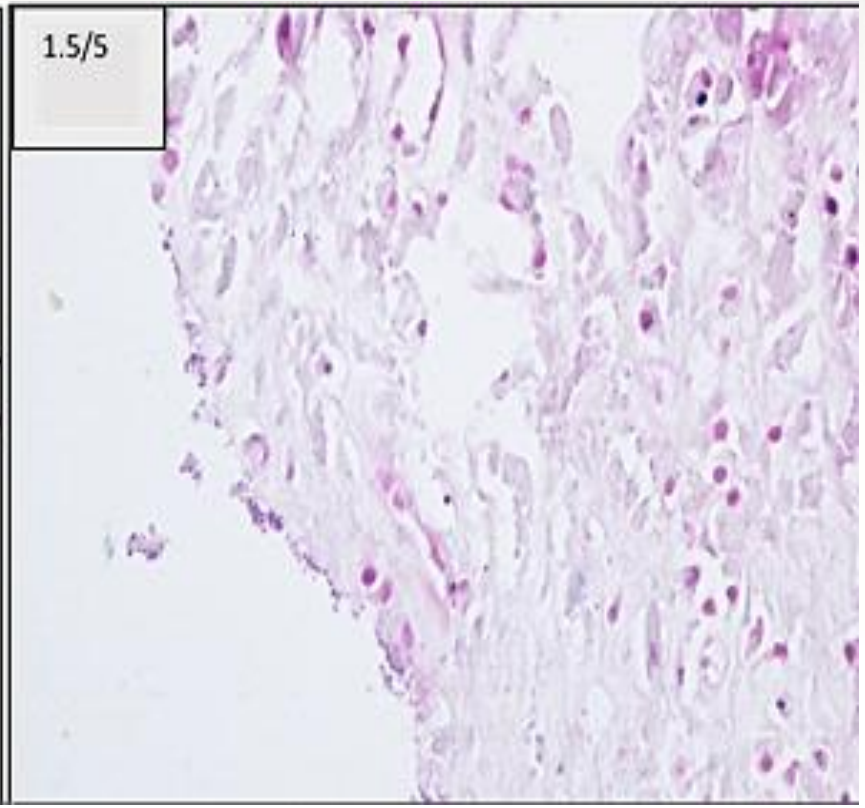


Decreased percentage and weak staining of gram negative organisms. Brown and Hopps method. 40x.

Results from the Gram Assessment

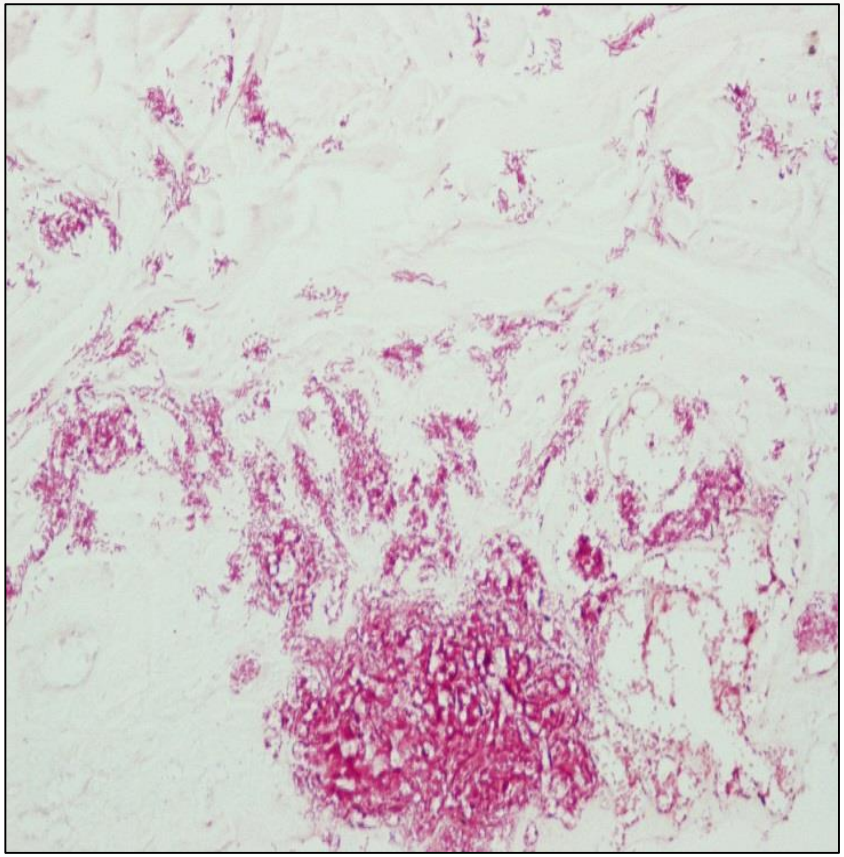


Optimal staining of gram positive organisms. Brown Brenn method. 40x.

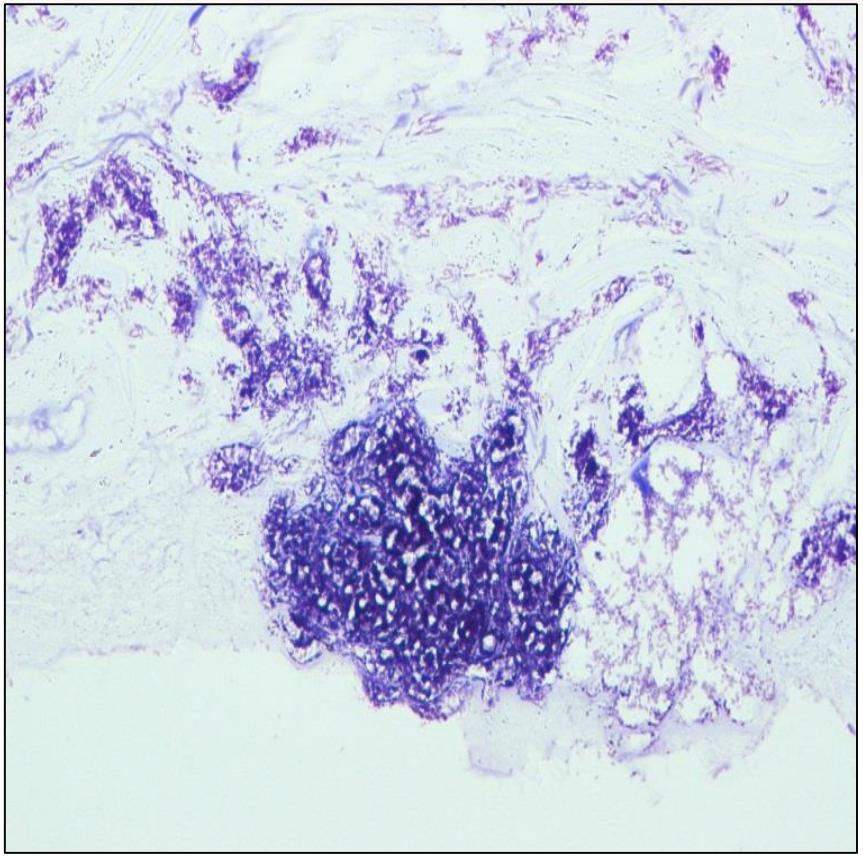


Weak and false negative staining of gram positive organisms. Brown Brenn method. 40x.

Results from the Gram assessment



Optimal staining of the Gram negative organisms.



Gram negative organisms staining falsely positive.

Gram Stain Workshop Overview

- Workshop booklet provided.
- Methods of staining required
  - ❖ Gram Twort stain
  - ❖ Modified Garvey stain
- Stain slides using both methods
- Select slide with optimal staining (Both positive and negative organisms)
- Self assessment. Complete assessment scoring sheet and submit together with slides for review.
- Delegate involvement in the assessment process.

References

- Bancroft J, Gamble M. Theory and Practice of Histology Techniques. 5th Edition.2002, Churchill Livingstone. 17:312-313.

- Richard W. Brown. Histologic preparations Common Problems and Their Solutions, CAP press 2009. 5:49-56.

- Peck M, Badrick T. A review of contemporary practice and proficiency with Gram staining in anatomical pathology laboratories. *J. Histotechnology* 2017; 40:54-61

Acknowledgements

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