S1050 Pap EA 65 (Modified) GCC Diagnostics

General & useage information

PAP EA65 contains a higher percentage of Green stain component that EA50, it is ofter refered to as ' PAP EA65 enhanced green '.
There are many different formulations for this product across the World. Almost every manufacturer has a slightly different formula. To add to this, many laboratories have slightly different methods and staining procedures which give rise to slightly different results . The lack of an International Standard in this area has led to a lack of clarity on what a good Pap Stain should be.

After the work of G.W Gill especially related to the chemical conflict between the traditional Pap Stain components Phosphotungstic acid and Bismark Brown Y or R, where according to Gill one negatively interferes with the other, creating precipitation , altering colouration over time and reducing shelf life , our product is formulated without Bismark Brown. It also contains Acetic acid to maintain the required pH for optimal staining.

We have formulated our product to provide the basic requirements of a Pap Stain , that is, when used with suitable Haematoxylin stain and counterstained with PAP OG6 results will be:

Blue Nucleus with optical density light enough to allow clear observation of chromatin particles in the lobes of polymorphonuclears (PMN's) and dark enough for clear observation of chromatin particles in intermediate squamous cell nuclei.

Eosiniphilic squamous cells will colour pink – red.

Nucleoli, cilia and Red Blood Cells (RBC's) will take up the eosin component (pink red)

Keratinised cells will colour orange.

All other cells will take up the (Basophilic) Fast Green component and colour blue-green.

All cells should be transparent and thick groups of cells are expected to be stained fairly uniformly throughout.

Although we publish a procedure for the Pap Stain, individual laboratories may need, because of past experience and what stain picture the technicians are used to , have a trial approach to the procedure and make adjustments where required to staining times, differentiation, rinses etc to achieve a stain picture that meets their requirements and achieve technically satisfactory staining.

Samples available on request.

Please see the Pap procedure below for results expected to be obtained.

METHOD : Set out at least 15 staining jars.
Fill staining jars in following order and follow staining and rinse times given.
Change reagents for fresh material where stated.

1. Cytopack fixative - Fix smears in reagent for 15-30 minutes. If smears are.delivered to the laboratory with a fixative/coating, remove coating with a short rinse in Alcohol rinse 95% and distilled water or follow fixative manufacturer's instructions.

2. Distilled water - Rinse smear for a few seconds.

3. Harris Haematoxylin - Immerse slide for 3 - 4 minutes.

4. Distilled water - Rinse slide for 5-6 seconds.

5. Haematoxylin differentiator - Dip slide in differentiator for 10 - 15 seconds
Control this stage microscopically. Inspecting the slide to ensure that the cell cytoplasm is clear of any background haematoxylin staining otherwise this will interfere with EA counter staining.

6. Haematoxylin bluing reagent - Dip slide 2 - 4 times in bluing reagent. (This stage may be omitted if required).

7. Distilled water - Rinse slide for a few seconds in distilled water.

8. Alcohol Rinse 70% - Rinse slide for a few seconds.

9. Alcohol Rinse 100% - Rinse slide for a few seconds.

10. Papanicolaou OG-6 - Immerse slide for 1 minute.

11. Alcohol Rinse 95% - Rinse slide for a few seconds each in 2 changes.
12. Papanicolaou EA-65 - Immerse slide for 1½ - 2 minutes.

13. Alcohol rinse 95% - Rinse slide for a few seconds each in 2 changes of the rinse.

14. Alcohol rinse 100% - Rinse and dehydrate in 2 changes of rinse for 2-3 seconds each.

15. Alcohol-Xylene rinse - Rinse slide for 2-3 seconds.

16. Xylene - Clear slide in xylene for 3 - 5 seconds.

17. Mount slide with cytopack synthetic mountant.

RESULTS:

Nuclei	Blue
Basophilic cells	Blue-green
Eosinophilic cells	Pink-red
Keratinised cells	Orange

Material safety Data Section

Reagents in this kit are harmfull to eyes, skin and by inhalation. Many reagents are highly flammable (individual details below).Keep and use these reagents away from sources of ignition and use in containers that have a tight closure to prevent evaporation. Do not consume the reagents. Danger of cumulative effect especially from xylene. Wear suitable protective clothing ie: skin, eye & face protection and use reagents in a well ventilated place or under a protective hood. Wear appropriate type organic vapour mask for breathing protection. In all cases take off contaminated clothing and wash with soap & water. In case of contact with skin and eyes, rinse with large volumes of water or rinse from an eye bath for several minutes and seek medical attention. In all cases of skin contact wash with soap and water, if soreness persists seek medical attention. In cases of inhalation, move patient to a clear air zone to recover. If patients feels unwell seek immediate medical attention. Show this sheet to medical professionals if needed.

Cytopack Fixative – is based on Methanol and is HIGHLY FLAMMABLE and TOXIC by inhalation an ingestion and possible skin absorption. UN 1230.

Alcohol rinse 70% , 95% and 100% is based on isopropyl alcohol and is HIGHLY FLAMMABLE. Do not breathe vapour. UN 1219

Alcohol-xylene rinse (Isopropyl alcohol + xylene mix) is HIGHLY FLAMMABLE. HARMFULL. Danger of cumulative effect. Xylene is a carcinogenic compound. Do not breathe vapour.

Xylene is HIGHLY FLAMMABLE and HARMFULL. Carcinogenic compound. Do not breathe vapour or get in contact with eyes or skin.

Cytopack Synthetic Mountant is based on plastic polymers and Xylene. Treat as for Xylene as detailed above.

Harris Haematoxylin contains haematoxylin dyes , inorganic aluminium salts and harmless organic solvents and water. HARMFULL by ingestion and eye contact. Will irritate eyes and discolour skin. Follow guidelines above.

Haematoxylin Differentiator contains dilute hydrochloric acid solution <5%. HARMFULL if in contact with skin and eyes and by ingestion. May cause burns to skin , eyes and internal organs.. Follow guidelines above.

Haematoxylin Bluing reagent is harmless aqueous solution of alkaline buffer salts. No real risk in normal use.

Papanicolaou OG6 & EA50 reagents are based on organic dyes dissolved in and water <20%'Ethanol <60% /Methanol <20% solvent containing small amounts of inorganic and organic acidifying agents. HIGHLY FLAMMABLE and HARMFULL by ingestion, inhalation and skin contact. Will irritate eyes and skin. Follow guidelines above.

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Accidental Spillage & Waste Disposal

For all reagents listed – mop up spillage with cloth ,rinsing the cloth under tap water diluting the spill to Public Sewer or mop up with absorbent paper or granules and dispose of through a waste disposal contractor allowing the final residue to evaporate to air. Consult local regulations if required.

Unsatisfactory Performance & Limitations

As part of our duty to monitor product performance and our policy of continual improvement. Please report to us any unsatisfactory performance you may experience with this product. If any reagent degrades before expiry date of shelf life we will replace that reagent free of charge.
GCC Diagnostics guarantees that the highest quality reagents are supplied with this product and that the product conforms to the information contained in this leaflet.
The user should however, determine the suitability of this product for their particular use.
If you wish to report any findings to us or if you require help or further information on the use of this product please contact us.

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