GCC Diagnostics SP300 DIFF-3 Procedure

Rapid differential staining kit for blood & cerebrospinal fluid smears. Detection of Helicobacter pylori in smears & sections. Staining of microorganisms and spermatozoa.

Principle of method

The Diff-3 kit enables the user to stain blood and cerebrospinal fluids in less than 1 minute, cleanly and reliably. The staining picture produced is very similar to that obtained using traditional Romanowsky stains but with the results being obtained in a fraction of the time.
The kit can also be used to stain H. Pylori , spermatozoa and basophilic / eosinophilic micro-organisms.

Each kit contains the following reagents:

Fixative solution 500m l (Methyl alcohol base – FLAMMABLE-TOXIC)
Solution A 500ml
Solution B 500ml
Rinse buffer concentrate pH 6.80 – 7.00
Dispenser pipettes 1ml x 4

If you require help using DIFF-3, please contact us at the address at the end of this leaflet.

DIFF-3 Stain for Haematology

Single slide method:

Using a pipette
Flood slide with Fixative (1-2 ml) After 10-20 seconds pour off excess apply 1ml of Soln A for 5 seconds, pour off excess apply 1ml Soln B for 5-10 seconds. Pour off excess stain, rinse in buffer or tapwater until slide is pink or check microscopically to obtain optimum differentiation.

Staining Jar Method:

Dispense sufficient reagents into minimum sized staining vessels set up in the following order:
1-Fixative solution 2 – Solution A 3 – Solution B 4 – Rinse solution

Use staining vessels with a closure to prevent evaporation when not in use. Do not return used reagents to their kit containers.
Blood smears should be air dried for a few minutes.

Stage 1 – Dip the smear in the Fixative for 5 times for 1-2 seconds each. Shake off the excess fixative.

Stage 2 – Dip the smear 2-3 times in solution A for 1 second each time. Shake off the excess.

[Optional short rinse here – see note below]

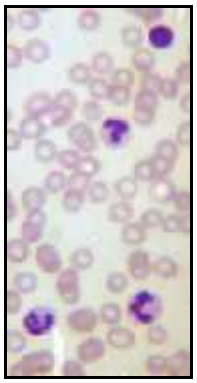
Stage 3 – Dip the smear 3-4 times in solution B for 1 second each time. Shake off the excess.

Stage 4 – Rinse in the rinse solution or under running tapwater for several minutes or until slide is pink (check microscopically that differentiation is satisfactory) and allow to dry. Examine or mount in synthetic mountant such as DPX.

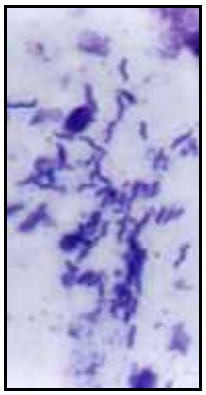
Discard rinse when excessively contaminated. Replace with fresh solution. The method given in this leaflet is designed to be a general guide. In cases where the user wishes to increase or decrease the level of eosinophilic or basophilic staining the number of dips in the particular stain solution should be increased or decreased accordingly.

NOTE: 2 or 3 dips in rinse to remove excess 'A' can enhance differentiation, clear the stain picture and avoid precipitation and overstaining. We recommend a trial and error approach to obtain optimal performance.

	NUCLEUS	CYTOPLASM	GRANULES
Erythrocytes		Pink	
Platelets		Pink	violet purple
Neutrophils	Purple	Pink-grey brown	Red-lilac
Eosinophils	Blue – purple	Pink- grey brown	Red orange
Basophils	Purple-blue		Dark purple-black
Bands	Purple	Pink- grey brown	Violet
Lymphocytes	Purple	Blue	Red lilac



Blood smear (Diff3)



H. pylori (Diff3)

Detection of Helicobacter pylori in smears & sections

The DIFF-3 stain has proven to be reliable in the detection of H Pylori in patients with ulcer and non-ulcer dyspepsia. Air dried smears from fresh biopsy tissue or sections from paraffin wax processed tissue are stained with the DIFF-3 technique taking about 30 seconds. This method has proven to be rapid, simple and clean enabling slides to be prepared , stained and examined in minutes and an immediate report given.

Method : Preparation of smears.

Fresh biopsy fragments should be ground in a sterile grinder and air dried smears prepared.

Preparation of sections.

Gastric biopsy specimens should be processed in formalin and 4 um sections prepared and dewaxed.

Prepared air dried slides from smears or sections should be stained as follows:

Stage 1 – Dip slide in Fixative , 5 x 1 second each.

Stage 2 – Dip slide in Solution A , 4 x 1 second each.

Stage 3 – Dip slide in solution B , 5 x 1 second each.

Stage 4 – Rinse excess stain from slide in rinse solution and allow to dry.

Clear in xylene & mount with DPX.

The characteristic morphology of Helicobacter pylori will be easily identified as blue-black or purple blue-black bacilli against a pale blue or clear background.

Staining of microorganisms and spermatozoa.

Micro-organisms

For microbiological specimens, slides should be prepared , air dried and stained using the 4-stage DIFF-3 procedure for Haematology.

Spermatazoa

Dilute ejaculate should be carefully spread on a clean glass slide using as little manipulation as possible to avoid damage to the specimen.

Air dry the smears and apply the 4 stage DIFF-3 procedure for Haematology. Mount and examine.

Spermatazoa stained dark blue-gray / violet.

Safety Data Sheet Section

DIFF-3 Fixative contains Methyl alcohol and a thiazin dye <0.05% which is Highly Flammable & Toxic by inhalation and ingestion. Do not consume. Avoid sources of ignition when using. Long term exposure may cause blindness. If ingested seek immediate medical attention on what treatment is appropriate. Wash with soap & water if in contact with skin. If in contact with eyes, bathe with eye-bath for 5 minutes. Seek medical attention if soreness persists. Wear suitable eye, face & body protection when using. Use in a suitable ventilated area.

DIFF-3 Solutions A and B and the buffered rinse reagent pose no significant risk in normal use. They contain organic dyestuffs in a harmless aqueous phosphate buffer. Skin staining may occur from the dyestuffs. Wash with soap & water if in contact with skin. Eye bath for 5 minutes if in contact with eyes. Seek medical attention if soreness or other problems remain after contact with eyes. Do not consume. Wear hand and face protection when using.

H225 HIGHLY FLAMMABLE liquid & vapour. H301+ H311+H332 Toxic if swallowed, by skin contact and inhalation. P210 Keep away from heat sources, sparks, hot surfaces – No Smoking. P260 Do not breathe fumes. P233 Keep container tightly closed. P262 Do not get in eyes, skin, clothing. P285 In case of inadequate ventilation, wear respiratory protection. P301 IF SWALLOWED – rinse mouth with wate, seek urgent medical attention. IF INHALED – move to clear air zone. IF ON SKIN or IN EYES – rinse with soap and water and apply eye bath.

For more complete information on Health & Safety, Storage, Firefighting, Transport , Spillage etc please read the MSDS for this product.

Storage

Keep all kit reagents in the product outer packaging and store in a darkened cupboard at room temperature. Replace bottle caps immediately after use to prevent air oxidation. Kit will remain usable during its shelf life in these conditions.

Accidental spillage & waste disposal

The only hazardous component is the DIFF-3 Fixative which is Flammable & Toxic. It is volatile in air so spillage vapours may present an explosion hazard in small spaces if mixed with air. Mop up spillage immediately with absorbent granules or paper towel and remove to outside area for proper disposal (can be evaporated to atmosphere). For fixative residue and all other reagents in this kit clean the spillage area with detergent & water and run the waste to sewer drains.
Unused reagents can be disposed of in the following manner:

DIFF-3 Fixative – evaporated to atmosphere or incinerated.
All other kit reagents – diluted to Public Sewer with water.

Unsatisfactory Performance

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 of shelf life we will replace that reagent free of charge.

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