GCC Sudan Black B Stain Kit SP960

USE
Cytochemical demonstration of Sudanophilia in Leukocytes.

PRINCIPLE OF TEST
Sudan Black B was used by Sheehan and Storey in 1947 to stain the granules of neutrophils, many of which appear to contain phospholipids. The pattern of this Sudanophilia usually parallels myeloperoxidase. Myeloid and monocytoid cells stain positive in this technique, thus it finds use in the differential diagnosis and classification of the acute leukaemias.

REAGENTS
1 – Sudan Black B stain reagent 10x20ml
2 – Buffered Phenol reagent 10x20ml
3 – Formaldehyde fixative 2 x 30ml
4 – Haematoxylin counterstain 2x50ml

Reagent components in this kit are for 'In-Vitro Diagnostic use only'. Wear full body protection when using these reagents. Use them in a well ventilated place or ventilated hood. Standard precautions in the handling of laboratory reagents should be followed.
Refer to Material Safety data Section before use.

STORAGE AND STABILITY
Store the reagents in a safe place in the kit box provided and keep the kit at cool – room temperature in a dark place.
After preparation store Glutaraldehyde-Acetone fixative in refrigerator 2-6 Deg C.

ADDITIONAL REAGENTS REQUIRED
Acetone
Ethanol (IMS) 70%

SPECIMEN COLLECTION AND STORAGE
Use freshly prepared whole or anticoagulated blood or bone marrow films. Fix as soon as possible.

REAGENT PREPARATION AND SET-UP

Prepare smears
Prepare the following solutions.

1 – To a 100ml beaker add 20ml (1 bottle) Sudan Black B reagent and 20 ml (1 bottle) Buffered Phenol reagent. Mix well, stand for 1-2 minutes and filter through Whatman No.1 with suction and transfer filtered reagent to a Coplin jar.

2 - Prepare fixative by adding 3 – 5ml of Formaldehyde fixative to a Coplin jar. Fit the lid tightly and place in a fume cupboard. Allow a small amount of time for the Formaldehyde vapour to fill the Coplin jar.

3 - Beaker (200ml) De-ionised water (2 changes required)

4 - Beaker (200ml) 70% Ethanol (IMS)

5 - Coplin jar (40ml) Haematoxylin Gill No 3. Return to bottle after use. Continue using reagent from same bottle until acceptable staining exceeds 6-8 minutes. Discard and commence use of fresh bottle. Kit is supplied with 2 x 50mls.

TECHNIQUE

1 - Place air dried smears into the Formaldehyde vapour Coplin jar – attach the lid securely and fix smears for 10 minutes at room temperature in the Formaldehyde vapour.

2 - Rinse well in de-ionised water (2 changes).

3 - Stain in filtered Sudan Black B/buffered Phenol reagent in Coplin jar for 5-6 minutes.

4 - Rinse in 70% Ethanol (IMS) until dye stops running from slide.

5 - Rinse well in de-ionised water.

6 – Counterstain in Haematoxylin counterstain for 5-6 minutes.

7 - Rinse well in tap water, air dry, examine or mount in DPX or similar and examine under oil immersion.

QUALITY CONTROL
Smears from known healthy donors should be processed along with patient samples as a control and method performance measure.

EXPECTED RESULTS

Neutrophils and precursors	Blue-black intracellular granulation.
Monocytes	Less intense blue-black granulation.
Lymphocytes	No reaction usually. [Although documented reports of rare instances of sudanophilic acute lymphoblastic leukaemia are known].

French-American-British Classification.

FAB Classification	Sudan Black B Reaction
M1	Positive (Auer rods or azurophilic granules present)
M2	Positive
M3	Positive
M4	Positive (often mixed population of cells observed)
M5	Sometimes positive
M6	Positive myelocytic cells
M7	Positive myelocytic cells

Results with Sudan Black B (positive or negative) should be confirmed by other cytochemical tests, esterases, PAS, NAP etc. Only when a complete cytochemical profile is available and used in conjunction with clinical history can a presumptive diagnosis be made.

MATERIAL SAFETY DATA SECTION

H301+H311+332 TOXIC IF swallowed, by skin contact. H225 HIGHLY FLAMMABLE liquid + vapour. P210 Keep away from sources of heat. P262 DO NOT get in eyes, on skin, or on clothing. P281 Use personal protective equipment as required. P304+P340 IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing. P302+P352 IF ON SKIN Wash with plenty of soap and water. P305+P351+P338 IF IN EYES Rinse cautiously with water for several minutes. P308+P313 IF exposed or concerned:Get medical advice/attention. P314 Get medical attention if you feel unwell. P403+P233 Store in a well-ventilated place. Keep container tightly closed. P501 Dispose of contents/ container to licensed waste disposal contractor.

1 - Sudan black B reagent contains the azo-dye Sudan Black in an alcoholic solvent system. Azo dyes are harmful or Toxic in larger amounts. Reagent may irritate eyes and stain skin.

2 - Buffered Phenol reagent – this contains Phenol <20% with harmless inorganic buffering salts in aqueous solution. Phenol can cause serious eye-damage and is Toxic by ingestion, inhalation and by skin absorption. Wear appropriate protection when in use. Work in fume hood. If in contact with eyes use immediate eye bath for several minutes and seek immediate medical advice on what treatment to administer. Show this sheet.

3 - Formaldehyde Fixative – this contains approx 38% formaldehyde. This reagent is TOXIC by ingestion, inhalation and skin absorption. It will irritate and cause burns especially to the eyes. Wear appropriate protection when in use. Work in fume hood. In case of accident follow above instructions. Show this sheet.

4 - Haematoxylin solution - Maybe harmfull if taken internally. Will discolour skin. Will irritate eyes, skin and internal organs. Contains aluminium salts and haematoxylin dye.

ACCIDENTAL SPILLAGE & WASTE DISPOSAL

Sudan Black B reagent – mop up spillage with cloth, rinsing cloth under tap water diluting to public sewer or mop up with absorbent paper or granules and evaporate to air or dispose of through waste disposal contractor.

Buffered Phenol Reagent –Consult local regulations . In the quantities supplied and used in the test, it is unlikely to pose a significant danger to the environment – therefore dispose of small test quantities to the public sewer diluting greatly with water .Larger spills should be taken up on absorbent granules and disposed of through a licenses waste disposal contractor. Local water authorities may be consulted about local regulations for the release of this substance to the environment (bearing in mind only 20ml is used in each test, < 4gm phenol). If local regulations prohibit its release then any spillage must be absorbed into paper or suitable absorbent and any spent incubation mixture kept in a suitable container for disposal by a licensed waste disposal contractor.

Formaldehyde Fixative – Consult local regulations . In the quantities supplied and used in the test, it is unlikely to pose a significant danger to the environment – therefore dispose of small test quantities to the public sewer diluting greatly with water. .Larger spills should be taken up on absorbent granules and disposed of through a licenses waste disposal contractor. If local regulations prohibit its release then any spillage must be absorbed into paper or suitable absorbent and any spent incubation mixture kept in a suitable container for disposal by a licensed waste disposal contractor.

Haematoxylin counterstain – Mop up with damp cloth., rinsing cloth with tapwater to the public sewer.

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 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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