1.7 Parasitological stains and reagents

Laboratory diagnosis of parasitic infections can be carried out by detection and identification of parasites or a stage in the life cycle of the parasite (trophozoites, cysts, egg or larva) in various specimens (feces, blood, bone marrow, urine and biopsy material from spleen, liver lymph nodes) by various application of stains and reagents. Some of the reagents and stains used are;

1.7.1 Lugol's iodine

Stool is emulsified in a drop of five time's diluted solution of Lugol's iodine in a glass slide.

To prepare Lugol's iodine, 10g of potassium iodide is dissolved in 100ml distilled water and 5g of iodine crystals are then added slowly. The solution is filtered and kept in a stoppered bottle of amber color.

Iodine wet mount is used for the study of nuclear character of cysts and trophozoites for the identification of the species.

1.7.2 Formal saline

Fecal samples cal be preserved and parasite in them can be sedimented in 10% formalin saline or formal water. (100ml formaldehyde in 900ml 0.85%sodium chloride or distilled water)

Formal water adequately preserves cysts, eggs and larvae.

One disadvantage of formalin -fixed stool specimens is the unsuitability for the preparation of permanent stained smears

1.7.3 Normal saline

This is a solution prepared by dissolving one gram equivalent weight of sodium chloride in a quantity of distilled water and making up the volume

To 1000ml mark. A normal saline will contain 58.5g Nacl /liter.

Saline wet preparation is made by mixing small quantity of feces (about 2mg) in a drop of saline placed on a glass slide.

1.7.4 Zinc sulphate solution

Prepared by mixing 33gms of zinc sulphate powder in a quantity of distilled water and toping up with same distilled water to 100ml mark. Specific gravity should be 1.180 – 1.200.

Used to carry out Zinc sulphate centrifugal floatation technique.

1.7.5 Brine

It is used to preserve vegetables, fish, and meat, in a process known as brining (now less popular than historically). Brine is also commonly used to age Halloumi and Feta cheeses. Brine is a common fluid used in large refrigeration installations for the transport of heat from place to place. It is used because the addition of salt to water lowers the freezing temperature of the solution and the heat transport efficiency can be greatly enhanced for the comparatively low cost of the material. At a concentration of 23.3%, the freezing point of Nacl brine is lowered to −21°C (252.15 K, −6°F), and that of $CaCl_2$ brine down to -40°C (233.15 K, -40°F).

At 15.5°C (288.65 K, 60°F) saturated sodium chloride brine is 26.4% salt by weight (100 degree SAL). At 0°C (273.15 K, 32°F) brine can only hold 26.3% salt. 0°F was set as the zero point in the Fahrenheit temperature scale, as it was the coldest temperature that Daniel Gabriel Fahrenheit could reliably reproduce — by freezing brine. Brine is also used for pickling foodstuffs, as a means of preserving them (or increasing for taste). Almost anything can be pickled - cucumbers (most common), eggs, peppers. Common pickling flavors include garlic, dill, mustard seed, etc.

1.7.6 Giemsa stain

0.75g of Giemsa powder is placed in a motor and 25ml of glycerol is added to it and grinded with pestle. To this add methanol 75ml and stirred. Then pour in a dark colored bottle and incubate at 37°c for 24hrs. Used for staining both thick and thin blood films.

1.7.7 Leishman

Prepared by dissolving 0.15g of Leishman dry powder in 100ml of absolute methyl alcohol in a bottle. Shake until powder dissolves and allowed to stand for 48hrs with frequent shaking in between.

1.7.8 Field stain

This is a quick method of staining of malaria parasites in thick films (without fixation). Requires two solutions.

Solution A

Methylene blue	0.8g
Azure I or B	0.5g
Disodium hydrogen phosphate	5.0g
Potassium hydrogen phosphate	6.25g
Distilled water	500ml

Solution B

Eosin	1.0g
Disodium hydrogen phosphate	5.0g
Potassium hydrogen phosphate	6.25g
Distilled water	500ml

- Phosphate salts are first dissolved in water, then stain is added. Solution of azure I is facilitated by grinding in a mortar with the phosphate solvent. The solutions are set aside for 24hrs and after filtration are ready for use.

1.7.9 Eosin

Direct mount with eosin is used to distinguish cyst of intestinal protozoa from background. Cysts are seen as distinct particles
against a stained pink background.

1.7.10 Malachite green

Malachite green is prepared by mixing 5g of the powder in a quantity of solvent until powder dissolves. Top-up with the solvent to a 100ml mark..It is used as a counter-stain in modified zielh – Neelsen method for staining oocysts.

1.7.11 Indian ink

Basic India ink is composed of a variety of fine soot known as lampblack, combined with water to form a liquid. A binding agent such as gelatin or, more commonly shellac, may also be added, to make the ink more durable once dried. India ink is occasionally sold not as a liquid, but in solid form (most commonly, a stick), which must be moistened before use.

In pathology laboratories, India ink is applied to surgically removed tissue specimens to maintain orientation and indicate tumor resection margins. The painted tissue is sprayed with acetic acid, which acts as a mordant, "fixing" the ink so it doesn't track. This ink is used because it survives tissue processing, during which tissue samples are bathed in alcohol and xylene and then embedded in paraffin wax. When viewed under the microscope, the ink at the tissue edge informs the pathologist of the surgical resection margin or other point of interest.

Microbiologists use India ink to stain a slide containing bacteria. The background is stained while the bacteria remain clear. This type of staining is called a negative stain. India ink, along with other stains, can be used to determine if a cell has a gelatinous capsule.