SP709 Parasite trichrome Staining (Wheatley) GCC Diagnostics

GCC Diagnostics Aug 2003.

Permanent Stains.

Preparation of smears – 3 methods.

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1 – Unpreserved specimens with Schaudinn fixative.

To prepare thin uniform smears, place a drop of saline on a glass slide. With an applicator stick transfer a small representative portion of the specimen to the drop of saline, and mix the two. Spread the solution into a film by rolling the applicator stick along the surface. Remove any lumps.
Before watery specimens are smeared , apply an adhesive such as serum or albumin to the slide. Liquid specimens may be centrifuged and the sediment used to prepare the smear.

Fixation – place fresh smears immediately into Schaudinn fixative. Do not allow the smears to dry at any time before they are stained. Smears should fix for at least 1 hour at room temperature or 5 minutes at 50 Deg C, however, they can be left in the fixative for several days if required. After fixation slides may be kept in 70% alcohol indefinitely before they are stained.

2 – Unpreserved specimens with PVA fixative.

On a slide thoroughly mix 1 drop of unfixed specimen with 1 drop of PVA fixative..

Spread the specimen over the centre third of the slide by rolling the specimen with an applicator stick. Remove any lumps. The film should extend to both the top and bottom edges of the slide, as this helps prevent peeling.

Allow the smear to dry thoroughly before it is stained.

3 – PVA fixative preserved specimens.

Preserve 1 part specimen in 3 parts PVA fixative.
Mix thoroughly. Fix for at least 1 hour. Specimens will keep indefinitely.

Add 1 drop of PVA fixed to a slide.
(a). If there is little sediment in the sample remove a portion of the sediment for sample with a pasteur pipette.
(b). If there is a lot of sediment, mix the specimen thoroughly and before settlement add 1 drop of mixed specimen to the slide with a pasteur pipette or applicator stick.

Spread the drop over the centre third of the slide by rolling the specimen with an applicator stick. Remove any lumps. The film should extend to both the top and bottom edges of the slide, as this helps prevent peeling.

Allow the slide to dry overnight at room temperature or in an urgent situation dry the slide at 30-35 Deg C for 4 hours and then stain.

Trichrome Staining Proceedure.

	Reagent	Staining Time PVA Fixative	Staining Time Schaudinn Fixative
1	70% alcoholic iodine	10 min	1 min
2	70% alcohol	5 min	1 min
3	70% alcohol (fresh)	5 min	1 min
4	Trichrome stain	6 – 8 min	2 – 8 min
5	90% acid alcohol	5 – 10 seconds	2 seconds
6	95% alcohol	Rinse 20 secs	Rinse 10 secs
7	95% alcohol (fresh)	3-4 mins	Rinse 10 secs
8	Carbol-xylene	5 min	1 min
9	Xylene	5-10 min	2-3 min

Mount with cover slip (DPX or similar) or dry slide and examine directly under oil.

Scan under low power to find optimal areas then examine in detail under high magnification.

Stain reactions:

In a good prep the cytoplasm of cysts and trophozoites is blue-green tinged with purple.
Entamoeba coli cysts cytoplasm is often more purple than that of other species.
Nuclear chromatin, chromatoid bodies, erythrocytes and bacteria stain red or purplish red.
Other ingested particles such as yeasts often stain green.
Parasite eggs and larvae usually stain red.
Inflammatory cells and tissue cells stain in a manner similar to that of protozoa.
However colour reaction may vary from the above.

Incompletely fixed cysts may stain red and organisms which have degenerated before fixation often stain pale green.
Poor fixation due to an inadequate mixing of the specimen in the fixative may result in both of these appearances.

It should be noted that in some specimens degeneration may have occurred before the specimen is placed in the fixative either in the patient before the specimen was evacuated or because of a delay in fixing the specimen.

Restaining if necessary.

If the staining is weak or unsatisfactory the slide can be restained as follows.
Place slide in xylene to remove cover slip.
Place in 50% alcohol/water to hydrate the specimen for 10 minutes
Destain in 10% Acetic acid for several hours until destained.
Wash through water then 50% alcohol .
Then restain for 8-10 minutes (or longer if required) in Trichrome and continue the rest of the rinsing procedure.