

STAINING PROCEDURES FOR STOOL SPECIMENS

1. Modified Acid-Fast Staining Procedure

This technique is useful for the identification of oocysts of the coccidian species (*Cryptosporidium*, *Cystoisospora*, and *Cyclospora*), which may be difficult to detect with routine stains such as trichrome. Unlike the Ziehl-Neelsen Modified Acid-Fast Stain, this stain does not require the heating of reagents for staining.

Specimen:

Concentrated sediment of fresh or formalin-preserved stool may be used. Other types of clinical specimens such as duodenal fluid, bile, pulmonary samples (induced sputum, bronchial wash, biopsies) may also be stained.

Reagents:

There are four steps to this procedure requiring the following solutions:

Absolute Methanol

Acid Alcohol: 10 ml Sulfuric Acid + 90 ml Absolute ethanol. Store at room temperature.

Kinyoun's Carbol fuchsin: may be purchased commercially.

3% Malachite green: dissolve 3 g of malachite green in 100 ml of distilled water. Store at room temperature

Procedure:

Prepare a smear with 1 to 2 drops of specimen on the slide and dry on a slide warmer at 60°C until dry. Do not make the smears too thick!

Fix with absolute methanol for 30 seconds.

Stain with Kinyoun's carbol fuchsin for one minute. Rinse briefly with distilled water and drain.

Destain with acid alcohol for 2 minutes. Rinse with distilled water and drain.

Counterstain with Malachite green for 2 minutes. Rinse briefly with distilled water and drain. Dry on a slide warmer at 60°C for about 5 minutes. Mount with a coverslip using desired mounting media.

Examine 200 to 300 fields using 40× or higher objectives. To confirm internal morphology, use 100× oil immersion objective.

Quality Control:

A control slide of *Cryptosporidium* spp. from a 10% formalin preserved specimen should be included with each staining run. *Cryptosporidium* spp. stains a pinkish-red color. The background should stain uniformly green.

2. Chromotrope Staining Procedure

This staining method was developed at CDC using various components of the trichrome staining method to differentiate microsporidia spores from background fecal elements.

Specimen:

Prepare a thin smear using approximately 10 µl of 10% formalin fixed stool suspension (unconcentrated) on a glass slide. Formalin concentrates may also be used but the number of organisms will be essentially the same as before concentration. Heat fix on a slide warmer at 60°C until completely dry (5-10 minutes).

Reagents:

There are six steps to this procedure requiring the following solutions:

A Primer In Neurological Staining Procedures

J Elliott



A Primer In Neurological Staining Procedures:

A Primer in Neurological Staining Procedures George Clark, Margaret P. Clark, 1971 *Neuroanatomical Research Techniques* Richard T. Robertson, 2013-10-22 *Neuroanatomical Research Techniques* discusses developments in major neuroanatomical research techniques The book is organized into four parts Part I deals generally with the preparation and study of brain tissue It includes a chapter on the microscope discussing optical magnification limitations of microscopy and optical contrasting methods Other chapters summarize basic techniques for tissue preparation and sectioning present guidelines for a number of standard but essential staining procedures and present sophisticated and contemporary computer techniques that are proving to be invaluable as neuroanatomy evolves from a qualitative to a quantitative discipline Part II deals with techniques often used for the study of normal tissue These include the Golgi method fluorescence histochemistry techniques for staining single neurons and the use of the electron microscope Part III presents techniques for studying intrinsic connections of the nervous system These include techniques for silver impregnation of degenerating fibers autoradiographic technique for studying axonal projections and somatopetal movement of horseradish peroxidase as a tool for studying connections and neuron morphology Part IV discusses the interpretation of results from neuroanatomical research techniques and presents examples of the applications of neuroanatomical methods to major problems in physiological psychology

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