Practice Number: 5200296

Instructions for Participants

05 May 2021
01-2021

These are the clinical scenarios and response form for the NHLS Parasitology Stool PT Scheme. Detailed instructions can be found in the new Instruction Booklet for all NHLS Proficiency Testing Schemes.

NHLS Proficiency Testing Scheme – Parasitology Stool

NHLS Stool Parasites PT Scheme 0121

PTS LAB NO: _____ **LABORATORY NAME:** _____

CHALLENGE:	ANSWER CODE/S:	CLINICAL HISTORY:	INSTRUCTIONS:
PS01/21 Stool/urine concentrate		Patient complaining of foul-smelling, greasy poop that can float.	Vortex/mix the specimen well. Make a few wet preparations and examine the slide for parasites using 10x and 40x objectives.
PS02/21 Stool/urine concentrate		Patient complaining of mild abdominal cramping, including loose stools	Vortex/mix the specimen well. Make a few wet preparations and examine the slide for parasites using 10x and 40x objectives.
PS03/21 Stool/urine concentrate		Patient complaining of severe abdominal pain and fatigue.	Vortex/mix the specimen well. Make a few wet preparations and examine the slide for parasites using 10x and 40x objectives.
PS04/21 Stool/urine concentrate		Patient complaining of abdominal pain and mild abdominal cramps	Vortex/mix the specimen well. Make a few wet preparations and examine the slide for parasites using 10x and 40x objectives.
PS05/21 Stool smear	Stain used:	Patient complaining of bloating, flatulence and loss of appetite	You are provided with a fixed stool smear; stain with an appropriate stain and examine the slide for parasites using the appropriate objective. Please **do not** return your slide.

IMPORTANT INFORMATION

- Please read the **instruction booklet** on internet for detailed instructions.
- Codes for completion of the response form can be found on internet
- The closing date for Survey 01 2021 is the **28 May 2021.**
- Submit results and queries to **parapts@nhls.ac.za** or 086 225 2460.

TEACHING SERIES:

SOP: MODIFIED ZIEHL-NEELSEN ACID-FAST HOT STAIN

(Q-pulse document #: NIC0169)

PURPOSE

This SOP details the method of performing a modified Ziehl-Neelsen acid-fast hot stain on a stool smear.

PRINCIPLE

The modified Ziehl-Neelsen acid-fast hot stain is suitable for the staining of *oocysts* of *Cryptosporidium* species*, Isospora belli* and *Cyclospora cayetanensis.*

BACKGROUND AND INTRODUCTION

Cryptosporidium species, *Cyclospora cayetanensis* and *Isospora belli* can cause severe diarrhoea in immunocompromised and immunocompetent patients. *Oocysts* in clinical specimens may be difficult to detect without special staining. Modified acid-fast stains are recommended and the application of heat to carbol-fuchsin helps the stain to penetrate better into the *oocysts*.

RESPONSIBILITY

The medical technologist or scientist is responsible for performing the staining technique, for microscopy and the results.

SAFETY PRECAUTIONS

Cryptosporidium species *oocysts* are immediately infective when passed in the stool and gloves should be worn during all phases of specimen processing. Oocysts of *Cyclospora* and *Isospora* are not immediately infectious. Sulphuric acid can cause severe burns, so should be handled with care. Methanol is highly flammable. Methylene blue is harmful if swallowed. Carbol fuchsin is toxic if in contact with skin and if swallowed. It causes burns and is an eye irritant. Care should be taken when heating the carbol-fuchsin.

FREQUENCY OF TESTING

The turnaround time is 24 hours from specimen receipt in the laboratory to result issuing. This test is not batched and is performed as soon as a specimen is received.

SPECIMEN TYPE, COLLECTION AND HANDLING

Fresh stool smears are made from the concentrated sediment of all stool specimens received for parasite investigations. Other specimens include duodenal fluid and bile, as well as respiratory specimens, such as sputum. Stool specimens should be sent in preservative, ideally 10% formalin (volume for volume).

EQUIPMENT AND MATERIALS

Microscope, Pasteur pipettes, Slides and coverslips, 100% methanol, Carbol-fuchsin stain, 5% sulphuric acid, Methylene blue and Tap water

PREPARATION AND STORAGE OF REAGENTS AND CONTROLS

Positive control specimens are made from concentrated sediments of stool specimens. Smears are prepared, allowed to dry then fixed in methanol for 30 seconds. Once dry the slides are packed into boxes and stored at room temperature.

PROCEDURE

1. Smear one to two drops of concentrated stool specimen onto the slide and allow it to air dry for 20 minutes or dry on a 70°C heating block for 5 minutes. Do not make the smears too thick (one should be able to read print through the wet material before it dries). Prepare two smears in case the first one is not usable. Stain a positive control slide with every batch of specimens.
2. Fix in methanol for 30 seconds if smears were not heat fixed.
3. Place the slide on a staining rack and flood it with carbol-fuchsin stain.
4. With an alcohol lamp or a Bunsen burner, gently heat the slide to steaming by passing the flame under the slide. Discontinue heating as soon as the stain begins to steam. Do not boil the stain on the slide.
5. Allow the slide to stain for five minutes. If the slide begins to dry, add more stain (without additional heating).
6. Rinse the slide thoroughly with tap water and drain it.
7. Decolourise the slide with 5% sulphuric acid for 30 seconds. The timing is critical. Ziehl-Neelsen decolourizer (acid alcohol) should not be used.
8. Rinse the slide with tap water and drain it.
9. Flood the slide with methylene blue for 30 seconds to 1 minute.
10. Rinse the slide with water; drain it, and allow it to air dry.
11. Examine the slide using the 10X objective first, then examine under the 50X oil objective. To see the internal morphology of oocysts and for measurement, use the 100X oil immersion objective.

INTERPRETATION OF TEST RESULTS

Cryptosporidium oocysts are round and range in size from 4-6µm. The oocysts stain pink, to red, to deep purple and contain four sporozoites, which may or may not be visible. *Cryptosporidium* oocysts will sometimes stain pale pink, whilst some oocysts will not stain at all and will appear clear.

Cyclospora cayetanensis oocysts are larger than *Cryptosporidium*, with a size range of 8-10µm. The oocysts stain acid-fast variable and may range from red-purple to clear. They often appear wrinkled with no internal structure. Not every oocyst stains.

Isospora belli oocysts are spindle-shaped, measuring 30µm x 12µm. When mature they each contain 2 sporocysts, which measure 11µm x 9µm; immature *oocysts* have a single sporoblast. The oocysts stain pink, to red, to deep purple. Some immature oocysts are entirely stained, whilst the sporocysts of mature ones take up the stain and have a clear area between the stained sporocysts and the oocyst wall.

REPEAT EXAMINATION DUE TO ANALYTICAL FAILURE

- Prepare and stain a new smear if the specimen has washed off.
- If the positive control or specimen stain poorly, restain the second smear.

LIMITATIONS OF METHOD AND MEASUREMENT UNCERTAINTY

- The number of *oocysts* in the stool varies from day to day, so multiple specimens should be examined. A series of three specimens submitted on alternate days is recommended.
- It is important to ensure that smears are not too thick because thicker smears may not adequately destain.
- A concentrated specimen is essential for demonstration of organisms.
- Some specimens require treatment with 10% KOH because of their mucoid consistency. Add 10 drops of 10% KOH to the sediment and vortex until it becomes homogeneous. Rinse with 10% formalin and centrifuge without decanting the supernatant, take one drop of the sediment and smear it thinly onto a slide.
- Do not boil the carbol-fuchsin stain. Gently heat it until steam rises from the slide and do not allow the stain to dry on the slide.
- Various concentrations of sulphuric acid (0.25% to 10%) may be used; however, destaining time varies according to the concentration used. Generally 1-5% solutions are used.
- There is some debate as to whether organisms lose their ability to take up the acid-fast stain after long-term storage in 10% formalin.
- PVA-preserved specimens are not suitable for this stain.
- Other organisms, such as bacteria and *Nocardia* species, may stain acid-fast, so attention should be paid to the size and features of *Cryptosporidium* species, *Cyclospora cayetanensis* and *Isospora belli* oocysts*.*

INTERNAL QUALITY CONTROL AND EXTERNAL QUALITY ASSESSMENT PROCEDURES

A positive control slide of either *Cryptosporidium* species, *Cyclospora cayetanensis* or *Isospora belli* should be stained with each batch and when new reagents are opened. The oocysts should stain pink-red, but variably acid-fast and the background a uniform light blue.

Check the smear macroscopically for adherence to the slide.

The microscope should have been serviced and calibrated within the last 12 months.

REFERENCES

NCCLS. (1997). Procedures for the recovery and identification of parasites from the intestinal tract; Approved guideline. NCCLS document M28-A, vol 17 no 23, pp 28-29. NCCLS, Pennsylvania USA.