Performing parasitological tests

NTQF Level III

Learning Guide #46

Unit of competence: performing parasitological tests

Module Title: performing parasitological tests

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LO4. Perform parasitological tests

Instruction Sheet	Learning Guide #4

This learning guide is developed to provide you the necessary information regarding the following content coverage and topics –

Process parasitological tests

4. Diagnostic Techniques in Medical Parasitology
    - 4.1. Authorizing the requested test
    - 4.2. Performing quality control procedures
    - 4.3. Recording of individual results
    - 4.4. Result verification before release
    - 4.5. Sample storage for further use

This guide will also assist you to attain the learning outcome stated in the cover page. Specifically, upon completion of this Learning Guide, you will be able to –

- select authorized tests that are indicated for the requested investigations
- perform quality control *procedures*
- conduct individual tests according to documented methodologies
- record all results by noting any phenomena that may be relevant to the interpretation of results
- verify results before releasing for clinician/client
- discuss with colleague when result interpretation is outside parameters of authorized approval
- store unused sample or sample components for possible future reference, under conditions suitable to maintain viability
- store tested sample or sample components according to organizational sample retention policy for retesting when requested

Learning Instructions:

1. Read the specific objectives of this Learning Guide.
2. Follow the instructions described in number 3 to 19.
3. Read the information written in the “Information Sheets 1”. Try to understand what are being discussed. Ask your trainer for assistance if you have hard time understanding them.
4. Accomplish the “Self-check 1” in page 6.
5. Ask from your trainer the key to correction (key answers) or you can request your trainer to correct your work. (You are to get the key answer only after you finished answering the Self-check 1).
6. If you earned a satisfactory evaluation proceed to “Information Sheet 2”. However, if your rating is unsatisfactory, see your trainer for further instructions..
7. Submit your accomplished Self-check. This will form part of your training portfolio.
8. Read the information written in the “Information Sheet 2”. Try to understand what are being discussed. Ask your trainer for assistance if you have hard time understanding them.

9. Accomplish the “Self-check 2” in page 9.
10. Ask from your trainer the key to correction (key answers) or you can request your trainer to correct your work. (You are to get the key answer only after you finished answering the Self-check 2).
11. Read the information written in the “Information Sheets 3 and 4”. Try to understand what are being discussed. Ask your trainer for assistance if you have hard time understanding them.
12. Accomplish the “Self-check 3” in page 10.
13. Ask from your trainer the key to correction (key answers) or you can request your trainer to correct your work. (You are to get the key answer only after you finished answering the Self-check 3).
14. If you earned a satisfactory evaluation accomplish the “Self-check 4” in page 12
15. Ask from your trainer the key to correction (key answers) or you can request your trainer to correct your work. (You are to get the key answer only after you finished answering the Self-check 4).

Information Sheet-1	Diagnostic Techniques in Medical Parasitology

4. Diagnostic Techniques in Medical Parasitology

4.1. Authorizing the requested test

Introduction

The reasons for performing laboratory tests and follow-up investigations must be clear. The tests performed in laboratories must reflect the common and emergency health needs of the area and provide information that can be easily interpreted. The tests must also be efficient, i.e. provide sufficient benefit to justify their cost and any risks involved in their performance. Medical officers should encourage qualified experienced laboratory staff to provide maximum information from laboratory tests and to proceed to further testing when this is obviously indicated and will lead to better and earlier treatment for a patient.

In deciding which tests and test methods are appropriate it is important to consider:

- the clinical and public health needs of the laboratory,
- wellbeing of patients,
- laboratory technical aspects,
- costs involved

Self-check 1	Written test

Write True if the statement is correct and False if it is incorrect

1. Medical Parasitology deals with parasites that cause disease. (2 points)
2. A parasite is an organism that can survive without help of other organism.(2 points)
3. A vector can transmit an infective stage of a parasite.(2 points)

Note: satisfactory rating is 4 points, unsatisfactory 2 points. You can ask your instructor for copy of correct answer.

Score = _____

Rating: _____

Answers:

1. _____
2. _____
3. _____

4.2. Performing quality control procedures

- **Stool Sample Collection**

- Provide the patient with a suitable wide-mouthed, container with a lid.
- Ask the patient to collect a walnut size piece or about 10ml of a watery specimen.
- It is not necessary to fill the whole container.
- Ask the patient to keep the outside of the container clean, health hazard!

- This is especially true of protozoa

- Amebic trophozoites will begin to degenerate 1- 2 hours after passage and alterations in appearance may result in erroneous identification

- Flagellate trophozoites may also undergo changes that would make differentiation difficult

- Cysts will deteriorate if fecal specimens are left standing for many hours or overnight, especially if the temperature is high

- Helminth eggs and larvae are less affected by the age of the specimen than are protozoa

- Nevertheless, changes may occur that would affect identification

- Hookworm eggs, for example, may become embryonated and larvae amy hatch from the eggs

- Even Ascaris eggs may develop to multicellular stages

- In addition, larvae may degenerate in old stools making it impossible to identify the species

- To ensure that good specimens are provided for examination, pay attention to the following points:

- Use clean, dry containers for collecting feces

- Dirt will interfere with examinations and may introduce free – living organisms from the soil.

- Urine and water will destroy trophozoites, if present

- Have the specimen brought to the laboratory as soon as it is passed to prevent deterioration of protozoa and alterations in the morphology of protozoa and helminths.

- Note the patient's name and the date and time of passage on the specimen

• Some reagents will last indefinitely if kept properly stoppered and out of direct sunlight.

– Examples are formalin solutions, isotonic saline, fixatives, and alcohol solutions (unless evaporation occurs)

• The "life" of each solution is indicated in the direction for preparing it

- Label all reagents with the date of preparation. Keep records for each solution. Review these every week and discard outdated solutions

- Many of the solutions used in the method for trichrome stain need to be changed at regular intervals.

- No procedure used for examining fecal specimens is 100% effective – that is the procedures will not always recover all the species present and, if a particular species is present in only very low numbers, they may fail to demonstrate them when used on a single specimen.

- Because the techniques are not perfect, you should perform them as carefully as possible for optimum results. Also, be sure to use techniques that are appropriate for the material you are examining.

- **BLOOD PARASITES**

- **Sample collection time:**

- The number of certain parasites in the blood depends on the time of collection.

- **Malaria**

- ⇒ Highest number of parasites is found during fever attacks and before the start of treatment

- **Microfilaria**

- ⇒ W.bancrofti and Brugia malayi – take specimen at night between 10 p.m. – 2 a.m.
- Smear for Malaria Parasites
- Follow proper collection procedures.

- Glass slides must be clean and free from grease.

- Thick films and thin films must be prepared properly.

- While drying protect blood films from dust, flies and insects.

- Do not dry exposed to direct sun light

- When fixing the thin film, be careful not to let methanol touch the tick film.
- Performance of a blood film examination on a poor quality film achieves inaccurate results.
    - A poor quality film caused by:

- Poor spreading technique
- Poor leukocytes distribution
- Too small a working area
- Red cell morphology altered by the spreading process.

- Use of the wrong anticoagulant, resulting in altered blood cell size and/or morphology

- Patient sample mix up.
- Transcriptional error.
- Inadequate mixing of the sample with the anticoagulant, resulting in the formation of small fibrin strands and a decrease in the platelets count.
- Poor stain quality etc

- Smear for Microfilaria

- Filaria are seldom found in the early and in the late stage of the disease.
- The proper time of collection is important (10 p.m. to 2 a.m.)
- Un sheated non-pathogenic filaria can be found any time of the day.
- Patients with filaria in the blood show also eosinophilia in the blood.

Self-check 2	Written test

Write True if the statement is correct and False if it is incorrect

1. Providing a quality service to patients and those requesting tests is part of total laboratory quality management.
2. A quality test result of clinical laboratory should be from right patient with right procedure for testing
3. Quality assurance is a onetime action which can evaluate the performance of the laboratory.

Note: satisfactory rating is 4 points, unsatisfactory 2 points. You can ask your instructor for copy of correct answer.

Score = _____
Rating: _____

Answers:

1. _____
2. _____
3. _____

4.3. Recording of individual results

In clinical laboratories, records of test results can be kept by retaining carbon copies of reports, using work sheets, or recording test results in registers (exercise books). Whichever system is used it must be reliable and enable patients' results to be found quickly. Test records are also required when preparing work reports and estimating the workload of the laboratory. If carbon copies or work sheets are used these must be dated and filed systematically each day. If registers are used, backing cards which are headed and ruled can be placed behind pages to avoid having to rule and head each page separately. The cards must be heavily ruled with a marker pen so that the lines can be seen clearly. Separate registers, each with its own cards, can be prepared to record the results of parasitological tests.

Write True if the statement is correct and False if it is incorrect

1. A laboratory record book can be used as a carbon copy for the test results reported.
2. The laboratory record should be with components that specify the patient demographic informations, type of the test and test results.

Note: satisfactory rating is 4 points, unsatisfactory 2 points. You can ask your instructor for copy of correct answer.

Score = _____

Rating: _____

Answers:

1. _____
2. _____
3. _____

4.4. Result verification before release

Laboratory staff should provide as much relevant information as possible to assist those requesting tests to interpret the results of tests correctly and use the information in the best possible way to benefit patients and the community. Reports should be clearly and neatly written (particularly figures). A patient's notes must contain the signed reports issued by the laboratory. In the use and interpretation of laboratory test results it is important to understand the limitations of tests, e.g. the ability of tests to indicate when disease is present or absent or whether the value in a report is normal or abnormal for a patient.

Self-check 4	Written test

Write True if the statement is correct and False if it is incorrect

1. The test results should be properly verified before the release of the test. (2 points)
2. Results should be clearly written and verified by the lab supervisor.(points)
3. Always Lab tests should provide reliable test results with clinical examinations. (2 points)

Note: satisfactory rating is 4 points, unsatisfactory 2 points. You can ask your instructor for copy of correct answer.

Score = _____

Rating: _____

Answers:

1. _____
2. _____
3. _____

Preservative	Advantages	Disadvantages
10% Formalin	**All-purpose fixative** **Easy to prepare** **Long shelf life** **Good preservation of morphology of helminth eggs, larvae, protozoan cysts, and coccidia** **Suitable for concentration procedures and UV fluorescence microscopy** **Suitable for acid-fast, safranin, and chromotrope stains** **Compatible with immunoassay kits and UV fluorescence microscopy**	**Not suitable for some permanent smears stained with trichrome** **Inadequate preservation of morphology of protozoan trophozoites** **Can interfere with PCR, especially after extended fixation time**
MIF merthiolate-iodine-formaldehyde)	**Components both fix and stain organisms** **Easy to prepare** **Long shelf life** **Useful for field surveys** **Suitable for concentration procedures**	**Not suitable for some permanent smears stained with trichrome** **Inadequate preservation of morphology of protozoan trophozoites** **Iodine interferes with other stains and fluorescence** **Iodine may cause distortion of protozoa**
LV-PVA (low viscosity polyvinyl-alcohol)	**Good preservation of morphology of protozoan trophozoites and cysts** **Easy preparation of permanent smears stained with such as trichrome (solution both preserves organisms and makes them adhere to slides)** **Preserved samples remain stable for several months**	**Inadequate preservation of morphology of helminth eggs and larvae, coccidia, and microsporidia** **Contains mercuric chloride** **Difficult and expensive to dispose of** **Difficult to prepare in the laboratory** **Not suitable for concentration procedures** **Cannot be used with immunoassay kits** **Not suitable for acid-fast, safranin and chromotrope stains**

4.5. Sample storage for further use

SAF (sodium acetate-acetic acid-formalin)	**Suitable for both concentration procedures and preparation of permanent stained smears** **Easy to prepare** **Long shelf life** **Suitable for acid-fast, safranin, and chromotrope stains** **Compatible with immunoassay kits**	**Requires additive (e.g., albumin-glycerin) for adhesion of specimens to slides** **Permanent stains not as good as with PVA or Schaudinn's fixative**
Schaudinn's Fixative	**Good preservation of morphology of protozoan trophozoites and cysts** **Easy preparation of permanent stained smears**	**Less suitable for concentration procedures** **Contains mercuric chloride** **Inadequate preservation of morphology of helminth eggs and larvae, coccidia, and microsporidia** **Poor adhesion of liquid or mucoid specimens to slides**
Modified PVA copper or zinc	**Permanent smears can be made and stained with trichrome** **Zinc is preferred over copper** **No mercuric chloride**	**Staining not consistent** **Organism morphology may be poor** **Copper-morphology of cysts and trophozoites is poor** **Zinc-better morphology but not comparable to LV-PVA**
One-Vial Fixatives (such as Ecofix, Parasafe, Unifix, Proto-fix, STF, and others that may be available)	**Concentrate and permanent smear can be made out of one vial** **Immunoassays can be done on most** **No mercuric chloride**	**Certain one-vial fixatives must use certain stains** **Color difference of stain** **Staining not always consistent** **Sometimes more expensive than formalin and LV-PVA**

Preservation of specimens is necessary when stool specimens cannot be examined within the prescribed time interval. Various preservatives are available (see table), with the two most commonly used being 10% aqueous formalin and PVA (polyvinyl-alcohol). Because 10% formalin and PVA have complementary advantages (see table 4.1. below), it is recommended that the specimen be divided and preserved in both types of preservatives (add one volume of stool to three volumes of the preservative). Preserved specimens can be stored for several months.

Self-check 5	Written test

Write True if the statement is correct and False if it is incorrect

1. Stool specimen should be preserved always.(2 points)
2. Formol ether is all purpose preservative but not suitable for PCR technique. .(2 points)
3. SAF (sodium acetate-acetic acid-formalin) can be used both for direct and concentration techniques. (2 points)
4. Merthiolate-iodine-formaldehyde is Suitable for acid-fast, safranin, and chromotrope stains. (2 points).
5. Preserved specimens can be stored for several months and can be used for teaching purpose. (2 points).

Note: satisfactory rating is 4 points, unsatisfactory 2 points. You can ask your instructor for copy of correct answer.

Score = _____

Rating: _____

Answers:

1. _____
2. _____
3. _____
4. _____
5. _____

List of Reference Materials

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- Jeffrey H.C and Leach R.M. 1975. Atlas of Medical Helmintology and Protozoology
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- Strickland GT.Hunter's tropical medicine. 7th ed. Philadeliphia: WB Saunders, 1991.
- Sullivan J.T, 1997. A color Atlas of Parasitology. Taticheeff , Seyoum, Yahya A,

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