

LIVERPOOL SCHOOL OF TROPICAL MEDICINE



CLINICAL DIAGNOSTIC PARASITOLOGY LABORATORY



Accredited Medical Laboratory
Reference No: 4001

LABORATORY USER HANDBOOK 2016

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CONTENTS		PAGE
1	INTRODUCTION	4
	1.1 Introduction to the laboratory	4
	1.2 Quality policy	4
	1.3 Using this handbook	5
2	LABORATORY INFORMATION	5
	2.1 Opening times	5
	2.2 Turnaround times	6
	2.3 Contact details	6
3	ADVICE FOR USERS	6
	3.1 Specimen containers	6
	3.2 Request forms	7
	3.3 Patient identifiers	7
	3.4 Packaging and Transport	7
	3.5 Urgent requests / High risk specimens	8
	3.6 Specimen retention times	8
	3.7 Protection of personal information policy	9
	3.8 Complaints procedure	9
4	TESTS OFFERED AND KEY FACTORS AFFECTING TESTS	9
	4.1 Blood Parasite diagnosis	9
	4.1.1 Malaria	9
	4.1.2 African Trypanosomes	10
	4.1.3 Filariasis	10
	4.2 Faecal Parasite diagnosis	11
	4.2.1 Faecal microscopy (including hot stool)	11
	4.2.2 Culture for Strongyloides / Hookworm	11
	4.2.3 Faecal staining methods	11
	4.2.4 Enterobius microscopy	12
	4.3 Urine techniques	12
	4.4 Leishmania diagnosis	13
	4.4.1 Microscopy	13
	4.4.2 PCR	13

4.5	Faecal Antigen detection	13
4.5.1	<i>Entamoeba histolytica</i>	13
4.5.2	<i>Giardia lamblia</i>	14
4.6	Antibody detection	14
4.7	Other samples	15
4.7.1	Semen	15
4.7.2	Sputum	15
4.7.3	Histology	15
4.7.4	Insects	16
4.7.5	Whole worms / proglottids	16
4.7.6	Amoebiasis	16
4.7.7	Cyst fluid for Hydatid disease (<i>Echinococcus</i>)	16
5	CHARGES	17
6	RESULTS AND REPORTS	17
6.1	Written reports	17
6.2	Telephoned / faxed reports	17
6.3	Interpretation and advice (including out of hours)	17

1 INTRODUCTION

1.1 Introduction to the laboratory

The Clinical Diagnostic Parasitology laboratory is a CPA accredited laboratory located in the Department of Clinical Sciences at The Liverpool School of Tropical Medicine. The laboratory offers a referral service for the identification of a wide range of human parasites from clinical specimens. The laboratory participates in the external NEQAS quality assurance schemes for blood parasitology, faecal parasitology, malaria rapid diagnostic tests (RDT's) and parasite serology.

The laboratory accepts samples from NHS and PHE laboratories, private hospitals, and private laboratories.

The laboratory cannot accept samples directly from patients; samples must be referred to the laboratory by a health professional.

1.2 Quality policy

Our Quality Policy presented below aims to offer a first class nationwide service for the laboratory diagnosis of parasitic infections.

The Clinical Diagnostic Parasitology Laboratory is committed to providing a high quality diagnostic service responsive to the needs and requirements of its users and will:

- Provide a high quality diagnostic parasitology service encompassing; blood and faecal parasitology, parasite serology and other diagnostic parasitology tests according to individual patient needs as appropriate.
- Operate a quality management system to integrate the procedures, processes and resources of the services provided.
- Set quality objectives and plans in order to implement this quality policy.
- Ensure that all personnel are familiar with the quality manual and all policies and procedures relevant to ensure user satisfaction.
- Commit to the health, safety and welfare of all staff.
- Treat visitors to the unit with respect and provide for their safety.
- Maintain good professional practice and conduct including compliance with relevant environmental legislation.

The Clinical Diagnostic Parasitology Laboratory will comply with standard ISO15189:2012 and is committed to:

- Staff training and development to provide an appropriate and effective service to users.

Page 5 of 17

- Procurement and maintenance of equipment and resources necessary for provision of the service.
- Appropriate handling of specimens to ensure correct performance of laboratory examinations.
- Examination procedures that will ensure the high quality of all tests offered.
- Reporting results in a timely, confidential, accurate and clinically useful manner.
- Regular assessment of user satisfaction, in addition to internal audit and external quality assessment, in order to produce continual quality improvement.

Signed on behalf of the Clinical Diagnostic Parasitology Laboratory, LSTM,



1.7.15

**Prof. David Lalloo MB,BS,MD,FRCP,FFTM,RCPS
Clinical Director**



1.7.15

**Mrs Jayne Jones BSC,MSc
Laboratory Manager**

1.3 Using this handbook

This handbook aims to provide help and advice for users of our service. Information contained in this handbook should help the user understand the requirements needed for the diagnostic parasitology services offered by the laboratory. Also included is information regarding interpretation of results. This handbook should assist with pre-examination and post-examination information needed by the user. If users require any additional information that is not provided in this handbook then they should contact the laboratory for advice.

2 LABORATORY INFORMATION

2.1 Opening times

Please note that the Laboratory is open between the hours of 8am to 5pm Monday to Friday. Specimens received after 4pm may not be processed until the following day.

The laboratory is closed at weekends and on Bank Holidays.

The laboratory does not provide an on-call / out of hours service.

The laboratory is a department of LSTM which is closed over the Christmas and New Year period therefore a limited service is offered. Christmas closures are sent to users informing dates of closure.

2.2 Turnaround times

TEST REQUEST	MAXIMUM TURNAROUND TIME
Malaria / other blood parasite microscopy	Same day
Blood filtrations	Same day
Faecal parasite microscopy	2 working days
Faecal parasite staining	3 working days
Faecal parasite antigen tests	7 working days
Strongyloides stool culture	7 working days
Urine microscopy / filtration	2 working days
Parasite antibody serology tests	7 working days
Leishmania PCR	7 working days (if speciation required up to 10 working days)
Leishmania microscopy	3 working days

Turnaround times for some additional tests are variable – please contact the laboratory for advice.

2.3 Contact details

Please see the information on page one of this handbook for all contact details.

3 ADVICE FOR USERS

3.1 Specimen containers

The majority of procedures used in this laboratory require standard specimen containers such as plastic stool pots and plastic serum tubes.

Leishmania PCR is the only test where use of buffer and Eppendorf tubes supplied by our laboratory are preferred. Information is available under the “Leishmania” section of this handbook (page 13).

3.2 Request forms

It is essential that a request form accompanies all samples. The current version of our request form can be found on the internet, address on page one of this handbook.

All available patient identifiers must be completed along with hospital reference numbers. Clinical details and travel history are helpful regarding diagnosis and interpretation and should be completed where possible. The requesting laboratory must include contact details on the form for return of results. Test requested and type of sample must be completed along with the priority status and high risk information.

If users send their request form it must contain all information required such as patient identifiers, sending location, clinical details, sample sent and test requested.

3.3 Patient identifiers

In order to comply with specimen acceptance policy, samples sent to the laboratory for diagnosis plus accompanying forms must be labelled with a minimum of 3 out of the 6 sample identification criteria:

1. Family name (or reference number)
2. First name(s)
3. Date of Birth
4. Referring laboratory reference number
5. Hospital number
6. Specimen date

Unfortunately specimens not stating at least 3 of these criteria will be rejected.

3.4 Packaging and Transport

Specimens must be packaged in compliance with UK postal regulations for Diagnostic Biological samples – UN3373

Specimens should be in an appropriate container surrounded by absorbent material and then placed in a leak proof sample bag. The request form should be placed in a separate pocket of that bag; all should be placed inside a plastic container which is then placed into an appropriate cardboard box. The cardboard box should be labelled with recipient's full address along with senders contact details. The box should be clearly marked as UN3373 – Biological substance category B, pathological specimen fragile with care.

Specimens not packed in this way may leak in transit and be unsuitable for testing.

Samples should be sent by first class mail, or courier to:

The Clinical Diagnostic Parasitology Laboratory
Liverpool School of Tropical Medicine
Pembroke Place
Liverpool
L3 5QA

OR

If in the Hays DX scheme:
Liverpool School of Tropical Medicine
Diagnostic Laboratory
DX6966301
Liverpool 92L

The sender is responsible for ensuring health and safety of any courier or taxi service that is used to transport specimens to the laboratory.

3.5 Urgent requests / High Risk specimens

Please ensure that the laboratory is informed if samples are High Risk and / or in need of urgent testing. The laboratory cannot process samples from patients with TB or VHF. The laboratory does not knowingly deal with specimens from patients with Category 3 infections such as viral haemorrhagic fevers.

Urgent testing can be performed if required. If this results in a test being performed in addition to its routine timescale, for example antibody testing, then an additional cost will be incurred. Requests must be marked urgent and ideally the laboratory telephoned in advance.

3.6 Specimen retention times

If there is need of additional testing of a sample already sent to the Diagnostic Parasitology laboratory please note the following sample retention times:

- EDTA blood - 1 day
- Faecal samples - 2 days

- Serum samples - 1 year
- Negative blood films – 3 months
- Positive blood films – 1 year

3.7 Protection of personal information policy

The Clinical diagnostic parasitology laboratory has a storage of information policy that ensures patient data and information, either electronically or as a hard copy, is kept confidential and secure at all times. All levels of staff adhere to this policy.

3.8 Complaints procedure

Any complaints received by the Clinical Diagnostic Parasitology Laboratory are dealt with as a matter of urgency. The laboratory has a written policy on dealing with complaints. Please contact the laboratory manager if a complaint arises.

4 TESTS OFFERED AND KEY FACTORS AFFECTING TESTS

If a sample is received for a test the laboratory does not offer the user will be contacted and if requested the sample forwarded to the relevant laboratory.

If the sample has been home-collected by the patient the date and time of specimen still needs to be recorded. Please see information for each individual test regarding sample age and storage procedures.

Specimens will be rejected if they do not meet any special requirements for the test requested.

4.1 Blood Parasite diagnosis

4.1.1 Malaria

Samples are screened using thick blood films, thin films are examined on positive samples. If *P.falciparum* is found, parasitaemia is performed. Rapid diagnostic tests (RDT's) are available.

Sample requirements:

- Thick and thin films (unstained but thin film methanol fixed) made from fresh blood sample.
- Thick and thin films made and stained from fresh blood sample.
- Original EDTA sample.

Key factors affecting tests: malaria parasites collected into anticoagulants such as EDTA deteriorate and morphological changes occur within a few hours.

We would be grateful if the malaria reference laboratory form could be completed, along with our report form, and enclosed with the sample. Sample date and patient travel history should be stated. Malaria reference laboratory form is available on the internet, address can be found on page one of this document.

4.1.2 African Trypanosomes

Thick blood films and concentration technique if films are negative. Blood samples for concentration should be examined on the day of collection.

Sample requirements:

- unstained thick/thin films
- EDTA blood sample

Key factors affecting tests: Trypanosomes may not survive for more than 1 day in EDTA tube therefore posted samples could show false negative results. **PHONE LAB FOR ADVICE BEFORE SENDING SPECIMEN.**

If a CSF sample is to be examined for trypanosomes it should ideally be examined within 20 minutes of L.P.

4.1.3 Filariasis

A wet preparation is examined and samples are filtered using a 3.0µm Nuclepore membrane. If *W.bancrofti* is suspected blood collection time should be between 22.00 and 02.00 hours, for *Loa loa* diurnal blood sample (noon) is preferred.

Sample requirements: EDTA blood. Sample size is not critical but ideally between 5ml and 10ml should be sent.

Key factors affecting tests: sheathed microfilariae may ex-sheath if blood sample is not examined within 2 to 3 days of collection, all microfilariae may last up to 72 hours in EDTA blood before disintegration.

4.2 Faecal Parasite diagnosis

4.2.1 Faecal microscopy (including hot stool)

A concentration technique is routinely performed on all faecal samples for the presence of cysts, ova and larvae. Direct saline smears are examined on samples that are 24 hours old or less where trophozoite stages of protozoan parasites are suspected.

Sample requirement: Minimum of ¼ specimen pot of faecal sample

Key factors affecting test: trophozoites may only survive for up to 24 hours in voided faecal samples

If *Entamoeba histolytica* trophozoites are suspected a “hot” stool must be examined.

Sample requirement: specimen must reach the laboratory within 30 minutes of sample being produced. If this is not possible thin faecal smears should be made, air dried, fixed in methanol for 1 minute and sent to laboratory for staining for *E.histolytica* trophozoites.

4.2.2 Culture for Strongyloides / Hookworm

Faeces are cultured for filariform larvae using the charcoal technique.

Sample requirements: At least 50g (half pot) of faeces is required for this test.

Key factors affecting tests: samples should NOT be stored in a refrigerator/cold room following collection as this may inhibit subsequent larval growth.

4.2.3 Faecal staining methods

Cryptosporidia

Slides from concentrated faecal deposit are stained using the Z-N technique for identification of oocysts.

Sample requirement: minimum of ¼ pot of faecal sample

Dientamoeba fragilis

Romanowsky stained direct smears are examined for the identification of *D.fragilis* trophozoites.

Sample requirements: please send a fresh (dated) unfixed sample of faeces (minimum of ¼ pot) plus a **thin** faecal smear -made without saline and fixed once dry for 1 minute in methanol/IMS

Key factors affecting test: ideally sample should not be more than 1 day old when sent for testing as trophozoites degenerate.

4.2.4 Enterobius microscopy

Enterobius vermicularis

The rectal swab method is used in preference to sellotape slides

Sample requirements: a cotton bud dampened with physiological saline is wiped around the peri-anal area, and placed in a small bottle of physiological saline. The bottle is sent to the laboratory for examination.

Key factors affecting test: ideally the swab should be taken in the morning before washing the peri-anal area.

4.3 Urine techniques

Schistosoma haematobium

Urine is tested by dipstick for the presence of blood /Hb, and RBC's. Urine is routinely filtered using 12.0µm polycarbonate filters.

Sample requirement: ideally total 10am – 2pm urine collection or terminal midday sample

Key factors affecting test. Random urine samples may give false negative results

4.4 Leishmania diagnosis

4.4.1 Microscopy

4.4.2 PCR

Sample requirements: See downloadable sampling notes on website, address can be found on page one of this document.

Cutaneous Leishmaniasis

- Unstained, fixed aspirate or biopsy impression smears for microscopy.
- Giemsa-stained histology slides.
- Biopsy material in PCR (ATL) buffer (available from laboratory on request), OR in dry, sterile container OR in 10% ethanol. If histological wax block of tissue is the only material available please cut 10µm thick wax sections and float onto microscope slides (3/slide, two or 3 slides/sample), send these unstained for DNA extraction. A biopsy should ideally be around the size of a grain of rice.

Visceral leishmaniasis

- THIN marrow smears (please fix for 1 minute in methanol before sending.)
- Marrow /blood in EDTA for PCR -minimum of 500µl

Key factors affecting test: Use of aspirate, wax block or samples smaller than the size of a grain of rice MAY cause false negative or insufficient DNA results. The use of Iodine during sampling will inhibit the PCR amplification and may cause false negative results. The use of Lithium heparin instead of EDTA will inhibit the PCR amplification and may cause false negative results.

4.5 Faecal Antigen detection

4.5.1 *Entamoeba histolytica*

E.histolytica adhesin ELISA

To differentiate between cysts of *E.histolytica/dispar* as seen by faecal microscopy.

Sample requirement: samples must not be bloody. The test must be performed on FRESH (< 48 hour old) NOT FIXED specimens **OR** unpreserved specimens should be kept at 2-8°C and tested within 48 hours of defaecation. **If the test cannot be performed within 48hours** store sample at -20°C or lower until tested. Do NOT send preserved specimens. Minimum of ¼ pot of faecal specimen.

Key factors affecting test: test not validated on samples older than specified

4.5.2 *Giardia lamblia*

Giardia lamblia (intestinalis) Quik Chek

If cysts cannot be identified by routine microscopy faecal antigen detection is a sensitive method of diagnosis.

Sample requirement: specimens should be fresh (< 72 hours old), **or** sample may be freshly-frozen at -20°C for up to 90 days if test cannot be performed within 72 hours. Minimum of ¼ pot of faecal specimen.

4.6 Antibody detection

Interpretations of all results are subject to clinical and travel information. A positive result may reflect previous or current infection. Different serology tests have differing cut off points therefore more information regarding tests and interpretation is available from the laboratory staff.

Sample requirement: 0.5ml serum OR 10ml of clotted blood (minimum 0.1ml serum or 5ml clotted sample)

Key factors affecting tests. Serum samples ideally should not be sent “on the clot”. Please use serum gel or similar tubes to avoid haemolysis of serum if delayed in transit as there is a possibility that the test result may be affected/invalid due to storage temperature.

Please ensure geographical location / travel history has been stated on the form if known, this is particularly important for African Trypanosomiasis testing.

Parasite antibody serology testing is available for:

- **Amoebiasis (Amoebic Liver abscess)**
- **Malaria**
- **Leishmaniasis**
- **African Trypanosomiasis** (CSF samples may also be tested for antibodies to African trypanosomiasis)
- **South American T.cruzi**
- **Schistosomiasis**
- **Strongyloidiasis**
- **Hydatid** (*Echinococcus granulosus*)
- **Filariasis**
- **Fasciola**

4.7 Other samples

4.7.1 Semen

Seminal fluid microscopy is performed for schistosome ova.

Specimen requirement: Age and volume of sample is not critical.

4.7.2 Sputum

Sputum microscopy is performed for parasitic ova.

Specimen requirement: Age and volume of sample is not critical. The laboratory does not process samples when a diagnosis of TB cannot be excluded.

4.7.3 Histology

Histology sections should be sent stained for parasite microscopy. Ideally H&E stained sections plus Giemsa stained section if querying Leishmaniasis or a PAS stained section if querying Amoebae.

4.7.4 Insects

Specimens query insects or ectoparasites must be sent in a clean dry container – not attached to sellotape.

4.7.5 Whole worms / proglottids

Identification of whole worms/proglottids

Sample requirements: Whole worms such as *Ascaris* may be stored in 10% formol saline. Suspected *Taenia* species proglottids are best stored in **physiological saline** and sent to the laboratory as soon as possible. The use of 10% formol saline should be avoided unless the sample cannot be sent to the reference laboratory within 3 days.

Key factors affecting tests: Identification may be difficult if formalin-fixed proglottids are sent for identification, however proglottids may disintegrate if in saline for 3 days or more.

4.7.6 Amoebiasis

Examination of cyst aspirate/pus samples for amoebae.

Specimen requirement: Requests for “amoebae” in samples of pus from liver abscesses should either be examined within one hour of collection or fresh THIN smears of pus should be made (minimum of 3), fixed when dry (1 minute in methanol) and sent for staining together with pus sample.

Key factors affecting test: About 30 minutes after aspiration of cyst pus/fluid *E.histolytica* trophozoites become indistinguishable from macrophages.

4.7.7 Cyst fluid for Hydatid disease (*Echinococcus* species)

Examination of cyst fluid for protoscolices and/ or hooklets of *Echinococcus* species.

Specimen requirement: Cyst fluid should ideally be examined within 2 days of sample being taken. No minimum sample requirement.

5 CHARGES

A charge is made for all laboratory services, for current prices please telephone laboratory staff on 0151 705 3220

6 RESULTS AND REPORTS

6.1 Written reports

Reports are processed and issued as soon as results are available and have been authorised. Copies can be issued if required please contact the laboratory if this is needed.

6.2 Telephoned / faxed reports

Positive malaria film results are telephoned as soon as a diagnosis is available. If the report is to be telephoned to a specific individual then please add their details to the request form.

Other results may be telephoned if the laboratory manager considers it appropriate.

Other requests for telephoned results are reported when a result is available. Please state on request form if telephoned results are required.

Once a result has been telephoned, a hard copy is also sent, and it will be indicated on the form that the result has already been telephoned.

Results may be faxed if requested. The user must have access to a secure fax, patient names are not included on faxed transmissions but all other identifiers remain.

6.3 Interpretation and advice

For technical advice regarding samples/tests and for interpretation of results please contact the diagnostic laboratory manager 0151 705 3220/3290

For clinical advice on treatment of parasitic diseases please contact one of the consultant physicians via on call mobile telephone 07909910899. This telephone is in operation Monday to Friday 9am to 5pm. Out of hours this number will transfer to Infectious diseases unit at the Royal Liverpool University Hospital. If any issues occur regarding transfer of call out of hours please telephone the RLUH switchboard and ask for Infectious diseases 0151 7062000.