LIVERPOOL SCHOOL OF TROPICAL MEDICINE

CLINICAL DIAGNOSTIC PARASITOLOGY LABORATORY

LABORATORY USER HANDBOOK 2026

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UKAS ACCREDITED FOR TESTS PUBLISHED ON SCHEDULE OF ACCREDITATION. REFERENCE 9362.
Please see UKAS website for further details on scope of practice. Details also listed within contents list and section 4 of this handbook.

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1 INTRODUCTION

1.1 Introduction to the laboratory

The Clinical Diagnostic Parasitology laboratory (CDPL) is a UKAS accredited laboratory working to ISO15189 standard, accredited for all tests placed on scope of practice. See section four of this handbook for details of tests accredited by UKAS. The CDPL is 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 laboratories, private hospitals, and private laboratories. For samples from GP's please see section 3.5.

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

1.2 Quality policy

The Quality Policy of the Clinical Diagnostic Parasitology Laboratory, LSTM

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 and is committed to:

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



SW

Date....22.9.26.....
Dr Stephen Woolley, Clinical Director, Consultant in Infectious Diseases, Medical Microbiology and Tropical Medicine and Medical Microbiology, Liverpool University Hospitals NHS Foundation Trust

Date.....22.9.26.....
Mrs Jayne Jones BSC, MSC, MIBMS, Unit 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.

Samples received out of hours are stored and processed on the next working day.

2.2 Turnaround times

TEST REQUEST	MAXIMUM TURNAROUND TIME
Malaria and other blood parasite microscopy	Same day (if samples received by 4pm)
Blood filtrations	Same day (if samples received by 4pm)
Faecal parasite microscopy	2 working days
Faecal parasite staining	3 working days
Faecal parasite PCR	7 working days
Strongyloides stool culture	7 working days
Urine microscopy / filtration	2 working days
Parasite antibody serology tests (excluding Fasciola)	7 working days
Fasciola serology	10 working days
Leishmania PCR	7 working days (if speciation required up to 10 working days)
Leishmania microscopy	3 working days
Skin / worm / other microscopy	4 working days

Turnaround times for some additional tests are variable – please contact the laboratory for advice. Turnaround times to result available are noted above. The unit is currently using a manual reporting system that can take longer to process.

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.

Stool samples requiring helminth culture should not be stored in a refrigerator, room temperature storage is required for these samples.

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, listed as "Laboratory services request form" address as follows **https://lstmed.ac.uk/clinical-services/cdpl/**

Royal Liverpool and Broadgreen NHS Trust users – requests can now be made via ICE, however, the lab is not yet able to access ICE, therefore your ICE form must still be printed and accompany the samples to the lab. You will be updated once the lab systems can communicate with ICE.

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 user 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.

3.3 Patient identifiers / Specimen acceptance

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 Advice for sending samples directly from GP surgeries

Please be aware that due to potential issues with packaging, posting and invoicing, we would recommend that samples to be sent from a GP practice would be best sent via your local hospital pathology department, marked “to be sent to the Liverpool School of Tropical Medicine for testing”. Please do not hesitate to contact the laboratory if more information on this is required.

3.6 High Risk specimens

Please ensure that the laboratory is informed if samples are High Risk and / or in need of urgent testing. The laboratory is a Category2 facility and therefore cannot process certain samples from patients with TB, VHF or other pathogens above Cat2. For example, sputum or samples for DNA amplification cannot be processed from patients with proven or suspected TB, samples will be retained until a negative TB result is gained, they can then be processed if still required. With regard to VHF, the laboratory does not knowingly deal with specimens from patients with Category 3 infections such as viral haemorrhagic fevers, the laboratory can be contacted for protocol to make safe these samples before they are sent to LSTM for examination. If a sample is marked high risk, please indicate the reason for this on the request form.

3.7 Urgent requests

Urgent testing may be performed if required. If this results in a test being performed outside of 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. If a request is marked urgent please state the clinical justification for this on the request form.

3.8 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 - 7 days
- Serum samples - 1 year
- Negative blood films – 3 months
- Positive blood films – 1 year

Other sample types may have differing retention periods.
If there is clinical need to request a longer retention period for a sample, please contact the laboratory on 01517053220 to discuss.

3.9 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. There is a local privacy notice for all users, available on the CDPL website.

3.10 Complaints procedure

Any complaints received by the Clinical Diagnostic Parasitology Laboratory are dealt with within an applicable timeframe. The laboratory has a written policy on dealing with complaints. Please contact the laboratory manager if a complaint arises.

3.11 Service agreement

The Clinical Diagnostic Parasitology Laboratory (CDPL) at the Liverpool School of Tropical Medicine considers every request received for a test carried out within our department as a service agreement between the requestor and the CDPL. Once a specimen is accepted for testing a service agreement is established and the specimen processed according to appropriate SOP's. The service agreement will be reviewed upon receipt of sample and during issue of results. The request, specimen received, suitability of test carried out, recommendations for further tests, interpretation of result and the final report all constitute part of that service agreement review. Tests offered in the CDPL have been validated and evaluated for use in the unit. The CDPL management are confident that the tests offered are appropriate to the service provided. Staff employed by the CDPL are in roles specific to their qualifications and HCPC registration and regular staff competency testing is carried out.

3.12 Add on tests

Please inform the lab ASAP if add on tests are required, a written request is needed for add on tests.

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 sample requirements for the test requested.

4.1 Amoebiasis (*Entamoeba histolytica*)

4.1.1 Faecal microscopy – UKAS accredited

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

Hot stool sample requirement: specimen must reach the laboratory within 30 minutes of sample being produced and must be marked as hot stool, this will then be examined with priority. Methanol fixed faecal smears can be made at requesting site if the sample cannot arrive at CDPL within 30 minutes, these smears can then be sent to CDPL for staining and examination.

A direct examination and concentration technique are routinely performed on all faecal samples for the presence of cysts, ova and larvae.

Sample requirement: Minimum of ¼ specimen pot of faecal sample. Ova, cysts and parasites may be passed intermittently therefore three samples may be required to be examined. Ova, cysts and parasites will diminish over time therefore it is more ideal for the sample to be less than 2 days old upon receipt at LSTM, however older stools will not be rejected.

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

4.1.2 Faecal PCR – not UKAS accredited

The parasites included in the PCR panel are; *Giardia intestinalis, Cryptosporidium* species*, Entamoeba histolytica, Entamoeba dispar, Strongyloides stercoralis, Necator americanus, Ancylostoma duodenale, Schistosoma* species*, Ascaris lumbricoides, Enterobius vermicularis and Trichuris trichiura.*

From our intensive evaluations we have concluded that one stool sample received and tested using stool concentration, faecal PCR and helminth culture (if required), will yield the same results as the recommended three stool sample request for stool concentration and helminth culture (if required). The introduction of the faecal PCR has therefore eliminated the need for requesting three stool samples from patients for parasitology testing and a move to a one stool sample request for those pathogens on the faecal PCR panel. This will be of benefit to the patient plus enhance the sensitivity and confidence in results from a one-off stool sample test for users of our service.

The faecal PCR is validated on faecal samples, however, we have had excellent results utilising cyst fluid samples for Amoebiasis.

Sample requirements: Minimum of ¼ specimen pot of faecal sample.

Key factors affecting test: Addition of formalin to the sample will hinder DNA extraction and qPCR amplification.

4.1.3 Cyst fluid / aspirate microscopy – UKAS accredited

Specimen requirement: Requests for *Entamoeba histolytica* in samples of pus from liver abscesses should either be examined within 30 minutes 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, *E.histolytica* trophozoites become indistinguishable from macrophages.

Entamoeba histolytica is detected on the faecal PCR panel, cyst fluids can be run on this PCR panel if required.

4.1.4 Amoebic serology – not UKAS accredited

Sample requirement: 0.8ml serum OR 10ml of clotted blood

ELISA used as initial screen, urgent or positive ELISA samples will have a secondary test performed. Up to 95% of Amoebic liver abscess patients are sero-positive by two weeks' post infection. Lower-level positive results may be obtained if tested earlier than two weeks' post infection. In these cases, a low-level positive result may be significant and a repeat test should be performed at 14 days' post infection. Titre will decrease in 1 to 2 months after successful treatment but may remain low level positive for an extended time.

Entamoeba histolytica antigen used. IgG antibodies detected.

	SENSITIVITY %	SPECIFICITY %
AMOEBIASIS ELISA	100	98
SECONDARY TEST (in house verification)	100	91.6

4.2 Babesia

4.2.1 blood film microscopy – UKAS accredited

Thick and thin blood films are examined for Babesiosis.

Sample requirements:

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

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

4.3 Cryptosporidia

4.3.1 ZN stained smear microscopy – UKAS accredited

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. Ova, cysts and parasites may be passed intermittently therefore three samples may be required to be examined.

4.3.2 Faecal PCR – not UKAS accredited

The parasites included in the PCR panel are; *Giardia intestinalis, Cryptosporidium* species*, Entamoeba histolytica, Entamoeba dispar, Strongyloides stercoralis, Necator americanus, Ancylostoma duodenale, Schistosoma* species*, Ascaris lumbricoides, Enterobius vermicularis and Trichuris trichiura.*

From our intensive evaluations we have concluded that one stool sample received and tested using stool concentration, faecal PCR and helminth culture (if required), will yield the same results as the recommended three stool sample request for stool concentration and helminth culture (if required). The introduction of the faecal PCR has therefore eliminated the need for requesting three stool samples from patients for parasitology testing and a move to a one

stool sample request for those pathogens on the faecal PCR panel. This will be of benefit to the patient plus enhance the sensitivity and confidence in results from a one-off stool sample test for users of our service.

Sample requirements: Minimum of ¼ specimen pot of faecal sample.

Key factors affecting test: Addition of formalin to the sample will hinder DNA extraction and qPCR amplification.

4.4 Dientamoeba fragilis

4.4.1 *Dientamoeba fragilis* microscopy – Not UKAS accredited

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

Sample requirements: In order to detect D.fragilis, a fresh stool sample less than 24 hours old is required. Please send a fresh unfixed sample of faeces (minimum of ¼ pot). In cases where examination is not possible with the first 24 hours, please also send **thin** faecal smears spread onto a glass microscope slide and fixed once dry for 1 minute in IMS.

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

4.5 Ectoparasites

4.5.1 Macro and Microscopic examination -Not UKAS accredited

Sample requirement: Samples for diagnosis of arthropods or ectoparasites must be sent in a clean dry container.

Key factors affecting test: Please do not attach sample to Sellotape, this hinders microscopy.

4.6 Enterobiasis (Enterobius vermicularis)

4.6.1 Faecal microscopy - UKAS accredited test

A direct examination and concentration technique are routinely performed on all faecal samples for the presence of cysts, ova and larvae.

Sample requirement: Minimum of ¼ specimen pot of faecal sample. Ova, cysts and parasites may be passed intermittently therefore three samples may be required to be examined. Ova, cysts and parasites will diminish over time therefore it is more ideal for the sample to be less than 2 days old upon receipt at LSTM, however older stools will not be rejected.

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

4.6.2 Faecal PCR – not UKAS accredited

The parasites included in the PCR panel are; *Giardia intestinalis, Cryptosporidium* species*, Entamoeba histolytica, Entamoeba dispar, Strongyloides stercoralis, Necator americanus, Ancylostoma duodenale, Schistosoma* species*, Ascaris lumbricoides, Enterobius vermicularis and Trichuris trichiura.*

From our intensive evaluations we have concluded that one stool sample received and tested using stool concentration, faecal PCR and helminth culture (if required), will yield the same results as the recommended three stool sample request for stool concentration and helminth culture (if required). The introduction of the faecal PCR has therefore eliminated the need for requesting three stool samples from patients for parasitology testing and a move to a one stool sample request for those pathogens on the faecal PCR panel. This will be of benefit to the patient plus enhance the sensitivity and confidence in results from a one-off stool sample test for users of our service.

The faecal PCR is validated on faecal samples, however, we have had excellent results utilising peri-anal swab samples and whole worms for Enterobius.

Sample requirements: Minimum of ¼ specimen pot of faecal sample.

Key factors affecting test: Addition of formalin to the sample will hinder DNA extraction and qPCR amplification.

4.6.3 Peri-anal swab microscopy – UKAS accredited

The rectal swab method is used in preference to sellotape slides, however both means of sample collection will be examined.

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. OR adhesive tape is applied to the area, attached sticky slide down to a microscope slide and sent to the laboratory for examination.

Key factors affecting test: Swab is preferred to Sellotape due to difficulty in examination of the Sellotape sample. Ideally the swab / sellotape sample should be taken in the morning before washing the peri-anal area.

Enterobius diagnosis is also available via our faecal PCR; this can be performed on swab samples if required.

4.7 Faecal parasites

Some faecal / intestinal parasites have their own section in this handbook. Those not mentioned specifically will be covered by this section.

Please note, in addition to the microscopy and PCR discussed below, there are also serology tests available for some of the faecal parasites, please see specific disease sections for further details.

4.7.1 Faecal microscopy - UKAS accredited test

A direct examination and concentration technique are routinely performed on all faecal samples for the presence of cysts, ova and larvae.

Sample requirement: Minimum of ¼ specimen pot of faecal sample. Ova, cysts and parasites may be passed intermittently therefore three samples may be required to be examined. Ova, cysts and parasites will diminish over time therefore it is more ideal for the sample to be less than 2 days old upon receipt at LSTM, however older stools will not be rejected.

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

4.7.2 Faecal PCR – Not UKAS accredited

The parasites included in the PCR panel are; *Giardia intestinalis, Cryptosporidium* species*, Entamoeba histolytica, Entamoeba dispar, Strongyloides stercoralis, Necator americanus, Ancylostoma duodenale, Schistosoma* species*, Ascaris lumbricoides, Enterobius vermicularis and Trichuris trichiura.*

From our intensive evaluations we have concluded that one stool sample received and tested using stool concentration, faecal PCR and helminth culture (if required), will yield the same results as the recommended three stool sample request for stool concentration and helminth culture (if required). The introduction of the faecal PCR has therefore eliminated the need for requesting three stool samples from patients for parasitology testing and a move to a one stool sample request for those pathogens on the faecal PCR panel. This will be of benefit to the patient plus enhance the sensitivity and confidence in results from a one-off stool sample

test for users of our service.

Sample requirements: Minimum of ¼ specimen pot of faecal sample.

Key factors affecting test: Addition of formalin to the sample will hinder DNA extraction and qPCR amplification.

4.8 Fasciola

4.8.1 Faecal microscopy - UKAS accredited test

A direct examination and concentration technique are routinely performed on all faecal samples for the presence of cysts, ova and larvae.

Sample requirement: Minimum of ¼ specimen pot of faecal sample. Ova, cysts and parasites may be passed intermittently therefore three samples may be required to be examined. Ova, cysts and parasites will diminish over time therefore it is more ideal for the sample to be less than 2 days old upon receipt at LSTM, however older stools will not be rejected.

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

4.8.2 Fasciola serology – UKAS accredited

Sample requirement: 0.8ml serum OR 10ml of clotted blood

Commercial ELISA used. This utilises *Fasciola* species antigen and detects IgG antibodies.

Antibody levels detected around 2 to 3 months' post exposure.

Antibody levels should revert to negative around 6 months' post successful treatment.

	SENSITIVITY %	SPECIFICITY %
ELISA (In house verification)	100	77

4.8.3 Fluke examination – Not UKAS accredited

If the adult fluke is extracted and confirmation is required, the adult can be examined

microscopically. Please send whole fluke in 70% Ethanol for examination.

4.9 Filariasis

4.9.1 blood microscopy – UKAS accredited

A definitive diagnosis of filariasis is usually made by the demonstration of microfilariae in the peripheral blood. The exception to this is the diagnosis of *Onchocerca volvulus* which is diagnosed by demonstration of microfilariae in skin snips.

Blood filariasis:

A wet preparation is examined, and samples are filtered using a Nuclepore membrane. Microfilariae exhibit a marked periodicity depending on the species involved, therefore the time of specimen collection is critical. If a filarial infection is suspected, the optimal collection time for demonstrating microfilariae is:

- Loa loa—midday (10am to 2pm)
- Brugia or Wuchereria—at night, after 10pm and before 2am
- Mansonella—any time
- Onchocerca—any time

Sample requirements: EDTA blood. Sample size is not critical but ideally between 5ml and 10ml should be sent, the larger the sample filtered the greater chance of demonstrating the microfilariae.

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.

4.9.2 Skin snips for *Onchocerca volvulus* – UKAS accredited

Please contact the laboratory before sending skin snips for microfilariae diagnosis, contact details on page one of this handbook.

4.9.3 Filariasis serology – UKAS accredited

Sample requirement: 0.8ml serum OR 10ml of clotted blood

Commercial ELISA used. This utilises *Acanthocheilonema viteae* antigen, that will cross react with human filariasis species and detects IgG antibodies. Sensitivity and specificity of the test is dependant on filaria species being queried.

Antibody levels detected around 2 to 3 months' post exposure.

Antibody levels negative from 1 year or longer post successful treatment.

	SENSITIVITY %	SPECIFICITY %
ELISA (in house verification)	100	90

Cross reactions may be seen with Strongyloides and Hookworm infections.

4.10 Hookworm

4.10.1 Faecal microscopy - UKAS accredited test

A direct examination and concentration technique are routinely performed on all faecal samples for the presence of cysts, ova and larvae.

Sample requirement: Minimum of ¼ specimen pot of faecal sample. Ova, cysts and parasites may be passed intermittently therefore three samples may be required to be examined. Ova, cysts and parasites will diminish over time therefore it is more ideal for the sample to be less than 2 days old upon receipt at LSTM, however older stools will not be rejected.

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

4.10.2 Faecal PCR – Not UKAS accredited

The parasites included in the PCR panel are; *Giardia intestinalis, Cryptosporidium* species*, Entamoeba histolytica, Entamoeba dispar, Strongyloides stercoralis, Necator americanus, Ancylostoma duodenale, Schistosoma* species*, Ascaris lumbricoides, Enterobius vermicularis and Trichuris trichiura.*

From our intensive evaluations we have concluded that one stool sample received and tested using stool concentration, faecal PCR and helminth culture (if required), will yield the same results as the recommended three stool sample request for stool concentration and helminth culture (if required). The introduction of the faecal PCR has therefore eliminated the need for requesting three stool samples from patients for parasitology testing and a move to a one stool sample request for those pathogens on the faecal PCR panel. This will be of benefit to the patient plus enhance the sensitivity and confidence in results from a one-off stool sample test for users of our service.

Sample requirements: Minimum of ¼ specimen pot of faecal sample.

Key factors affecting test: Addition of formalin to the sample will hinder DNA extraction and qPCR amplification.

4.10.3 Helminth culture – UKAS accredited

Faeces are cultured for filariform larvae using the charcoal technique.

Sample requirements: At least 50g (half pot) of faeces is required for this test. Ova, cysts and parasites may be passed intermittently therefore three samples may be required to be examined.

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

4.11 Hydatid (*Echinococcus*)

4.11.1 Hydatid serology – UKAS accredited

Sample requirement: 0.8ml serum OR 10ml of clotted blood

Samples are screened with commercial ELISA. IHA is performed on ELISA positive samples.

Amongst proven cases of hydatid disease, 92% show a positive ELISA test. Sensitivity depends on cyst site: Liver 96%, pulmonary 76%, skeletal 60%, other sites vary. Brain hydatid rarely shows positive serology. Serological cross-reactions, giving rise to false positives, can occur with other parasitic infections, particularly larval cestodes and filarial worms and with some neoplasms. Less than 3% of non-infected controls are positive.

False negatives may occur (about 8%) and are more common in patients with extra-hepatic cysts. False negatives can be due to calcified cysts. Patients with cysts occurring in the brain are usually serologically negative.

Antibody levels are detected at variable timescales post infection due to nature of disease.

Antibody levels may remain positive for life post successful treatment / surgery.

IHA is performed on all samples with positive hydatid ELISA serology

	SENSITVITY %	SPECIFICITY %
ELISA (in house verification)	100	97.6
IHA (manufacturers analysis)	93	94.9

The sensitivity of the ELISA is estimated to be 97%, the cases with negative serology but subsequently proven hydatid disease have mainly been those patients with extra-hepatic lesions.

Samples found equivocal by ELISA are re-tested by IHA. We consider results significant if both tests are positive. If sample shows equivocal results, we suggest repeating serology in 1 months' time.

ELISA utilises *Echinococcus* species antigen and detects IgG antibodies.

IHA uses *Echinococcus granulosis* antigen.

4.11.2 Cyst fluid microscopy – UKAS accredited

Aspiration of cyst should only be considered after taking expert advice.

Specimen requirement: Cyst fluid should ideally be examined within 2 days of sample being taken. No minimum sample requirement. If viability testing is required sample must be examined within an hour or being extracted from the cyst – contact the laboratory for further information.

We are currently evaluating a PCR for Echinococcus speciation on cyst fluids, we anticipate this will be available for use in 2027.

4.12 Leishmania

There is a downloadable sampling guide on our website if required.

4.12.1 Leishmania microscopy – UKAS accredited

Microscopy can be performed on varying sample types for Leishmania depending on the nature of the disease.

Cutaneous Leishmaniasis sample types as follows – skin biopsy impression smears, biopsy histology sections.

Visceral Leishmaniasis sample types as follows – EDTA blood smear, EDTA bone marrow smear, splenic biopsy dabs, splenic biopsy histology sections, bone marrow trephine histology sections.

Sample requirements:

Cutaneous – Fresh skin biopsy pressed against clean microscope slide and allowed to dry, fixed in methanol and sent for staining. Stained histology sections for examination, preferred stains for Leishmania are Giemsa and H&E.

Visceral – Thin EDTA smear (blood or bone marrow) fixed in methanol. Splenic biopsy pressed against a clean microscope slide and allowed to dry, fixed in methanol and sent for staining. Stained histology sections for examination, preferred stains for Leishmania are Giemsa and H&E.

4.12.2 PCR – not UKAS accredited

Initially a pan screening PCR is used to detect if Leishmania DNA is present or not.

If pan screening PCR is positive, a speciation PCR is performed.

We have two speciation PCRs available, one old world and one new world. It is essential that we are informed of travel history to enable the correct PCR to be performed.

Sample requirements:

Cutaneous Leishmaniasis - 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.

Visceral leishmaniasis - Marrow /blood in EDTA for PCR -minimum of 500µl

Key factors affecting test:

A biopsy should ideally be around the size of a grain of rice.
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.
EDTA bone marrow is the preferred sample rather than EDTA blood for visceral cases.
EDTA blood may provide uncertain results.
Please list travel history with all Leishmania PCR requests.

4.12.3 Leishmania serology – not UKAS accredited

Sample requirement: 0.8ml serum OR 10ml of clotted blood

ELISA used as screening test.
RK39 serology also performed in non-cutaneous queries.

Ab levels detected around 1 month post exposure.

Titres should seroconvert to negative within 6-9 months of successful treatment.

Antibody detection is useful in cases of suspected Visceral Leishmaniasis who do not have HIV infection. In suspected Visceral Leishmaniasis patients who are HIV infected sera is often non-reactive.

In Cutaneous Leishmaniasis, serology is of little value.

Cross-reactions may occur with trypanosomiasis and autoimmune conditions.

	SENSITIVITY %	SPECIFICITY %
LEISHMANIA ELISA	90.91	92.59
RK30	100	100

ELISA IgG antibodies detected.

4.13 Malaria

4.13.1 Blood film microscopy – UKAS accredited

If malaria is suspected a diagnosis must be made urgently via blood film microscopy.

Samples are screened using thick blood films, thin films are examined on positive samples. A percentage parasitaemia is given with all *P.falciparum* and *P.knowlesi* results.

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. Both forms available from our website, address as follows **https://lstmed.ac.uk/clinical-services/cdpl/** Sample date and patient travel history should be stated.

4.13.2 Rapid diagnostic tests (RDT's) – Not UKAS accredited

Blood film microscopy is gold standard method for malaria diagnosis but RDT's are available. RDTs are used, when required, as an aid to diagnosis.

Sample requirement: EDTA blood sample.

Key factors affecting test:

False positives and negatives may occur.

Antigens may circulate for up to 28 days post successful treatment leading to false positive results.

Patients may be infected with the *P.falciparum* strain that is HRPII deficient and therefore a false negative result may occur.

Some genetic variations of *P.ovale* may also cause false negative results.

Prozone effect (overloading of high levels of antigen) can cause false negative results.

There are some other circulating proteins that can cause a false positive result, such as rheumatoid factor.

4.13.3 Malaria serology – Not UKAS accredited

Sample requirement: 0.8ml serum OR 10ml of clotted blood

ELISA used.

If current acute infection is suspected blood films must be examined urgently. Antibody testing may be useful for retrospective diagnosis.

Antibodies are detected about 7 to 15 days from initial infection.

In a non-immune traveller treated for a single infection Ab levels should fall within 3-6 months of successful treatment.

IgG antibodies of all human infecting *Plasmodium* species detected.

	SENSITIVITY %	SPECIFICITY %
MALARIA ELISA	96.6	100

4.13.4 Malaria PCR – Not UKAS accredited

Malaria PCR is available but is only used, when required, as an aid to diagnosis alongside blood film microscopy.

Sample requirements: EDTA blood sample.

4.14 Paragonimus

4.14.1 Sputum microscopy – UKAS accredited

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.

If pathogens such as strongyloides or ascaris are suspected, a sputum sample can be run on the faecal PCR panel if required.

4.15 Schistosomiasis

4.15.1 Faecal microscopy - UKAS accredited test

A direct examination and concentration technique are routinely performed on all faecal samples for the presence of cysts, ova and larvae.

Sample requirement: Minimum of ¼ specimen pot of faecal sample. Ova, cysts and parasites may be passed intermittently therefore three samples may be required to be examined. Ova, cysts and parasites will diminish over time therefore it is more ideal for the

sample to be less than 2 days old upon receipt at LSTM, however older stools will not be rejected.

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

4.15.2 Faecal PCR – Not UKAS accredited

The parasites included in the PCR panel are; *Giardia intestinalis, Cryptosporidium* species*, Entamoeba histolytica, Entamoeba dispar, Strongyloides stercoralis, Necator americanus, Ancylostoma duodenale, Schistosoma* species*, Ascaris lumbricoides, Enterobius vermicularis and Trichuris trichiura.*

From our intensive evaluations we have concluded that one stool sample received and tested using stool concentration, faecal PCR and helminth culture (if required), will yield the same results as the recommended three stool sample request for stool concentration and helminth culture (if required). The introduction of the faecal PCR has therefore eliminated the need for requesting three stool samples from patients for parasitology testing and a move to a one stool sample request for those pathogens on the faecal PCR panel. This will be of benefit to the patient plus enhance the sensitivity and confidence in results from a one-off stool sample test for users of our service.

Sample requirements: Minimum of ¼ specimen pot of faecal sample.

Key factors affecting test: Addition of formalin to the sample will hinder DNA extraction and qPCR amplification.

Urine, semen and bladder biopsies may be run on the faecal PCR if schistosomiasis is suspected, please let the lab know if this is required.

4.15.3 Urine filtration – UKAS accredited

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

Sample requirement: ideally total 10am – 2pm urine collection or terminal midday sample (the last 10 to 20ml of urine passed).

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

4.15.4 Semen microscopy – UKAS accredited

Seminal fluid microscopy can be performed for schistosome ova if required.

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

4.15.5 Schistosome serology – UKAS accredited

Sample requirement: 0.8ml serum OR 10ml of clotted blood

Commercial ELISA used. Test utilises *Schistosoma mansoni* antigen, however this does cross react with other Schistosoma species. IgG antibodies are detected.

	SENSITIVITY %	SPECIFICITY %
ELISA (in house verification)	100	60

Antibody levels may not be detected until 3 months' post exposure. In patients with a negative ELISA test up to 3 months but significant freshwater exposure we would recommend further testing from 4 months after the last exposure.

Antibody levels may be positive for 18 months or longer post successful treatment.

4.16 Strongyloides

4.16.1 Faecal microscopy - UKAS accredited test

A direct examination and concentration technique are routinely performed on all faecal samples for the presence of cysts, ova and larvae.

Sample requirement: Minimum of ¼ specimen pot of faecal sample. Ova, cysts and parasites may be passed intermittently therefore three samples may be required to be examined. Ova, cysts and parasites will diminish over time therefore it is more ideal for the sample to be less than 2 days old upon receipt at LSTM, however older stools will not be rejected.

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

4.16.2 Faecal PCR – Not UKAS accredited

The parasites included in the PCR panel are; *Giardia intestinalis, Cryptosporidium* species, *Entamoeba histolytica, Entamoeba dispar, Strongyloides stercoralis, Necator americanus, Ancylostoma duodenale, Schistosoma* species, *Ascaris lumbricoides, Enterobius vermicularis and Trichuris trichiura.*

From our intensive evaluations we have concluded that one stool sample received and tested using stool concentration, faecal PCR and helminth culture (if required), will yield the same results as the recommended three stool sample request for stool concentration and helminth culture (if required). The introduction of the faecal PCR has therefore eliminated the need for requesting three stool samples from patients for parasitology testing and a move to a one stool sample request for those pathogens on the faecal PCR panel. This will be of benefit to the patient plus enhance the sensitivity and confidence in results from a one-off stool sample test for users of our service.

Sample requirements: Minimum of ¼ specimen pot of faecal sample.

Key factors affecting test: Addition of formalin to the sample will hinder DNA extraction and qPCR amplification.

4.16.3 Helminth culture – UKAS accredited

Faeces are cultured for filariform larvae using the charcoal technique.

Sample requirements: At least 50g (half pot) of faeces is required for this test. Ova, cysts and parasites may be passed intermittently therefore three samples may be required to be examined.

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

4.16.4 Strongyloides serology – Not UKAS accredited

Sample requirement: 0.8ml serum OR 10ml of clotted blood

Commercial ELISA used. IgM and IgG antibodies are detected.

Cross-reactions are known to occur with patients with filarial infections and with heavy

Hookworm infections.

HIV status has no effect on Strongyloides antibody test

Antibody levels normally detected 2 to 3 months post exposure, however, there are occasions when no antibody response will be detected.

Antibody levels revert to negative approximately 6 months to 1 year post successful treatment.

	SENSITIVITY %	SPECIFICITY %
ELISA (in house verification)	93	85

A negative Strongyloides antibody result does not exclude infection, particularly in immunosuppressed patients, in whom serological sensitivity may be reduced.

In patients with relevant epidemiological exposure who are receiving or are expected to start corticosteroids or other significant immunosuppression, specialist advice is recommended regarding further investigation and/or presumptive treatment, given the risk of potentially life-threatening Strongyloides hyperinfection. Please contact the laboratory or on call clinician for advice.

4.17 Trypanosomiasis

4.17.1 African Trypanosomiasis

4.17.1.1 African Trypanosomiasis blood film microscopy – UKAS accredited

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

Sample requirements: unstained thick/thin films, EDTA blood sample received at LSTM within 24 hours of blood being taken.

Key factors affecting tests: Trypanosomes may not survive for more than a few hours in EDTA tube therefore posted samples could show false negative results. **PHONE LAB FOR ADVICE BEFORE TAKING SPECIMEN.** We would recommend making at least 4 thick and thin blood films if you are unable to get the EDTA sample to LSTM within 24 hours of

the sample being taken. Fix and stain the thin films, stain the thick films, as you would for a malaria request. Send these films with the EDTA sample ASAP. There is the possibility of adding some glucose to the EDTA sample to keep Trypanosomes active for longer than a few hours, please contact the laboratory if you would like advice on this.

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

4.17.1.2 African Trypanosomiasis serology – Not UKAS accredited

Sample requirement: 0.8ml serum OR 10ml of clotted blood

IFA used for *T.b.rhodesiense* queries. IgG antibodies detected.

CATT used for *T.b.gambiense* queries:

	SENSITIVITY %	SPECIFICITY %
CATT	100%	98%

Travel history and clinical details are required to determine which test is performed.

Cross reactions may occur between trypanosome & leishmania serology.

CSF samples can also be tested for African trypanosomiasis antibodies.

4.17.2 South America Trypanosomes, Trypanosoma cruzi, Chagas

4.17.2.1 T.cruzi blood film microscopy – UKAS accredited test
Blood film examination may be useful but only within the acute phase of the infection. Please contact the laboratory for advice before sending sample.

4.17.2.2 T.cruzi serology – Not UKAS accredited

Sample requirement: 0.8ml serum OR 10ml of clotted blood

Serology is useful, Ab's are detected around 1-month post- infection and remain positive for life.

Commercial T.cruzi ELISA used.

	SENSITIVITY %	SPECIFICITY %
in house verification ELISA	99.13	98.3 to 99.6 depending on patient demographic

Positive results are always repeated for quality purposes, firstly at LSTM, then LSTM clinician would recommend user to obtain a repeat test at another site.

4.18 Whole worms

Samples of whole worms or proglottids may be sent for diagnosis.

Sample requirements: Whole worms such as Ascaris may be stored in 10% formol saline. Suspected Taenia species (or other cestode) 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.

5. USEFUL INFORMATION REGARDING ALL SEROLOGY TESTS

Interpretations of all results are subject to clinical and travel information provided.

Antibody detection may not be useful as a test of cure.

A positive result may reflect previous or current infection. We can examine samples for parasites following a positive antibody test result if required.

Different serology tests have differing cut off points therefore more information regarding tests and interpretation is available from the laboratory staff.

Sample requirement: 0.8ml serum OR 10ml of clotted blood (minimum 0.3ml 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 long or delayed incorrect storage temperatures, serum is able to be sent via Royal mail or couriers at room temperature and the

integrity will not be compromised. Plasma can be tested, although due to the fact that there are more proteins present in plasma samples than in serum samples the risk of an incorrect result is increased.

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

6. CHARGES

A charge is made for all laboratory services, for current prices please contact the laboratory using the contact details on page one of this handbook.

7. RESULTS AND REPORTS

7.1 Written reports

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

7.2 Telephoned / emailed 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 emailed if requested. This is performed using NHS secure email addresses. Please contact the laboratory if you would like to utilise this service.

8. INTERPRETATION AND ADVICE

For **technical advice** regarding samples/tests and for interpretation of results please contact the laboratory using stated contact details.

For **clinical advice** from one of the laboratory clinicians please contact the laboratory using stated contact details. These contact details are in operation Monday to Friday 9am to 5pm.

Out of hours please contact infectious diseases unit at the Royal Liverpool University Hospital using the following details – telephone RLUH switchboard on 0151 706 2000 and ask for Infectious disease registrar.

Tel: 0151 705 3220
Email: CDPL@lstmed.ac.uk
Or when sending patient details: LSTMLab@liverpoolft.nhs.uk