LONDON SCHOOL of HYGIENE &TROPICAL MEDICINE

UKAS MEDICAL 9148

DIAGNOSTIC LABORATORY PARASITOLOGY

LABORATORY USER HANDBOOK

2019

Faculty of Infectious and Tropical Diseases
London School of Hygiene & Tropical Medicine
Keppel Street
London WC1E 7HT

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A. INTRODUCTION

1. The Laboratory and outline of services

The Diagnostic Parasitology Laboratory is a UKAS accredited medical laboratory No. 9148, based at the London School of Hygiene and Tropical Medicine, which is itself a centre of excellence for scientific research and postgraduate education. The Parasitology Laboratory provides a reference facility offering a wide range of parasitological investigations for enteric parasites, blood parasites and acanthamoebae. We also offer technical advice on methodology and laboratory procedures.

The services above are offered to all hospitals, NHS and PHE laboratories, General Practitioners and private medical laboratories throughout the UK and abroad. The Parasitology Laboratory also has considerable expertise in the diagnosis of parasites of non-human primates and offers this service to veterinary practitioners.

Advice on the investigation of malaria and other parasitological diseases is always available; technical teaching sessions can also be arranged for small or large groups.

The laboratory processes around 2000 specimens annually, participates in the NEQAS quality assurance schemes for blood and faecal parasitology.

Please note that all parasitology serology is performed at Clinical Parasitology, HSL, LLP Analytics at the Hospital for Tropical Diseases, see page 15 for contact details.

Please note that entomology investigations are conducted by the LSHTM Medical Entomologist and are not within the scope of accreditation of the Diagnostic Parasitology Laboratory

2. Laboratory policy

Our policy is to offer a first class diagnostic and reference facility for parasitic infections. We are also strongly committed to active research into parasites and their diagnosis and to the provision of training for pathology staffs, other healthcare professionals and those working in the control of infectious diseases.

3. Using this handbook

This handbook is designed to aid and advise the user on the appropriate use of the facilities to include diagnostic, teaching and advisory services. The sections are indexed to assist in understanding the structure of the laboratory, identification of staff, the diagnostic and advisory services offered and the specimens and investigations carried out by the laboratory.

Parasites and diseases are listed alphabetically and each section gives an outline of the specimens required for investigation and the tests carried out. This is not an exhaustive list and users not finding a specific requirement should contact the laboratory for help and advice. Further copies of this handbook can be downloaded from the DPL website at www.parasite-referencelab.co.uk
where referral forms and other useful information and related links can also be found.

4. Malaria Reference Laboratory requests

The PHE Malaria Reference Laboratory is also located in the same department and will deal with all malaria requests. A separate handbook is available to download from the MRL website at www.malaria-reference.co.uk

Specimens for malaria diagnosis should be sent to the Malaria Reference Laboratory where they will be dealt with appropriately.

Please note that malaria serology is performed at Clinical Parasitology, HSL, LLP Analytics at the Hospital for Tropical Diseases, see page 15 for contact details.

5. Customer Satisfaction

We encourage users of our services to make any suggestions with regard to quality improvement and customer satisfaction.
Suggestions, queries or complaints should be made to:-

Dawn Britten, Quality Manager
Dawn.Britten@lshtm.ac.uk

and/or

Claire Rogers, Principal BMS.
Claire.Rogers@lshtm.ac.uk

B. LABORATORY AND STAFF

1. Laboratory opening times

The diagnostic laboratory is open between the hours of: -

Monday to Friday 9:00 am to 5:00 pm

when staff are available for advice, information, specimen reception and processing.

Most routine specimens are sent to us by post or the DX system, any urgent specimens are usually delivered by courier and accepted during the hours stated**. Please telephone urgent requests prior to despatch to inform us of their impending arrival and priority status.**

2. Out of hours and public holidays

We offer foremost a reference facility and therefore do not provide an on-call service. The majority of specimens we receive will have had a preliminary diagnosis made by the sender or primary laboratory and so are usually non-urgent. The laboratory is usually closed on public holidays; when there is an extended holiday period, for example Christmas and New Year, limited cover is arranged to deal with non– urgent postal specimens, and all users are informed of these arrangements prior to the holidays.
Any specimens delivered to the Diagnostic Parasitology Laboratory out of normal hours and where no prior arrangement with us has been made will be held and dealt with the next working day.

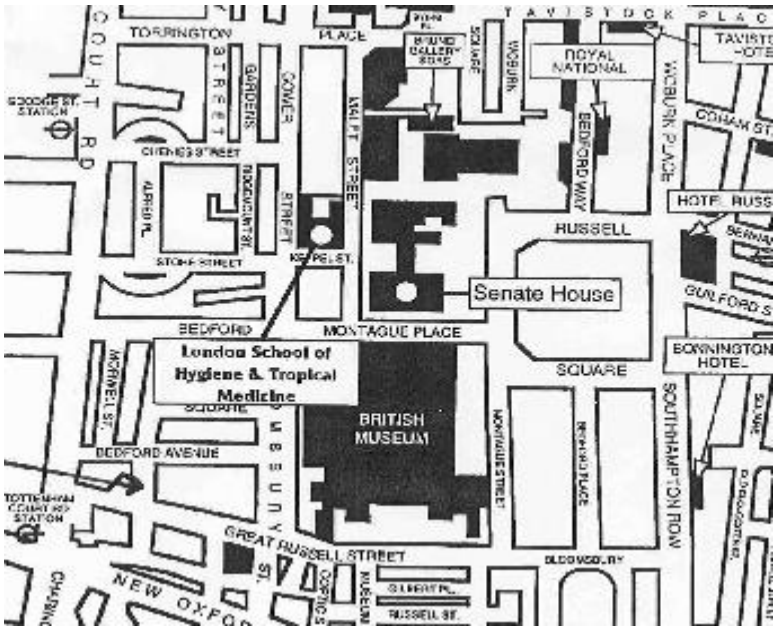
Where urgent diagnosis is required out of normal hours for malaria, Babesia or African trypanosomiasis only, specimens should be referred to: -

Clinical Parasitology at HSL, LLP Analytics at the Hospital for Tropical Diseases, telephone number: 0845 155 5000 (UCLH switchboard) and ask for the on-call Biomedical Scientist in Parasitology

Please note that this service will incur a charge.

3. Location

The LSHTM is situated close to Tottenham Court Road, Goodge Street and Russell Square underground stations and is a 10 minute walk from Euston Main line station. Car parking is very restricted in the local area. The LSHTM has no parking facilities of its own.



4. Visitors

Visitors who have arranged an appointment should report to the reception desk in the LSHTM entrance foyer, reception staff will then inform the laboratory of their arrival. Please note that we are not able to see members of the general public and all patient referrals must be made by a registered health professional to the Hospital for Tropical Diseases, where the clinical service is delivered. There are no clinics at LSHTM.

5. Staff and telephone numbers

Role	Position	Name	Telephone number
Director	Consultant Parasitologist	Professor P L Chiodini	020 7927 2427
Head of Teaching & Diagnostics Unit/Training Officer	Principal BMS/ Head TDU	Claire Rogers	020 7927 2318
Lead BMS, Molecular Diagnostics	Lead BMS	Dr Debbie Nolder	020 7927 2343
Lead BMS, Main Laboratory	Lead BMS	Juliana Tucker	020 7927 2427
Lead BMS, Quality Manager	Lead BMS	Dawn Britten	020 7927 2427
Biomedical Scientists	Senior BMS	Sarah Cheeseman	020 7927 2427
	Senior BMS	Emma Victory	020 7927 2427
	Senior BMS	Helen Liddy	020 7927 2427
	Trainee BMS	Lindsay Stewart	020 7927 2427
Medical Laboratory Assistants	MLA	Susan Passarelli	020 7927 2427
	MLA	Karen Osborne	020 7927 2427
	MLA	Helena Stone	020 7927 2427
	MLA	Amy Gallimore	020 7927 2427
	MLA	Saba Entezam	
Results/enquiries; (technical advice and forms, guidelines, kits, containers, isolation media, etc.)	Laboratory		020 7927 2427
Laboratory fax number			020 7637 0248
Medical Entomologist		Cheryl Whitehorn	020 7927 2344
Member of the LSHTM Faculty of Infectious & Tropical Diseases entomology staff, who provides expertise to diagnostic laboratory)			

C. DIAGNOSTIC AND ADVISORY SERVICES

1. Information and enquiries

For consultation on the investigation and diagnosis of parasitological disease, interpretation of results and general information, please contact the laboratory on 020 7927 2427 from where enquiries can be answered or referred to appropriate personnel.

2. Specimens and containers

For the majority of investigations, the submitting laboratory or institution will refer specimens in an appropriate container of their choice.
For certain investigations or where special conditions are required, kits and containers are available from us upon request.

For example: -

- A collection kit containing 1 plain vial and 3 fixative vials (SAF) with collection instructions is available for investigation of both intestinal cysts and trophozoites including *Dientamoeba fragilis & Blastocystis hominis.*

See from page 16 for guidelines as to appropriate specimen type for specific investigations

Please telephone us if in any doubt as to which container to use or to discuss specific requirements.

3. Referral forms

A referral form must accompany all specimens referred to the laboratory and we ask that requesting practitioners use our referral form wherever possible. Hard copies can be supplied upon request or the form may be downloaded electronically, as a pdf, from the laboratory website at: www.parasite-referencelab.co.uk

4. Minimum Data Set

As a guide, the following table sets our minimum data requirements to ensure patient safety. Where specimens/referral forms are received that do not meet this criteria, we shall contact the sender for more information but reserve the right to reject the sample.

	ESSENTIAL	**DESIRABLE**
SAMPLE	**1**. Full name OR other coded patient identifier **2**. DOB &/or Hosp no./ Unit no./ **NHS No**.	**1**. Sending laboratory's ref no. **2**. Date specimen taken **3.** Nature of specimen inc. qualifying details e.g. right/left
REQUEST FORM	**1**. Full name OR other coded patient identifier **2**. DOB &/or Hosp no. / Unit no./ **NHS No**. **3**. Investigation required **4**. Name and address of requesting practitioner and name and address for reports if different	**1**. Sending laboratory's ref no. **2**. Date specimen taken **3.** Nature of specimen inc. qualifying details e.g. right/left **4.**Telephone/bleep no. of requesting practitioner **5**. Gender **6**. Sending lab's diagnosis **7**. Clinical information

Please note the following extract from IBMS Professional Guidance:
*"Use of the **NHS or CHI** Number on paper and electronic patient records is now a mandatory requirement included within the NHS Operating Framework 2008/9. Patient data should be used to identify the sample up to the point where a NHS or CHI Number is allocated whereupon this becomes the primary identifier."*

5. Clinical Information

All relevant clinical details to include, where available

- antimicrobial therapy
- travel history
- risk status if applicable
- date of onset and duration of illness
- anatomical sites from which biopsies, foreign bodies, insects, other specimens taken
- epidemiological information, for example family/institutional outbreaks

6. Packaging

Packaging must comply fully with UK transport regulations for clinical specimens (UN 3373 regulations).

Specimens should be in an appropriate container, securely fastened and the accompanying referral form should be placed in a separate area of the packaging so as not to be in direct contact with the specimens.

The outside of the package must be appropriately marked and clearly state:-

‘**BIOLOGICAL SUBSTANCE - CATEGORY B’**

In those circumstances where specimens are unlabelled or inadequately labelled and the patient’s identification is unclear, or if they have leaked or are contaminated, they may be unsuitable for testing. In such instances the requesting laboratory or doctor will always be informed immediately by telephone to discuss the matter and to arrange for repeat specimens if necessary.
As a reference laboratory we do appreciate that some specimens cannot be repeated and every effort is made to avoid the need for repeat requests.

7. High risk specimens and safety

Specimens are regarded as HIGH RISK if taken from patients known, suspected, or at risk of having serious infectious disease.
Of note are blood-borne agents such as Hepatitis, HIV, or various viral haemorrhagic fevers or other infectious diseases such as tuberculosis or typhoid. Parasitological high risks include *Echinoccocus spp.* and ova/segments of *Taenia solium*.

For further advice on patient management, please contact the Imported Fever Service on 0844 778 8990.

Hazard Group 3 risks:- In addition to the standard packaging instructions given, all high risk specimens must be labelled as HIGH RISK both on the container and the request form, with a standard yellow 'DANGER OF INFECTION' sticker, and placed in a Biohazard bag.

The precise nature of the infection risk should be clearly given in the clinical details.

Hazard group 4 risks:-

This is essentially referring to viral haemorrhagic fevers but also similar human infectious diseases of high consequence.

Specimens from a patient with a **confirmed** hazard group 4 pathogen.

Specimens containing HG4 organisms cannot be dealt with in this laboratory. The referring clinicians must not send any specimens and must discuss the case with the Imported Fever Service on 0844 778 8990.

Samples from a patient with a **possible risk** of a hazard group 4 pathogen.

The referring clinicians must first discuss the case with the Imported Fever Service on 0844 778 8990. The risk assessment and any VHF screening required as a result of this should have taken place before specimens are sent to us and we may receive specimens once the risk has been downgraded.

8. Specimen transport & reception

Most specimens are received via post, DX or courier. During normal working hours, all specimens are received at LSHTM reception from where they are forwarded to the reference laboratory. Outside normal working hours, non-urgent specimens may be left at reception and will be dealt with on the next working day.

9. Urgent investigations

Urgent specimens are usually delivered by courier and accepted during normal working hours. Please telephone urgent requests before you send the samples so that we know they are coming, also ensure that you have provided full and correct contact details so that we can telephone the results back to you. Please package as described above and mark clearly on the outside that it is urgent.

When an urgent malaria, Babesia or African trypanosomiasis diagnosis is required outside of normal hours, specimens should be referred to Clinical Parasitology, HSL Analytics LLP at the Hospital for Tropical Diseases (HTD). Call 0845 155 5000 (UCLH Switchboard) and ask for the on-call Biomedical Scientist in Parasitology.

Please note that this service will incur a charge.

Please note that if a specimen is en route to LSHTM and you wish it to be re-directed to HTD, you must arrange this directly with the courier, as LSHTM staff cannot authorise a courier to re-route.

Any specimens delivered to us out of normal working hours, if special arrangements have not been made with us, will remain securely at LSHTM Reception until being transferred to the laboratory on the following working day.

D. SPECIMENS AND INVESTIGATIONS

1. Specimen collection

General

As a reference laboratory, many specimens received have already had preliminary investigations carried out by a primary laboratory and are sent to us in due course.

In general, specimens should be collected or transferred into an appropriate container such as a sterile universal, with an accompanying referral form giving all relevant information, including laboratory findings and sent to us with minimal delay.
Please inform us of any known infection risks.

Please provide a specimen representative of the condition under investigation and in sufficient quantity to permit a full examination – see specific guidelines on pages 17-39.

Where a specimen is submitted for a general screen with no specific parasitological investigation requested we may, as a result of our findings or as indicated to us by the specimen type or clinical details given, proceed to further investigations as deemed appropriate.

Faeces

Unless otherwise requested, faecal specimens are given a general parasitological screen to include:-

examination for adult worms and segments
formol-ether concentration and microscopy for ova, cysts and larvae
amoebic culture

If the presence of trophozoites is to be investigated, faeces must either be fresh or collected into SAF preservative (available upon request).
See also guidelines for intestinal parasites and specific parasitic diseases.

Blood

See specific parasitic diseases for guidelines on volume, anticoagulants, blood smears etc.

Serology

We do not perform serological tests, all parasitology serology is performed by Clinical Parasitology, HSL Analytics LLP at the Hospital for Tropical Diseases (HTD) and those received by us will be forwarded to the HTD. Referring laboratories should send specimens directly to the HTD:

Clinical Parasitology, HSL Analytics LLP
Hospital for Tropical Diseases (HTD)
Mortimer Market
Capper Street
London
WC1E 6JB

Telephone 020 7307 9400 and ask for Parasitology Serology

Tissues and biopsies

Under aseptic conditions, transfer material to a sterile universal container. If the sample is very small, add 0.5ml of sterile saline to prevent drying. Please refer to specific investigations for more detail.

Worms and worm segments

Adult worms and tapeworm segments should be sent without preservative in a sterile universal container. If there is likely to be a delay of more than 24 hours, then 10% formol water should be added to the specimen.

Ectoparasites and Entomology

Arthropods, larvae, etc. should be sent without preservative if 'living' or otherwise in 70% alcohol in a suitable container. Where extracted from a body site and there is a risk of infection, specimen may be fixed in 10% formol water, rinsed in water and transferred to 70% alcohol. Do not leave in formal water as this hardens some specimens. Please allow specimen to remain intact if possible, giving full clinical details including travel history and site of extraction if relevant. If in any doubt please call to discuss.

2. Specimen retention

Specimens are retained for varying periods of time according to type. Should additional tests be requested after the initial referral, please observe the following guidelines:-

Faeces/urine/blood/body fluids/CSF:- minimum of 2 weeks at 2-8°C after receipt

Worms, segments, insects:- minimum of 2 weeks after receipt

Tissues, biopsies:- 4 weeks after final report, at 2-8°C

Blood sent for malaria or T.cruzi PCR :– after DNA extraction, 1.0 mL aliquots of blood are held at -20°C indefinitely (where sample volume permits).

NB The above guidelines and the following specific parasitic diseases are not intended as an exhaustive list of parasitological investigations available; please contact the laboratory to discuss individual cases, specific requirements or investigations not listed.

3. Parasitic diseases and their laboratory investigation

Please refer to the following pages where all are listed in alphabetical order.

All parasitology serology is now performed at Clinical Parasitology at HSL, LLP Analytics at the Hospital for Tropical Diseases.

Amoebiasis

Intestinal amoebiasis/ amoebic dysentery and invasive amoebiasis

Causative organism: *Entamoeba histolytica* (pathogenic) *or Entamoeba dispar* (non-pathogenic)

Diagnosis:formol-ether concentration and microscopical examination of faeces for cysts

direct microscopy of fresh faeces /pus for trophozoites

amoebic culture from faeces or pus

specific antigen ELISA for the detection of *E. histolytica* adhesin in faeces and culture

Specimens: faeces in plain container for concentration and culture

for OCP screen, minimum 1g faeces or 2mL if liquid
for ELISA, minimum 0.2g faeces or 400 µL if liquid

fresh faeces or rectal scrapings for trophozoite investigation to be carried out within 1 hour of sample being taken – please inform laboratory in advance

*pus from liver or lung abscess – please inform laboratory in advance

* Advice on collection of these samples is available from the Doctor on Duty in Infectious & Tropical Diseases at the Hospital for Tropical Diseases – telephone 0845 155 5000 (UCLH switchboard)

N.B. Due to the intermittent nature of passage of trophozoites and cysts in faeces, it may be necessary to examine 3 or more samples, collected on different days, to confirm a diagnosis.

Free living amoebae:

Amoebic keratitis

Causative organism: *Acanthamoeba spp.*

Diagnosis: microscopy and culture
PCR

Specimens: contact lens and/or wash fluids

corneal scrapes/biopsies/swabs

submitted cultures

Please note further detail below:

The greatest diagnostic sensitivity for AK is achieved through use of both *in vitro* culture / microscopy and a probe-based qPCR.

Suitable sample types:

Clinical samples (corneal scrapes, biopsies, fluids, swabs etc.) should be submitted for testing together with a completed ***Acanthamoeba*** **DPL referral form** (available to download and print from: www.parasite-referencelab.co.uk).

We can also perform culture and PCR on **non-clinical samples** (i.e. contact lenses and associated contact lens fluid). Isolation of *Acanthamoeba* from these specimens, whilst suggestive, does not necessarily implicate the amoeba as causing the patient's symptoms. Amoebic genera (other than *Acanthamoeba*), flagellates, ciliates and other organisms may regularly be found in contaminated washing fluids and on lenses, particularly with poor lens hygiene. Non-clinical samples should also be submitted for testing together with a completed *Acanthamoeba* DPL referral form (available to download and print as above).

Please note: we do not test commercial contact lens solutions (other than that already in the patients' contact lens case).

Notes re sample preparation:

All **clinical samples** should be sent in a small volume (200 - 1000µL) of sterile saline or sterile distilled water in a small (<5mL) sterile vial / tube. Please ensure vials / tubes are tightly screwed and use Parafilm (NOT Sellotape) to prevent leakage during transit.

Material from a needle or blade scrape should be rinsed into the saline / water.
NB: Please remove blades or needles after rinsing. Do NOT leave the blade in the tube as it rusts: this inhibits our PCR and may have a detrimental effect on culture isolation.

Swabs or washings appear to be less efficient in detecting the organism. NB: Please do not send dry swabs: if swabs must be sent then please add a small volume of sterile saline or sterile distilled water to the swab to prevent drying.
Continued on next page

Punch biopsies or portions of excised cornea may also be submitted: put sample into a small volume of sterile saline / distilled water in a small sterile vial.

For **non-clinical samples**, please submit contact lens(es) in their lens cases (ie in used contact lens fluid.) Please ensure cases are tightly screwed and use Parafilm (NOT Sellotape) to prevent leakage.

Culture-positive samples: please send original culture plate if possible, or blocks of agar from the plate in a sterile vial. Please seal plates or tubes with Parafilm (NOT Sellotape) to prevent leakage.

Free living amoebae:

Granulomatous Amoebic Encephalitis (GAE)

Primary Amoebic Meningoencephalitis (PAM)

Causative organisms; *Acanthamoeba spp*., *Balamuthia spp*. (GAE), *Naegleria fowleri* (PAM)

Diagnosis: microscopy, culture, PCR

Specimens: CSF and biopsy material

NB These infections require urgent diagnosis; telephone advice should be obtained as soon as infection is suspected.

Investigations for GAE and PAM are currently performed at:

Clinical Parasitology, HSL Analytics LLP
Hospital for Tropical Diseases (HTD)
Mortimer Market
Capper Street
London
WC1E 6JB

Telephone 020 7307 9400 and ask for Parasitology Microscopy

Babesiosis

Causative organism: *Babesia spp.*

Diagnosis: microscopical examination of thin and thick blood films for parasites stained with Giemsa and Field's

Specimens: 2 thin (methanol-fixed) and 2 thick (unfixed) blood films sent in a slide container.
Blood taken into anticoagulant (EDTA should be used) and films made with a minimum of delay and preferably within 2 hours of taking the blood.

Blood should be taken at peak of parasite density as indicated by fever; however parasites may be found in the absence of fever and the examination of blood films should NOT be delayed. Repeat blood films may be necessary to demonstrate infection.

NB Travel history and splenic status is important in the diagnosis of this infection.

Cryptosporidiosis

Causative organism: *Cryptosporidium spp.*

Diagnosis: microscopy after acid fast staining of faecal smears using a modified Ziehl-Neelsen stain or fluorescent microscopy after phenol auramine staining for detection of oocysts.

immunochromatographic rapid antigen test

Specimen: faeces in a plain container or fixed in SAF
Minimum 1mL for antigen test

N.B. Due to the intermittent nature of passage of oocysts in faeces, it may be necessary to examine 3 or more samples, collected on different days, to confirm a diagnosis.

Cyclosporiasis

Causative organism: *Cyclospora cayetanensis*

Diagnosis: microscopical examination of faecal smears stained by modified Ziehl-Neelsen for oocysts and direct microscopy following formol-ether concentration

Specimens: faeces in plain container

N.B. Due to the intermittent nature of passage of oocysts in faeces, it may be necessary to examine 3 or more samples, collected on different days, to confirm a diagnosis.

Cystoisosporiasis (Isosporiasis)

Causative organism: *Cystoisospora belli (Isospora belli)*

Diagnosis: microscopical examination of faecal smears stained by modified Ziehl-Neelsen for oocysts and direct microscopy following formol-ether concentration

Specimens: faeces in plain container

N.B. Due to the intermittent nature of passage of oocysts in faeces, it may be necessary to examine 3 or more samples, collected on different days, to confirm a diagnosis.

Enterobiasis

Threadworm or Pinworm

Causative organism: *Enterobius vermicularis*

Diagnosis: microscopical examination for ova

Specimens: adhesive tape smears of perianal skin

perianal swab

faeces in clean container (low sensitivity)

Adhesive tape or swab preferred.

Cut a 10cm strip of sellotape, or similar, and press middle 3-5cm firmly against the right and left perianal folds, sticky side down. Stick tape onto a microscope slide and place in a slide box.

or

Moisten a swab in sterile saline and repeatedly roll over the whole of the perianal area; break off into a small volume of saline in a sterile universal.

Carry out either procedure first thing in the morning before bathing or defaecation. Repeated samples over 4 to 6 consecutive days may be necessary to confirm a diagnosis.

BEWARE eggs are highly infectious and resistant to drying!

Filariasis

Causative organisms: of particular importance are *Loa loa, Wuchereria bancrofti. Brugia malayi, Onchocerca volvulus*

Diagnosis: membrane filtration and microscopical examination of peripheral blood for microfilaria (except *O. volvulus*)

examination of skin snips for microfilariae of *O*. *volvulus*

examination of histological material for adults

Specimens: 10 – 20 mLof citrated (preferred anticoagulant) blood (observe periodicity* if known, if not take at any time).

Additionally 4x unfixed thick films to be made from the blood with a minimum of delay.

Skin snips for *O. volvulus* placed into physiological saline; advice should be sought before taking skin snips.

*Depending on clinical and travel history, possible periodicity should be observed when taking blood.

Species	**Geographical Distribution**	**Collection time**
Wuchereria bancrofti Periodic, nocturnal	Asia, Africa, Caribbean, South America, West Pacific	22:00-04:00 peak 24:00
Wuchereria bancrofti Subperiodic, nocturnal	Thailand, Vietnam	20:00-22:00 peak 21:00
Wuchereria bancrofti Subperiodic, diurnal	South East Pacific	14:00-18:00 peak 16:00
Brugia malayi Periodic, nocturnal	South & East Asia	22:00-04:00 peak 24:00
Brugia malayi Subperiodic, nocturnal	South East Asia	20:00-22:00 peak 21:00
Brugia timori Nocturnal	Lesser Sunda islands of Indonesia, inc Timor	22:00-04:00 peak 24:00
Loa loa Diurnal	West & Central Africa equatorial rainforests	10:00-15:00 Peak 13:00
Mansonella. ozzardi	Central and South America, Caribbean	Non-periodic
Mansonella perstans	Tropical Africa, South America	Non-periodic

Giardiasis

Causative organism: *Giardia intestinalis* (syn., *G. lamblia, G. duodenalis*)

Diagnosis: microscopy of fresh faeces for trophozoites and cysts

formol-ether concentration and microscopical examination for cysts

immunochromatographic rapid antigen test

Specimens: faeces in plain container (minimum 1mL for antigen test)

if fresh and for trophozoites, examination should be carried out within 4 hours of specimen being produced.

N.B. Due to the intermittent nature of passage of trophozoites and cysts in faeces, it may be necessary to examine 3 or more samples, collected on different days, to confirm a diagnosis.

Hydatid infection

Causative organisms: *Echinococcus granulosus* and *E. vogeli* for cystic hydatid and *E. multilocularis* for alveolar hydatid.

Diagnosis: microscopical examination for hooks and protoscoleces in hydatid sand

Specimens: fluid/contents of cysts (fixed in formalin)

NB Clinical advice on the management of hydatid disease should be sought before considering aspiration of a cyst as leakage of fluid may cause further dissemination or an anaphylactic reaction. Advice is available from the Doctor on Duty in Infectious & Tropical Diseases at the Hospital for Tropical Diseases– telephone 0845 155 5000 (UCLH switchboard)

Insects and other arthropods

Living insects, ticks and mites for identification should be sent in a plain tube without fixation.
If specimen is not living then it should be sent in 70% ethanol.

Larvae (maggots) etc. should be sent live if possible, or in 70% ethanol.

Where specimen has been excised from the patient and there is a risk of infection, the specimen should be fixed in 10% buffered formalin (as used for histology) or 10% formol water/saline, then rinsed in distilled water and transferred to 70% ethanol.

Allow specimen to remain intact if possible, giving full clinical details including travel history and site of extraction if relevant.

Please note that entomology investigations are conducted by the LSHTM Medical Entomologist and are not within the scope of accreditation of the Diagnostic Parasitology Laboratory

Intestinal parasitic infections with helminths and protozoa (in general)

A wide range of nematodes, cestodes, trematodes and protozoa are dealt with; some are listed individually e.g. amoebiasis, cryptosporidiosis, cyclosporiasis, giardiasis, microsporidiosis, schistosomiasis, strongyloides.

See also pages for individual parasites

Diagnosis: macroscopical examination of faeces for adult worms and segments

direct microscopy of fresh faeces for trophozoites

formol-ether concentration of faeces and microscopy for ova, cysts and larvae

iron-haematoxylin staining and microscopy of SAF-fixed faeces for protozoal trophozoites - volume according to instructions on

Specimens: faeces in plain container for adult worms, segments and concentration- for OCP screen, minimum 1g faeces or 2mL if liquid.
NB do not refrigerate if Strongyloides culture required.

SAF fixed faeces for trophozoites (especially suitable when a fresh sample is not practical and for fragile organisms e.g. *Dientamoeba fragilis & Blastocystis hominis*) sample volume as indicated on collection pot

fresh faeces for trophozoites

N.B.

1. Due to the intermittent nature of passage of parasites in faeces, it may be necessary to examine 3 or more samples, collected on different days, to confirm a diagnosis.
2. We can supply kits for the collection of faeces in SAF – please contact us if required

Leishmaniasis

Visceral, Cutaneous & Mucosal leishmaniasis

Causative organism: Leishmania spp.

Diagnosis: microscopy of stained aspirates.and smears.

histological examination of ready-stained tissue sections for presence of parasites

Specimens: This laboratory only performs microscopy of impression smears or tissue sections from e.g. bone marrow, lymph, spleen, liver, tissue. Ideally the sending laboratory should send at least two smears, fixed and unstained, although we can also examine ready-stained smears.

For a full investigation of cutaneous or visceral leishmaniasis please contact Clinical Parasitology, HSL Analytics LLP, telephone 020 7307 9400 and ask for Parasitology which offers a range of investigations including culture, serology (visceral) and PCR.

Malaria

Please refer to the separate PHE Malaria Reference Laboratory handbook for full details of this service, referral forms etc.
www.malaria-reference.co.uk

Causative organism: of human importance are *Plasmodium falciparum*, *P. vivax*, *P. malariae, P. ovale* spp. and *P.knowlesi*.

Diagnosis:

using the following methodologies, selected according to individual circumstances :

- microscopical examination of thin and thick blood films for the detection and species identification of malaria parasites (using Giemsa and Field's stain)
- real-time PCR (screening) and nested PCR (gold standard) for malaria confirmation and species determination. PCR is not performed routinely on all specimens received for malaria diagnosis but at the discretion of MRL staff and according to laboratory algorithms
- immunochromatographic techniques for the detection of malaria antigen in blood
- for P.falciparum and P.knowlesi, parasitaemia estimation to indicate severity of infection and effectiveness of treatment, please note that this is only performed upon request

Specimens: 2 thick (unfixed) and 2 thin (methanol fixed) ready made films sent in a slide box and a sample of EDTA blood from which the initial diagnosis was made (minimum 3 mL– if possible) for PCR.

Blood is ideally collected during fever, however parasites are found at all stages of the infection and therefore blood films **without delay** are mandatory in all cases of suspected malaria. If the first films are negative, blood should be taken and films made and checked on at least two occasions over the first 24 hours and further films examined every 12 hours after that if strongly clinically indicated.

Blood taken into anticoagulant (EDTA should be used) should have films made as soon as possible to minimise morphological changes in the parasites, and certainly within 2 hours. However, parasites can be detected even after extended exposure to anticoagulant (exceptionally up to 24 hours) and no sample will be rejected unexamined.

NB **Serology** is occasionally useful in detecting evidence of past infection, but its main indication in the UK is for blood donor screening. **It has no place in the diagnosis of acute malaria, for which blood films are mandatory.**

Serology is no longer performed by this laboratory, but may be obtained from the Clinical Parasitology, HSL Analytics LLP at the Hospital for Tropical Diseases (HTD) see page 15

BLOOD FILMS ARE ESSENTIAL IN CASES OF ACUTE FEVER OR OTHER SYMPTOMS WHERE MALARIA IS SUSPECTED

Other investigations are offered where appropriate- each case to be discussed with the Clinical Scientist or Laboratory Clinical Director.

Molecular markers for drug sensitivity in treatment / prophylaxis failure such as anti-folate resistance, atovaquone proguanil resistance or ACT/artemisinin failure to clear

Discrimination of parasite species *P. ovale curtisi* and *P. ovale wallikeri* that cause ovale malaria

Outbreak investigations:
Clusters of malaria cases of any *Plasmodium* species seen to be clustered in time or location can be investigated for genetic relatedness of the parasites responsible.

Microsporidiosis

Causative organisms: including *Enterocytozoon bieneusi*, *Encephalitozoon intestinalis*

Diagnosis: microscopical examination of strong trichrome stained faecal/urine smears for microsporidial spores

Specimens: faeces

urine

N.B. Due to the intermittent nature of passage of spores, it may be necessary to examine 3 or more samples, collected on different days, to confirm a diagnosis.

Schistosomiasis

Bilharzia

Causative organism: *Schistosoma mansoni, S. haematobium, S. japonicum, S. intercalatum, and S. mekongi*

Diagnosis: formol-ether concentration and microscopy of faeces for ova

microscopical examination of urine after concentration or filtration

microscopical examination of squash preparations of tissue for ova

examination of stained histology sections

Specimens: faeces in plain container, minimum 1g faeces or 2mL if liquid

urine in a plain, sterile container.
either a midday urine specimen (between 10.00 and 14 00hrs) or a 24-hour collection of terminal urine
If a single urine specimen is to be submitted there should ideally be a minimum volume of 10mL
1 mL of undiluted formaldehyde should be added to the specimen to preserve any eggs that may be present.

NB peak egg excretion occurs between noon and 3pm, eggs may be found trapped in the blood and mucus in the terminal portion of the urine specimen.

Tissue, unfixed biopsy material (rectal, sigmoid, bladder) material for squashes

ready-stained sections for histological examination.

NB when screening after return from an endemic area, it is advisable to examine both urine and faeces.

N.B. Due to the intermittent nature of passage of parasites, it may be necessary to examine 3 or more samples, collected on different days, to confirm a diagnosis.

Strongyloidiasis

Causative organism: *Strongyloides stercoralis*

Diagnosis: microscopical examination for larvae/adults
isolation of larvae

Specimens: faeces, for OCP screen minimum 1g faeces or 2mL if liquid
duodenal/jejunal aspirate

For isolation of strongyloides, 8-10 g faeces and as much as possible for aspirates

N.B. Please do not refrigerate specimen if isolation is required

Due to the intermittent nature of passage of parasites in faeces, it may be necessary to examine 3 or more samples, collected on different days, to confirm a diagnosis.

Trichinosis

Causative organism: *Trichinella spiralis*

Diagnosis: microscopical examination for larvae by squash

Specimens: *unfixed/fixed muscle biopsy

NB it is widely considered unnecessary to perform biopsy for the diagnosis of this parasite, the alternative being serology (performed at Clinical Parasitology, HSL, HTD, see page 15)

Advice on collection of samples is available from the Doctor on Duty in Infectious & Tropical Diseases at the Hospital for Tropical Diseases – telephone 0845 155 5000 (UCLH switchboard)

Trypanosomiasis - African

Sleeping sickness

Causative organism: *Trypanosoma brucei rhodesiense*, *T.b. gambiense*

Diagnosis: microscopical examination of blood films for trypomastigotes

microscopical examination of cerebrospinal fluid where neurological involvement *****

Specimens: 2x methanol-fixed thin and 2x unfixed thick blood films for microscopy; blood taken into anticoagulant (preferably heparin) should be used and films made with a minimum of delay and preferably within 2 hours of taking the blood.

*CSF

*Advice should be sought before attempting to take CSF sample for diagnosis, due to risk of introducing trypanosomes into the CNS from the blood, please contact the Doctor on Duty in Infectious & Tropical Diseases at the Hospital for Tropical Diseases - telephone 0845 155 5000 (UCLH switchboard)

Trypanosomiasis – South American

Chagas Disease

Causative organism: *Trypanosoma cruzi*

Diagnosis: microscopy of blood film in the acute stage (extremely rarely seen in the UK)

PCR

Specimens: Blood collected into EDTA, 10mL from adult, 2mLfrom child

PCR is essential in the investigation of suspected acute cases (e.g. neonates, travellers, reactivations).

Before sending samples please discuss the case with the Doctor on Duty in Infectious & Tropical Diseases at the Hospital for Tropical Diseases – telephone 0845 155 5000 (UCLH switchboard)

NB Serology is the usual method for diagnosis in the chronic phase and this should be performed before PCR is considered. *T. cruzi* serology is performed at Clinical parasitology, HSL at the Hospital for Tropical Diseases, see p 15 for full details.

Worms - general

Diagnosis: macroscopical examination of adult worms and segments

formol-ether concentration of faeces and microscopy for ova and larvae

Specimens: faeces in plain container for adult worms, segments and concentration
minimum 1g faeces or 2mL if liquid
Whole worms and segments

4. Laboratory Schedule & Turnaround Times

Generally, specimen processing is begun on the day of receipt. Specimens that require microscopy only may have results available that same day whereas investigations processed in batches, those requiring culture or worm identification will take variable periods of time. As a reference laboratory some investigations are highly unusual and so target turnaround times serve as a guide only. When complete, final reports are produced and posted the same or next working day – interim or final telephone reports are always available upon request.

Results of any urgent investigations will be telephoned to the requesting laboratory immediately.

Turnaround time guideline:-
from receipt of specimen to release of report (telephoned or posted letter), in working days).

Malaria:-
Diagnosis/confirmation by blood film and/or immunochromatographic techniques: 1-2 working days. Telephoned results available within 2 hours of receipt of specimen, upon request.
Diagnosis/confirmation by PCR:- 1-4 working days.

Intestinal Parasitology:-
Specimen processing takes between 1 and 6 days, depending upon the range of investigations required for each specimen (for example, concentration, specific staining, microscopy, Techlab ELISA, amoebic culture).

Acanthamoeba culture:-
Culture usually takes up to 7 working days; microscopy results, where applicable, available in the interim. All positive results telephoned
NB a further 2 days is required where dry lens cases are received as a pre-culture stage is needed

Acanthamoeba PCR:-
Up to 7 working days. All positive results telephoned.

T.cruzi PCR
Suspected acute (neonate, travel, transplant-associated, needle-stick injuries) are treated as urgent and results are usually available within 1-2 working days. Please contact us to discuss the case before the sample is sent.
Chronic cases: assay run 1-2 times monthly, batched – please contact us if regarded as more urgent e.g possible reactivation

Worm Identification
1-2 working days but allow up to 5 days if clearing necessary

Microscopy of stained/unstained specimens
e.g hydatid, blood parasites, pus: 1-2 working days

Entomology-
This is variable depending upon specimen. The turnaround time is normally 2 - 5 working days but may take longer. If your request is urgent, we advise that you contact us before sending the sample to discuss.

5. Charges

For current scale of charges please contact the laboratory for information.

Should there be any change to this, all laboratories will be given, wherever possible, a minimum of 2 months advance notice.

E. RESULTS AND REPORTS

1. Written reports

Reports are printed and dispatched by Royal Mail each working day.

In most cases it can be assumed that the written report is final, however if further results are to follow, or if a repeat specimen is required, this will be clearly stated. Interim reports, where necessary, will normally be given by telephone and confirmed in a full and final written report.
Interpretation of results and comments on individual cases will be given where required.

Please contact the laboratory on 020 7927 2427 to obtain results or to arrange for copies of paper reports if not received.

2. Telephone reports

Results of urgent investigations, those which may aid immediate patient management, or any results specifically requested by the sending laboratory will be telephoned as soon as they become available.
The name and status of the person to whom results are given will be required for our records. The Clinical Laboratory Director will telephone to discuss results where clinical interpretation or advice is required.

The results of some investigations may be rapidly available and to aid the management of certain infections will be telephoned immediately. Examples are:

- Primary diagnosis of malaria by microscopy and/or immunochromatographic techniques and PCR
- *P. falciparum* or *P.knowlesi* where undiagnosed by the requesting laboratory
- *T.cruzi* PCR positive
- Trypanosomes in blood films
- Diagnosis of *E. histolytica* by microscopy and antigen–specific ELISA
- *Giardia intestinalis*
- Hydatid
- Babesia
- *T.solium*
- Any other pathogen where prompt initiation of treatment is considered necessary.

All telephoned reports, whether initiated by the reference laboratory or the requesting laboratory, will be confirmed with a written report.

Please contact the laboratory on 020 7927 2427 to obtain results.

3. Fax results

Should the submitting laboratory require, the reference laboratory can fax final reports to a secure fax line (safe-haven).

4. Archiving of reports & security of information

All reference laboratory copies of written reports (to which the original request form is attached) are held in secure, locked storage for a minimum period of 5 years.

All staff have a duty of patient confidentiality and as part of the induction process are aware that all patient-related information is confidential and all data is held in accordance with the Data Protection Act 2018 and GDPR. Staff should adhere to the School's Data Protection policy and related guidance. It is a mandatory requirement that all staff undertake the LSHTMs on-line training and guidance in security of information and GDPR.

5. Obtaining information and results

Staff are always available during laboratory opening hours to discuss results and to give advice and information.

Please contact the laboratory on 020 7927 2427 from where queries can be answered or referred to appropriate personnel.

Updates to the Handbook

Please note that this guidance is valid on the day that you print or download it. Always check for the latest version at www.parasite-referencelab.co.uk