



DIAGNOSTIC LABORATORY PARASITOLOGY

LABORATORY USER HANDBOOK

2016

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A. INTRODUCTION

1. The Laboratory and outline of services

The Diagnostic Parasitology Laboratory is a CPA accredited laboratory, 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 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 malaria and other blood parasites. We are also strongly committed to active research into malaria and its 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 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

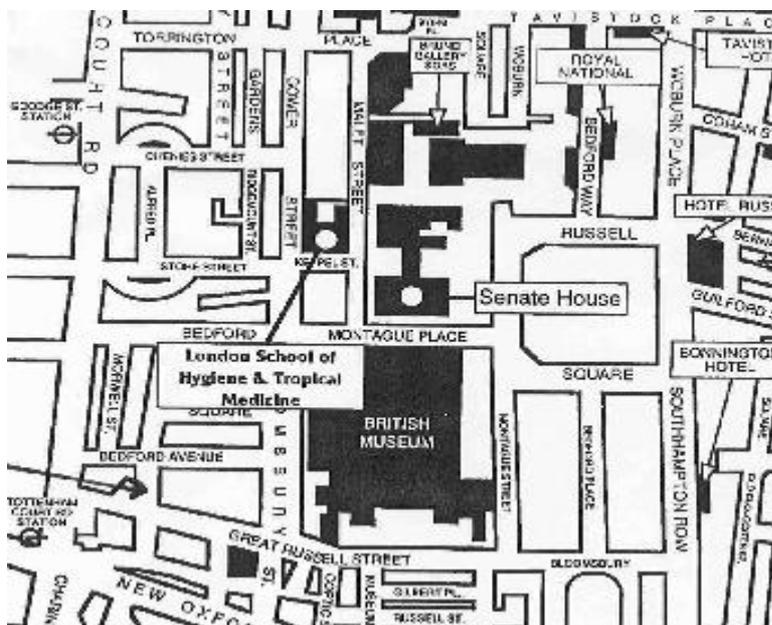
We offer foremost a reference facility and therefore do not provide an on-call service. The majority of specimens we receive will have had a preliminary diagnosis made by the sender or primary laboratory and so are usually non-urgent. The laboratory is usually closed on public holidays; when there is 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 of out of normal hours for malaria or African trypanosomiasis only, specimens should be referred to: -

The Department of Clinical Parasitology at the Hospital for Tropical Diseases (HTD), telephone number: 0845 155 5000 and ask for the duty doctor in Infectious & Tropical Diseases, who will liaise with the on-call Biomedical Scientist.
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 should report to the reception desk in the LSHTM entrance foyer, reception staff will then inform the laboratory of their arrival. It is advisable to make appointments in advance. 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	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	Paul Lansdell	020 7927 2427
	Senior BMS	Emma Victory	020 7927 2427
	BMS	Susan Ndimah	020 7927 2427
Medical Laboratory Assistants	MLA	Susan Passarelli	020 7927 2427
	MLA	Karen Osborne	020 7927 2427
	MLA	Helena Stone	020 7927 2427
Results/enquiries; (technical advice and forms, guidelines, kits, containers, isolation media, etc.)	Laboratory		020 7927 2427
Laboratory fax number			020 7637 0248
Medical Entomologist (Member of the LSHTM Faculty of Infectious & Tropical Diseases entomology staff, who provides expertise to diagnostic laboratory)		Cheryl Whitehorn	020 7927 2344

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, containers and transport media 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*
- Culture and isolation media for acanthamoebae

See also pages 17-39 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	<ol style="list-style-type: none"> 1. Full name OR other coded patient identifier 2. DOB &/or Hosp no./ Unit no./ NHS No. 	<ol style="list-style-type: none"> 1. Sending laboratory's ref no. 2. Date specimen taken 3. Nature of specimen inc. qualifying details e.g. right/left
REQUEST FORM	<ol style="list-style-type: none"> 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 	<ol style="list-style-type: none"> 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 *Echinococcus spp.* and ova/segments of *Taenia solium*.

For further advice on patient management, please contact the 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 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 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 prior to despatch to inform us of their impending arrival and priority status.**

Please package as described, and additionally mark clearly on the outside as 'URGENT'

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

The Department of Clinical Parasitology at the Hospital for Tropical Diseases (HTD), telephone number: 0845 155 5000 and ask for the duty doctor in Infectious & Tropical Diseases, who will liaise with the on-call Biomedical Scientist.

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 the Diagnostic Parasitology Laboratory out of normal working hours and **where no prior arrangement** with us has been made, will not be dealt with until 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

All parasitology serology is now performed at the Parasitology Reference Laboratory at the HTD. Please send samples directly to:

The Department of Clinical Parasitology, Hospital for Tropical Diseases, Mortimer Market, Capper Street, London, WC1E 6JB.

Tel. 0845 155 5000 ext. 75413. or direct 020 344 75413.

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:- >2 weeks at 2-8°C after receipt

Worms, segments, insects:- >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

Parasites requiring special methods and/or sampling techniques are listed separately; others are listed under general headings. All are listed in alphabetical order.

All parasitology serology is now performed at the Parasitology Reference Laboratory at the HTD.

Amoebiasis

Intestinal amoebiasis/ amoebic dysentery and invasive amoebiasis

Organisms investigated: *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 differentiation of
E. histolytica from *E. dispar* from faeces and culture

Specimens: faeces in plain container for concentration and culture

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 for microscopy and culture*

* Please telephone laboratory for advice before taking sample.

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

NB Comprehensive specimen collection and transport details are available upon request in a separate document but please observe the following:-

Material from a blade should either be directly inoculated onto a plate, or rinsed with a small volume of sterile saline/distilled water into a small sterile vial.

Please do not leave the blade in as it rusts and can have a detrimental effect on culture conditions.

We prefer not to receive needles and request that you flush out the eye of the needle with a small volume of sterile saline/distilled water into a small sterile vial.

*PCR assay currently under development; if this investigation is required, please telephone the laboratory to discuss

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 the Department of Clinical Parasitology, Hospital for Tropical Diseases, Mortimer Market, Capper Street, London, WC1E 6JB

Tel. 0845 155 5000 ext. 75414 or direct line 020 344 75414

For out of hours contact details see page 7

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 (or less suitable, formalin)

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

NB Due to the intermittent nature of oocyst excretion 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

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 exclude diagnosis.

BEWARE eggs are highly infectious and resistant to drying!

Filariasis

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 ml of 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.

for *Loa loa* take sample between 12 noon and 2 pm

for *W. bancrofti* & *B. malayi* take around midnight

skin snips for *O. volvulus* placed into physiological saline

Advice should be sought before taking skin snips.

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

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

NB Due to the intermittent nature of cyst shedding 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

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 - see page 7.

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 (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.

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

Specimens: faeces in plain container for adult worms, segments and concentration

SAF fixed faeces for trophozoites (especially suitable when a fresh sample is not practical and for fragile organisms e.g. *Dientamoeba fragilis*)

fresh faeces for trophozoites

NB It may be necessary to submit 3 samples collected on different days to confirm a diagnosis.

Leishmaniasis

Visceral and Cutaneous leishmaniasis

Causative organism *Leishmania* spp. (several species involved in both forms of the disease)

Diagnosis: microscopy of stained marrow/spleen/liver impression smears.

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

Specimens: This laboratory only performs microscopy of impression smears or tissue sections.

For a full investigation of cutaneous or visceral leishmaniasis please contact the Department of Clinical Parasitology at the Hospital for Tropical Diseases, telephone number 0845 155 5000 ext. 75414, direct line 020 344 75414 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

Of human importance are *Plasmodium falciparum*, *P. vivax*, *P. malariae* and *P. ovale* spp. There have been two reported cases in the UK of travel-acquired *P. knowlesi*.

Diagnosis: microscopical examination of thin and thick blood films for malaria parasites and for species identification using Giemsa and Field's stain

parasitaemia estimation to indicate severity of infection and effectiveness of treatment

immunochromatographic techniques for the detection of malaria antigen in blood

Real-time qPCR and nested PCR (gold standard) for malaria confirmation and species determination, when warranted

serology is no longer performed by this laboratory, but may be obtained from the Department of Clinical Parasitology at the Hospital for Tropical Diseases, see page 15

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

For example:-

Molecular markers for drug sensitivity in treatment / prophylaxis failure, these include:

Anti-folate resistance

Atovaquone proguanil resistance

ACT/artemisinin failure to clear

Discrimination of parasite species causing ovale malaria:

P. ovale curtisi and *P. ovale wallikeri*.

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.

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.**

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

Microsporidiosis

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

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

Specimens: faeces

urine

NB if histology is required on biopsy material please contact us for advice

Schistosomiasis

Bilharzia

Parasite: *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

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

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.

Strongyloidiasis

Causative organism *Strongyloides stercoralis*

Diagnosis: microscopical examination for larvae/adults
culture for larvae

Specimens: faeces
duodenal/jejunal aspirate

NB Please do not refrigerate specimen if culture is required

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 HTD, see page 15)

If histology is required on biopsy material please contact us for advice.

Trypanosomiasis - African

Sleeping sickness

Causative organisms *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 Dr on duty in Infectious & Tropical Diseases at HTD– see page 7..

Trypanosomiasis – South American

Chagas Disease

Causative organism *Trypanosoma cruzi*

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

PCR

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

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 the Hospital for Tropical Diseases, tel. 0845 155 5000 ext. 75413. or direct 020 344 75413 see p 15 for full details.

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

Before sending samples please discuss the case with the Dr on duty in Infectious & Tropical Diseases at HTD– see page 7..

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
 Whole worms and segments

3. Laboratory Schedule & Turnaround Times

Generally, specimen processing is begun on the day of receipt. Specimens that require microscopy only will often 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:- 1-3 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, ELISA/PCR, amoebic culture).

Acanthamoeba culture:-

Culture generally takes 5 – 7 working days; microscopy results, where applicable, available in the interim.

T.cruzi PCR

Suspected acute (neonate, travel): 1-2 working days, on demand

Chronic cases (unless deemed urgent e.g. reactivation): assay run monthly, batched

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, microsporidia 1-2 working days

Entomology-

This is variable depending upon specimen but normally 2 - 5 working days

4. 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 3 months advance notice.

E. RESULTS AND REPORTS

1. Written reports

Reports are printed and dispatched 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 reports.

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 member of staff who has undertaken the investigation will usually give the telephone report; the name and status of the person to whom results are given will be required for our records. The Laboratory Director will telephone to discuss results where 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* 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 patient information is handled securely in accordance with the Data Protection Act 1998.

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.