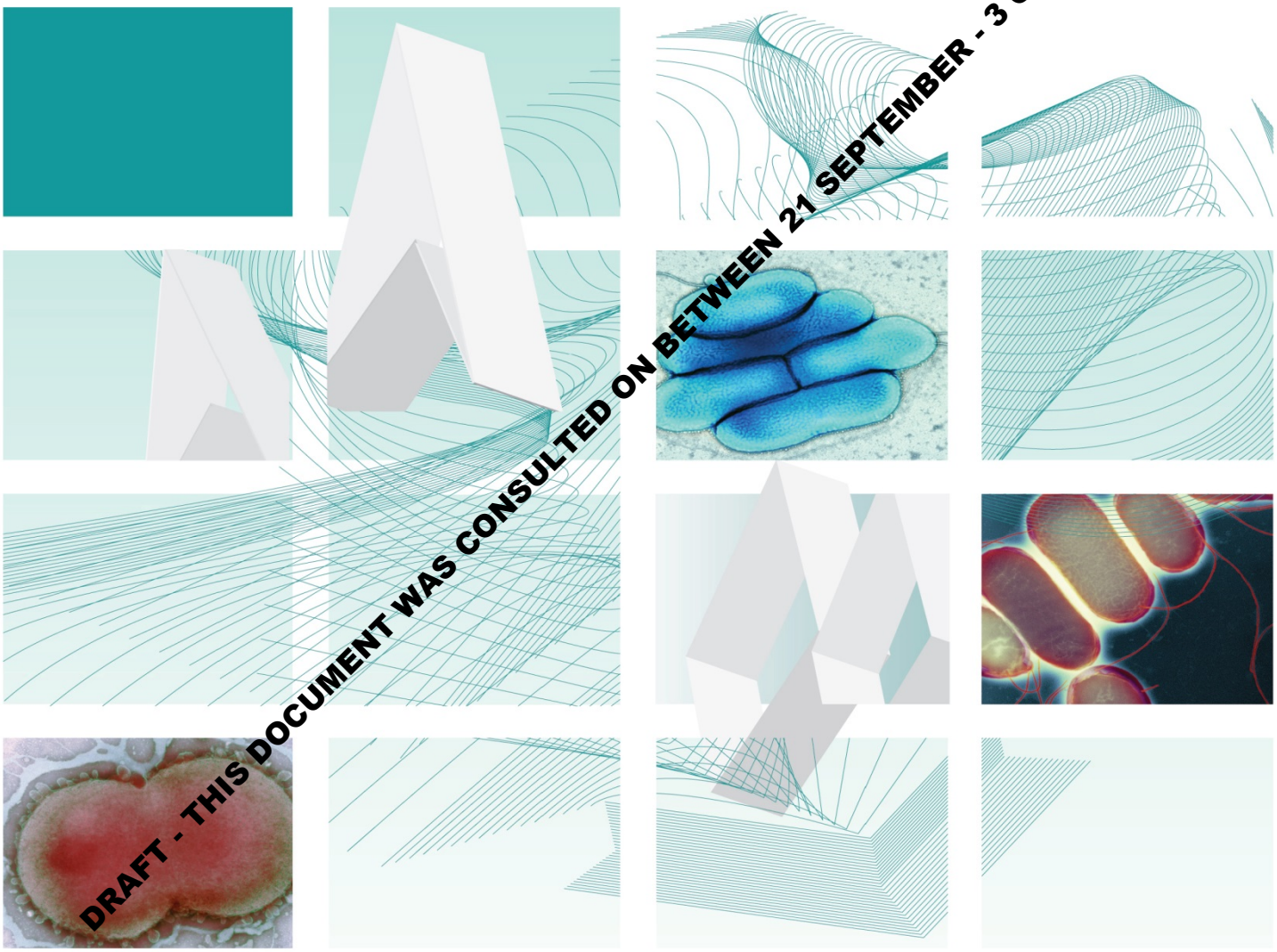
Public Health England

NHS

UK Standards for Microbiology Investigations

Investigation of specimens other than blood for parasites



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Amendment table

Each SMI method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from standards@phe.gov.uk.

New or revised documents should be controlled within the laboratory in accordance with the local quality management system.

Amendment no/date.	9/dd.mm.yy <tab+enter>
Issue no. discarded.	
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Section(s) involved	**Amendment**
Whole document.	Updated the scope of the document on the current molecular and traditional methods used for detection of parasites. Updated Technical limitations section. Updated safety considerations section. More diagrams added ion the appendices.

UK SMI[#]: scope and purpose

Users of SMIs

Primarily, SMIs are intended as a general resource for practising professionals operating in the field of laboratory medicine and infection specialties in the UK. SMIs also provide clinicians with information about the available test repertoire and the standard of laboratory services they should expect for the investigation of infection in their patients, as well as providing information that aids the electronic ordering of appropriate tests. The documents also provide commissioners of healthcare services with the appropriateness and standard of microbiology investigations they should be seeking as part of the clinical and public health care package for their population.

Background to SMIs

SMIs comprise a collection of recommended algorithms and procedures covering all stages of the investigative process in microbiology from the pre-analytical (clinical syndrome) stage to the analytical (laboratory testing) and post analytical (result interpretation and reporting) stages. Syndromic algorithms are supported by more detailed documents containing advice on the investigation of specific diseases and infections. Guidance notes cover the clinical background, differential diagnosis, and appropriate investigation of particular clinical conditions. Quality guidance notes describe laboratory processes which underpin quality, for example assay validation.

Standardisation of the diagnostic process through the application of SMIs helps to assure the equivalence of investigation strategies in different laboratories across the UK and is essential for public health surveillance, research and development activities.

Equal partnership working

SMIs are developed in equal partnership with PHE, NHS, Royal College of Pathologists and professional societies. The list of participating societies may be found at https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories. Inclusion of a logo in an SMI indicates participation of the society in equal partnership and support for the objectives and process of preparing SMIs. Nominees of professional societies are members of the Steering Committee and working groups which develop SMIs. The views of nominees cannot be rigorously representative of the members of their nominating organisations nor the corporate views of their organisations. Nominees act as a conduit for two way reporting and dialogue. Representative views are sought through the consultation process. SMIs are developed, reviewed and updated through a wide consultation process.

Quality assurance

NICE has accredited the process used by the SMI working groups to produce SMIs. The accreditation is applicable to all guidance produced since October 2009. The process for the development of SMIs is certified to ISO 9001:2008. SMIs represent a good standard of practice to which all clinical and public health microbiology

[#] Microbiology is used as a generic term to include the two GMC-recognised specialties of Medical Microbiology (which includes Bacteriology, Mycology and Parasitology) and Medical Virology.

laboratories in the UK are expected to work. SMIs are NICE accredited and represent neither minimum standards of practice nor the highest level of complex laboratory investigation possible. In using SMIs, laboratories should take account of local requirements and undertake additional investigations where appropriate. SMIs help laboratories to meet accreditation requirements by promoting high quality practices which are auditable. SMIs also provide a reference point for method development. The performance of SMIs depends on competent staff and appropriate quality reagents and equipment. Laboratories should ensure that all commercial and in-house tests have been validated and shown to be fit for purpose. Laboratories should participate in external quality assessment schemes and undertake relevant internal quality control procedures.

Patient and public involvement

The SMI working groups are committed to patient and public involvement in the development of SMIs. By involving the public, health professionals, scientists and voluntary organisations the resulting SMI will be robust and meet the needs of the user. An opportunity is given to members of the public to contribute to consultations through our open access website.

Information governance and equality

PHE is a Caldicott compliant organisation. It seeks to take every possible precaution to prevent unauthorised disclosure of patient details and to ensure that patient-related records are kept under secure conditions. The development of SMIs is subject to PHE Equality objectives https://www.gov.uk/government/organisations/public-health-england/about/equality-and-diversity.

The SMI working groups are committed to achieving the equality objectives by effective consultation with members of the public, partners, stakeholders and specialist interest groups.

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While every care has been taken in the preparation of SMIs, PHE and the partner organisations, shall, to the greatest extent possible under any applicable law, exclude liability for all losses, costs, claims, damages or expenses arising out of or connected with the use of an SMI or any information contained therein. If alterations are made by an end user to an SMI for local use, it must be made clear where in the document the alterations have been made and by whom such alterations have been made and also acknowledged that PHE and the partner organisations shall bear no liability for such alterations. For the further avoidance of doubt, as SMIs have been developed for application within the UK, any application outside the UK shall be at the user's risk.

The evidence base and microbial taxonomy for the SMI is as complete as possible at the date of issue. Any omissions and new material will be considered at the next review. These standards can only be superseded by revisions of the standard, legislative action, or by NICE accredited guidance.

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Scope of document

Type of specimen

Faeces, urine, sellotape slide/perianal swab, CSF, bile, pus from abscesses, duodenal/jejunal aspirates, tissues, hydatid cyst, sputum/ bronchoalveolar lavage, biopsies from colonoscopy or surgery

This SMI describes the detection and isolation of a range of parasites from a variety of clinical samples, excluding blood. It discusses the traditional and molecular methods of detection in detail.

This SMI should be used in conjunction with other SMIs.

Introduction

Although faecal specimens are the most commonly received samples for the detection of parasites, parasites may also be detected in a variety of other clinical specimens submitted to the laboratory. Clinical presentation, specimen preparation and organism identification of the parasites most commonly seen in the laboratories are described here, but others may be found. For completeness, uncommon species are also described.

Reference laboratories should be used to identify those parasites outside the laboratory's normal experience. Haematology, histopathology, and serology laboratories may also contribute significantly to the laboratory diagnosis of parasitic infections.

This introduction covers:

- protozoa
    - amoebae
    - flagellates/Ciliates
    - coccidia
- nematodes
- trematodes
- cestodes

Carriage rate

The carriage rate will vary among parasites and this will largely depend on:

- infecting species of the parasite
- geographic distribution
- seasons
- migration and travelling
- residents
- age

- poor hygiene
- previous exposure
- predisposing illness such as in immunocompromised patients eg AIDS

Protozoa[2]

Intestinal amoebae

Amoebae that may be isolated from the human gastrointestinal tract include *Entamoeba histolytica, Entamoeba dispar, Entamoeba moshkovskii, Entamoeba coli, Entamoeba hartmanni, Endolimax nana* and *Iodamoeba butschlii*. All except *E. histolytica* are usually non-pathogenic (see Appendices 9-11).

E. histolytica and *E. dispar* are morphologically indistinguishable by light microscopy. Of the two species, only *E. histolytica* is capable of causing invasive disease. When the diagnosis is made by light microscopy, cysts should be reported as *E. histolytica/E. dispar*[3]. *E. histolytica* may cause ulcerative and inflammatory lesions in the colon. It spreads to extraintestinal sites, most commonly the liver, where marked tissue destruction occurs, leading to abscess formation. Inflammation of the colon produces symptoms of dysentery which include lower abdominal pain, increased frequency of bowel movements and liquid stools. Infection can lead to perforation of the colon, toxic megacolon, amoeboma, and perianal ulceration[3].

Appropriate specimens include liquid aspirated or scraped from an area of inflamed bowel tissue or rectal scrape in dysentery, and freshly passed faeces for wet microscopy and formol-ether / ethyl acetate concentration. Motile trophozoites with ingested red blood cells found in stools, or trophozoites in tissue biopsies, both strongly suggest infection with *E. histolytica*[2]. Alternative diagnostic methods include antigen detection by enzyme immunoassay or DNA detection by PCR[2,4].

Free-living amoebae

Human infection with free-living amoebae is uncommon. Such protozoa include: *Acanthamoeba* species, *Naegleria fowleri* , *Balamuthia mandrillaris* and *Sappinia diploidea*[5]. Infections include central nervous system invasion, mostly in immunocompromised individuals and acanthamoeba keratitis, common in contact-lens wearers.

Primary amoebic meningoencephalitis (PAM) is caused by *N. fowleri*. It occurs in adults and children who have recently been swimming in infected warm water. Organisms gain access to the central nervous system by direct invasion through the nasal mucosae. There is no effective and reliable treatment available and the disease is rapidly fatal[5].

When patients present with purulent meningitis or meningoencephalitis and a history of recent exposure to fresh water, PAM should be considered. The peripheral white blood cell count may be low early on in the disease, but will increase in time. The cerebrospinal fluid (CSF) appearance is haemorrhagic, glucose is low or normal and protein is elevated.

Laboratory diagnosis may be made by examining a wet mount and a stained preparation of the CSF for amoebic trophozoites (Appendix 9 and 11).

Granulomatous amoebic encephalitis (GAE) is caused by *Acanthamoeba* species and *Balamuthia mandrillaris*. It is a chronic opportunistic infection, occurring more often in

immunosuppressed/AIDS patients; spreading haematogenously from pulmonary or skin lesions to the central nervous system and it is often fatal. Acanthamoeba keratitis is associated with the use of soft contact lenses and ocular trauma; if not treated promptly may lead to corneal ulceration, and eventually to blindness. However, *Acanthamoeba* species may be isolated from contact lens fluid from individuals with no signs or symptoms of disease (B 2 – Investigation of bacterial eye infections)[5].

GAE may be diagnosed by examining brain biopsy material. Acanthamoeba infection of the skin is frequently present with GAE: skin nodules or ulcers may be biopsied and examined by wet microscopy and a stained preparation (Appendix 9). Serological methods are also available.

Flagellates

Giardia duodenalis (synonymous with *Giardia intestinalis and Giardia lamblia)* [6]

This organism may cause waterborne outbreaks of diarrhoea and is primarily spread from person to person, or zoonotically, via the faecal-oral route[2]. Infection may present as self-limiting diarrhoea or a syndrome of chronic diarrhoea, steatorrhoea, malabsorption and weight loss. Symptoms include diarrhoea, abdominal cramps, bloating and flatulence. Vomiting, fever and tenesmus can also occur, but infection may also be asymptomatic[2].

Laboratory diagnosis for faeces is made by either antigen detection by enzyme immunoassay (EIA) followed by confirmation using microscopy/stains (especially where borderline positives and questionable negatives are obtained with this technique) or DNA detection by PCR[6]. Evidence suggests a doubling of detection rates using automated EIA [7]. Due to the variable shedding of organisms, several stool specimens should be examined. Ideally a total of three specimens should be taken 2-3 days apart.

Diagnosis could also be made by microscopic examination of faeces, duodenal or jejunal aspirates, and biopsies (see Appendix 11). Motile trophozoites can be seen in the direct examination of fresh faeces, duodenal and jejunal aspirates while cysts can be seen in a direct saline preparation or a formol-ether /ethyl acetate concentration of faeces.

Note: It should be noted it is not possible to identify trophozoites or cysts to species level by light microscopy.

Serological tests are available, although these are not reliable**.**

Dientamoeba fragilis[2]

The pathogenicity of *Dientamoeba fragilis* is debatable; however it has been documented in cases of non-invasive diarrhoea with fatigue. Unlike other protozoa it does not have a cystic stage, and is now regarded as amoebae/flagellate. The role of dientamoeba in patients with HIV and bowel disorders is unclear and more research is required[8].

The trophozoite stage is very difficult to detect in wet saline preparations, and will not be seen in formol-ether/ethyl acetate concentrations. They can be detected by staining the faecal sample with trichrome, Giemsa or Field stains (TP 39 - Staining procedures). Alternative methods including DNA detection by PCR have been developed[8].

Trichomonas vaginalis and *Pentatrichomonas hominis*

Both *Trichomonas vaginalis* and *Pentatrichomonas hominis* may be found in human infections. The majority of infections caused by *T. vaginalis* are sexually transmitted whereas *P. hominis* inhabits the large bowel and is usually regarded as a non-pathogen, although it may cause mild gastro-intestinal symptoms when present in large numbers (B 28 – Investigation of genital tract and associated specimens)[9].

Laboratory diagnosis is usually made by observing the motile trophozoites in the microscopic examination of a wet saline preparation. Preparations with stained acridine-orange may also be used, although this will involve fluorescence microscopy and consequential loss of immediacy.

Ciliates

Balantidium coli

B. coli is a ciliate parasitising numerous mammals including humans and pigs and is found worldwide. Humans are usually resistant to *B. coli* infection, but achlorhydria or poor nutrition may increase the risk of colonisation. Colonisation is often asymptomatic. Patients may develop intermittent watery diarrhoea or an acute dysenteric colitis with stools containing mucus and blood[2].

Rapidly motile, large trophozoites may be observed microscopically in fresh faeces (Appendix 10). In preserved samples, diagnosis is by microscopy of a wet preparation, as neither the trophozoites or the cysts stain clearly with iodine or permanent stains[1].

Coccidia

Cryptosporidium

Cryptosporidium species can cause profuse watery diarrhoea in humans[10]. Children are particularly vulnerable due to lack of acquired immunity and poor personal hygiene. Infection shows seasonal variation with peak incidence in the spring and autumn. Infection is a particular problem for patients who are severely immunocompromised[2]. The usual manifestation is severe, chronic diarrhoeal disease with signs of malabsorption, but other presentations include atypical gastrointestinal disease such as cholangitis, cholecystitis, pancreatitis and hepatitis. Respiratory tract disease has also been reported. Primary laboratory diagnosis is based on antigen detection by enzyme immunoassay followed by confirmation using microscopy/stains or DNA detection by PCR [11,12]. Specialist tests include sensitive immunofluorescence microscopy and PCR-based tests for species/genotype identification.

Cryptosporidium diagnosis may also be done by microscopic demonstration of oocysts in appropriate specimens, usually stool, with auramine-phenol stain, modified Ziehl-Neelsen stain (TP 39 - Staining procedures) or antibody techniques. The sensitivity of modified Ziehl-Neelsen microscopy for detecting cryptosporidia has been shown to be significantly less than for other tests[12-14].

Cyclospora

Cyclospora infection occurs in many countries and may be associated with drinking or bathing in contaminated water. Large outbreaks affecting travellers and foreign residents have being known to occur during the rainy season in South Asia and North America[15,16]. Foodborne outbreaks have been reported in Europe associated with the consumption of salad and vegetables[17,18]. Infection with *Cyclospora cayetanesis* also occurs in HIV-infected patients[19].

Symptoms of *Cyclospora* infection include watery diarrhoea with weight loss, nausea, vomiting and abdominal pain.

Laboratory diagnosis is by appearance of oocysts in wet or stained preparations. Concentration methods can be used and modified Ziehl-Neelsen may be used as a stain. *Cyclospora cayetanesis* stains poorly with auramine-phenol stain, but gives a characteristic blue autofluorescence at 340-360nm (see TP 39 - Staining procedures and Appendices 3 and 6).

Whole genome sequencing has been used to detect and subtype *Cyclospora cayetanensis* especially in outbreak investigations[20].

Cystoisospora belli (formerly *Isospora belli*)[2]

Cystoisospora belli infection is relatively uncommon in developed countries, but is endemic in some parts of the world. It is usually transmitted by the ingestion of contaminated food or water. In immunocompetent patients it may cause non-specific, self-limited watery diarrhoea with malaise, anorexia, abdominal cramps and weight loss whereas severe infection can occur in immunocompromised hosts causing severe diarrhoea with electrolyte disturbances and morbidity [21]. AIDS patients are particularly susceptible to *Cystoisospora belli* infection (this being particularly common in underdeveloped countries especially Africa and the Middle East where the incidence ranges from 0.2% to 20% in patients with AIDS[22].

Laboratory diagnosis is achieved by microscopy of stool samples or intestinal mucus. Formol-ether concentration of faeces is recommended; as with *Cyclospora* species, *Cystoisospora* autofluoresce at 340-360nm (see TP 39 - Staining procedures and Appendices 3 and 6). PCR assay has been developed to detect *Cystoisospora belli* in stool samples[23].

Sarcocystis

Infections with *Sarcocystis* species are zoonotic in origin. *Sarcocystis* species differ from other coccidia in that they require two hosts to complete their life cycle. Humans become the intermediate host after eating undercooked meat of the primary host which contains sarcocysts. Most infections of this type are asymptomatic, but symptoms such as abdominal pain, nausea, bloating and diarrhoea can be associated with presence of sporocysts in the faeces. Muscular sarcocystosis results when humans become infected by eating undercooked meat or infected cats. Symptoms then include muscle soreness or swelling.

Laboratory diagnostic techniques are the same as for *Cystoisospora* species, with mature sporocysts being present in the faeces from nine days post-infection. They cannot easily be distinguished from *Cystoisospora* species microscopically; therefore identification should be performed by a Reference Laboratory.

Toxoplasma

Toxoplasma gondii infection is asymptomatic in most immunocompetent patients; however symptoms of pyrexia, malaise, lymphadenopathy, encephalitis and myalgia may be experienced. Ocular disease also occurs. Infections may be primary or reactivations. Reactivation of latent infection occurs in patients who become severely immunocompromised. Foetal infection may result from an acute maternal infection during pregnancy. For more information on investigation of *Toxoplasma* in pregnancy, it is advised that the appropriate specialist Reference Laboratory should be contacted.

Laboratory diagnostic procedures include direct microscopic observation of *Toxoplasma gondii* tachyzoites in bronchoalveolar lavage fluid (BAL) stained with Giemsa stain and PCR. PCR has been found to provide better results especially in laboratories where there is less microbiological experience[24].

Alternative methods include serological tests, culture and histology. Serological tests for *T. gondii* give poor results in immunocompromised patients.

Microsporidia

There are over 150 genera and almost 1500 species of 'microsporidia'. The genera that are implicated in human diseases are *Anncaliia* (formerly *Brachiola*), *Nosema, Enterocytozoon, Encephalitozoon, Microsporidium, Pleistophora*, *Trachipleistophora Tubulinosema* and *Vittaforma* [25]. There are at least 15 microsporidian species that have been identified as human pathogens: *Anncaliia algerae, Anncaliia connori, Anncaliia vesicularum, Encephalitozoon cuniculi, Encephalitozoon hellem*, *Encephalitozoon intestinalis*, *Enterocytozoon bieneusi, Microsporidium ceylonensis*, *Microsporidium africanum*, *Nosema ocularum*, *Pleistophora* species, *Trachipleistophora hominis*, *Trachipleistophora anthropophthera*, *Vittaforma corneae*, and *Tubulinosema acridophagus*. These organisms are obligate intracellular organisms occurring in body fluids, tissues and the gastrointestinal tract. Originally classified as a parasite, these organisms are now known as fungi[26].

Microsporidiosis presents a particular problem in HIV-infected patients[2]. These and other immunocompromised patients are frequently infected with opportunistic parasites that do not usually produce symptoms in immunocompetent individuals[27]. Chronic diarrhoea is a major clinical feature in HIV infection and is a leading cause of morbidity and mortality[28]. Microsporidial keratoconjunctivitis has recently been recognised in patients with AIDS.

Histological stains, immunological techniques and electron microscopy may be used to identify these organisms in urine, sputum, bronchoalveolar lavages, bile, duodenal aspirates, faeces, tissues and scrapes from the cornea and conjunctiva. Microsporidia can be stained with modified trichrome stain (see TP 39 – Staining procedures Appendices 3 and 6).

Molecular identification of *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis* (formerly *Septata intestinalis*), *Encephalitozoon hellem* and *Encephalitozoon cuniculi* can be done using species-specific PCR assays that are commercially available.

Protozoa of uncertain taxonomic status

Blastocystis hominis

Previously described as a flagellate, a yeast, a coccidian, and an amoeba, this organism is part of a diverse group of organisms called stramenopiles[29]. The importance of *B. hominis* as a human pathogen is debatable[30]. Large numbers present in stools (more than five per high power field) may be associated with symptoms of nausea, abdominal pain, anorexia, flatus and diarrhoea[2].

Laboratory diagnosis by microscopy of primary wet smears (after formol-ether /ethyl acetate concentration), and smears stained with Giemsa and Field stain or differential staining kits may be used to maximise recovery of cyst-like forms[31] (see TP 39 – Staining procedures). Alternative methods include culture, PCR, ELISA and serological tests.

Culture techniques are, most likely, more sensitive than direct smears. Amplification of *Blastocystis*-specific DNA by polymerase chain reaction directly from stool has been reported and permits identification of the *Blastocystis* subtypes. However, serologic testing is not currently used for diagnosis of infection by this organism[32].

*Pneumocystis jirovecii (*formerly *Pneumocystis carinii)*[33]

Pneumocystis jirovecii was thought to be a protozoan, but has been reclassified and is now considered to be a parasitic fungus based on nucleic acid and biochemical analysis[34]. It is an extracellular organism that causes interstitial plasma cell pneumonia found in humans, and generally causes disease in immunocompromised and immunosuppressed patients and in premature, sick and malnourished infants[35]. The symptoms of *Pneumocystis* pneumonia include dyspnea, non-productive cough, and fever.

P. jirovecii pneumonia is a common opportunistic infection of patients with AIDS (for more information, see B 57 – Investigation of bronchoalveolar lavage, sputum and associated specimens).

Laboratory diagnosis is made by staining specimens from induced sputum or bronchoalveolar lavage (BAL) for the microscopic identification of *P. jirovecii* trophozoites and cysts. This can be performed using Giemsa stain, a silver stain, or more specifically by direct or indirect immunofluorescent antigen detection (see TP 39 – Staining procedures). Molecular methods (PCR assays) have been shown to be extremely useful for the detection of *P. jirovecii* in clinical specimens[36,37].

Nematodes (roundworms)

The Nematodes belong to the phylum 'Nematoda'. There are over 25,000 described species and of which many are parasitic. Laboratory diagnosis of nematode infestations relies mainly on the identification of eggs or larvae passed in the faeces. A wet preparation of faeces in saline following a formol-ether/ethyl acetate concentration permits the demonstration of ova microscopically (Appendix 5). The macroscopic presence of worms in the sample is also of diagnostic value.

Charcot-Leyden crystals in the stool can be seen with a wide range of parasitic bowel infections including amoebic dysentery. These crystals are hallmarks of eosinophil involvement in certain tissue reactions and are the result of eosinophil breakdown. Hexagonal bipyramidal crystals of Charcot-Leyden crystal protein can be seen by light microscopy or by fluorescence microscopy as bright yellow green fluorescing needles[38,39].

The occurrence of tissue and peripheral blood eosinophilia in travellers returning from long-term residence in, or a visit to, developing countries, or in immigrants from tropical areas, suggests the possibility of an infection with a helminth[40]. Some protozoal infections also cause eosinophilia. The greatest numbers of eosinophils in tissue and blood occur when the association of a parasite and host tissue is the closest, for example with migrating larvae or after their extended retention in tissue. Examples include trichinosis, visceral larval migrans, ascaris pneumonia, strongyloidiasis, filariasis and acute schistosomiasis. Organisms, such as tapeworms, that remain in the bowel, and do not invade the intestinal mucosa, cause little or no eosinophilia.

Enterobius vermicularis

Also known as the thread or pin worm, it causes perianal and perineal pruritus, mainly in children[41]. Migrations of the parasite enable it to be found in the appendix, salpinges and in ulcerative lesions in the small or large bowel, but the causal relationship to clinical pathology is uncertain (Appendix 5).

Laboratory diagnosis is usually made by microscopy of a Sellotape preparation and/or perianal swab sample.

Trichuris trichiura

Also known as the whip worm, the infection can often be asymptomatic[41]. The loss of blood caused by adult worms embedding their heads in the intestinal mucosa is usually negligible, but heavy infections can cause a mild anaemia, bloody diarrhoea, dehydration, growth retardation and rectal prolapse (see Appendix 5). Diagnosis is by detection of eggs in faeces.

Laboratory diagnosis is by identifying eggs in the faeces. In heavy infections, the stools are frequently mucoid and contain Charcot-Leyden crystals while in light infections, concentration methods are required for diagnosis.

Ascaris lumbricoides[40,41]

This nematode infection is usually asymptomatic. However the worms can cause serious pulmonary disease and obstruct the biliary and intestinal tracts. Larvae migrate through the lungs and may cause peripheral blood eosinophilia and symptoms associated with pulmonary infiltration. Third stage larvae may be seen in sputum. In children with heavy infections, the mass of worms can obstruct the lumen of the small bowel. This causes abdominal distension, vomiting and cramps. It may also invade the biliary duct and cause epigastric pain, nausea and vomiting (Appendix 5).

Laboratory definitive diagnosis is usually made by microscopic examination of a faecal smear. Concentration techniques involving floatation or sedimentation of eggs may also be used. Diagnosis can also be made visually by the presence of the adult stage of *Ascaris lumbricoides* worm in freshly-passed faeces.

Hookworms

The two species of hookworm that cause human infection are *Ancylostoma duodenale* and *Necator americanus*[41]. Larvae penetrate the skin causing intense pruritus, erythema and papular, vesicular rash. Larvae migrate through the lungs and may cause respiratory symptoms and eosinophilia in the sputum and peripheral blood. *A. duodenale* can cause infection by the oral route whereas *N. americanus* cannot. Symptoms include anaemia, chronic protein deficiency, abdominal pain, diarrhoea, weight loss and malabsorption. Hookworm eggs may be seen in faecal samples, but it is not possible to distinguish between species without hatching the eggs (see Appendices 5 and 8).

In older faecal samples infected with hookworm, first-stage larvae may be seen which must be differentiated from those of S*trongyloides* (Appendix 8).

Diagnosis is made by direct microscopic examination showing eggs in stool specimens. It should also be noted that the two species (ie both *Ancylostoma duodenale* and *Necator americanus*) cannot be distinguished on the basis of their eggs but a formol-ether/ethyl acetate concentration method should be used in most cases

Trichostrongylus species

They are also known as hairworms. These nematodes are distributed worldwide, but are rarely seen in Europeans. They may cause disease in humans and are associated with rural areas where herbivorous animals are raised. Infections in humans occur through ingestion of infective larvae from contaminated water or vegetables. Symptoms include abdominal pain, weight loss, diarrhoea, nausea, flatulence and generalised fatigue. Eosinophilia is seen frequently in symptomatic patients. Diagnosis is made by direct microscopic examination showing eggs in stool specimens.

Strongyloides stercoralis

Strongyloides stercoralis invade the intestinal mucosa and deposit thin-walled eggs which hatch to rhabditiform larvae[40]. These larvae may be passed in the faeces or develop within the lumen of the bowel to infective larvae that can autoinfect the host. It may cause an overwhelming autoinfection in immunocompromised patients[41]. Pulmonary symptoms resemble those seen in hookworm infections. Other symptoms include burning or colicky abdominal pain, diarrhoea and passage of mucus, nausea, vomiting, weight loss, and malabsorption. Patients may also develop a generalised or localised urticarial rash beginning perianally and extending to the buttocks, abdomen and thighs.

First stage larvae (rhabditiform) are usually seen in faeces and eggs are only seen when diarrhoea is severe (see Appendix 8)[42]. Third stage larvae (filariform) can be seen in the sputum in cases of autoinfection. Direct smear or concentration methods of stool examination are useful for diagnosis of strongyloidiasis. However, serological tests are a valuable aid in diagnosis.

Unusual nematode infections

Laboratory diagnosis of the following diverse infections may be beyond the scope of most routine diagnostic laboratories. As many of these infections are rare and only occur in tropical countries, it is recommended that samples from patients be dealt with by a reference laboratory:

Trichinella species are ingested in raw or undercooked meat[40,43]. Most infections are asymptomatic, but large numbers of adult worms in the intestines can cause diarrhoea, abdominal discomfort and vomiting. Larvae burrow into skeletal muscles and cause fever, periorbital oedema and myositis with pain and swelling. Infection is confirmed by serology or a skin test for *Trichinella* sp. Muscle biopsy is usually unnecessary.

Visceral larval migrans (VLM) – This is also called Toxocariasis. Larvae migrate from the intestine to the liver, lung and trachea[40,44]. The most frequent agent is *Toxocara canis*. *Toxocara* species and other helminths (*Ascaris lumbricoides, Gnathostoma spinigerum*) may also be associated with the syndrome. VLM occurs mainly in children under six years of age. Most infections are asymptomatic, however patients may present with cough, fever, wheezing and hepatomegaly. Rarely, larvae may localise in the eye. Eosinophilia accompanied by leucocytosis is suggestive of VLM. Serological tests are available.

Ocular larval migrans (OLM) is the invasion of the eye by *T. canis* (and less frequently *T. cati*) larvae which become trapped in the eye and result in an eosinophilic inflammatory mass[44].

Cutaneous larva migrans, or creeping eruption, is commonly caused by *Ancylostoma braziliense*[44]. It presents as serpiginous, reddened, elevated, pruritic skin lesions. Other parasites such as *S. stercoralis* may also cause it.

Larvae of *Anisakis* species (associated with eating raw fish eg sushi), *Phocanema* species and other genera, may penetrate the stomach and small intestine and cause abdominal symptoms which mimic appendicitis[44].

Laboratory diagnosis may be made by endoscopy, radiographic studies, or pathological examination of tissues. Serological tests are helpful in the diagnosis of Toxocariasis and Trichinellosis.

Angiostrongylus

Angiostrongylus cantonensis larvae can invade the brain and cause meningitis associated with eosinophilic pleocytosis in the CSF and peripheral eosinophilia[44]. The larvae may rarely be seen in the CSF. *Gnathostoma spinigerum* may cause a similar illness. Humans become infected through ingestion of larvae in raw or undercooked snails, or contaminated water and vegetables. Abdominal angiostrongyliasis is caused by *A. costaricensis*[44,45]. The larvae penetrate and develop in the lower small bowel and colon.

Laboratory diagnosis may be made by examining biopsy specimens.

Capillaria and *Paracapillaria*[35,44]

Capillariasis is a parasitic infection caused by new nematodes species namely, *Paracapillaria philippinensis* (previously known as *Capillaria philippinensis*) [46] and *Capillaria hepatica* (also known as *Calodium hepaticum*). *P. philippinensis* may present as diarrhoea, vomiting, weight loss and malabsorption, while *C. hepatica* can cause acute/subacute hepatitis with peripheral eosinophilia and has similar symptoms as the former including ascites and hepatolithiasis. The main source of acquiring intestinal capillariasis is by eating of raw fresh water fish. This infection is seen in raw fish eating areas like the Philippines[47].

For the laboratory diagnosis of this infection, examination of faecal material by formol-ether/ ethyl acetate concentration or biopsy material is required. Alternative methods include immunodiagnosis which may be a supplementary diagnostic tool which helps to detect *P. philippinensis* infection[47] as well as PCR which has been used successfully in the rapid diagnosis of *P. philippinensis,* thereby avoiding the delay in management and possible complications[48].

Dracunculus

Dracunculiasis (Guinea worm disease) is caused by the nematode parasite *Dracunculus medinensis* (known commonly as the Guinea worm). This infection is characterised by a chronic cutaneous ulcer from which the worm protrudes[43]. Some patients have a generalised reaction with urticaria, nausea, vomiting, diarrhoea and dyspnea. Painful ulcers develop which discharge fluid containing larvae. This may be examined microscopically to confirm the diagnosis.

Onchocerca volvulus

Onchocerciasis (known as river blindness) is caused by the filarial parasite *Onchocerca volvulus*. This parasite is transmitted by blackflies[43]. It causes an itchy dermatitis, subcutaneous nodules, keratitis and chorioretinitis. Laboratory diagnosis

may be made by microscopic detection of microfilariae in skin snips, indirect detection of microfilariae with the diethylcarbamazine patch test, detection of antibodies to onchocercal antigens, or detection of *O. volvulus* DNA in skin snips by PCR[49].

Adult worms can be found in nodule biopsy specimens (Appendix 13).

Mansonella streptocerca

This particular species is transmitted by midges and blackflies[43]. Other species are generally found in blood. *Mansonella streptocerca* is usually diagnosed by finding microfilariae in skin snips.

Dirofilaria immitis[44]

D. immitis causes lung nodules or subcutaneous abscess in humans. Migrating filariae die and cause local vasculitis which leads to pulmonary infarcts. Other species may cause subcutaneous masses. Laboratory diagnosis may be made by examining biopsies.

Trematodes (flukes)[50]

Generally, the laboratory diagnosis of trematodes is achieved by microscopic identification of eggs passed in the faeces; exceptions to this will be listed. Blood samples may be required especially when the parasite burden is low and stools and urine samples may be negative. A wet preparation of the sample in saline followed by a formol-ether /ethyl acetate concentration permits the demonstration of ova microscopically.

Blood flukes

Schistosoma

There are five species of *Schistosoma*: *Schistosoma haematobium, Schistosoma intercalatum, Schistosoma japonicum, Schistosoma mansoni,* and *Schistosoma mekongi.* They may cause schistosomiasis[51]. The adult worms inhabit the portal and mesenteric blood vessels (except for *S. haematobium* which inhabits the vesical venous plexus). Major disease syndromes include dermatitis, Katayama fever and chronic fibro-obstructive sequelae. These syndromes coincide with and are related to three different stages of development of the parasites in the host: cercariae; mature worms; and eggs. Penetrating cercariae cause a papular pruritic itch called swimmer's itch or schistosome dermatitis. When worms have matured and begin depositing eggs, Katayama fever or acute schistosomiasis develops. Some eggs remain in the body of the host. These can lead to granuloma formation and tissue damage which may obstruct portal blood flow to the liver and pulmonary blood flow to the lungs, as well as urine flow through the ureters and bladder. Haematuria is the most common presentation of *S. haematobium* infection[40]. Chronic infection with *S. haematobium* can lead to bladder cancer[45].

Laboratory diagnosis is made by demonstrating eggs in the faeces (urine or semen for *S. haematobium*). Rectal snips or biopsies may also be examined. Serological tests can be of value when eggs cannot be found in clinical samples.

Liver flukes

Opisthorchis sinensis (formerly known as *Clonorchis sinensis*)

Also known as the Chinese liver fluke. Clonorchiasis is an infection caused by *Opisthorchis sinensis*. These may cause localised obstruction of the bile ducts and thickening of the walls in heavy infections, as well as cholangitis and cholangiohepatitis[51].

The standard diagnostic method is microscopic examination of faeces / duodenal aspirate thereby identifying eggs. Alternative methods include ELISA, which has become the most important method. Detecting DNAs from eggs in faeces can be done using PCR and LAMP assays, which are highly sensitive and specific. Imaging diagnosis is also to be very useful and is now widely used[52].

Opisthorchis viverrini and Opisthorchis felineus

Also known as Southeast Asian liver fluke and cat liver fluke respectively.These liver fluke parasites generally cause the infection, Opisthorchiasis and this is acquired by humans ingesting raw or undercooked infected fish which contain metacercariae. *O. viverrini*

Symptoms caused include abdominal pain, diarrhoea or constipation. Chronic symptoms include obstruction of the biliary tract, inflammation and fibrosis of the biliary tract, liver abscesses, pancreatitis, and suppurative cholangitis. In rare cases, they cause liver cholangiocarcinoma[50].

Diagnosis is as shown above in *Opisthorchis sinensis*.

Fasciola hepatica

Infestation has two distinct clinical phases corresponding to a hepatic migratory phase of the life cycle and to the presence of worms in their final habitat, the bile duct[51]. The early phase is characterised by an enlarged liver with fever and pain in the upper quadrant with obstruction of the bile duct and biliary cirrhosis.

Ova may be demonstrated in the faeces or the bile. Serological tests are also available.

Intestinal flukes

These include *Fasciolopsis buski, Heterophyes heterophyes, Nanophyetus salmincola, Metagonimus yokogawai* and *Echinostoma* species[44,51]. Most *F. buski* infections are asymptomatic. Heavy infections can cause diarrhoea, abdominal pain and malabsorption. *H. heterophyes* causes abdominal pain and diarrhoea. *Echinostoma* species infection is rare but has been documented. *N. salmincola* causes diarrhoea, abdominal pain, bloating and eosinophilia.

Ova and parasites may be demonstrated in the faeces.

Lung flukes

Paragonimus species

There are almost 50 species and subspecies of *Paragonimus*, of which over 13 of these are known to infect humans causing the condition, “paragonimiasis”. The most common is *Paragonimus westermani*, also known as the ‘oriental lung fluke’. Humans get infected when they eat uncooked or undercooked seafood such as freshwater

crabs or crayfish that contain the parasites. They encapsulate within the lung parenchyma, usually close to the bronchioles[51]. Eggs are deposited which pass into the bronchioles and are coughed up. These may then be detected in the sputum or, if they are swallowed, in the faeces. Patients develop eosinophilia and experience chest complaints; they may cough up brownish sputum and have intermittent haemoptyses. This leads on to chronic bronchitis or bronchiectasis with profuse expectoration and pleuritic chest pain.

Laboratory diagnosis may be made microscopically by demonstrating the presence of eggs (ova) in the sputum or faeces. Other alternative tests include EIA and serology tests, which has been useful for diagnosis in early infections [53].

Cestodes (tapeworms)

Generally, the laboratory diagnosis of cestodes is achieved by microscopic identification of eggs passed in the faeces. Exceptions to this will be listed. A wet preparation of sample in saline followed by a formol-ether concentration permits the demonstration of ova microscopically. The macroscopic presence of proglottids in the sample is also of diagnostic value (Appendix 5 and 7).

In humans, cestode infestations occur in one of two forms: mature tapeworms within the gastrointestinal tract, or as one or more larval cysts (called hydatidosis, cysticercosis, coenurosis and sparganosis) embedded in the liver, lung, muscle, brain, eye or other tissues.

Diphyllobothrium latum

Also known as the fish tapeworm, it is associated with cold, clear lakes found in Scandinavia, northern Europe, northern Japan, Canada, Alaska and North America. It causes a parasitic infection called ‘diphyllobothriasis’ in humans which is acquired by eating raw fish infected with the parasite. Infestations are usually asymptomatic, however when the parasite reaches a large size it may cause mechanical obstruction of the bowel resulting in diarrhoea and abdominal pain. Prolonged or heavy infections cause vitamin B12 deficiency. In some cases it can lead to neurological symptoms[54].

Hymenolepis nana

Hymenolepis nana is the smallest of the tapeworms that infect humans. It is also known as the ‘dwarf’ tapeworm due to its particularly small size. It is the most common tapeworm infection of humans worldwide[54]. This infection may cause mild abdominal discomfort, irritability, anorexia and diarrhoea. Although humans may acquire infection by accidental ingestion of infected beetles (often found in dry cereals), direct infection is more common and usually occurs in familial and institutional settings where hygiene is poor. *Hymenolepsis diminuta*, primarily a parasite of rats, is an occasional human parasite via the ingestion of beetles in cereals. *Dipylidium caninum* is commonly found in dogs and cats. Children in particular may become infected through close contact with the animals and their fleas.

Diagnosis can be achieved by recovery and identification of the characteristic ova in a formol-ether concentrate of faeces. Adult worms and proglottids are rarely seen in stool samples.

Taenia saginata

Also known as the beef tapeworm. It is more common in developing countries where hygiene is very poor. Humans become infected when raw or undercooked meat is

ingested. They cause abdominal discomfort and patients experience proglottid migration from the anus[54].

Taenia solium

Also known as the pork tapeworm. The adult worms cause minimal symptoms, but the larval cysts cause local inflammation[54]. Migration of these into the central nervous system causes seizures, hydrocephalus and arachnoiditis.

Cysticercosis (this is tissue infection with cysticerci of *T. solium*) can develop in man by autoinfection from the adult worm. Involvement of the central nervous system is called neurocysticercosis[54].

Diagnosis can be achieved by serology, immunodiagnostic methods or biopsy[55].

Taenia multiceps, *Taenia serialis* and *Taenia brauni*

Taenia multiceps and *Taenia serialis* are more commonly seen in Europe and USA while *Taenia brauni* is common in Africa. These species cause a cyst infection called coenurosis, and usually develop into tapeworms in dogs[54]. Symptomatic disease in humans involves the eye, central nervous system, sub-cutaneous and muscular tissues. Laboratory diagnosis is by microscopic examination of appropriate material for the presence of protoscolices (similar to those of *T. solium*).

Spirometra mansonoides

The tissue infection, sparganosis is usually caused by *Spirometra mansonoides*. It is a tissue infection with plerocercoid cysts of several different cestode species, and symptoms include local inflammation of the skin at the site of invasion[54]. Tissue injury may be severe, particularly in the eye, because some forms of the parasite spread to other areas of the body.

Laboratory diagnosis is by histological techniques only.

Echinococcus granulosus, Echinococcus vogeli and Echinococcus multilocularis

These tapeworms cause the parasitic disease called Echinococcosis which has two forms: hydatid or unilocular cyst disease caused by *Echinococcus granulosus* or *Echinococcus vogeli*, and alveolar cyst disease caused by *Echinococcus multilocularis*[54]. Eggs hatch to form oncospheres that penetrate the gut mucosa and enter the circulation. These encyst in the host viscera and develop to form mature larval cysts. Symptoms occur due to the mechanical effects of enlarging cysts in confined spaces.

Laboratory diagnosis can be made by

1. Imaging and serodiagnosis are the mainstay of diagnosis. Serological tests include Enzyme linked immunosorbent assay (ELISA), an indirect haemagglutination test and a complement fixation test

2. Microscopic examination of the cyst fluid to look for the characteristic protoscolices which can be either invaginated or evaginated. The cyst fluid will also reveal free hooklets

Note: Diagnostic aspiration is contraindicated.

3. Histological examination of the cyst wall after surgical removal

Occasionally pulmonary cysts containing *E. granulosus* may rupture and intact protoscolices and hooks can be coughed up in the sputum, and seen in microscopic preparations. Serological tests are also available.

Technical information/limitations

Limitations of UK SMIs

The recommendations made in UK SMIs are based on evidence (eg sensitivity and specificity) where available, expert opinion and pragmatism, with consideration also being given to available resources. Laboratories should take account of local requirements and undertake additional investigations where appropriate. Prior to use, laboratories should ensure that all commercial and in-house tests have been validated and are fit for purpose.

Specimen containers[56,57]

SMIs use the term, “CE marked leak proof container,” to describe containers bearing the CE marking used for the collection and transport of clinical specimens. The requirements for specimen containers are given in the EU in vitro Diagnostic Medical Devices Directive (98/79/EC Annex 1 B 2.1) which states: “The design must allow easy handling and, where necessary, reduce as far as possible contamination of, and leakage from, the device during use and, in the case of specimen receptacles, the risk of contamination of the specimen. The manufacturing processes must be appropriate for these purposes.”

Rapid diagnostic tests

A variety of rapid identification methods of varying sensitivities and specificities are available. These techniques may have potential advantages/disadvantages and should therefore be evaluated and validated prior to use. Molecular methods (eg multiplex PCR) and enzyme immunoassays (EIA) may perform better than conventional methods, and should therefore be considered for use where available, following validation to ensure appropriate clinical interpretation[13,58].

Commercial test kits

The quality of reagents in commercially available test kits may be variable or deteriorate under storage conditions; for that reason in-house as well as external controls are necessary to determine whether the kit is fit for purpose. Laboratories should use test kits according to manufacturers’ instructions.

Concentration methods

Faecal specimens should be submitted fresh, without formalin, as concentration is not required routinely prior to staining. Where concentration is deemed necessary, modified methods should be used to minimise oocyst losses and prevent interference with the adhesion of oocysts to slides and with staining.

Toxoplasma testing

Several commercial kits for *Toxoplasma* serologic testing are available. However, the sensitivity and specificity of these kits may vary widely from one commercial brand to another. This is of concern because serology results can influence decisions on continuation or termination of pregnancies.

Enzyme immunoassays (EIA)

Specimens (faeces) to be used for EIA or other rapid assays should not be concentrated prior to testing because antigens are lost during the procedure such as in diagnosis of *Giardia duodenalis* and *Cryptosporidium* species. EIA tests require preferably, the use of fresh or frozen stool specimens[11]. However, there are now some commercially available test kits that use preserved faecal specimens for detection of antigens.

Preservation of specimens

Preservation in 10% formalin is necessary when faeces cannot be examined within the prescribed time interval. These specimens can be stored for several months. However it should be noted that it has its drawbacks which include its interference with PCR especially after extended fixation time, its inadequate preservation of morphology of the protozoan trophozoites as well as its non-suitability for some smears stained with trichrome stain [59].

Problems with identification

There are many microscopic artefacts that can be found in faeces which may be confused with trophozoites, cysts or eggs. See Appendix 4 for more information.

1 Safety considerations[56,57,60-74]

1.1 Specimen collection, transport and storage[56,57,60-63]

Use aseptic technique.

Collect specimens in appropriate CE marked leak proof containers and transport in sealed plastic bags, with the exception of Sellotape slide/perianal swab for *E. vermicularis* ova which should be transported in a sealed plastic bag[1].

In the case of CSF, any inoculated plates should also be transported in a robust, CE marked leak proof container.

Compliance with postal, transport and storage regulations is essential.

1.2 Specimen processing[56,57,60-74]

All sample types

Containment Level 2 unless otherwise specified (see below).

Laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet[66].

Disposable gloves should be worn for all parasitology investigations.

Refer to current guidance on the safe handling of all organisms documented in this SMI.

Faeces

Containment Level 3 is not required for investigation of *Echinococcus* species and *Taenia solium* but a Class 1 microbiological safety cabinet is essential.

Unpreserved tapeworm proglottids submitted to the laboratory for identification are hazardous due to the possibility of accidental infection, and the possibility of cysticercosis[75].

Care should be taken even with faecal specimens that have been fixed in preservatives as they could potentially be infectious. Fixation in formalin can take days to weeks to kill some parasite cysts or oocysts, eg eggs of *Ascaris lumbricoides* may continue to develop and are infectious even when preserved in formalin.

Specimens for microscopy only should be prepared in 10% (v/v) formalin in water (this would not be suitable for the examination of trophozoites).

For formol-ether concentrations, ethyl acetate should be used in place of diethyl ether for safety reasons[35]. Procedures should be carried out in a well-ventilated area with no naked flames.

CSF

Containment Level 3 and a safety cabinet are required for the investigation for *Naegleria fowleri.*

Tissues, biopsies, hydatid cyst and pus from abscesses

Process specimens from the lung and pleural cavity, and hydatid cysts, in a Class 1 exhaust protective cabinet in a Containment Level 3 room.

Sputum/bronchoalveolar lavage

All specimens must be processed in a Class 1 exhaust protective cabinet in a Containment Level 3 room.

The above guidance should be supplemented with local COSHH and risk assessments.

2 Specimen collection

2.1 Type of specimens

Faeces, urine, sellotape slide/perianal swab, CSF, bile, pus from abscesses, duodenal/jejunal aspirates, tissues, hydatid cyst, sputum/bronchoalveolar lavage, biopsies from colonoscopy or surgery

2.2 Optimal time and method of collection[76]

For safety considerations refer to Section 1.1.

Collect specimens before antimicrobial therapy where possible[76].

Faeces

Faeces should be presumably collected before antimicrobial or anti-diarrhoeal therapy where possible and between 10pm and midnight, or early in the morning, before defecation or bathing.

Sellotape slide/perianal swab should be collected for *E. vermicularis* ova[1].

Fresh faeces specimens are essential for the examination of trophozoites.

Faeces may be passed directly to a sterile wide-mouthed CE marked leak proof container or may be passed to a clean, dry bedpan or similar container and transferred to a CE marked leak proof container.

Fresh, unpreserved specimens should be transported immediately. Cysts will not form once the specimen has been passed.

Protozoan trophozoites will not survive if the specimen dries out. 10% formalin will kill trophozoites and renders them immotile. Liquid stool should therefore be examined ideally within 30 minutes from the time of collection without the addition of formalin (usually with a drop of saline) if trophozoites are sought. If delays cannot be avoided, the specimen should be preserved to avoid disintegration of the trophozoites.

Soft stools (which may contain both trophozoites and cysts) should preferably be examined within 1hr of passage[77].

Formed specimens (less likely to contain trophozoites) can be kept for up to one day, with overnight refrigeration if needed, prior to examination [78].

Sellotape slide/perianal swab for *E. vermicularis* ova[1]

Sellotape slide

Apply clear Sellotape to the perianal region, pressing the adhesive side of the tape firmly against the left and right perianal folds several times; the tape can be wrapped around a tongue depressor to aid specimen collection. Smooth the tape back on the slide, adhesive side down.

Perianal swab

Perianal specimens are best obtained in the morning before bathing or defecation. Three specimens should be taken on consecutive days before pinworm infection is ruled out.

Cotton-wool swab in dry container should be used for collection.

Spread buttocks apart, and rub the moistened cotton wool swab over the area around the anus, but do not insert into the anus. Place cotton wool swab back in its container (no transport medium required). Occasionally, an adult worm may be collected from a patient and sent in saline or water for identification.

Urine (for *S. haematobium*)

It is preferable to obtain total urine collected over the time period between 10am and 2pm as it has been shown that a maximum concentration of eggs is excreted at this time[79]. Alternatively, a 24hr collection of terminal samples of urine may be obtained. Sterile containers without boric acid must be used. In patients with haematuria, eggs may be found trapped in the blood and mucus in the terminal portion of the urine specimen.

If the urine cannot be examined within an hour of collection it is advisable to add 1ml of undiluted formalin to preserve any eggs that may be present.

CSF

Specimens will be obtained by specialist collection according to local protocols.

Tissues, Biopsies, Hydatid cyst and Pus from abscesses, Bile, Duodenal/jejunal aspirates

Specimens will be obtained by specialist collection according to local protocols.

Sputum/bronchoalveolar lavage

Sputum from the lower respiratory tract expectorated by deep coughing is required. When the cough is dry, physiotherapy, postural drainage or inhalation of an aerosol before expectoration may be helpful.

2.3 Adequate quantity and appropriate number of specimens[76]

Faeces

Ideally three stool specimens collected over no more than a 10-day period. It is usually recommended that specimens are collected every other day. Unless the patient has severe diarrhoea or dysentery, no more than one specimen should be examined within a single 24 hour period, as shedding of cysts and ova tends to be intermittent.

If *E. histolytica* is suspected and the first three specimens are negative, consideration should be made for referral where available for molecular tests.

There are no prescribed limits for the size of sample required, as some laboratory procedures will require larger quantities than others.

Sellotape slide/perianal swab for *E. vermicularis* ova[1]

It is recommended that samples should be taken for at least four to six consecutive days. If the results of all these are negative the patient can be considered free from infection. In practice, more than one specimen is rarely received.

Urine (for *S. haematobium*)

Ideally, a minimum volume of 10ml is required.

CSF

Ideally, a minimum volume of 1ml is required.

Tissues, biopsies, hydatid cyst and pus from abscesses

Pus

Ideally, the entire volume of pus or a minimum of 1ml is required.

Tissues/biopsies

Ideally, the specimen should be large enough to carry out all microscopical preparations and cultures.

Bile, duodenal/jejunal aspirates

Ideally, a minimum volume of 1ml is required.

Sputum/bronchoalveolar lavage

Ideally, a minimum volume of 2ml is required.

3 Specimen transport and storage[56,57]

3.1 Optimal transport and storage conditions

For safety considerations refer to Section 1.1.

Specimens should be transported and processed as soon as possible[76].

Faeces

If prompt examination of stools cannot be carried out, the use of 10% formalin-water preservative is necessary to prevent deterioration of protozoan morphology, the hatching of first-stage hookworm larvae, and overgrowth of yeasts[80].

Sellotape slide/perianal swab for *E. vermicularis* ova[1]

Refrigeration or store at room temperature (20 - 25°C) for up to 48hr.

Urine (for *S. haematobium*), Bile, Duodenal/jejunal aspirates and Sputum/bronchoalveolar lavage

If processing is delayed, refrigeration is preferable to storage at room temperature (20-25°C).

Tissues, biopsies, hydatid cyst and pus from abscesses

If specimen of tissue/biopsy is small, place it in sterile water to prevent desiccation.

If processing is delayed, refrigeration is preferable to storage at room temperature (20-25°C).

4 Specimen processing/procedure[56,57]

4.1 Test selection

Faeces

Select a representative portion of specimen for appropriate procedures such as culture for bacterial pathogens, testing for Clostridium difficile toxins (B 10 – Laboratory investigation of *Clostridium difficile* infection) and virological examination, depending on clinical details.

Faecal concentrations are carried out on all specimens where examination of parasites is specifically requested, where there are definite clinical indications and when advised by senior laboratory staff.

All faecal samples from symptomatic individuals should be stained for *Cryptosporidium* oocysts[81].

Stain for microsporidia in symptomatic, HIV positive and immunocompromised patients.

For all other specimens

N/A

4.2 Appearance

N/A

4.3 Sample preparation

For safety considerations refer to Section 1.2.

4.3.1 Pre-treatment

N/A

4.3.2 Specimen processing

Faeces

Sample parts of faeces samples that contain blood, pus, or mucus for direct examination as wet preparations or for staining.

If sampling formed faeces, collect and examine material from various parts of the faecal sample for concentration, wet preparations and for staining.

Faeces for examination of protozoa

Standard

If specimen is fresh, examine for motile trophozoites as follows:

1. Place one drop of 0.85% saline on the left-hand side of a clean microscope slide, and one drop of double-strength Lugol's iodine on the right-hand side (the distance between the drops should be sufficient to enable coverslips to be placed over each drop).

2. Using a different swab stick for each preparation, take a small amount of unfixed faeces and thoroughly emulsify in the saline and in the Lugol's iodine.
3. Place cover slips over each preparation on the slide. Examine both entire areas with a low power objective. Use a medium power objective to identify any suspicious morphological features.
4. If required, prepare smears on clean microscope slides for auramine-phenol and/or Giemsa staining (see TP 39 - Staining procedures).
5. Also, concentrate the specimen with the formol-ether* concentration technique described below.

Faeces for examination of *Cryptosporidium* species[77,82]

1. Prepare a medium to thick smear of faeces on a clean microscope slide and air dry.

   Note: If specimen is dry or solid, 10% formalin may be added.
2. Fix in methanol for three minutes.
3. Smears can be stained by either auramine phenol or modified cold Ziehl-Neelsen (see TP 39 - Staining procedures).

Modified formol-ether* concentration[83,84]

***Ethyl acetate (not diethyl ether) must be used in a well-ventilated area with no naked flames**

The following method is the recommended technique for faecal concentration. There are many commercial kits for the concentration of faeces available which are based on the Ridley Allen method described below, which is the method of choice used by most clinical laboratories. Commercial concentration kits are often used.

1. Take a sample of faeces about the size of a large pea (approximately 1g) with a swab stick and emulsify it in 7ml of 10% formalin (one volume of 40% formaldehyde diluted with nine volumes of distilled water) in a clean universal container.
2. Sieve by pouring the whole contents of the universal through a sieve (a nylon tea-strainer or a square of wire gauze is suitable) and collect in a suitable container. Sieves are washed in copious amounts of clean water and re-used. Sieving the faeces and formalin mixture prior to centrifugation helps eliminate large pieces of faecal matter from the suspension.
3. Transfer the filtrate into a stoppered glass or polypropylene (ether resistant) container appropriate for centrifugation.
4. Add 3ml of ethyl acetate and a small drop of 0.1% Triton X 100 (helps emulsify the faecal specimen) and vortex for 15secs, or shake vigorously for 60secs.
5. Centrifuge the specimen at 1200 x g for 3min.
6. Loosen the fatty layer with a swab stick by passing it around the inside circumference of the tube, removing all residues of the fat from the tube.
7. Tip away the contents of the tube, allowing the last few drops to return to the bottom of the tube to cover the remaining deposit.

8. Resuspend the deposit in the remaining fluid. Place a drop of this on a clean microscope slide and place a coverslip over it.
9. Double strength iodine may be added to a separate preparation to enhance and facilitate comparison of morphological details.
10. Search the entire area using a low power objective; use a medium power objective to examine morphological features.

Commercially available concentrator kits containing sieves of varying pore sizes are available; the size of the pore affects the yield of parasite stages and the amount of debris present[85-88]. A larger pore size may result in a higher yield of parasite stages, however the increase in debris leads to a denser deposit, making it more difficult to examine the slide; ova and cysts may therefore be obscured. If the pore size is too small, despite having a cleaner slide which is easy to examine, the yield of parasite stages will be reduced. Commercially available faecal concentrator kits should be validated prior to use, and manufacturers' instructions should be followed.

To maximise the recovery of parasites it is important to sieve the faecal formalin mixture, use a solvent, ie, ethyl acetate with triton X and centrifuge for the correct time and at the correct centrifugal force[86]. Recovery of parasite stages may be greatly diminished if a solvent (for example ethyl acetate) as an extractor of fat and debris is not used[88]. A recent study confirms and recommends that 1200 x g for 3min is optimal for parasite recovery[88,89].

Sellotape slide

1. Before examining the slide, it may be advantageous to lift the tape and place one drop of immersion oil or glycerol/alcohol under the middle of the tape and replace in position. This will improve the transparency of the tape.
2. Examine the slide using a low power objective.

Perianal swab

1. Add enough saline to cover the swab in its container and replace the cap.
2. Shake vigorously.
3. Withdraw the swab from the saline, rolling it against the side of the container to squeeze out the saline. Discard the swab.
4. Concentrate the resultant fluid by centrifugation at 800 x g for 2min.
5. Remove the supernatant with a disposable pipette, without disturbing the sediment.
6. Agitate the tube to resuspend the residue.
7. Using a disposable pipette, place a drop of sediment on a microscope slide, apply a coverslip and use a low power objective to examine the entire area.

Urine (for *S. haematobium*)

24 hour terminal urine specimens are preferred[82].

For large volumes of urine (>25ml)

1. Allow the specimen to sediment for 1hr.

2. Decant and discard the supernatant, then transfer the sediment along with some residual urine (approximately 1ml) to conical-bottom containers for centrifugation.
3. Centrifuge at 500 x g for 2min.
4. Decant and discard the supernatant then mix the sediment using a pipette.
5. Place 1- 2 drops of the whole deposit on several clean microscope slides and apply coverslips. Examine the entire area of each slide preparation with a low power objective.

Other urine specimens

If specimen is already in a conical-bottom container, proceed as from number 3 above, if not, transfer the entire specimen to conical-bottom containers and proceed as from number 3 above.

Filtration method (recommended for non-cellular, crystal-free urine specimens)

1. Draw ≥10ml urine into a syringe, and then connect to a Swinnex filter (pore size 12µm).
2. Gently ease the urine through the filter.
3. Draw in 20ml of air and ease this through the filter.
4. Remove the top of the filter and place the membrane on a microscope slide.
5. Add a drop of saline, apply a coverslip and view microscopically with a low power objective.

CSF[90]

1. Perform direct microscopy of the CSF as soon as received (use a low or medium power objective).
2. Concentrate the CSF by centrifuging at 100 x g for 10min.
3. With a sterile pipette transfer the supernatant, leaving approximately 0.5ml, to another sterile container for additional testing if required (protein content, virology, etc.).
4. Resuspend the centrifuged deposit in the remaining fluid and place two drops in the centre of a bacteria-coated agar plate.
5. After the fluid has been absorbed, incubate and examine the plate as described for corneal scrapings (refer to B 2 - Investigation of bacterial eye infections).

Note: 35°C–37°C incubation will give better results for *Naegleria* species.

1. Also, place one drop of the centrifuged deposit on a clean microscope slide and place a cover slip over it. Screen the entire area with a low power objective; use a medium objective to identify any morphological features.

Hydatid Cyst and Pus from abscesses

For pus and hydatid cyst contents (including hydatid sand), prepare wet preparations and air-dried smears for staining (if required) (see TP 39 - Staining procedures).

Tissues and biopsies[90]

1. In addition to standard histological preparations make impression smears, teased and squash preparations.
2. Place specimen in a sterile Petri dish to examine it macroscopically and to select a sample for microscopic examination.
3. Select an area that appears to look different from normal. For example, select grey consolidated or granulomatous portion of a lung, or the ulcerated area of intestinal tissue.

Impression smears

1. If the sample is large enough, cut the tissue and use the cut surfaces to touch the slide.
2. Press the tissue against a clean microscope slide, lift and press again.
3. Turn the sample over and press the area of the cut surface against the slide to make two more impressions. This leaves three impressions in a row on one microscope slide.
4. If several tissue samples were supplied, make a row of impressions with each of the samples.
5. Air dry and fix in methanol for 1min before staining by Giemsa (see TP 39 - Staining procedures).

Squash preparations

Squash preparations - for tissue parasites such as *Trichinella*:

1. Cut selected tissue portions into very fine fragments in a Petri dish, placing a fragment on a clean microscope slide.
2. Add one drop of sterile saline or sterile distilled water.
3. Cover with a second clean microscope slide and press the slides firmly together.
4. Examine microscopically with a low power objective.

Note: Care should be taken when squash preparations are performed as they can release lots of eggs increasing the risk to the operator.

Bile, duodenal/jejunal aspirates

Standard

1. Centrifuge specimen at 800 x g for 2min.
2. Decant supernatant to disinfectant.
3. Resuspend the centrifuged deposit in the remaining drops of supernatant.
4. Use the resuspended pellet to prepare smears for staining.
5. Stain using Ziehl-Neelsen and auramine-phenol for *Cryptosporidium*, Giemsa for *Cyclospora cayetanesis* and *C. belli* (see TP 39 - Staining procedures), and iodine or plain wet preparation for *S. stercoralis* and *G. duodenalis.*

Supplementary

For examination of microsporidia:

1. Centrifuge specimen at 800 x g for 2min.
2. Decant supernatant to disinfectant.
3. Resuspend the centrifuged deposit in the remaining drops of supernatant.
4. Use the resuspended pellet to prepare smears for staining.
5. Stain with the modified trichrome stain (see TP 39 - Staining procedures).

Sputum[90]

1. Select any blood-tinged viscous areas for sampling.
2. Place 1ml of sputum in a centrifuge tube.
3. Add 1ml dithiothreitol and agitate gently for approximately 10sec. Allow to stand at room temperature for 15min.
4. Centrifuge at 1500 x g for 2min.
5. Decant the supernatant to a discard jar.
6. Resuspend the deposit in the few remaining drops of supernatant.
7. Place one drop of this on a clean microscope slide and apply a cover slip. Examine the entire area with a low power objective.

Induced sputum / BAL (for *P. jirovecii*)

Several methods exist for the staining and identification of *P. jirovecii*. Histological stains may be used, however immunofluorescence methods with monoclonal antibodies are used in many microbiology laboratories. Manufacturers' recommendations should be followed when using commercial kits and reagents. For more information, see B 57 – Investigation of bronchoalveolar lavage, sputum and associated specimens.

4.4 Microscopy

See Section 4.3.2 for all specimens.

4.5 Culture and investigation

See Section 4.3.2 for all specimens.

4.6 Identification

Minimum level

Identify parasites to species level where possible.

4.7 Antimicrobial susceptibility testing

NA

4.8 Referral for outbreak investigations

For information regarding outbreak investigation referral, contact specific reference laboratory.

4.9 Referral to reference laboratories

For information on the tests offered, turnaround times, transport procedure and the other requirements of the reference laboratory click here for user manuals and request forms.

Organisms with unusual or unexpected resistance, and whenever there is a laboratory or clinical problem, or anomaly that requires elucidation should, be sent to the appropriate reference laboratory.

Contact appropriate devolved national reference laboratory for information on the tests available, turnaround times, transport procedure and any other requirements for sample submission:

England and Wales

https://www.gov.uk/specialist-and-reference-microbiology-laboratory-tests-and-services

Scotland

http://www.hps.scot.nhs.uk/reflab/index.aspx

Northern Ireland

http://www.publichealth.hscni.net/directorate-public-health/health-protection

5 Reporting procedure

5.1 Microscopy

Report on any parasites seen.

Faeces

Include comment on the presence of all parasites seen, whether they are pathogenic or non-pathogenic.

Microscopy reporting time

Written report 16–72hr stating, if appropriate, that a further report will be issued.

Urgent microscopy: telephone when available.

5.2 Culture

CSF

Report presence or absence of *Acanthamoeba* species and/or *Naegleria fowleri.*

Culture reporting time

CSF

Written report on day four stating if appropriate, that a further report will be issued.

Clinically urgent requests: telephone when available.

5.3 Antimicrobial susceptibility testing

N/A

6 Notification to PHE[91,92], or equivalent in the devolved administrations[93-96]

The Health Protection (Notification) regulations 2010 require diagnostic laboratories to notify Public Health England (PHE) when they identify the causative agents that are listed in Schedule 2 of the Regulations. Notifications must be provided in writing, on paper or electronically, within seven days. Urgent cases should be notified orally and as soon as possible, recommended within 24 hours. These should be followed up by written notification within seven days.

For the purposes of the Notification Regulations, the recipient of laboratory notifications is the local PHE Health Protection Team. If a case has already been notified by a registered medical practitioner, the diagnostic laboratory is still required to notify the case if they identify any evidence of an infection caused by a notifiable causative agent.

Notification under the Health Protection (Notification) Regulations 2010 does not replace voluntary reporting to PHE. The vast majority of NHS laboratories voluntarily report a wide range of laboratory diagnoses of causative agents to PHE and many PHE Health protection Teams have agreements with local laboratories for urgent reporting of some infections. This should continue.

Note: The Health Protection Legislation Guidance (2010) includes reporting of Human Immunodeficiency Virus (HIV) & Sexually Transmitted Infections (STIs), Healthcare Associated Infections (HCAIs) and Creutzfeldt–Jakob disease (CJD) under 'Notification Duties of Registered Medical Practitioners': it is not noted under 'Notification Duties of Diagnostic Laboratories'.

https://www.gov.uk/government/organisations/public-health-england/about/our-governance#health-protection-regulations-2010

Other arrangements exist in Scotland[93,94], Wales[95] and Northern Ireland[96].

Appendix 1: Specimen types and possible parasites detectable

Specimen types	Possible parasites present
Bile	*Fasciola hepatica, Opisthorchis sinensis, Cryptosporidium* species
CSF	*Acanthamoeba* species, *Angiostrongylus cantonensis*, *Balamuthia mandrillaris,* Microsporidia - *Encephalitozoon cuniculi, Naegleria fowleri*, any nematodes producing VLM (Visceral Larva Migrans)
Duodenal and jejunal aspirates	*Cryptosporidium* species*, Cyclospora cayetanesis, Giardia duodenalis* Microsporidia - *Enterocytozoon bieneusi*, *Strongyloides* species
Faeces	*Ancylostoma duodenale, Ascaris lumbricoides,* adult *Acari* sp. worms and ova, *Balantidium coli*, *Blastocystis hominis, Paracapillaria philippinensis, Chilomastix mesnili, Cryptosporidium* species, *Cyclospora cayetanesis, Dientamoeba fragilis, Diphyllobothrium latum, Echinostoma* species, *Endolimax nana*, *Entamoeba histolytica*, other *Entamoeba* species, *Enteromonas hominis, Fasciola hepatica, Fasciolopsis buski, Giardia duodenalis, Heterophyes heterophyes, Hymenolepis nana*, *Iodamoeba butschlii, Cystoisospora belli*, Microsporidia [*Enterocytozoon bieneusi and Encephalitozoon (Septata) intestinalis*], *Metagonimus yokogawai*, *Nanophyetes salmincola, Necator americanus, Opisthorchis sinensis*, *Paragonimus* species*, Retortamonas intestinalis, Sarcocystis* species, *Schistosoma* species *Strongyloides stercoralis, Taenia saginata* worms and ova*, Taenia solium*, *Trichuris trichiura, Enterobius* species adult worms and ova
Liver and spleen aspirates Hydatid cyst Pus	*Entamoeba histolytica, Leishmania* species, *Echinococcus granulosus*
Sellotape slide	*Enterobius vermicularis*
Sputum / BAL	*Ascaris lumbricoides*, *Cryptosporidium s*pecies, Microsporidia, *Paragonimus westermani, Strongyloides stercoralis, Pneumocystis jirovecii*
Swabs	*Trichomonas vaginalis* – genital swab Microsporidia – eye swab
Tissues and biopsies	*Acanthamoeba* species - brain biopsy, skin nodules and ulcers; *Angiostrongylus costaricensis*, *Anisakis* species, *Cryptosporidium* species – small bowel and liver biopsy; Filarial worms, *Giardia duodenalis* - duodenal biopsy; *Leishmania* species - lymph node biopsy, cutaneous ulcers; *Anncaliia* species*, Nosema* species*, Vittaforma corneae, Microsporidium africanum and Microsporidium ceylonensis* - Cornea ulcer Microsporidia [*Pleistophora, Nosema*, *Trachipleistophora* and *Phocanema* species], *Schistosoma* species, *Taenia solium*, *Trichinella* and other tissue nematodes - muscle biopsy
Urine	*Schistosoma haematobium,* Microsporidia

Serology testing	*Entamoeba histolytica, Acanthamoeba* species*,* Cysticercosis (*Taenia solium*), *Echinococcus granulosus, Fasciola hepatica*, Filaria, *Giardia duodenalis*, *Cryptosporidium* species, *Leishmania* species, *Schistosoma* species, *Strongyloides stercoralis*, *Toxocara* species, *Toxoplasma gondii*, *Trichinella* species*,* any nematodes producing VLM (Visceral Larva Migrans),

Appendix 2: Geographic distribution of parasitic infections

Infection / Infective organism	Geographic distribution
Amoebiasis/ *Entamoeba histolytica*	Worldwide
Free-living amoebae - *Acanthamoeba*, *Naegleria* sp.	Worldwide
Flagellates:	
Giardiasis, Trichomoniasis	Worldwide
Coccidia:	
Cryptosporidiosis/*Cryptosporidium* sp.	Worldwide
Cyclosporiasis/*Cyclospora* sp.,	Worldwide, United states
Microsporidia	Worldwide
Cystosporiasis/*Cystoisospora* sp.,	Worldwide
Sarcocystosis/*Sarcocystis* sp., Toxoplasmosis/ *Toxoplasma gondii*, *Pneumocystis jirovecii*	Worldwide
Nematode infections (GI-tract) – *Enterobius, Trichuris, Ascaris*	Worldwide
Hookworms:	
Ancylostoma duodenale	Europe, S America, India, China, SE Asia, Indonesia, Australia, some Pacific isles
Strongyloides sp.	Tropics and subtropics
Trichostrongylus species	Worldwide
Necator americanus	N and S America, sub-Saharan Africa, India, China, SE Asia, Indonesia, Australia some Pacific isles
Unusual nematodes:	
Trichinellosis, Toxocariasis	Worldwide
Gnathostomiasis (fever and pulmonary infiltrates)	Asia
Gnathostomiasis *Angiostrongylus* sp. (myeloencephalitis)	Africa, America, Asia, Australasia
Capillariasis	Africa, America, Asia, Europe
Dracunculiasis	Africa, Asia
Onchocerciasis	Africa, America (central and south), Asia
Trematodes (blood flukes):	
Schistosomiasis	Africa, America (central and south), Asia
Liver flukes:	
Fascioliasis	Worldwide
Opisthorchiasis	America (south), Asia, Europe
Intestinal flukes:	
Fasciolopsiasis	Southern and Eastern Asia

Lung flukes:	
Paragonimiasis/ *Paragonimus* species	Far East, Indian subcontinent, Africa, some Pacific Isles
Cestodes (Tapeworms):	
Diphyllobothrium latum	America (north), Canada, Europe, Japan, Russia, Scandinavia
Hymenolepsis nana, Taeniasis/ *Taenia saginata/solium*, Cystercercosis/ *Taenia solium*	Worldwide
Echinococcosis/ *Echinococcus* sp.	Africa, America, Asia, Europe, Australasia
Sparganosis/ *Spirometra mansonoides*	America, Asia (particularly China and Japan)
Coenurosis/ *Taenia multiceps/serialis*	Africa, America, Europe

Appendix 3: Calibrating the microscope for measurement

Calibrating the microscope for measurement

The size of ova and cysts is an important identifying feature of many parasites.

Size can be determined with an eyepiece micrometer. The calibration of the microscope is carried out as follows:

Equipment required includes an eyepiece micrometer and a stage micrometer.

1 The eyepiece scale is divided in 100 small divisions.

2 The stage micrometer scale extends over 1mm being divided in 0.1mm divisions, each being divided again in 0.01mm divisions.

3 Insert the eyepiece graticule to the eyepiece, and replace into the microscope.

4 Place the stage micrometer on the microscope stage.

5 Focus the low-power objective on the stage scale.

6 Adjust the eyepiece and stage scales until they are parallel and overlap.

7 Note the number of eyepiece divisions and its corresponding stage measurement, eg 10 eyepiece divisions = 0.20mm on the stage scale.

8 Calculate value of one eyepiece division as follows:

10 eyepiece divisions = 0.20

1 eyepiece division = 0.20/10 = 0.020mm = 20µm

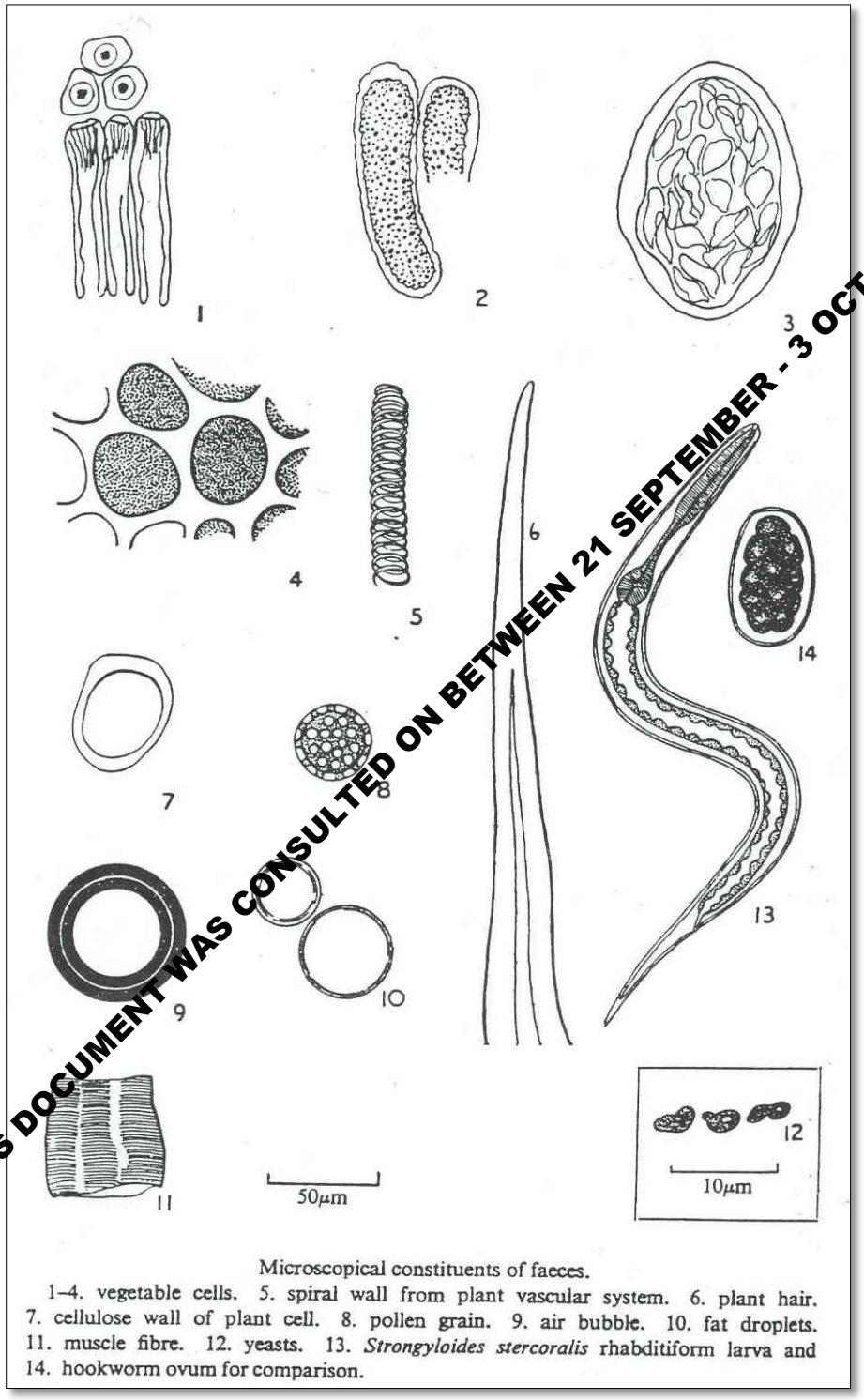
Note: To convert the calculated value (0.020mm) above to µm, it should be multiplied by 1000 µm/mm to give 20µm

9 Repeat from step 5 with each objective, noting and recording the reading from each.

10 Calibration need only be done once for each microscope, and its objectives and eyepieces.

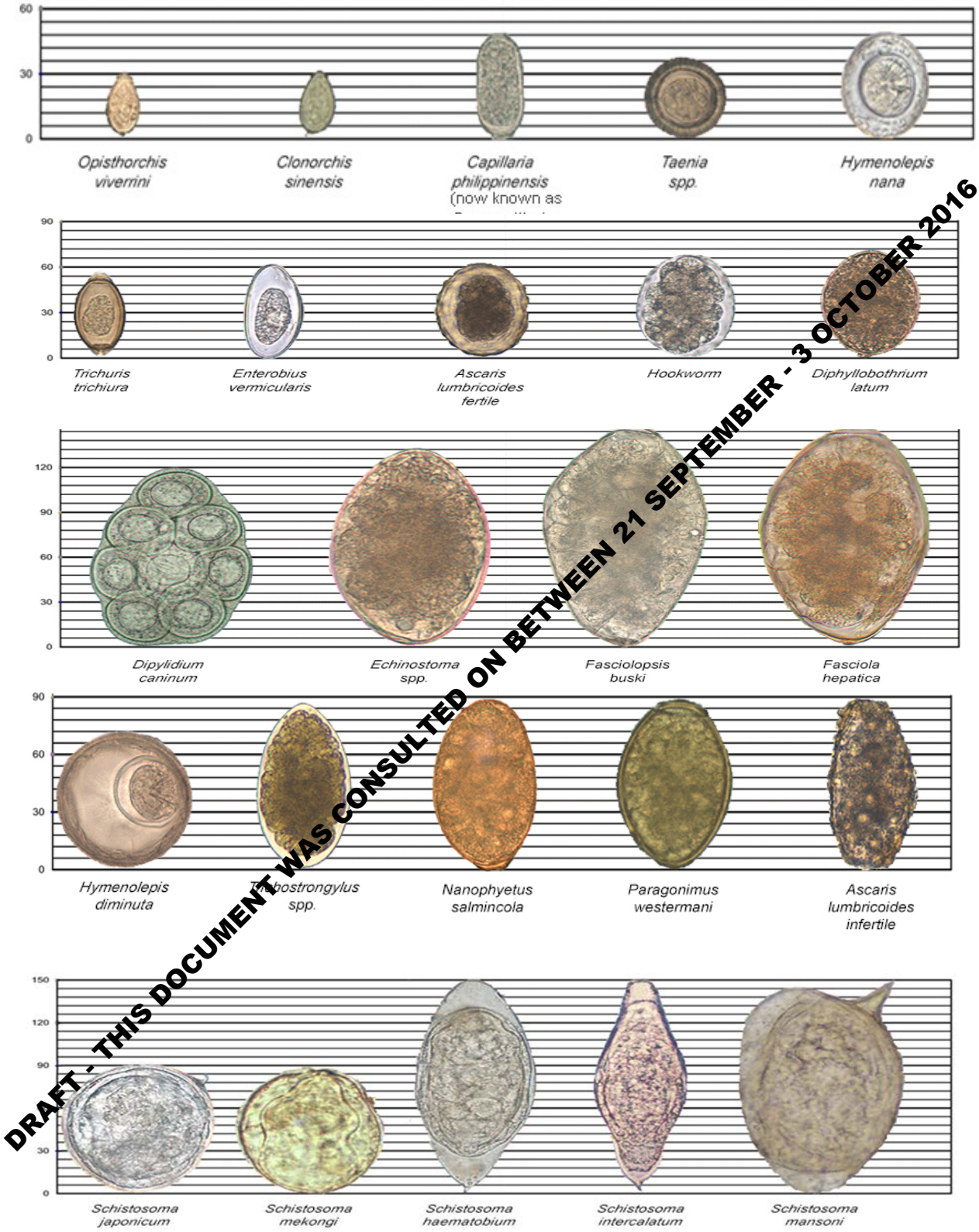
Courtesy of UK NEQAS Parasitology Teaching Programme 2003/2004; Faecal Parasites. Co-ordinated by Hilary Edwards.

Appendix 4: Common microscopic constituents of faeces[97]



Microscopical constituents of faeces.
1–4. vegetable cells. 5. spiral wall from plant vascular system. 6. plant hair. 7. cellulose wall of plant cell. 8. pollen grain. 9. air bubble. 10. fat droplets. 11. muscle fibre. 12. yeasts. 13. *Strongyloides stercoralis* rhabditiform larva and 14. hookworm ovum for comparison.

Appendix 5: Relative sizes of helminth eggs*



*Measurements in micrometres (µm)

Appendix 6: Oocysts of coccidian

Cystoisospora belli[1]

Immature oocyst (32 x 16µm) Mature oocyst

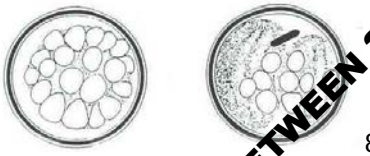
Oocysts are transparent. Reduced illumination is recommended. Modified Ziehl-Neelsen can be used for direct smears

***Cryptosporidium* species**

5µm

Auramine-phenol and modified Ziehl-Neelsen stains are recommended

***Cyclospora* species** (CLB)



8 – 10µm

unsporulated sporulated

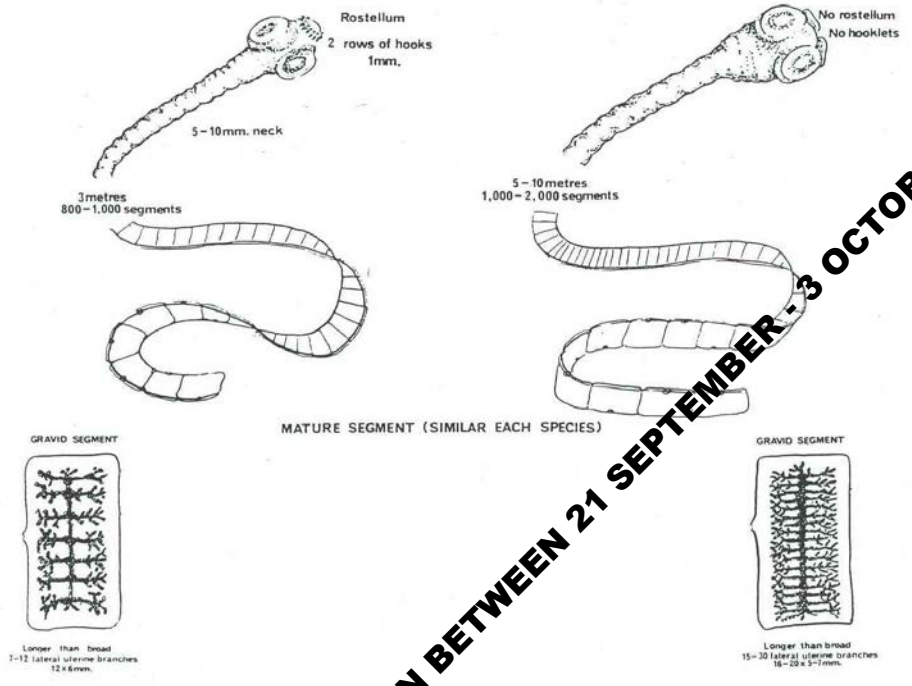
In an unstained wet preparation, a central morula contains several refractile spheres. In fresh water, the morula divides into 2 smaller structures. *Cyclospora cayetanesis* can be seen in formol-ether concentrations as refractile spheres which do not stain with iodine or auramine-phenol stain but are variably acid-fast, staining pink or not at all with modified Ziehl-Neelsen stain. It will auto-fluoresce blue at 340 – 360 nm

Microsporidia species

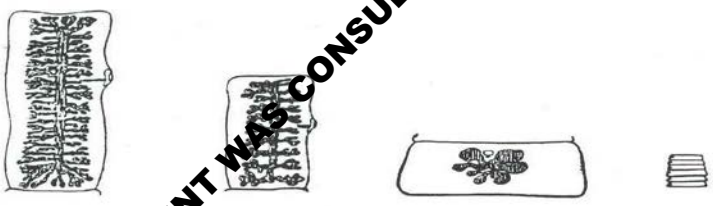
1-4µm depending on the species

Although microsporidia may appear acid-fast when stained with modified Ziehl-Neelsen, the trichrome stain is recommended. Microsporidial spores are ovoid and refractile and the spore wall stains bright pink-red. Occasionally the spores stain with a red `belt' across the centre of the spore, or show polar granules, which are both diagnostic features

Appendix 7: Comparison of tapeworms found in humans[98]



Appearance of gravid segments of tapeworms found in humans.



T. saginata *T. solium* *D. latum* *H. nana*

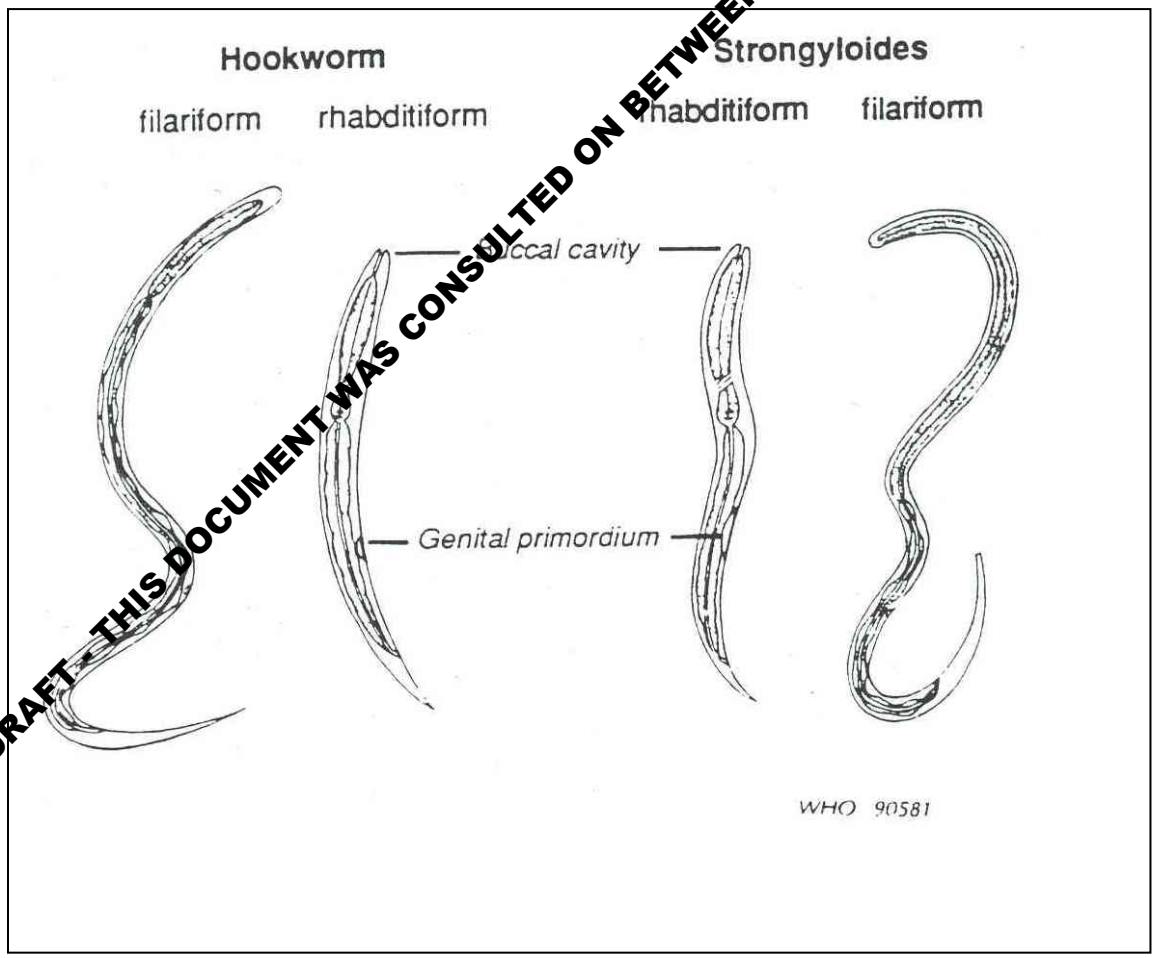
Note: The ova of both *T. solium* and *T. saginata* are identical and diagnosis is made by the recovery of the segments or scolex.

Appendix 8: Helminth larvae – characteristics[99]

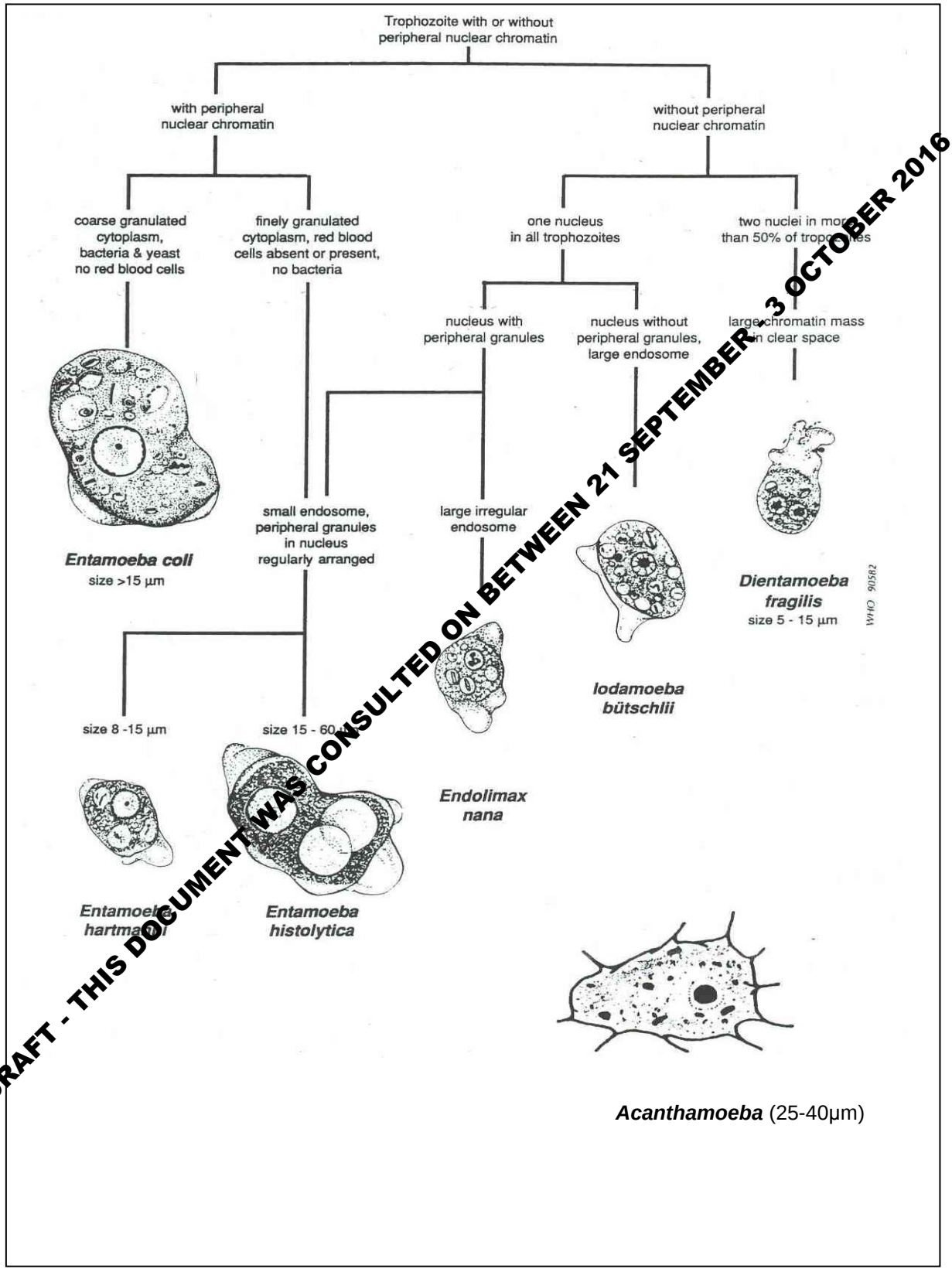
Hookworm	***Strongyloides***
Filariform larvae Size 500 x 14-20 µm Sheathed Tapered tail Oesophagus one-third of body length	*Filariform larvae* Size 500 x 14-20µm Unsheathed Blunt or forked tail Oesophagus half of body length
Rhabditiform larvae Size 100 - 150 x 15-17µm Long buccal cavity – 15µm Oesophagus one-third of body length with two swellings Genital primordium small – 7µm Anal pore 80µm from posterior end	*Rhabditiform larvae* Size 200 - 300 x 15-18µm Short buccal cavity – 4µm Oesophagus one-third of body length with two swellings Genital primordium large – 22µm Anal pore 50µm from posterior end

In fresh stool specimens, the most likely larvae to be seen are rhabditiform larvae of *Strongyloides stercoralis.* If the stool is >12 hours old, the larvae may develop into filariform larvae which must be differentiated from hookworm larvae (these may appear in the stool within 12–24hr).

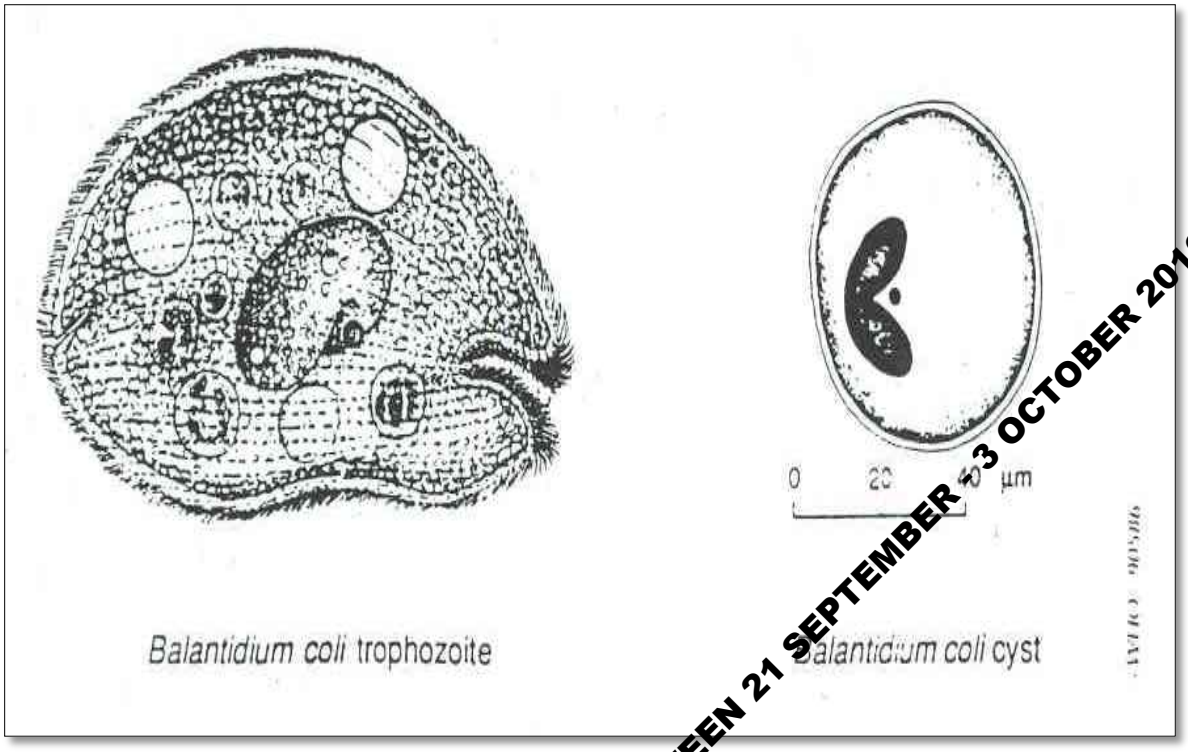
Helminth larvae



Appendix 9: Identification of amoebic trophozoites in stained smears[1]



Appendix 10: *Balantidium coli* - trophozoite and cyst[1]

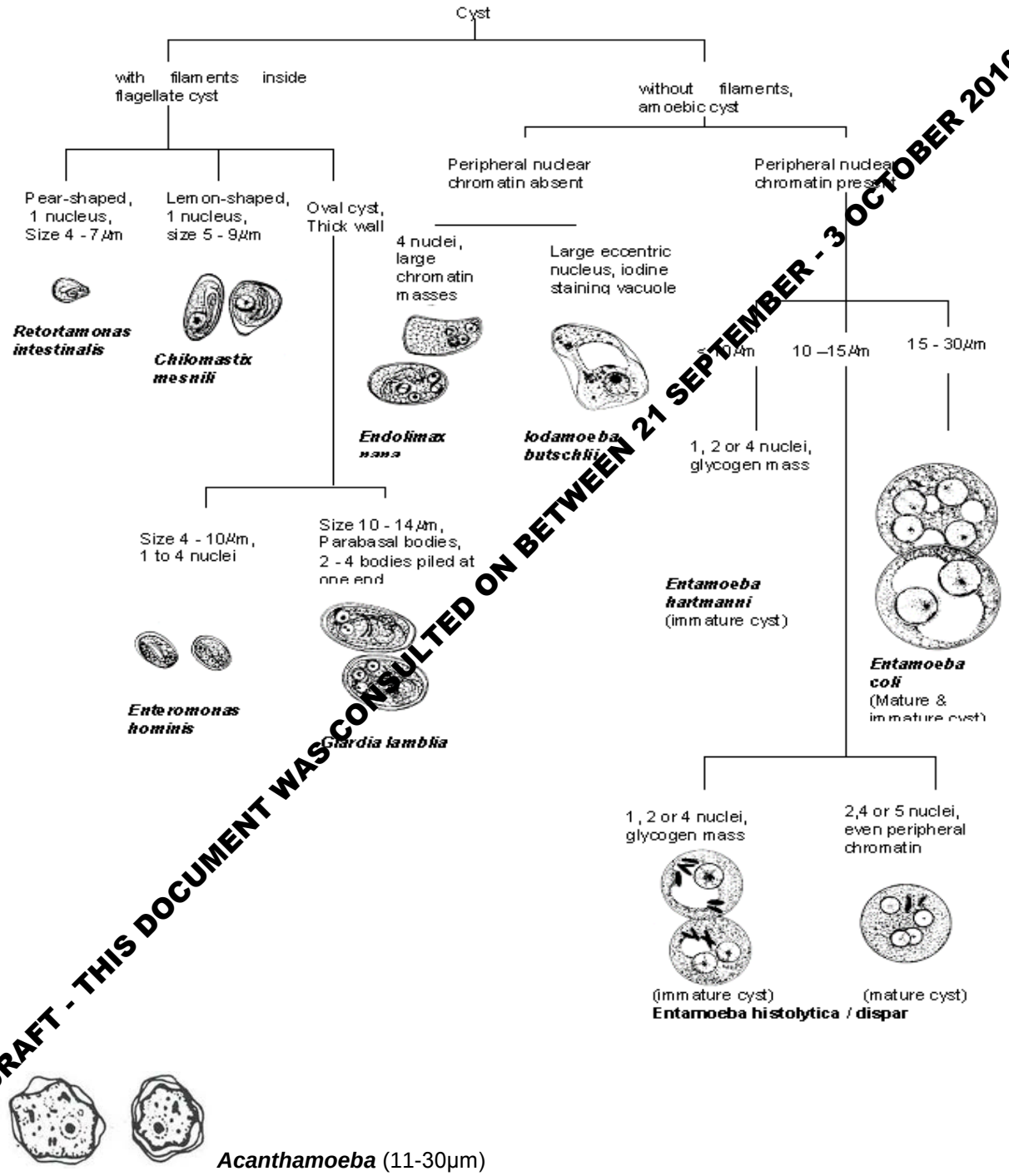


The characteristics between the *Balantidium coli* Trophozoite and *Balantidium coli* cyst

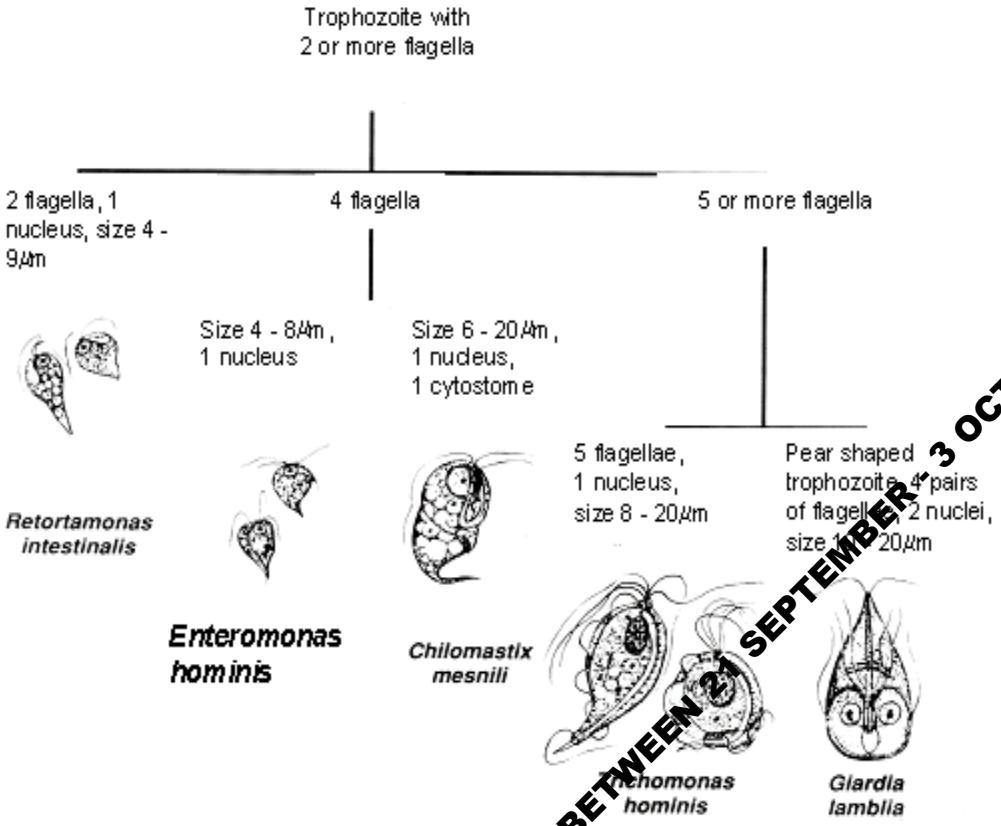
Trophozoite	Cyst
Oval and covered in short cilia measure approximately 30-150µm in length x 25-120µm in width but may attain lengths of up to 200µm A funnel shaped cytosome can be seen near the anterior end Micronucleus and macronucleus may be observed in stained preparations	Spherical or ellipsoidal Measures from 30-200µm by 20-120µm Contains 1 macronucleus and 1 micronucleus Presence of cilia in young cysts but disappear after prolonged encystment

Note: Wet preparations of fresh and concentrated stool samples reveal the characteristic cysts and motile trophozoites. They are easier to identify in direct-smear saline preparations than permanently stained faecal smears.

Appendix 11: Identification of amoebic and flagellate cysts



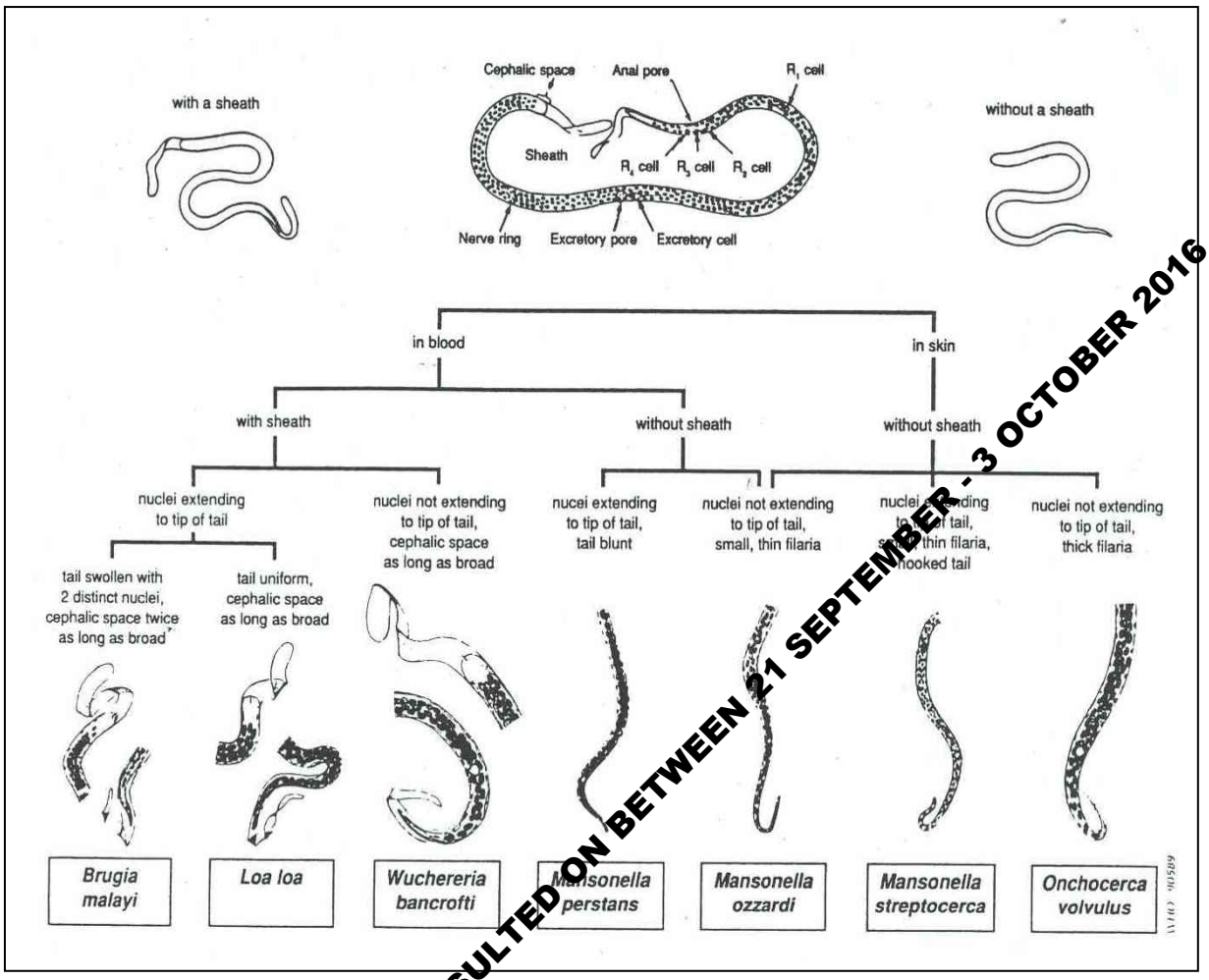
Appendix 12: Identification of flagellate trophozoites[1]



Note: It should be noted that *Trichomonas hominis* does not have a cyst stage.

(Adapted and redrawn, WHO, 1991)

Appendix 13: Microfilariae found in humans[1]



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