INDIRECT DEMONSTRATION

The differential diagnosis of infectious diseases is frequently complicated by the nonspecific nature of any disease symptoms (such as diarrhoea or fever), difficulties in detecting organisms in test samples (few present and irregular occurrence) and their variable characteristics (pleomorphy, virulence, growth requirements, drug sensitivity, etc.). Recourse is therefore often taken to the indirect demonstration of parasitic infections by the detection of specific clinical parameters (haematology and blood biochemistry), specific host antibodies or parasite antigens (immunology), or specific parasite proteins or DNA (molecular biology). However, correlation does not always imply causation; therefore the association between the test parameter and the parasitic disease needs to have been rigorously established by longitudinal, cross-sectional or experimental studies involving test accuracy, sensitivity and specificity.

CLINICAL HAEMATOLOGY

As given previously, several haematological tests have been developed to directly detect intracellular or extracellular parasites in host blood samples, including protozoa (haemosporidia, piroplasms, trypanosomes) and helminths (microfilariae). Clinical haematology can also be used to provide indirect presumptive evidence of parasitic infection by the demonstration of specific abnormalities in cellular and acellular components of blood, some being attributable to parasites. In addition, variations in the size, shape and number of blood cells provides an insight into the general functioning of the blood and the health of the host. Techniques have been developed to measure erythrocyte (red blood cell) characteristics (especially haemoglobin and haematocrit associated with oxygenation and perfusion potentials), leucocyte (white blood cell) characteristics (especially differential counts associated with infection and immuno-competency), and platelet (thrombocyte) characteristics associated with coagulopathies. Whole blood samples are usually collected by peripheral venepuncture into anticoagulant and screened for:

- haemoglobin (HGB): oxygen carrier [low levels associated with anaemia; high levels with polycythaemia]
- red blood cell count (RBC): quantifies erythrocytes [low counts associated with anaemia, excess body fluid and blood loss; high counts with dehydration, polycythaemia]
- haematocrit (HCT) or packed cell volume: percentage of blood occupied by erythrocytes [low levels associated with blood loss, reduced production and various anaemic conditions]

- red cell indices, such as mean cell volume (MCV), mean cell haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and red cell distribution width (RDW): provide details on volume and haemoglobin content of red blood cells [perturbations indicative of different types of anaemias; e.g. microcytic/hypochromic (often due to iron deficiency), normocytic/normochromic (due to chronic infections, inflammation, renal failure) or macrocytic (due to haemolysis, vitamin deficiency or liver disease); may also reveal haemozoin (HZ) pigment associated with malaria]

- white blood cell count (WBC): quantifies leucocytes [high levels associated with infection, stress; low levels with immuno-suppression, bone marrow failure]
- differential count: quantifies different types of white blood cells, including granulocytes (neutrophils (NEU), eosinophils (EOS), basophils (BAS)) and agranulocytes (lymphocytes (LYM), monocytes (MON)) [different levels provide information on host immuno-competency, many helminth infections associated with high EOS levels]

- platelet count (PLT): quantifies thrombocytes (coagulation agents) [high levels associated with inflammation and infection; low levels with spontaneous bleeding, disseminated intravascular coagulation (DIC), leukaemia]
- clotting indices, such as prothrombin time (PT), international normalized ratio (INR), activated thromboplastin time (APTT), thrombin clotting time (TCT), D-dimer, fibrinogen, anti-thrombin, bleeding time and viscosity [perturbations associated with various coagulopathies, including some caused by parasites]

Common haematological conditions include anaemias (nutritional, hypovolaemic, haemolytic, aplastic), cancers (lymphoma, leukemia, myeloma), haemorrhagic/clotting conditions (coagulopathies), immunodeficiencies (genetic/acquired, cellular/humoral) and microbial infections (viruses, bacteria, fungi, protozoa, helminths). Despite the excellent correlation between many haematological abnormalities and specific haemoparasites, similar abnormalities may be caused by other disease conditions therefore definitive diagnosis must be confirmed using alternative techniques. Nonetheless, a range of parasites may cause haematological conditions; either by feeding on blood, destroying blood cells, damaging vascular beds or eliciting hypersensitive responses.

Haematological changes associated with parasitic infections

Type of parasite	Disease	Site of infection	Changes to haematological parameters
protist (amoeba)	amoebiasis	gut	HGB ↓, RBC ↓, HCT ↓, WBC ↑
protist (kinetoplastid)	African trypanosomiasis	blood, brain	HGB ↓, RBC ↓, HCT ↓, MCV ↓, MCH ↓, WBC ↑, LYM ↑, MON ↑, PLT ↓, PT ↑, TCT ↑, APTT ↑, DIC
protist (kinetoplastid)	American trypanosomiasis	macrophages, tissues	RBC ↓, WBC ↑, LYM ↑, PLT ↓
protist (kinetoplastid)	leishmaniasis	skin, viscera	HGB ↓, RBC ↓, HCT ↓, WBC ↓, LYM ↓, EOS ↓, NEU ↑, MON ↑, PLT ↓, PT ↑, APTT ↑, DIC
protist (coccidian)	coccidiosis	gut	HGB ↓, RBC ↓, HCT ↓, MCV ↑, WBC ↑, LYM ↓, EOS ↑, NEU ↑
protist (coccidian)	toxoplasmosis	macrophages, brain, muscles	HGB ↓, RBC ↓, HCT ↓, WBC ↑, NEU ↑, LYM ↓, MON ↑, EOS ↑
protist (haemosporidian)	malaria	liver, erythrocytes	HGB ↓, RBC ↓, HCT ↓, MCV ↑, MCH ↑, MCHC ↑, HZ, WBC ↓, NEU ↓, MON ↓, LYM ↓, EOS ↓, PLT ↓, PT ↑, DIC
protist (piroplasm)	babesiosis	erythrocytes, spleen	HGB ↓, RBC ↓, HCT ↓, MCV ↓, MCHC ↓, WBC ↑, LYM ↑, MON ↑, NEU ↑, EOS ↑, PLT ↓, APTT ↓, DIC
protist (piroplasm)	theileriosis	blood cells	HGB ↓, RBC ↓, HCT ↓, WBC ↓
nematode (rhabditid)	strongyloidiasis	gut	HGB ↓, RBC ↓, HCT ↓, WBC ↑, EOS ↑
nematode (trichostrongyloid)	ostertagiasis	stomach	HGB ↓, RBC ↓, HCT ↓
nematode (trichostrongyloid)	haemonchosis	stomach	HGB ↓, RBC ↓, HCT ↓, WBC ↑, EOS ↑
nematode (trichostrongyloid)	trichostrongyliasis	stomach	HGB ↓, HCT ↓, WBC ↑, EOS ↑
nematode (ascaridid)	ascariasis	gut	HCT ↓, WBC ↑, EOS ↑, PLT ↑
nematode (ascaridid)	toxocariasis	gut, tissues	HGB ↓, RBC ↓, HCT ↓, MCV ↑, MCHC ↓, WBC ↑, EOS ↑, LYM ↓, BAS ↑
nematode (ancylostomatoid)	hookworm diseases	gut	HGB ↓, RBC ↓, HCT ↓, MCV ↓, WBC ↑, NEU ↑, LYM ↑, EOS ↑, PLT ↑
nematode (spiruroid)	gnathostomiasis	gut	WBC ↑, EOS ↑
nematode (enoplid)	trichinosis	gut, muscles	RBC ↓, HGB ↓, HCT ↓, WBC ↑, LYM ↑, EOS ↑
nematode (filarioid)	lymphatic filariasis	blood, lymphatics	RBC ↓, HGB ↓, HCT ↓, MCV ↑, MCH ↑, MCHC ↓, WBC ↑, EOS ↑, PLT ↑
nematode (filarioid)	onchocerciasis	skin, blood	WBC ↑, LYM ↑, EOS ↑, NEU ↓
cestode (cyclophyllidean)	cysticercosis	muscles, viscera	RBC ↓, HGB ↓, HCT ↓, WBC ↑, EOS ↑, LYM ↑, NEU ↓
cestode (cyclophyllidean)	echinococcosis	viscera	WBC ↑, EOS ↑, LYM ↑
trematode (digenean)	schistosomiasis	veins, viscera	HGB ↓, HCT ↓, WBC ↑, EOS ↑, MON ↑, NEU ↑, LYM ↓
trematode (digeneans)	liver flukes	liver	HGB ↓, RBC ↓, HCT ↓, MCV ↑, MCHC ↓, WBC ↑, MON ↑, LYM ↑, NEU ↑, EOS ↑
insects (flies, fleas, lice)	ectoparasites	skin	HGB ↓, RBC ↓, HCT ↓, MCHC ↓, WBC ↓, NEU ↓, PLT ↓
arachnids (ticks, mites)	ectoparasites	skin	HGB ↓, RBC ↓, HCT ↓, MCHC ↓, WBC ↓, NEU ↓, PLT ↓

CLINICAL BIOCHEMISTRY

A wide variety of chemical tests have been developed to detect small amounts of molecules present within blood samples; including electrolytes, blood gases, lipids, enzymes, hormones, toxins, tumour markers, acute phase proteins, antibodies and various medications. Blood is usually collected by peripheral venepuncture into colour-coded tubes containing different chemical preservatives and/or anticoagulants. Plasma or serum is harvested and micro-volume samples processed in automated systems for a predetermined panel of parameters. Blood samples are commonly analysed for:

- electrolytes: ions involved in membrane transport, water balance and acid-base regulation; including: sodium (Na), potassium (K), chloride (Cl), bicarbonate (HCO_3) [high levels associated with diarrhoea, kidney diseases; low levels with vomiting, excessive sweating, diabetes]; hydrogen ion concentration, pH [increased levels indicative of alkalosis; decreased levels with acidosis]; osmolality/osmolarity [increased levels of solutes associated with dehydration, uremia, kidney damage; decreased levels with hyponatremia].
- minerals: involved in numerous homeostatic mechanisms; including: calcium (Ca) [high levels associated with hyperparathyroidism, cancer; low levels with liver disease, malnutrition, pancreatitis, renal disorders]; magnesium [high levels associated with renal disorders, dehydration; low levels with malnutrition, diarrhoea, diabetes]; phosphorus/phosphate (P/PO_4) [high levels associated with kidney or liver disease, ketoacidosis; low levels with hyperparathyroidism, malnutrition, rickets]; copper [high levels associated with anaemia, jaundice, tremors; low levels with neutropenia, anaemia, osteoporosis]
- vitamins: essential for metabolic processes; including: vitamin A [deficiency associated with vision problems; excess with toxicities]; vitamins B9, B12 [low levels associated with malnutrition, anaemia, neuropathy, tapeworm infection, malabsorption, neural tube defects in developing foetus; high levels concomitant with cancer, diabetes, obesity, liver disease]; vitamin C [low levels associated with malnutrition, scurvy; high levels with erythrocyte fragility]; vitamin D [deficiency associated with bone malformation or weakness, some cancers, immune diseases]; homocysteine [increased levels associated with malnutrition, folate deficiency, homocystinuria, possibly heart attack, stroke]

- blood markers: involved in erythropoiesis; including: total serum iron (TSI) and ferritin [low levels associated with iron-deficiency anaemia, pica, chronic illness; high levels with haemolytic anaemia, haemochromatosis]; total iron-binding capacity (TIBC) and transferrin [low levels associated with haemolytic anaemia, haemochromatosis, chronic illness; high with iron deficiency anaemia]; zinc [increased levels indicate disruption to heme production, usually associated with iron deficiency or lead poisoning]
- blood gases: evaluate lung function; including: oxygen saturation, oxygen partial pressure (pO_2) [low levels indicate patient not getting enough oxygen]; carbon dioxide partial pressure (pCO_2) and bicarbonates [high levels associated with metabolic alkalosis (due to chronic vomiting, heart failure, cirrhosis) or respiratory acidosis (due to lung diseases); low levels with metabolic acidosis (due to kidney failure) or respiratory alkalosis (due to hyperventilation, anxiety, pain)]; lactate and pyruvate [increased levels associated with hypoxia (type A lactic acidosis due to respiratory insufficiency or ischaemia, type B due to excess demand due to disease)]
- cardiac markers; indicative of heart disorders; including: heart-type fatty acid binding protein (H-FABP), troponin (TRO) and brain natriuretic peptide (BNP) [high levels associated with myocardial ischaemia]

- lipid markers: fats involved in energy stores, cell membranes, metabolism; elevated levels of triglycerides (TRIG) and low-density lipoprotein (LDL) cholesterol (CHOL) associated with atherosclerosis and heart disease; elevated levels of high-density lipoprotein (HDL) CHOL considered cardio-protective.
- inflammation markers: parameters indicative of recent infection; including: C-reactive protein (CRP) and procalcitonin (PRO) [high levels associated with acute inflammation and/or infection]; alpha 1-antitrypsin (AAT) [low levels associated with acute systemic inflammation or emphysema]; erythrocyte sedimentation rate (ESR) [high levels associated with acute inflammation, infection, anaemia, pregnancy]

- liver markers: indicative of liver dysfunction; including: total protein (PROT), albumin (ALB), globulins (GLO), often expressed as ALB:GLO (A:G) ratio [low levels reflect overproduction of GLO (seen in multiple myeloma or autoimmune diseases), or underproduction of ALB (seen in cirrhosis) or loss of ALB from circulation (due to infection or nephrotic syndrome)]; glucose (GLU) [high levels associated with diabetes, kidney disease, pancreatitis; low levels with severe infections, liver disease, kidney failure]; ammonia [high levels associated with infant haemolytic disease, liver or kidney disease, encephalopathies, gastrointestinal bleeding]; bilirubin (BILI) [high levels of biliary pigment associated with poor liver function (haemolysis, cholestasis), jaundice]; aspartate transferase (AST) and alanine transferase (ALT) [high levels associated with hepatocellular damage]; gamma-glutamyl transferase (GGT) and alkaline phosphatase (ALP) [high levels associated with cholestasis]
- gastric markers: indicative of stomach disorders; including pepsinogen (PEP) [high levels associated with gastric mucosal infections, especially by nematodes]
- renal markers: used to assess kidney function; including: creatinine (CRE) [high levels associated with kidney disease, infection]; blood urea nitrogen (BUN) [hhigh levels associated with impaired kidney function, heart failure, dehydration];

uric acid (UA) [high levels seen in many disorders, gout, diabetes, kidney stones]; ketones (high levels associated with increased fat metabolism, ketoacidosis, diabetes]

- pancreas markers: indicate pancreatic problems; including: amylase (AMY) and lipase (LIP) [high levels associated with inflammation, disease]
- general markers: indicative of systemic/disseminated problems; including: lactate dehydrogenase (LDH) [high levels associated with tissue/cellular injury, anaemia, inflammation, infection: LDH-1 (heart, brain, erythrocytes); LDH-2 (reticulo-endothelial system); LDH-3 (lungs); LDH-4 (kidneys, pancreas, placenta); LDH-5 (liver, muscles); angiotensin-converting enzyme (ACE) [high levels associated with sarcoidosis, cirrhosis, infection; decreased levels with lung diseases, starvation]; eosinophilic cationic protein (ECP)(= ribonuclease 3) [high levels associated with inflammation, asthma, dermatitis, helminth infection]; creatine kinase (CK), myoglobin (MYO), aldolase (ALD) and carbonic anhydrase (CA-III) [high levels associated with muscle damage]

- endocrinology: levels determined for thyroid hormones (thyroxine, thyroid stimulating hormone, triiodothyroxine, thyroxine binding globulin, thyroglobulin), sex hormones (dihydrotestosterone, testosterone, hydroxyprogesterone, follicle-stimulating hormone, luteinizing hormone, estradoil, progesterone, dehydroepiandrosterone sulfate, beta human chorionic gonadotrophin) and others (adrenocorticotrophic hormone, cortisol, growth hormone, insulin-like growth factor, prolactin, parathyroid hormone, plasma renin activity, aldosterone)
- tumour markers: indicative of various cancers; e.g. alpha-fetoprotein (liver, testicular, ovarian cancer); carcinoembryonic antigen (colonic cancer); cancer antigen 19-9 (pancreatic cancer), CA-125 (ovarian cancer); calcitonin (thyroid cancer); acid phosphatase and prostate specific antigen (prostate cancer)

- immunoglobulin isotypes: antibody classes typed as IgA (secretory/mucosal), IgD (receptor), IgE (inflammatory/ allergenic), IgM (primary antibody responses, transient) or IgG (secondary antibody responses, persistent)
- auto-antibodies: a range of tests are available for auto-immune conditions (either systemic or organ-specific)
- toxins: blood tests are available for a range of toxic chemicals (e.g. lead, ethanol)

Parasites may damage tissues causing them to metabolize or release abnormal levels of particular chemicals, as evidenced by comparing levels with known reference ranges. Regrettably, few changes in chemical levels are specific for individual parasites, so biochemical screens are often used to supplement/complement clinical information by indicating which tissue systems have been compromised and to what extent. Some parasites may cause systemic or disseminated problems to the host, while others have more specific effects on particular organ/tissue systems involved in infection.

Blood biochemical changes associated with parasitic infections

Type of parasite	Disease	Site of infection	Changes to blood biochemical parameters
protist (amoeba)	amoebiasis	gut, liver, brain	ALP ↑, ESR ↑, CRP ↑, ALB ↓, AST ↑, ALT ↑
protist (diplomonad)	giardiasis	gut	CRE ↓, AMY ↓, GLU ↑, BUN ↑, PROT ↑, AST ↑, ALT ↑, Na ↑, K ↑
protist (kinetoplastid)	African trypanosomaisis	blood, brain	ESR ↑, PROT ↑, ALB ↓, IgM ↑, BUN ↑, GLO ↑
protist (kinetoplastid)	American trypanosomaisis	macrophages, tissues	CK ↑, PROT ↑, ALP ↑, ALD ↑, ALT ↑, GGT ↑, TRO ↑
protist (kinetoplastid)	leishmaniasis	skin, viscera	CHOL ↓, HDL ↓, LDL ↓, ALB ↓, TRI ↑, PROT ↑, GLO ↑, UA ↑, BUN ↑, CRE ↑, AST ↑, ALT ↑
protist (coccidian)	coccidiosis	gut	PROT ↓, ALB ↓, ALT ↓, AST ↓, ALP ↑
protist (coccidian)	toxoplasmosis	brain, muscles, viscera	PROT ↓, ALB ↓, ALT ↑, AST ↑, ALP ↑, CK ↑, BILI ↑, AMY ↑, LIP ↑, GLO ↑, ESR ↑, TRO ↑
protist (haemosporidian)	malaria	liver, erythrocytes	AST ↑, BILI ↑, GLU ↓, CRE ↑, BUN ↑, UA ↑, ESR ↑, ALB ↓, LDH ↓
protist (piroplasm)	babesiosis	erythrocytes, spleen	CK ↑, LDH ↑, AST ↑, ESR ↑, ALB ↓, BUN ↑, CRE ↑, CHOL (HDL, LDL) ↑, TRIG ↑, ALT ↑, BILI ↑, GLO ↓
protist (piroplasm)	theileriosis	blood cells	ALT ↑, AST ↑, ALP ↑, CK ↑, GGT ↑, UA ↑, BUN ↑, BILI ↑, P ↑, PROT ↓, ALB ↓, GLU ↓, CHOL ↓, Ca ↓
nematode (rhabditid)	strongyloidiasis	gut	ALB ↓, GLO ↑, CHOL ↓, GLU ↓, IgE ↑
nematode (trichostrongyloid)	ostertagiasis	stomach	PEP ↑, ALB ↓, PROT ↓, ALP ↑, AP ↑
nematode (trichostrongyloid)	haemonchosis	stomach, gut	PEP ↑, ALB ↓, PROT ↑
nematode (trichostrongyloid)	trichostrongyliasis	stomach, gut	PROT ↓, ALB ↓, GLO ↑, PEP ↑, Ca ↓, P ↓, vit. A ↓, ALP ↓
nematode (ascaridid)	toxocariasis	gut, tissues	ESR ↑, P ↓, Na ↓, K ↓, HCO_3 ↓, GLU ↓, PROT ↓, BUN ↓, CRE ↓, CHOL ↑, Ca ↑, Cl ↑, ALP ↑, AST ↑, ALT ↑
nematode (ancylostomatoid)	hookworm diseases	gut	CRP ↑, TSI ↓, IgE ↑, PROT ↓, ALB ↓, Na ↓, Cl ↓, ALP ↑, ALT ↑
nematode (enoplid)	trichinosis	gut, muscles	CK ↑, ALP ↑, AST ↑, ALT ↑, GLU ↑, K ↑, IgE ↑
nematode (filarioid)	lymphatic filariasis	blood, lymphatics	ESR ↑, ALT ↑, AST ↑, BILI ↑, PROT ↑, GLO ↑, GLU ↓, ALB ↓, BUN ↑, CRE ↑, P ↑, K ↑, Ca ↓, Na ↓
nematode (filarioid)	onchocerciasis	skin, blood	LDH ↑, ECP ↑, CRP ↑, CK ↑, ALD ↑
cestode (cyclophyllidean)	cysticercosis	viscera, muscles, brain	PROT ↓, AST ↑, ALT ↑, ALP ↑, CK ↑, LDH ↑, ALB ↓, GLO ↑
cestode (cyclophyllidean)	echinococcosis	viscera	GGT ↑, ALP ↑, BUN ↓, GLU ↓, AST ↑, CHOL ↓, BILI ↑, ALT ↑, AP ↑
trematode (digenean)	schistosomiasis	veins, viscera	GLO ↑, Ca ↑, K ↑, PROT ↑, ALB ↓, BUN ↑, UA ↓
trematode (digeneans)	liver flukes	liver	GGT ↑, BILI ↑, ALT ↑, AST ↑, LDH ↑, ESR ↑, PROT ↓, ALB ↓, ALP ↑
insects (flies, fleas, lice)	ectoparasites	skin	PROT ↑, GLO ↑, ALB ↓, GLU ↓, BILI ↓, AST ↓, Cu ↓, Zn ↓, Ca ↓, ALT ↑, TSI ↓, ALP ↓
arachnids (ticks, mites)	ectoparasites	skin	PROT ↑, GLO ↑, ALB ↓, GLU ↓, BILI ↓, AST ↓, Cu ↓, Zn ↓, Ca ↓, ALT ↑, TSI ↓, ALP ↓

IMMUNOLOGY

While most haematological and biochemical clinical parameters are non-specific for individual parasitoses, immunological techniques using highly specific antigen-antibody interactions can be used to diagnose infections by particular parasite species. Humans and animals respond to most infectious diseases by forming antibodies (Ab) against antigens (Ag) of the infecting pathogen as part of their immunological defenses. These antibodies (= gamma-globulins or immunoglobulins) are produced by plasma cells (transformed B lymphocytes) and are secreted into the blood stream to circulate through the body. When they come into contact with the relevant antigen, they bind to it and tag it for destruction.

Serum (or plasma) samples are usually tested for specific host antibodies, and sometimes for parasite antigens (antigenaemia usually associated with blood-borne parasites). More recently, techniques have been developed to test faecal samples for host secretory (mucosal) antibodies or parasite copro-antigens (usually associated with gastroenteric parasites). A range of immunological techniques are currently used to demonstrate host antibodies and/or parasite antigens; including complement fixation tests (CFT), dye tests (DT), direct or indirect haemagglutination test (HAT, IHAT), direct or indirect fluorescent-antibody tests (DFAT, IFAT), chemiluminescence analyses (automated PRISM system), enzyme-linked immunosorbent assays (ELISA) with various modifications (such as dot-ELISA, Falcon assay screening test ELISA (FAST-ELISA) and luciferase immunoprecipitation system (LIPS)], radioimmunoprecipitation assays (RIPA), rapid immunochromatographic antigen detection tests (RDT) and immunoblotting (IB).

In diagnostic tests, laboratory reagents (antigens or antibodies) are immobilized on substrates, incubated with test (and control) samples, an indicator system is added and the results best read objectively and quantitatively; i.e. without subjective interpretation by individual operators and with measurement of a related parameter, such as spectrophotometric absorbance (optical density), intensity of fluorescence, degree of haemolysis, etc. It is important to quantitate the amount of specific antibody present as this provides an indication of the severity of infection as well as the immunocompetence of the host. However, it is impossible to measure the absolute concentration (w/v) of specific antibodies by standard biochemical techniques, so the amount is estimated by serial dilution of test samples and reporting the 'end-titre' (last positive dilution); e.g. an end-point titre of 1/100 indicates there was enough specific antibody present to elicit a positive reaction when the blood was diluted 100 times.

Test results can be influenced by many factors which affect the integrity of the relationships between parameters (such as edge effects, detectable levels, accuracy, interference, competition, nonspecific background reactions, cross-reactivity with other microbes, reactions against vaccines previously given, poor test sensitivity and specificity). Positive and negative control samples can be used to indicate, and even mitigate, various problems, so the end-point titre of any particular sample can usually be given with a high degree of confidence. Interpreting the significance of the test results requires thorough knowledge of the kinetics (onset and duration) and dynamics (intensity) of the host response to infection. Longitudinal samples are often obtained to determine whether antibody titres in a particular individual remain stable or whether they are increasing or decreasing (plot titre over time).

Immunological tests available for parasitic infections in diagnostic pathology laboratories

Type of parasite	Disease	Test sample	Immunological tests*
protist (amoeba)	*Entamoeba histolytica*	serum (Ab)	ELISA
protist (amoeba)	*Entamoeba histolytica*	faeces (Ag)	ELISA, RDT
protist (amoebae)	*Naegleria, Acanthamoeba, Balamuthia* spp.	cerebrospinal fluid, tissues (Ag)	IFAT
protist (diplomonad)	*Giardia duodenalis*	faeces (Ag)	DFAT, ELISA, RDT
protist (trichomonad)	*Trichomonas vaginalis*	vaginal secretions (Ag)	DFAT, ELISA, IHAT
protist (kinetoplastid)	*Trypanosoma brucei*	serum (Ab)	HAT, IHAT, DFAT, IFAT, ELISA, dot-ELISA, IB
protist (kinetoplastid)	*Trypanosoma cruzi*	serum (Ab)	IFAT, ELISA, dot-ELISA, RIPA, PRISM, IB
protist (kinetoplastid)	*Trypanosoma cruzi*	serum (Ag)	RDT
protist (kinetoplastid)	*Leishmania* spp.	serum (Ab)	HAT, IHAT, ELISA, dot-ELISA
protist (coccidian)	*Cryptosporidium parvum, C. hominis*	faeces (Ag)	DFAT, IFAT, ELISA, RDT
protist (coccidian)	*Toxoplasma gondii*	serum (Ab)	DT, HAT, IHAT, DFAT, IFAT, ELISA, IB
protist (coccidian)	*Neospora caninum*	serum (Ab)	ELISA
protist (coccidian)	*Neospora caninum*	tissues, blood cells (Ag)	IFAT
protist (haemosporidian)	*Plasmodium falciparum, P. vivax, P. malariae*	serum (Ab)	IFAT, FAST-ELISA
protist (haemosporidian)	*Plasmodium falciparum, P. vivax, P. malariae, P. ovale*	serum, blood (Ag)	ELISA, RDT
protist (piroplasm)	*Babesia* spp.	serum (Ab)	IFAT, ELISA
protist (piroplasm)	*Babesia* spp.	tissues, blood cells (Ag)	IFAT
nematode (rhabditid)	*Strongyloides stercoralis*	serum (Ab)	HAT, IHAT, DFAT, IFAT, ELISA, IB, LIPS
nematode (trichostrongyloid)	carbohydrate larval antigens of *Haemonchus, Trichostrongylus, Telodorsagia, Cooperia, Nematodirus* spp.	saliva (Ab)	ELISA
nematode (ascarid)	*Toxocara canis*	serum (Ab)	ELISA
nematode (ascarid)	*Bayliascaris procyonis*	serum (Ab)	IB
nematode (metastrongyloid)	*Angiostrongylus cantonensis*	serum (Ab)	ELISA
nematode (filarial)	*Wuchereria bancrofti, Brugia malayi, B. timori, Loa loa*	serum (Ab)	HAT, IHAT, ELISA, dot-ELISA, LIPS
nematode (filarial)	*Wuchereria bancrofti*	serum, blood (Ag)	IHAT, ELISA, RDT
nematode (filarial)	*Dirofilaria immitis*	serum (Ab)	ELISA
nematode (filarial)	*Dirofilaria immitis*	serum (Ag)	ELISA, RDT
nematode (enoplid)	*Trichinella spiralis*	serum (Ab)	ELISA
cestode (cyclophyllidean)	*Taenia solium*	serum (Ab)	IB, ELISA
cestode (cyclophyllidean)	*Taenia solium*	faeces, serum (Ag)	ELISA
cestode (cyclophyllidean)	*Echinococcus granulosus, E. multilocularis*	serum (Ab)	HAT, IHAT, ELISA, dot-ELISA, IB
trematode (digenean)	*Fasciola hepatica, F. gigantica*	serum (Ab)	IB, ELISA, FAST-ELISA, dot-ELISA
trematode (digenean)	*Fasciola hepatica*	faeces (Ag)	ELISA
trematode (digenean)	*Paragonimus westermani*	serum (Ab)	IB
trematode (digenean)	*Schistosoma japonicum, S. mansoni, S. haematobium*	serum (Ab)	HAT, IHAT, ELISA, dot-ELISA, FAST-ELISA, IB
trematode (digenean)	*Schistosoma mansoni*	serum (Ag)	IB
insect (flea)	*Ctenocephalides* spp.	skin test (Ab)	intradermal inoculation
arachnid (mite)	*Sarcoptes scabiei*	serum (Ab)	ELISA

**cf. Ndao M 2009 Diagnosis of parasitic diseases: old and new approaches. Interdisciplinary Perspectives on Infectious Diseases doi:10.1155/2009/278246*

DPDx Laboratory Identification of Parasitic Diseases of Public Health Concern, Centers for Disease Control and Prevention (CDC) website www.cdc.gov

MOLECULAR BIOLOGY

Over the last several decades, molecular biology has undergone a dramatic revolution whereby techniques have become available to work directly with parasite proteins and nucleic acids (RNA and DNA). Research laboratories have utilized many different techniques to:

- characterize genetic variation within and between parasite species and different life-cycle stages;
- conduct disease screening and surveillance programs;
- explore parasite population genetics and molecular epidemiology;
- determine phylogenetic relationships between parasite taxa and assemblages;
- explore parasite biochemistry and protein expression (often related to drug resistance);
- produce defined immunoreagents (including vaccine development); and
- develop new diagnostic tests for field and laboratory use.

Early studies were conducted on parasite proteins and numerous electrophoretic systems were developed to detect species-specific polypeptides and isoenzymes by one- or two-dimensional electrophoresis in different gel matrices (polyacrylamide, cellulose nitrate, agarose) whereby molecules are separated on the basis of their size and charge. Specific molecules could be partly identified by their positions within the gels (with respect to molecular markers), by reacting them against specific substrates (for isoenzymes), or immunoblotting (IB) them against polyclonal or monoclonal antibodies. Rapid immunochromatographic techniques (RDT) have also been developed as dip-stick or spot-on tests to detect specific parasite proteins in test samples.

More recently, the advent of the polymerase chain reaction (PCR) allowed the rapid and specific amplification of specific parasite DNA or RNA sequences. Diagnostic PCRs work by using short synthetic (forward and reverse) primer pairs (around 20 bases) to amplify specific segments of known length and sequence of parasite DNA (usually 100-800 bases). Whole DNA is extracted from host samples (lysed blood/tissues) by precipitation and centrifugal filtration into elution buffer. [For RNA, reverse transcriptase is first used to synthesize cDNA which is then amplified by PCR (process known as reverse transcription PCR or RT-PCR)]. Eluted DNA (or cDNA) is then mixed with a solution containing the primers, *Taq* polymerase (from *Thermus aquaticus*), magnesium chloride and dinucleotide triphosphates (dNTPs). The solutions are then placed in a thermal cycler which starts with an initiation phase where high temperatures (~94°C) are used to denature double-stranded DNA into single strands. The temperature is then decreased to allow the primers to hybridize (anneal) to these single strands. The heat-stable polymerase then uses ambient nucleotides to copy (extend) the targeted sequence. These procedures are repeated through some 30 cycles thus facilitating the exponential amplification of parasite DNA (theoretically 2^{30} = 2 billion times). PCR products (amplicons) are then visualized, usually by agarose gel electrophoresis, where DNA bands are separated on the basis of their molecular size and charge. The agarose gel is treated with a fluorescent DNA stain that allows amplicons to be visualized under ultraviolet light. Smaller amplicons travel faster through the gel matrix than larger amplicons, and their final position can be used to determine band size by comparison with standard markers (DNA ladders). Positive results in a PCR test are indicated by the appearance of a specific band (the same size as the primer's targeted sequence) in the gel. Amplicons can also be examined by restriction fragment length polymorphism (RFLP) analysis (enzymes used to cleave DNA at specific sites) or by DNA sequencing if confirmation or further characterization is required.

Various PCR modifications have been examined in attempts to simplify the process, such as measuring amplification products in real-time, using single tubes, using constant temperatures, adopting multiplex formats, detecting amplicons by differential fluoroscopy, enzyme reactions or chromatography. Quantitative real-time PCR (qPCR) monitors DNA amplification in a single reaction vessel in real-time by measuring the fluorescence signal every cycle using a DNA-binding dye (e.g. SYBR-Green) or sequence-specific internal *Taq*Man probes (eliminating the need for subsequent gel electrophoresis). Loop-mediated isothermal amplification (LAMP) reactions are carried out at a constant temperature (eliminating the need for a thermal cycler) using six different primers recognizing eight distinct regions within a target gene. Luminex (LUM) technology is a bead-based flow-cytometric assay whereby probes (antigens, antibodies or oligonucleotides) are bound to microsphere beads and each emits a unique fluorescent signal when excited by laser. Quantitative nucleic acid sequence-based amplification (QT-NASBA) amplifies RNA sequences at a constant temperature using alternating treatments with reverse transcriptase to synthesize cDNA and then RNAse H to destroy RNA in RNA-DNA hybrids. Oligochromatography PCR (OC-PCR) visualizes amplicons on a dipstick through hybrization with a gold-conjugated probe. PCR-ELISA uses immunosorbent (or oligosorbent) assays to quantify amplicons after their immobilization on microtitre plates. Rather than being confined to research institutions, PCR technologies are now becoming more commonplace in diagnostic laboratories as they become easier and faster to use without elaborate specialized equipment.

The analytical sensitivities (limits of detection) of PCR for parasites have been variously reported to range from 50 ng to 10 fg of DNA, but it is impossible to convert these measurements into conventional parasite concentrations (such as numbers per weight or volume of sample, or percentage parasitaemia) without knowing many variables, including sample size, DNA extraction efficacy, PCR aliquot size, DNA purity and specificity, etc. Nowadays, experimental studies are conducted involving the serial titration of DNA extracted from samples spiked with known numbers of parasites, and analytical sensitivities (LODs) have been reported to be around one protozoan or helminth parasite per mL (or per gram) of sample; some even being as low as one cell (*T. cruzi*) per 20 mL of blood.

Molecular diagnostic tests for parasites

Type of parasite	Disease	Test sample	Type of test*
protist (amoebozoa)	*Entamoeba histolytica, E. dispar*	faeces	PCR, qPCR
protist (amoebozoa, heterolobosea)	*Naegleria, Acanthamoeba, Balamuthia* spp.	CSF, tissues	PCR
protist (kinetoplastid)	*Trypanosoma brucei*	blood	PCR, qPCR, OC-PCR, LAMP
protist (kinetoplastid)	*Trypanosoma cruzi*	blood	PCR, qPCR, LAMP
protist (kinetoplastid)	*Leishmania* spp.	blood	PCR, qPCR, OC-PCR, PCR-ELISA, QT-NASBA
protist (coccidian)	*Cryptosporidium parvum, C. hominis*	faeces, water	PCR, qPCR, LAMP, LUM
protist (coccidian)	*Cyclospora cayetanensis*	faeces	PCR, qPCR
protist (coccidian)	*Toxoplasma gondii*	blood	PCR, qPCR
protist (haemosporidian)	*Plasmodium falciparum, P. vivax, P. malariae, P. ovale*	blood	PCR, qPCR, PCR-ELISA, LAMP, LUM, QT-NASBA
protist (piroplasm)	*Babesia microti*	blood	PCR, qPCR
microsporidian (fungi)	various species	faeces	PCR
nematode (rhabditid)	*Strongyloides stercoralis*	faeces	PCR, qPCR
nematode (filarial)	*Wuchereria bancrofti, Brugia malayi, B. timori, Loa loa*	blood	PCR, PCR-ELISA
cestode (cyclophyllidean)	*Taenia solium*	faeces	PCR, LAMP
trematode (digenean)	*Schistosoma mansoni*	serum, faeces	PCR, qPCR, OC-PCR

**cf. DPDx 2017 Laboratory Identification of Parasitic Diseases of Public Health Concern, Centers for Disease Control and Prevention (CDC) website www.cdc.gov*
Ndao M 2009 Diagnosis of parasitic diseases: old and new approaches. Interdisciplinary Perspectives on Infectious Diseases dx.doi.org/10.1155/2009/278246

DNA sequencing of different gene fragments continues to fuel comprehensive genetic and phylogenetic analyses of both clinical and environmental isolates of many parasite species. Sequencing has facilitated the quantitation of genetic variation both between and within parasite species, leading to the recognition of particular 'genotypes' associated with clinical disease. Phylogenetic analyses use a range of computational algorithms (parsimony, maximum likelihood and Bayesian inference) to generate branching tree-like diagrams to represent estimated pedigrees of inherited characters. In many instances, molecular biology has validated conventional taxonomic classification systems (notably for families, genera and species), but in some cases they have led to the complete revision of classical classification systems, especially for higher taxa (kingdoms, phyla, classes and orders). While taxonomy underpins diagnosis, it must be remembered to be an ever-changing paradigm based on a constellation of characters (it is never absolute and cast in concrete).