Laboratory Diagnosis of Tropical Fever

Shalini Dewan Duggal, Tulsi Das Chugh

Tropical diseases are the diseases which are prevalent in the tropics but usually not seen in temperate climates. These may be vector borne or occur due to conditions of high humidity and temperature or sudden temperature changes which favour growth of particular microorganisms.

Diagnosis and management of tropical infectious diseases is important as they usually run a dramatic course if not treated. They may be caused by a variety of pathogens including bacteria, viruses, parasites and fungi; hence an accurate diagnosis leads to appropriate management. Diagnosis is also important from epidemiological purview to assess the burden of these diseases and monitor the effectiveness of national and international health programmes.

Recently the Indian society of Critical care medicine formulated certain guidelines and recommended a 'syndromic approach' to diagnosis and treatment of critical tropical infections. They have identified five major clinical syndromes: undifferentiated fever, fever with rash / thrombocytopenia, fever with acute respiratory distress syndrome (ARDS), fever with encephalopathy and fever with multi organ dysfunction syndrome.

Diagnosis of Tropical Diseases:

Diagnosis of a tropical infectious disease may require certain clues or hints which include a properly obtained history, epidemiological factors, recent travel and presenting clinical features 1. These infections have been categorised as arthropod-borne, rodent- associated, reservoir associated or human-human spread. An initial diagnosis may be based on the basis of interval between the exposure and the appearance of first symptom; which may be Short (≤ 10 days), Intermediate (7 – 28 days), Long (> 4 weeks) or Variable (weeks to years).

A full blood count and examination of blood smears is a nearly obligatory basic investigation. This may be accompanied by biochemical examinations like liver or kidney function tests or CSF examinations as the case may be. However, definitive diagnosis of any infectious disease relies on the microbiological investigations resulting in confirmation.

Laboratory Diagnosis in a Microbiology Laboratory:

Laboratory diagnosis may detect an organism directly by visualization under a microscope or by growing them in culture media. Culture of an organism and further identification by means of various tests proves the identity of the causative agent in an infectious disease, hence considered Gold standard. It also helps to test the organisms for susceptibility to antimicrobial agents under laboratory conditions. However, not all organisms can be cultured or identified routinely or results may not be available for days or weeks. For these agents, indirect methods of diagnosis are considered. These include serological or molecular methods. Serological tests include agglutination tests such as latex agglutination, enzyme immunoassays, Western blot,

precipitation tests, and complement fixation tests, and molecular tests may be nucleic and non-nucleic acid–based identification tests. In most Microbiology Laboratories, microscopic examination, culture facilities and some serological tests are available; other tests are done in special/ research Microbiology Laboratories.

A list of bacterial, viral, parasitic and fungal diseases along with their causative agents, source of infection and relevant investigations are presented in Table 1.

Table 1: Tropical Infectious Diseases:

Tropical Disease	Causative Agent	Source of Infection	Sample required	Investigations	Laboratory Type	Sensitivity (%)	Specificity (%)
Bacterial Tropical Diseases							
Legionellosis[2]	Legionella pneumophila	Cooling towers, Humidifiers, Respiratory therapy equipment, Potable/ hot water systems	Blood, Serum, Respiratory specimens, Urine	Direct fluorescent antibody staining	Special	25-70	90
				Indirect fluorescent antibody test (IFA)	Special	78-91	>99
				Cultures of sputum, lower respiratory tract secretions, tissue, blood	Special	10-80	100
				Urinary antigen	Routine	70-90	>99
				Polymerase chain reaction (PCR)	Special	30-100	>90

Leptospirosis[3]	Leptospira spp.	Urine, body fluids, or organs of infected animals, or by contaminated soil or water)	Blood, CSF, body fluids or tissues	Culture of body fluids or tissues like liver, muscle, kidney, skin, eyes. **GOLD Standard**	Special	6-28	100
				Microscopic agglutination testing (MAT)	Special	86-96	>98
				IFA	Special	64	>95
				Lateral flow immunochromatographic test	Routine	87	70
				PCR	Special	55	82
Melioidosis[4]	Burkholderia pseudomallei	Inoculation, inhalation	Blood, Serum, urine, sputum, skin lesions/ abscesses, throat/ rectal swabs	Culture of specimen : **GOLD Standard**	Special	51-68	100
				Gram stain, Immunofluorescence microscopy	Special	40-90	>90
				Indirect haemagglutination test, titres	Special	63-95	74-97
				IgM ELISA	Special	80	95
				PCR	Special		

Meningococcal Disease[5]	Neisseria meningitidis	Direct contact with droplets/discharges from nose and throat of patients and healthy carriers	Cerebrospinal fluid (CSF), Blood, Serum, Skin rash aspirates	CSF Gram stain	Routine	60-90	>97
				Blood and CSF cultures: **GOLD Standard**	Routine	70-85	100
				Antigen detection in CSF/Serum by latex agglutination	Routine	50-93	>99
				Smears/ culture from petechiae	Routine	60-70	>90
				PCR	Special	91-94	>96
Q fever[6]	Coxiella burnetii	Zoonosis. Cattle, sheep, goats or infected humans through Inhalation, tick bites, unpasteurized milk and milk products.	Whole blood, serum, , CSF, pleural fluid, bone/ liver biopsy/ excised heart valve, milk, placenta or foetal tissue	Culture of affected tissue: **GOLD Standard**	Biosafety level 3 (BSL 3) laboratories	15-53	100
				Increased Phase I and II IgM, IgG titres by Microimmunofluorescence	Special	58-100	92-99
				Increased Phase II IgM and IgG titres by ELISA	Special	80-84	>97
				Microagglutination	Special	81	98
				Immunohistochemistry of tissue	Special	71	>90
				PCR	BSL 3	84	100

Tuberculosis[7]	Mycobacterium tuberculosis	Infected person	Sputum & other respiratory specimens, abscess, blood, bone marrow, body fluids, urine, gastric lavage, faeces	Ziehl Neelsen	Routine	20-80	>90
				Fluorescence Microscopy	Special	30-90	>90
				Solid Culture-LJ Media	BSL 3	24	100
				Liquid Culture: **GOLD Standard**	BSL 3	41	100
				Mantoux test	Clinical	65-94	50-95
				PCR	Special	43-98	90-99
				Serological tests	Not recommended in India.	60-70	40-50
The presence of acid-fast-bacilli (AFB) on a sputum smear or other specimen often indicates TB disease. At least two sputum smears should be examined in a case of suspected pulmonary tuberculosis. A positive culture for M. tuberculosis confirms the diagnosis of TB. Culture examinations should be completed on all specimens, regardless of AFB smear results.							
Typhoid and Paratyphoid fever[8]	Salmonella enterica serotype Typhi, Paratyphi A, B or C	Water or food contaminated by faeces of an acutely infected / convalescent person or a carrier	Blood, bone marrow, urine, stool, Serum	Culture of blood, bone marrow-	Routine	40- 90	100
				Widal Test	Routine	88	70-80
				IgM Detection against S. Typhi	Routine	78-96	76-90
				Anti lipopolysachharide (LPS) haemagglutination	Routine	60	98.2
				Antigen detection by ELISA or co-agglutination	Routine		25-90
				PCR	Special	69-85	98-100

Culture is the **GOLD Standard** of diagnosis. Sensitivity of blood culture varies according to the amount of blood cultured, number of specimens, antibiotic therapy, and timing of specimen collection. The sensitivity of culture is 85–90% for bone marrow, 40–50% for blood, around 60% for rose spots, 40–60% and <10% for stool and urine cultures, respectively.							
Typhus: Scrub Typhus[9]	Orientia tsut-sugamushi	Trombiculid mites	Blood, serum, biopsy or smears from affected sites.	Weil–Felix OXK agglutination	Routine	89	89
				Scrub Typhus-Rapid Immunochromatographic test	Routine	74-96	86-99
Louse-borne or Epidemic Typhus caused by Rickettsia prowazekii by excrement of body louse (Pediculus corporis) inoculated into bite wound **Murine or Endemic Typhus** caused by Rickettsia typhi, transmitted by Bite or excreta of Rat Flea (Xenopsylla cheopis) scratched into skin				Murine Typhus Immunoblot test	Routine	51-91	87-100
				Histopathological examination of tissue sections by Giemsa or Gimenez staining	Special	53-75	100
				IFA: **GOLD Standard**	Special	46-100	78-100
				Indirect Immunoperoxidase staining	Special	50-100	80-100
				Cell Culture	Special	29-59	100
				PCR	Special	< 1 PFU/ml	100

Viral Tropical Diseases							
Avian influenza[10]	Influenza A H5N1	Direct or close contact with infected poultry	Throat /nasal swabs or aspirates	Viral Culture **GOLD Standard**	BSL 3	100	100
				Real-time reverse transcription-PCR	Special	100	100
				IFA test	Special	70-100	80-100
				Rapid Antigen Detection	Routine	70-75	90-95
Chikungunya[11]	CHIK virus of genus Alphavirus, family Togaviridae	Arboviral infection transmitted by bite of Aedes aegypti and Aedes albopictus mosquitoes	Serum, plasma or whole blood	IgM Antibody Capture (MAC) ELISA	Routine	84-100	>99
				IFA	Special	75-100	>99
				Lateral flow immunochromatography	Routine	10-100	>95
				Culture **GOLD Standard**	BSL 3	79-100	100
				PCR	Special	0.001-1 PFU /ml	100

Crimean-Congo Haemorrhagic Fever[12]	Genus Nairovirus, Family Bunyaviridae	Ticks and livestock animals, close contact with the blood, secretions, organs or other bodily fluids of infected persons	Serum, Blood, Body fluids, Tissue Biopsy	IgM ELISA	Routine	75-97	100
				Antigen detection	Special	50-100	100
				IFA	Special	75-100	97-100
				Pseudo-plaque reduction neutralization	Special	98	100
				Reverse transcriptase polymerase chain reaction (RT-PCR)	Special	79-83	100
				Virus isolation by cell culture **GOLD Standard**	BSL 3	Poor	100

Dengue[13]	Genus Flavivirus of the family Flaviviridae	Arboviral infection transmitted by bite of Aedes aegypti and Aedes albopictus mosquitoes	Whole Blood, Serum, Tissues	RT-PCR	Special	80-90	89-100
				MAC ELISA	Routine	90	98
				IgG ELISA	Routine	91	99
				IgM Rapid test	Not Recommended	21-99	77-98
				NS1 Antigen Detection	Routine	71-100	98-100
				Viral isolation **GOLD Standard**	BSL 3	<50%	100
				Plaque reduction and Neutralization test	Special	96	93-95
				Immunocytochemistry	Special	100	91
				Mosquito inoculation	Special	98-100	100
During the initial five days, the virus can be detected in serum, plasma, circulating blood cells and other tissues and virus isolation in cell culture, detection of viral RNA by nucleic acid amplification tests (NAAT), or by detection of viral antigens (NS1) by ELISA can be done. At the end of the acute phase of infection, IgM antibodies appear in 50% of patients by days 3-5 after onset of illness, increasing to 80% by day 5 and 99% by day 10. A four-fold or greater increase in antibody levels measured by IgG ELISA or haemagglutination inhibition test in paired sera indicates an acute or recent flavivirus infection. During a secondary dengue infection IgG is detectable at high levels, even in the acute phase. Early convalescent stage IgM levels are significantly lower in secondary infections than in primary ones.							

Haemorrhagic fever with renal syndrome[14]	Genus Hantavirus of family Bunyaviridae	Aerosolized rodent excreta	Blood, tissue	IgM Rapid immunochromatography test	Routine	80-97	90-100
				IgM ELISA	Special	94	99
				IgM IFA	Special	96-100	99
				Viral isolation by Cell culture **GOLD Standard**	Special	80-95	100
				RT-PCR	Special	94	100
Hepatitis A[1]	Genus Hepatovirus Family: Picornaviridae	Contaminated food or water	Serum, Faeces	IgM anti-HAV ELISA	Routine	100	99
				RT-PCR **GOLD Standard**	Special	-	100

Hepatitis B [1, 15]	Genus Orthohepadnavirus, Family Hepadnaviridae	Parenteral transmission, infected injection needles, vertical and sexual transmission	Blood-Serum or Plasma	HBsAg Rapid Immunochromatographic test	Routine	94.5-100	91-100
				HBsAg Latex Agglutination	Routine	66	98
				HBsAg ELISA	Routine	96-98	98-100
				Anti-HBs ELISA	Routine	94-98	98-100
				HBeAg ELISA	Routine	98-99	98-100
				Anti-HBe ELISA	Routine	90-96	98-100
				Anti-HBc ELISA	Routine	92-96	98-100
				HBV RT- PCR **GOLD Standard**	Special	90-95	100

Condition	**Laboratory Markers for Hepatitis B**					
	HBsAg	**HBeAg**	**HBV DNA**	**Anti HBs**	**Anti HBe**	**IgM Anti HBc**
Acute Infection	+	+	+	-	-	+
Chronic Infection	+	+	+/-	-	-	+
Fulminant hepatitis	+/-	+	+	-	-	+
Vaccinated person	+#	-	-	-	-	-
Infection immunity	-	-	-	+	+/-	-
Healthy carrier	+	-	-	-	+	+

Hepatitis C[16]	Flavivirus	Parenteral transmission, infected needles, vertical and sexual transmission	Blood	ELISA HCV Core Antigen	Routine	90-95	100
				Recombinant immunoblot assay	Special	78	90
				ELISA Anti-HCV (IV generation)	Routine	99-100	>99
				Saliva-based anti-HCV	Routine	87	99
				HCV RNA PCR **GOLD Standard**	Special	96	99-100
Hepatitis E[17]	Genus Hepevirus, Family Hepeviridae	Faecal-oral route, contaminated water.	Blood, stool	ELISA HEV IgM	Routine	52-91	74-100
				ELISA HEV IgG	Routine	60-91	96-98
				IgM HEV Immunoblot	Routine	95	100
				IgG HEV Immunoblot	Routine	97	85
				HEV PCR **GOLD Standard**	Special	83-100	100

Human Immunodeficiency virus (HIV)[18]	Lentivirus, family Retroviridae	an HIV infected person through sexual or vertical transmission, mucocutaneous or parenteral exposure	Whole blood, serum. Saliva and urine are not being used for testing in India.	HIV-1/2 Ab Rapid test	Routine	99-100	98-100
				Serum HIV-1/2 ELISA	Routine	99-100	97-100
				HIV-1and HIV-2 Ab/ HIV-1 p24 antigen	Routine	100	>99
				HIV-1/2 Ab (Oral fluids)	Routine	54-100	67-100
				HIV-1 Urine	Routine	92-100	95-100
				IFA anti-HIV-2	Special	93-99	98-100
				HIV Western Blot	Special	100	100
				HIV DNA PCR **GOLD Standard**	Special	90-96	54-100
After Pre-test counselling, NACO guidelines for testing are followed. Three different kits with different antigen system and / or different principles of tests are required. If the first test is negative, the patient is considered non-reactive. If the test serum is reactive with two tests and non-reactive with the third, it is reported as "indeterminate" and patient is called back for repeat testing after 2-4 weeks. The test used as the screening test is one with the highest sensitivity and the supplementary second and third tests are with the highest specificity. If all the 3 tests are reactive, post-test counselling is done and then the patient is referred to ART centre for treatment. For confirmation and viral load determination, molecular tests are done.							

Parasitic Tropical Diseases							
Amoebiasis[19]	Entamoeba histolytica	Food or water contaminated with faeces containing infectious cysts	Stool, Abscess fluid, serum	Stool Microscopy	Routine	10-60	10-50
				Microscopy (abscess fluid)	Routine	<25	10-50
				Culture with isoenzyme analysis **GOLD Standard**	Special	<60	100
				HK-9 antigen detection (ELISA)	Routine	65-100	>90
				Abscess antigen detection (ELISA)	Routine	100	>90
				Stool antigen detection (ELISA)	Routine	>95	>95
				Serum antibody detection (ELISA)	Routine	70-90	85-90
				PCR (stool)	Special	>70	>90

Leishmaniasis[20]	Leishmania donovani	Arthropod borne (Sand-fly bite), Zoonotic in some countries	Blood, bone marrow, lymphoid tissues, Serum	Microscopy of leucocytoconcentrates	Routine	<80	>80
				Histological and impression smear examination	Special	48-76	>80
				Culture from Buffy coats **GOLD Standard**	Special	<80	100
				Antigen detection ELISA	Routine	98	96-99
				IFA	Special	81	100
				Western blots	Special	88	100
				PCR	Special	88-95	100

Malaria[21]	Plasmodium vivax, P. falciparum, P. malariae, P. ovale, P. knowlesi	Bite of infected mosquitoes, rarely by transfusion.	Blood, Serum	Microscopy Thin blood film	Routine	100 parasites/ μl	100
				Microscopy Thick blood film	Routine	10-20 parasites/ μl	100
				Fluorescent Microscopy	Special	81-100	86-100
				Quantitative buffy coat examination	Special	41-93	93
				Immunofluorescence (1:128)	Special	> 90	> 90
				P. falciparum Anti-HRP-2 antibody test	Routine	77-98	83-98
				Plasmodium pLDH or Aldolase Rapid test at 100-500/ μL of blood	Routine	85-100	98-100
				Culture	Special	-	100
				PCR (1-100 parasites/ μl of blood)	Special	95-100	95-100
Microscopic examination of malarial parasites is considered **GOLD Standard** of diagnosis. Serological tests are approved only for emergencies and places where microscopy is not possible.							

Toxoplasmosis[22]	Toxoplasma gondii	Ingestion of oocysts shed in cat's faeces, vertical transmission, rarely infected blood/ organ donation	Serum, CSF, Blood, affected tissues	IgM ELISA	Routine	>93	90-100
				IgE ELISA	Routine	76	98
				IgG ELISA	Routine	>99	>99
				Western Blot	Special	99	100
				PCR for prenatal diagnosis	Special	90-92	>99
				PCR of placental tissue	Special	42-71	98-100
				PCR (Blood, CSF) in cerebral toxoplasmosis	Special	33-83	98-100
				IFN-γ release assay	Special	94	98
PCR is considered the **GOLD Standard** for Diagnosis.							
Fungal tropical diseases							
Cryptococcosis[23]	Cryptococcus neoformans	Inhalation or inoculation of basidiospores	CSF, Blood, Serum, Urine, Sputum	Microscopy (India ink preparation)	Routine	50-90	>90
				Culture **GOLD Standard**	Routine/ Special	50-90	100
				Cryptococcal antigen detection	Routine	83-97	93-100
				Antibody detection by ELISA	Routine	80-85	100
				PCR	Special	92-100	100

Depending on the provisional clinical diagnosis of the abovementioned diseases, relevant investigations can be done according to the available facilities.

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