MEDLAB BASICS · STUDY SHEET

Mycobacteria, Fungi, Parasites and Viruses

AFB stains and processing, Runyon groups, Nocardia, yeast and mold identification, dimorphic fungi, the O&P exam, blood parasites, viral specimens and tests, and the biosafety level for each

70 sources + more detail

medlabbasics.com/sheets/lab-micro-myco-fungi-parasites-virology.html
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1 Biosafety at a glance (CDC BMBL 6th edition)

WORK	LEVEL
Clinical specimens for TB, smears, non-aerosol handling	BSL-2 (aerosol-producing steps in a BSC)
Growing or manipulating M. tuberculosis complex cultures	BSL-3
Mycobacteria other than the MTB complex: specimens and cultures	BSL-2 (care with M. marinum and M. ulcerans on skin)
Clinical specimens and isolate identification for dimorphic fungi	BSL-2, cultures in a class II BSC
Sporulating mold cultures already identified as Coccidioides, Histoplasma or Blastomyces	BSL-3
Candida, Cryptococcus, dermatophytes, Aspergillus, Fusarium; all unknown molds	BSL-2; open unknown molds only in a class II BSC
Intestinal and blood parasites, helminth eggs	BSL-2 (Cryptosporidium oocysts are infectious when passed)
Seasonal influenza, human herpesviruses, routine clinical virology	BSL-2 (zoonotic avian influenza: BSL-3)

⚠ SAFETY Never make a slide culture or open a fuzzy white (hyaline) mold on the open bench: it may be Coccidioides, whose airborne arthroconidia make laboratory-acquired infection a documented hazard. Most exposures happen when a culture of unknown status is handled on the open bench: seal the plate and work in a class II BSC.

2 Mycobacteria: stains, specimens and processing

STAIN	HOW IT WORKS	POSITIVE LOOKS LIKE
Ziehl-Neelsen (hot)	Carbol fuchsin driven in with heat, acid-alcohol decolorizer, methylene blue counterstain	Thin red bacilli on a blue background; read with oil immersion
Kinyoun (cold)	Carbol fuchsin without heat; same decolorizer and counterstain	As Ziehl-Neelsen
Auramine-rhodamine (fluorochrome)	Fluorescent dyes bind mycolic acids; read at low power	Yellow-orange glowing bacilli on a dark field; more sensitive and faster to screen
Modified acid-fast (Kinyoun with 1-2% sulfuric acid)	Weaker decolorizer for partly acid-fast organisms	Nocardia: beaded, branching filaments that are weakly acid-fast

- Sputum for TB: at least 3 specimens 8-24 hours apart, at least one early morning. Smear result within 24 hours, graded 1+ to 4+.
- A smear needs about 10,000 bacilli/mL to turn positive; culture detects about 10/mL. A negative smear does not exclude TB, and every specimen is cultured whatever the smear shows.
- NAAT (for example Xpert MTB/RIF, which also detects rifampin-resistance mutations) on at least one respiratory specimen when TB is suspected; it does not replace smear, culture or growth-based susceptibility testing.
- Digestion-decontamination of sputum: NALC (mucolytic) with NaOH (kills normal flora), about 15 minutes, neutralize with phosphate buffer pH 6.8, centrifuge (3000 × g for 15 minutes), smear and culture the sediment. Sterile tissue and fluids are not decontaminated.
- Culture on solid (Lowenstein-Jensen, Middlebrook 7H10/7H11) and liquid media (MGIT); liquid is faster and more sensitive (results often in 2-3 weeks). Solid media are commonly read weekly for up to 8 weeks before a culture is called negative, and final confirmation can take longer.

EXAM TIP Contamination rate is the processing QC indicator: about 3-5% on solid media and up to about 7-8% in liquid (MGIT) is the usual target. A rate below about 3% with poor recovery and slower detection means the NaOH step is too harsh; a high rate means under-decontamination.

3 Mycobacteria: growth and identification

GROUP	DEFINING FEATURE	EXAMPLES
M. tuberculosis complex	Slow (about 2 weeks and more); rough, dry, buff, cauliflower-like colonies; serpentine cords in smears; inhibited by PNB. M. tuberculosis is niacin positive, nitrate positive and heat-stable (68 °C) catalase negative; M. bovis is niacin and nitrate negative	M. tuberculosis, M. bovis (including BCG), M. africanum
Runyon I, photochromogens	Slow; buff in the dark, turn yellow after light exposure	M. kansasii (pulmonary), M. marinum (fish tank granuloma, grows best at 30-32 °C)
Runyon II, scotochromogens	Slow; yellow-orange even in the dark	M. scrofulaceum (cervical lymphadenitis in children), M. gordonae
Runyon III, nonchromogens	Slow; no pigment	M. avium complex (lung disease; disseminated in advanced HIV), M. xenopi, M. ulcerans (Buruli ulcer, 30-33 °C, PCR preferred), M. haemophilum (needs hemin, 30-32 °C)
Runyon IV, rapid growers	Visible colonies in under 7 days	M. abscessus complex, M. fortuitum group, M. chelonae: wounds, devices, post-procedure infections, cystic fibrosis lungs

- Confirm MTB complex on growth by NAAT, DNA probe or MPT64 antigen; identify NTM by MALDI-TOF MS (extract and inactivate in the BSC, never spot live MTB colonies in the open), line probe assays or sequencing (16S rRNA, hsp65, rpoB).
- Susceptibility testing on the first MTB isolate from every patient; molecular resistance testing (for example CDC's MDDR service) for smear-positive or NAAT-positive specimens with drug-resistance risk.
- An NTM in one sputum may be colonization or contamination; MTB complex in any specimen is significant and is reported to public health.

⚠ SAFETY Call a new positive AFB smear, a positive MTB NAAT or an MTB complex culture to the care team and infection prevention the same day; TB is reportable to the health department.

4 Fungi: direct exam and yeasts

DIRECT METHOD	USE AND FINDING
KOH (10%)	Clears skin, hair and nail keratin so hyphae show; cannot tell living from dead or name the species
Calcofluor white	Binds chitin and cellulose; fungal walls glow under UV; often mixed with KOH
India ink (CSF)	Clear halo (capsule) around narrow-budding yeast: Cryptococcus; antigen testing is far more sensitive
Tissue stains	GMS and PAS outline fungi; mucicarmine or Fontana-Masson confirm Cryptococcus

YEAST	KEY TESTS AND FINDINGS
Candida albicans	Germ tube positive (true hyphal outgrowth in serum at 37 °C in 2-3 hours); chlamydoconidia on cornmeal agar; C. dubliniensis is also germ tube positive
Candida auris	No germ tube, rarely pseudohyphae, grows at 40-42 °C; misidentified by Vitek 2, API 20C, Phoenix and MicroScan (as C. haemulonii, C. famata, C. sake, Rhodotorula glutinis and others); identify by MALDI-TOF with an updated library or sequencing
Nakaseomyces glabratus (Candida glabrata); Pichia kudriavzevii (C. krusei)	Less susceptible to fluconazole; P. kudriavzevii is intrinsically fluconazole resistant
Cryptococcus neoformans and C. gattii	Encapsulated budding yeast; urease positive; brown (melanin) colonies on niger seed agar; capsular antigen by lateral flow on serum or CSF (preferred test, over 99% sensitive and specific)

⚠ SAFETY Suspect C. auris when a yeast is named C. haemulonii, C. duobushaemulonii, C. famata, C. sake or another species on CDC's misidentification list, or cannot be identified. Isolate the patient and report confirmed or suspected cases to public health at once.

5 Fungi: molds and dimorphic fungi

MOLD	IN TISSUE OR DIRECT EXAM	IN CULTURE
Aspergillus (hyaline)	Thin, septate hyphae with regular acute-angle (dichotomous) branching	A. fumigatus blue-green, uniseriate, columnar heads; A. flavus yellow-green; A. niger black; A. terreus cinnamon-brown
Mucorales (Rhizopus, Mucor and others)	Broad, ribbon-like, pauciseptate hyphae with right-angle branching	Fast-growing; mince tissue, never grind it (fragile hyphae)
Dematiaceous molds (for example Curvularia)	Brown-pigmented hyphae (melanin)	Dark colonies with dark reverse
Trichophyton	Hyphae in skin, hair or nail	T. rubrum: teardrop microconidia, rare pencil-shaped macroconidia. T. mentagrophytes group: grape-like microconidia, spirals, urease positive
Microsporum	Hair: ectothrix (M. canis); Wood's lamp fluorescence	Spindle-shaped, thick-walled macroconidia
Epidermophyton floccosum	Skin and nails, not hair	Smooth, club-shaped macroconidia in clusters; no microconidia

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5 Fungi: molds and dimorphic fungi CONTINUED

DIMORPHIC FUNGUS	WHERE ACQUIRED	MOLD AT 25-30 °C	TISSUE FORM AT 37 °C
Histoplasma capsulatum	Ohio and Mississippi River valleys; bird and bat droppings	Tuberculate macroconidia and small microconidia (infectious); weeks to grow	Small (2-4 µm) narrow-budding yeasts inside macrophages
Blastomyces	Ohio and Mississippi valleys, Great Lakes, St. Lawrence; Wisconsin, Minnesota, Ontario	Oval conidia on short stalks	Large (8-15 µm), thick-walled yeasts with broad-based buds
Coccidioides	Sonoran Desert: southern Arizona, central and southern California, western Texas, northern Mexico; also south-central Washington	Barrel-shaped arthroconidia alternating with empty cells (extreme lab hazard)	Spherules (20-200 µm) full of endospores (2-4 µm); no yeast
Paracoccidioides	South America	Slow, few conidia	Yeast with many buds around it ('pilot's wheel')
Sporothrix schenckii complex	Soil and plant material; skin inoculation	Delicate hyphae with rosettes of conidia	Small cigar-shaped yeasts
Talaromyces marneffei	Southeast Asia; mostly immunocompromised hosts (advanced HIV)	Red pigment diffusing into the agar	Yeasts that divide by fission, with a central septum

EXAM TIP 'Mold in the cold, yeast in the heat.' Labs no longer convert Coccidioides to its tissue form; confirm a dimorphic mold by DNA probe, sequencing or MALDI-TOF. Urine antigen is the fastest noninvasive test for Histoplasma and Blastomyces.

6 Parasites: stool exam

1. Collect three stools 2-3 days apart, before barium (wait 7-10 days), antibiotics (2-3 weeks) or antacids and mineral oil. One part stool to three parts preservative.
2. Examine fresh liquid stool within 30 minutes of passage and soft stool within 1 hour, or preserve it, or trophozoites disintegrate.
3. Concentrate a formalin portion by formalin-ethyl acetate sedimentation (500 × g for 10 minutes): best for eggs, larvae and cysts on a saline and iodine wet mount.
4. Make a permanent stained smear (trichrome) from PVA, SAF or a one-vial fixative: best for protozoan trophozoites and cysts and the only reliable way to see Dientamoeba.
5. Add special stains on request: modified acid-fast for Cryptosporidium, Cyclospora and Cystoisospora; chromotrope (modified trichrome) for microsporidia.

PROTOZOAN	DIAGNOSTIC FEATURE
Giardia duodenalis	Pear-shaped trophozoite, 10-20 µm, 2 nuclei, sucking disk, 8 flagella; oval cyst 8-19 µm with 4 nuclei
Entamoeba histolytica/dispar	Cyst 12-15 µm, up to 4 nuclei with central karyosome and fine even chromatin, blunt chromatoid bodies; species needs antigen or PCR
Entamoeba coli (nonpathogen)	Cyst 10-35 µm, usually 8 nuclei, eccentric karyosome, splinter-like chromatoid bodies
Endolimax nana; Iodamoeba buetschlii (nonpathogens)	E. nana: 4 nuclei with big central karyosomes. Iodamoeba: one nucleus and a large glycogen vacuole
Dientamoeba fragilis	Trophozoite only, 5-15 µm, usually 2 nuclei with fragmented karyosomes; missed by concentration
Blastocystis	Vacuolar (central body) form: a large central body inside a thin rim of cytoplasm, usually 8-10 µm in stool (range 5-40 µm); confirm on a permanent stain, because debris mimics it
Cryptosporidium	Round oocyst 4-5 µm, red on modified acid-fast; infectious when passed
Cyclospora cayetanensis	Round oocyst 7.5-10 µm, variably acid-fast, autofluoresces under UV; not infectious when passed
Cystoisospora belli	Ellipsoidal oocyst 25-30 µm with 1-2 sporoblasts; acid-fast; eosinophilia possible
Microsporidia	Spores 1-4 µm, pink with a dark equatorial band on chromotrope 2R

HELMINTH	DIAGNOSTIC STAGE
Ascaris lumbricoides	Fertile egg 45-75 µm, round, thick mammillated (bumpy) brown coat; infertile egg longer (up to 90 µm)
Trichuris trichiura	Barrel-shaped egg 50-55 µm with a plug at each end
Hookworm	Thin-shelled colorless egg 60-75 µm (Necator and Ancylostoma look the same)
Strongyloides stercoralis	Rhabditiform larvae in fresh stool: short buccal canal, prominent genital primordium; eggs rarely seen
Enterobius vermicularis	Egg 50-60 µm, flattened on one side; cellulose tape on the perianal skin in the morning before washing
Taenia	Round radially striated egg 30-35 µm with 6 hooks (species look alike); T. solium proglottid 7-13 uterine branches, T. saginata 12-30
Diphyllobothrium (Dibothriocephalus)	Oval operculated egg 55-75 µm with a small knob at the other end; proglottids wider than long
Hymenolepis nana	Egg 30-50 µm with polar filaments between the membranes (H. diminuta: larger, no filaments)
Schistosoma	S. mansoni: large lateral spine (stool). S. haematobium: terminal spine (urine collected noon to 3 PM). S. japonicum: small, rounder, tiny knob

SAFETY Handle Taenia proglottids and eggs with extreme care: swallowing T. solium eggs causes cysticercosis. Strongyloides larvae can penetrate skin; agar plate cultures are sealed and opened with care.

7 Parasites: blood

SPECIES	INFECTED RED CELL	WHAT ELSE IS SEEN
P. falciparum	Not enlarged; two or more rings in one cell is common; Maurer's clefts may show, depending on the stain	Fine, delicate rings about a fifth of the cell's width with 1 or 2 chromatin dots, some lying flat on the cell edge (appliqué forms); older trophozoites and schizonts are rarely seen in blood except in severe infection; crescent- or sausage-shaped gametocytes
P. vivax	Normal to 1.25 times with rings; 1.5-2 times and often misshapen with later stages; fine Schüffner's dots	Thick rings with one large chromatin dot; big amoeboid trophozoites with fine yellow-brown pigment; 12-24 merozoites per schizont; round to oval gametocytes; every stage circulates
P. ovale	Normal to 1.25 times; some cells oval with frayed (fimbriated) edges; Schüffner's dots	Sturdy rings and compact trophozoites; 6-14 merozoites per schizont; round to oval gametocytes
P. malariae	Normal or smaller (down to 0.75 times); Ziemann's stippling only rarely	Thick rings; band and basket trophozoites with coarse dark-brown pigment; 6-12 merozoites (usually 8-10), sometimes in a rosette; schizonts can be common
P. knowlesi	Normal or smaller (down to 0.75 times); several parasites per cell common	Rings that mimic P. falciparum (appliqué forms, 1-2 dots) and band forms that mimic P. malariae; up to 16 merozoites (about 10 on average); confirm by PCR
Babesia (B. microti in the US Northeast and Midwest)	Normal size	Varied, sometimes vacuolated ring forms; no pigment, no gametocytes or schizonts; dividing tetrads ('Maltese cross') and clumps outside red cells point to Babesia; PCR names the species. Spread by Ixodes ticks and by transfusion

- Thick smear (not fixed, red cells lysed) finds the parasite; thin smear (methanol-fixed) names the species and gives the parasitemia. Giemsa stain. At least 2 of each.
- Make smears from EDTA blood promptly; delay distorts parasites. Repeat every 8-12 hours for 2-3 days before calling malaria negative. Only one rapid antigen test is licensed in the US, and a positive or negative RDT is followed by smears.
- Microfilariae, all sheathed: Wuchereria bancrofti (nuclei stop short of the tail tip) and Brugia malayi (gap between the last two tail nuclei) draw blood at night; Loa loa (nuclei to the tail tip, eye worm, Calabar swellings) draw 10 AM to 2 PM. Concentrate with Knott's technique (2% formalin) or a membrane filter.
- Trypanosomes: T. cruzi (Chagas) is a C-shaped trypomastigote with a large kinetoplast in acute blood, amastigotes in tissue, and is diagnosed by two serologic tests when chronic. T. brucei has a small kinetoplast and undulating membrane; look in blood, chancre fluid, lymph node aspirate or CSF, using buffy coat concentration.

SAFETY Malaria is an emergency: read smears as soon as they are made, report any Plasmodium with the species (or 'P. falciparum cannot be excluded') and the percent parasitemia to the provider at once, and send for PCR when the species is uncertain.

8 Virology

- Respiratory swabs: synthetic fiber (flocked) on a plastic or wire shaft, in viral transport medium. Calcium alginate and wooden shafts can inactivate virus and inhibit PCR.
- NAAT (PCR, including multiplex panels) is the method of choice for most viruses. Rapid antigen tests are fast and specific but miss 30-50% of influenza; a negative result in a sick patient is followed by a molecular test.
- Viral culture is now kept for surveillance, typing and special cases; it is insensitive for CSF and healing herpes lesions.

SYNDROME	MAIN VIRUSES	SPECIMEN AND TEST
Respiratory infection	Influenza A and B, SARS-CoV-2 and other respiratory viruses	Nasopharyngeal swab (or nasal, oropharyngeal, lower respiratory); RT-PCR or panel
Aseptic meningitis, encephalitis	Enteroviruses (most common), HSV-2, VZV, West Nile and other arboviruses, HIV at seroconversion	CSF PCR; CSF IgM is more sensitive than PCR for West Nile virus
Genital ulcers	HSV-1 and HSV-2	Swab of the lesion for type-specific NAAT; Tzanck smear not recommended
Fever and rash after travel or exposure	Measles	Nasopharyngeal or throat swab and urine for RT-PCR plus serum IgM; notify public health immediately
Bloodborne	HBV, HCV, HIV	Serology and viral load: see the Immunology and Serology page

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