

II Microbiology

1 Contact information

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Immunocytometry	2319 8234
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Senior Medical and Health Officer (2)	2319 8211
Facsimile (Bacteriology)	2776 2744
Facsimile (Mycobacteriology)	2776 0344
Facsimile (Immunocytometry)	2319 8235

2 Scope of service

The list of specific examinations for individual sample types available from Microbiology Division together with description of the test methods used can be accessed from the following HOKLAS website: <http://www.itc.gov.hk/en/doc/quality/hkas/hoklas/804.pdf>.

3 Materials provided on request

- (a) Aerobic (BacT / ALERT SA) and anaerobic (BacT / ALERT SN) blood culture bottles for bacteria and fungi
- (b) Sterile containers for tissue, body fluids and pus
- (c) Sterile bijou bottles
- (d) Sterile urine culture bottles with preservatives
- (e) Sterile containers for sputum culture
- (f) Sterile swabs with bacterial transport medium
- (g) Modified Thayer Martin agar for *Neisseria gonorrhoeae* (provided to Social Hygiene Clinics, to be kept at 4°C)
- (h) Sterile nasopharyngeal swabs with transport medium for culture of *Bordetella pertussis*
- (i) Sterile swabs with Amies transport medium with charcoal for culture of *Neisseria gonorrhoeae* from male urethral specimens
- (j) Plain urine bottles
- (k) Plastic containers for stool examination
- (l) Specimen transport system for fungal examination
- (m) Biological indicators (for testing adequacy of autoclaves, to be kept at room temperature)
- (n) BACTEC MYCO / F Lytic Culture vials (provided to AIDS Clinics, to be kept at 4°C and

- used before expiry), an AFB culture broth for direct inoculation of blood and bone marrow
- (o) Plastic containers for sputum AFB smear and culture
 - (p) 1 Litre containers for early morning urine for AFB culture
 - (q) Plain clotted bottles / vacutainers for serology
 - (r) Specimen collection and transport kits for *Chlamydia trachomatis* (provided to Social Hygiene Clinics)
 - (s) EDTA blood vacutainers

Note: All materials provided should not be used after expiry if applicable.

4 Guideline for specimen collection, storage and transportation

- (a) Collect specimens with safety precautions / personal protective equipment in accordance with infection control guidelines of your institution.
- (b) Dispose of any potentially contaminated materials used for specimen collection in accordance with infection control guidelines of your institution and Code of Practice promulgated by the Environmental Protection Department.
- (c) Specimens should be transported as soon as possible to the laboratory preferably within the same session of the day. If delay in despatch is unavoidable, keep specimen at room temperature for up to 48 hours unless otherwise stated.
- (d) Requests other than those listed below are not routinely available. Pre-arrangement with the laboratory is required.

Specimen type	Collection		Replication limits	Comments
	Procedures	Recommended specimen container(s) * and quantity		
Sterile sites				
Blood culture	<ol style="list-style-type: none"> 1. Wipe stopper of culture bottle with 70% alcohol and wait 1 min. 2. Palpate for vein and cleanse venipuncture site with 70% alcohol. 3. Swab concentrically from centre with an iodine preparation. 4. Allow the iodine to dry and do not palpate vein at this point. 5. Collect blood and transfer into the anaerobic culture bottle first to avoid introduction of air into the anaerobic bottle. 6. After venipuncture, remove iodine from the skin with alcohol. 	Bacteria/fungi: (a), AFB: (n) 10 ml/bottle	Bacteria/ fungi: 3 sets in 24 h, AFB: 1/day	If delay in dispatch is unavoidable, keep specimen at either 35-37°C or room temperature. Please indicate on request form for suspected infective endocarditis.
Bone marrow	Prepare puncture site as for surgical incision.	Bacteria/fungi: (a), AFB: (n) 10 ml/bottle	None	If delay in dispatch is unavoidable, keep specimen at either 35-37°C or room temperature.
CSF	<ol style="list-style-type: none"> 1. Disinfect site with 2% iodine tincture. 2. Insert a needle with stylet at L3-L4, L4-L5, or L5-S1 interspace. 3. Upon reaching the subarachnoid space, remove the stylet and collect 1 to 2 ml of fluid into each of 3 leak-proof tubes. 	Bacteria/fungi: (c) 1-2 ml, AFB: (b) ≥2 ml	None	Transport to laboratory without delay. Obtain blood for culture also. Aspirate of brain abscess or a biopsy may be necessary to detect anaerobic bacteria or parasites. Keep specimens for AFB at 4°C.
Body fluids	<ol style="list-style-type: none"> 1. Disinfect overlying skin as blood culture 2. Obtain specimen via percutaneous needle aspiration or surgery. 3. Always submit as much fluid as possible; never submit a swab dipped in fluid. 	Bacteria: culture (a), 10 ml, microscopy (b), Fungi: culture (a), ≥10 ml, microscopy (b), AFB: culture (b) ≥10 ml	None	Transport to laboratory without delay. If delay in dispatch is unavoidable, keep AFB specimen at 4°C.
Tissue	<ol style="list-style-type: none"> 1. For small samples, add several drops of sterile saline to keep moist. 2. Do not allow tissue to dry out. 	Bacteria/fungi: (b), AFB: (b)	None	Send as much tissue as possible. Never submit a swab that has simply been rubbed over the surface.
Closed abscess	<ol style="list-style-type: none"> 1. Remove surface exudates by wiping with sterile saline. 2. Aspirate abscess material with needle and syringe. 	(b) ≥1 ml, (f)	1/day/source	

Specimen type	Collection		Replication limits	Comments
	Procedures	Recommended specimen container(s) * and quantity		
Wound / ear / eye				
Wound	1. Remove surface exudates by wiping with sterile saline. 2. Pass a swab deep into the lesion and firmly sample the lesion's advancing edge.	(b) ≥1 ml, (f)	1/day/source	Sampling of surface area can introduce colonizing bacteria not involved in the infective process.
Decubitus ulcer	1. Cleanse surface with sterile saline. 2. If a sample biopsy is not available, aspirate inflammatory material from the base of the ulcer.	(b), (f)	1/day/source	A tissue biopsy sample or a needle aspirate is the specimen of choice. Superficial swab is not desirable.
Outer ear	1. Use moistened swab to remove debris or crust from ear canal. 2. Obtain a sample by firmly rotating swab in the outer canal.	(f)	1/day/source	
Eye: conjunctiva	Sample both eyes with separate swabs (pre-moistened with sterile saline) by rolling over each conjunctiva.	(f)	None	If possible, sample both conjunctiva, even if only one is infected, to determine indigenous microflora.
Genital tract				
Genital: female or male lesion	1. Clean the lesion with sterile saline. 2. Remove lesion's surface with a sterile scalpel blade if necessary and allow transudate to accumulate. 3. While pressing the base of the lesion, firmly rub base with a sterile swab to collect fluid.	(f)	1/day	
Male urethral swab	Insert a urethrogenital swab 2-4 cm into the urethral lumen, rotate swab, and leave it in place for at least 2 seconds to facilitate absorption.	<i>Chlamydia trachomatis</i> by PCR: (r) Bacterial culture: (i)	1/day	<i>Neisseria gonorrhoeae</i> is found in exudates, whereas <i>Chlamydia trachomatis</i> infects specific cells.
Vaginal swab	1. Wipe away excessive amount of secretion or discharge. 2. Obtain secretions from the mucosal membrane of the vaginal vault with a sterile swab or pipette.	(f)	1/day	For intrauterine devices, place entire device into a sterile container.
Cervical swab	1. Visualize the cervix using a speculum without lubricant. 2. Remove mucus/secretions from cervix with swab and discard. 3. Firmly yet gently sample the endocervical canal with a fresh sterile swab.	<i>Chlamydia trachomatis</i> by PCR: (r) Bacterial culture: (f)	1/day	<i>Neisseria gonorrhoeae</i> is found in exudates, whereas <i>Chlamydia trachomatis</i> infects specific cells.
Culture for <i>Neisseria gonorrhoeae</i>	1. Collect female cervical and male urethral specimens with sterile loops by the above methods. 2. Inoculate specimens onto (g) directly. 3. Incubate plates in candle jar / 5% CO ₂ at 35-37°C for up to 24 h before sending to the laboratory.	(g)	1/day	This procedure is performed in Social Hygiene Clinics. Special arrangement with the laboratory is required in long holidays.
Prostate	1. Cleanse the glans with soap and water. 2. Massage prostate through rectum. 3. Collect fluid on a sterile swab or in a sterile tube.	(f), (b)	1/day	Urine specimens obtained immediately before and after massage may indicate urethral/bladder organisms. Ejaculate may also be cultured.

Specimen type	Collection		Replication limits	Comments
	Procedures	Recommended specimen container(s) * and quantity		
Respiratory tract				
Oral lesion	<ol style="list-style-type: none"> 1. Remove oral secretions and debris from the surface of lesion with a swab and then discard. 2. Using a second swab, vigorously sample the lesion, avoiding any areas of normal tissue. 	(f)	1/day	Sampling of superficial tissue for bacterial evaluation is not useful. Tissue biopsy specimens or needle aspirates are the specimens of choice.
Nasal	<ol style="list-style-type: none"> 1. Insert a swab, pre-moistened with sterile saline, ≈2 cm into the nares. 2. Rotate the swab against the nasal mucosa. 	(f)	1/day	Anterior nose cultures are reserved for detecting staphylococcal carriers or for nasal lesions. A nasal speculum may be appropriate.
Nasopharyngeal (pernasal) swab	<ol style="list-style-type: none"> 1. Gently insert the swab into the posterior nasopharynx via the nose. 2. Rotate swab slowly for 5 seconds to absorb secretions. 3. Remove swab and place swab in transport medium. 	(h)	1/day	For culture of <i>Bordetella pertussis</i> , inform the laboratory before delivery of specimen for preparation of fresh medium.
Throat	<ol style="list-style-type: none"> 1. Depress tongue with a tongue depressor. 2. Sample the posterior pharynx, tonsils, and inflamed areas with a sterile swab. 	(f)	1/day	Throat swab cultures are contraindicated for patients with an inflamed epiglottitis.
Sputum, expectorated	<ol style="list-style-type: none"> 1. Collect specimen under the direct supervision of a nurse or physician. 2. Have patient rinse or gargle with water to remove superficial flora. 3. Instruct patient to cough deeply to produce a lower respiratory specimen (not postnasal fluid). 	>1 ml Bacteria/fungi: (e), AFB: (o)	1/day	For pediatric patients unable to produce a specimen, a respiratory therapist should collect a specimen via suction.
Sputum, induced	<ol style="list-style-type: none"> 1. Have patient rinse mouth with water after brushing gums and tongue. 2. With the aid of a nebulizer, have patient inhale ≈25 ml of 3-10% sterile saline. 3. Collect the induced sputum into a sterile container. 	Bacteria/fungi: (e), AFB: (o)	1/day	
Bronchoalveolar lavage, bronchial brush or wash, tracheal aspirate	<ol style="list-style-type: none"> 1. Place aspirate or washing into a sputum trap. 2. Place brush into a sterile container with 1 ml saline. 	Bacteria/fungi: (b), AFB: (b)	1/day	

Specimen type	Collection		Replication limits	Comments
	Procedures	Recommended specimen container(s) * and quantity		
Urine				
Female, midstream	1. Thoroughly cleanse the urethral area with soap and water. 2. Rinse area with wet gauze pads. 3. While holding the labia apart, begin voiding. 4. After several millilitres has passed, collect a midstream portion without stopping the flow of urine.	(d)	1/day	In case where insufficient urine is anticipated, collect fresh urine in specimen container (e) and send to the laboratory without delay.
Male, midstream	1. Cleanse the glans with soap and water. 2. Rinse with wet gauze pads. 3. While holding the foreskin retracted, begin voiding. 4. After several millilitres has passed, collect a midstream portion without stopping the flow of urine.	(d)	1/day	In case where insufficient urine is anticipated, collect fresh urine in specimen container (e) and send to the laboratory without delay.
Straight catheter	1. Thoroughly cleanse the urethral area with soap and water. 2. Rinse area with wet gauze pads. 3. Aseptically, insert a catheter into the bladder. 4. After allowing ≈15 ml to pass, collect urine to be submitted in a sterile container.	(d)	1/day	The procedure may introduce urethral flora into the bladder and increase the risk of iatrogenic infection.
Indwelling catheter	1. Disinfect the catheter collection port with 70% alcohol. 2. Use needle and syringe to aseptically collect 5-10 ml of urine. 3. Transfer to a sterile tube or container.	(d)	1/day	Do not collect urine routinely from indwelling catheter unless patients are symptomatic.
AFB smear and culture	Collect early morning whole stream urine (at least 50 ml).	(p)	1/day	Keep specimen at 4°C before transport to the laboratory.
Routine urinalysis, biochemical tests	Collect urine by any of the above methods or spot urine avoiding contamination with vaginal secretion in case of female patients.	(j)	1/day	
<i>Legionella</i> urinary antigen test	Collect urine by any of the above methods.	(d) or (j)	1/day	Detailed clinical information must be provided.
Pregnancy test (HCG immunoassay test)	It is preferable to collect early morning urine.	(j)	1/day	Specimens collected at any time of day may be used, however, early morning urine generally contains the highest concentration of hormone.

Specimen type	Collection		Replication limits	Comments
	Procedures	Recommended specimen container(s) * and quantity		
Stool				
Examination for ova and cysts	Stool (rectal swab of faecal material should only be sent if stool is not obtainable): 1. Cover the toilet bowl with plastic wrap. 2. Pass stool onto the plastic wrap. 3. Transfer ≥5g (approximately 1 tablespoon) of stool with wooden spatula or stick. Select blood and mucus portion.	(k)	1/day	
Direct microscopy for amoeba		(k)	1/day	Arrange with laboratory prior to specimen collection, and send specimen to laboratory without delay.
Culture for pathogenic organisms		(k)	1/day	
Culture for AFB		(o)	1/day	Keep specimen at 4°C before transport to the laboratory.
<i>Clostridium difficile</i> toxin test		(k)	1/day	Patients should be passing ≥5 liquid or soft stools per 24 h. Testing of formed stool is not recommended.
Fungal investigation				
Hair	1. Infected hair should never be cut but removed by plucking with epilating forceps. 2. Collect at least 10-12 affected hairs with the base of shaft intact.	(l)	1/day/site	Collect scalp scales, if present, along with scrapings of active borders of lesions. Note any antifungal therapy taken recently.
Nail	1. Wipe nail with 70% alcohol using gauze (not cotton). 2. Discoloured, dystrophic or brittle areas should be sampled using pincer type nail clippers. Where the distal edge is not involved, scrape the affected area with a scalpel blade.	(l)	1/day	
Skin	Using a curved scalpel blade, scrape across the inflamed margin of the lesion into the apparently healthy tissue.	(l)	1/day	

Specimen type	Collection		Replication limits	Comments
	Procedures	Recommended specimen container(s) * and quantity		
Serology				
Syphilis serology tests	Blood: Collect 3-5 ml of clotted blood and send to the laboratory as soon as possible.	(q)	1/day/test	Available serology tests: 1. Venereal Disease Research Laboratory (VDRL) 2. Fluorescent Treponemal Antibody Absorption test (FTA-ABS) 3. <i>Treponema pallidum</i> Passive Particle Agglutination test (TPPA) 4. Syphilis Enzyme Immunoassay (EIA)
	CSF: Collect 1 ml in a plain sterile bottle.	(c)	1/day/test	Only VDRL test is available for CSF. Keep specimen at 4°C before transport to the laboratory.
Miscellaneous serology tests	1. It is recommended to send acute and convalescent sera at least 10-14 days apart. 2. Collect 5 ml of clotted blood for each of these tests.	(q)	1/day/test	Available serology tests: 1. <i>Brucella</i> antibody 2. <i>Leptospira</i> antibody 3. Streptolysin O antibody 4. Weil-Felix test 5. Widal test 6. Amoeba antibody 7. <i>Aspergillus</i> antigen 8. <i>Cryptococcus</i> antigen
Miscellaneous investigations				
Malaria parasite screening test	Blood : Collect 3-5 ml EDTA blood and send to the laboratory immediately	(s)	1/day/test	
Lymphocyte immuno-phenotyping	Blood : Collect 3-5 ml EDTA blood and send to the laboratory immediately	(s)	1/day/test	Test only available for HIV infected cases. Prior arrangement with the Immunocytometry Laboratory should be made for new cases. Specimens should arrive at the laboratory before 10:30 am on Mondays to Fridays.
Biological indicators	1. Record relevant information on the request form (date of testing, temperature and time of autoclave cycle). 2. Place indicator in test position in the autoclave. 3. Carry out the autoclaving procedure.	(m)	1/day/site	This is a biological method of checking effectiveness of autoclaves.

*Refer to 3 (a) to (s) for type of containers

5 Laboratory request form & labeling of specimen containers

- DH2538 : For smear, culture, identification, antimicrobial susceptibility and other tests for AFB
- DH2543 : For syphilis serology / malaria parasite screening / immuno-phenotyping
- DH2544 : For investigations other than the above
- DH2546 : For paying cases for above investigations
- Appendix 1 : For laboratory isolates referred for identification, typing or other investigations

- (a) The following information must be available on the request form before the sample can be processed and the appropriate tests performed:
- Full patient particulars (name, date of birth / age, sex, HKID / identity document number)
 - Clinic / institution registration number
 - Requesting unit
 - Clinical diagnosis and / or specific clinical features
 - Antibiotics that the patient is taking if any
 - Date of specimen taking
 - Nature of specimen
 - Test requested
 - Signature and name of requesting staff
- (b) The specimen container must be labeled with unique identification of the patient (e.g. name / HKID number) matching that on the request form.
- (c) Whenever two or more similar specimens from a single patient are sent (e.g. joint fluid vs. peritoneal fluid, cervical swab vs. vaginal swab, L ear swab vs. R ear swab), the anatomic site of origin of the specimen must be clearly specified on the specimen containers.

6 Transport of specimen

- (a) Ensure container is properly capped without leakage.
- (b) Place the specimen container in a plastic bag with the request form outside the bag.
- (c) Keep specimens upright to minimize the possibility of spillage.

7 Average turnaround times (TATs)

- (a) The following TATs serve only as a general reference. In case confirmatory tests are required, the TAT may be lengthened accordingly.
- (b) Reports are normally despatched to requesting units through automated facsimile server in batches. One additional working day may be required for receipt of reports.
- (c) For urgent requests, please contact medical staff for special arrangements.

Category	Test	Average TAT (working days)	Frequency of test
Direct examination	Ova & cysts, malaria parasite and AFB smear examination	1 day	Daily
	Antigen detection tests	1 day	Daily
	Chlamydia PCR	1 day	Twice weekly
Culture	Blood / CSF / Body fluids	7 days	Daily
	Other specimens for general bacterial culture	2-3 days	Daily
	Fungi	7 days	Daily
	Mycobacteria	9 weeks	Daily
Serological tests	Bacterial / fungal / amoebic serology	1-2 days	Once per week
	Syphilis serology	3 days	Daily
Other tests	Routine urinalysis	1 day	Daily
	Pregnancy test	1 day	Daily
	<i>Legionella</i> urinary antigen	1 day	Daily
	Lymphocyte immuno-phenotyping	3 days	Daily
	Antimicrobial susceptibility test of <i>M. tuberculosis</i> by absolute concentration method	5 weeks	Once per week

8 Requests for additional tests

- (a) Additional tests on specimens previously sent to the laboratory may be requested via telephone followed by written request via facsimile.
- (b) Such tests will be performed on the following conditions:
- Test(s) requested by a medical staff
 - Appropriate clinical indications
 - Sufficient amount of the appropriate sample available in the laboratory
 - Tests results not affected by storage

Microbiology Division, Public Health Laboratory Services Branch (PHLSB)
Centre for Health Protection, Department of Health, HKSAR

Referral Form for Identification / Typing of Isolates

Please contact medical staff (tel. 2319-8254 / 300; fax 2776-5758) before sending isolates.

Date: _____

Referring hospital: _____

PHLSB staff contacted: _____

Patient identifier: (OR Gum Label)

Name _____

Sex / age _____

HKID no. _____

Clinical information:

Onset date of illness: _____

Clinical features: _____

Presumptive diagnosis: _____

Referred isolate: _____ Your laboratory number: _____

Test requested (please circle): Identification / Typing _____ / Others _____

Specimen type: _____

Medium sent: Name: _____ Incubated for: _____ hours

Incubation (please circle): Aerobic / MICROaerophilic / ANaerobic

Preliminary laboratory findings:

Gram stain morphology: _____

Results from biochemical tests / automated systems: _____

Other relevant results: _____

Presumptive identification of isolate: _____

Other relevant information:

Requested by: _____
 (Name of medical staff / Contact telephone number)