MEDIA INOCULATION AND CULTURE SETUP

☒ St. Joseph Medical Center, Tacoma, WA	☒ St. Anthony Hospital Gig Harbor, WA	☐ Harrison Medical Center, Bremerton, WA
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☒ St. Clare Hospital Lakewood, WA	☒ Highline Medical Center Burien, WA	☐ PSC

PURPOSE

To provide instructions for setting up Microbiology specimens to the proper media for the test requested.

SUPPLIES

The abbreviated names used below refer to the BBL/REMEL product names on the package label:

BA/MAC	TSA II 5% SB / MacConkey II
BA	TSA II 5% SB
CHOC	CHOC II
BEAA	Bile Esculin Azide agar
THIO	Thioglycollate medium (tube)
MLM	Martin-Lewis media
BBL LIM	LIM BROTH(tube)
MAC	MacConkey II
HE	Hektoin Enteric
CAMPY	Charcoal Selective Medium
MACSORB	MacConkey II with Sorbitol
GN	GN Broth
Yersinia agar	CIN
Diamonds Trich	Trichosel broth (tube)
BAC FRAG/LAKED	Bacteroides fragilis / Brucella LKB
PEA	PEA 5 % SB
CNA	Columbia CNA
IMA	Inhibitory Mold agar (tube)
MYCOSEL	Mycosel Agar (tube)
SABDEX	Saboraud Dextrose plate
CHROM	Chromagar/MRSA/Strep B
UREA	Urease slants
VTM	Viral Transport Media

TEST	SOURCE	MEDIA	NOTES
DIRECT SMEAR GS			Direct smears are performed & examined on all specimens with the exception of throat, urine and catheter tips. Gram stains from these sources are performed on request. At SCH,SFH, SAH, SEH or HCH please make an additional smear to be stained for review if needed.
BLOOD CULTURE BLDCULT	Blood Body fluids other than blood	Aerobic (green) & Anaerobic (purple), or for small volumes one aerobic or Pedi(yellow) (for body fluids other than blood, use green aerobic) (Critical value if positive) Use **SPS** tubes to draw for AFB blood cultures.	Check sensor on bottom of bottles - if yellow, subculture instead of loading to instrument. 1. If positive, clean top of bottle with alcohol, use venting needle or TB syringe to obtain a drop of blood for a gram stain slide. 2. Inoculate BA/MAC, BA or Choc. 3. Incubate the plates on the shelf of the incubator above the new plates. 4. Leave the bottles in tray on counter
FUNGAL BLOOD CULTURE FUNCBLD	Blood	Aerobic bottle(green)	Incubate in the BacTAlert. In BacTAlert change the incubation time from 5 days to 30 days
WOUND CULTURE WDGS EARCGS EYECGS	Swab specimen skin lesions, abcesses,burns, exudate, drainages, eye,ear,hip, knee, decubitis, tissues	BA/MAC,CHOC, Direct smear Grind tissues prior to culture setup	Incubate plates in CO2 incubator or incubate chocolate plates in candle jar before transport to SJMC.

BODY FLUID BFCGS	Aspirates,fluids,tissues from normally sterile body sites.Amniotic fluid, biopsies, tympanocentesis, bone,brain,fallopian tube, lymph node, paracentesis, pericardial fluid, pleural fluid,surgical spec., synovial fluid, prostatic fluids.Tissues	BA/MAC,CHOC, Direct Smear Grind tissues prior to culture setup.	If greater than 1ml, centrifuge 10 minutes and use sterile pipet for inoculating sediment to media and direct smear. If other tests are included (AFBC/SM, Fungal culture etc.), pour off samples prior to centrifugation. Fungal cultures will be plated at SJMC lab. Incubate plates in CO2 incubator or incubate chocolate plates in candle jar before transport to SJMC.
CSF SPECIAL HANDLING BFCGS	CSF, myelogram, CSF shunt fluids	BA/MAC, CHOC, Direct smear (Critical value if positive)	All samples are inoculated to media STAT regardless of ordering priority. Follow the procedure for body fluids. In addition, make two slides – one to be stained immediately and the other to be saved for later use if needed. Add CSFCOM comment to smear results if volume is <=0.5ml Bacterial antigen screens are no longer available. Incubate plates in CO2 incubator or incubate chocolate plates in candle jar before transport to SJMC
PERITONEAL EFFLUENT/ DIALYSIS FLUID BFCGS ANC	Peritoneal effulent, dialysis fluid	Green aerobic blood culture bottle & Purple anaerobic blood culture bottle. BA/MAC, CHOC, Direct smear	A liter bag is submitted. Prepare the tubing for removing the fluid by wrapping an iodine pledget around the tubing and allowing it to remain for 5 minutes. Cut the tubing at the iodine site using a sterile scissors. Hold the bag upside down, open the clamp and fill three 50 ml centrifuge tubes with aliquots of effluent. Centrifuge two 50 ml tubes for 10 minutes. Remove most of the supernatant using a sterile transfer pipet. Use 11-12 mls sediment to inoculate the aerobic green, the anaerobic bottle and culture plates and the direct smear. Load the bottle in the BacTAlert. In the LIS, alert the Microbiologists that a blood culture bottle was inoculated using the Micro comment field. Save and refrigerate the third aliquot.

ANAEROBIC CULTURE ANC	Must be collected in anaerobic container. Gel swabs are acceptable	BAC FRAGILIS/ LAKED BLOOD AGAR ANA PEA AGAR	An aerobic culture is <u>always</u> performed in addition to the anaerobic culture. Specimens are put in an anaerobe jar or in an anaerobic gas generation pouch for transport to SJMC. Anaerobe jars and pouches are incubated in the non-CO2 incubator. If only an anaerobe culture has been ordered, order a Wound Culture also.
CATHETER TIP CATHTIP	Catheter tips	BA	Using sterile forceps, gently roll the segment back and forth across the agar surface at least 4 times - discard segment. Do not streak. (Foley urinary catheter tips are not acceptable for culture due to contamination in removal).
LOWER RESPIRATORY CULTURE RESP GS	Sputum, ET aspirates, bronchial wash, ET brush	BA/MAC (P DISC) CHOC, Direct Smear	Sputum cultures (not ET aspirates) are screened for adequacy prior to inoculation. (For Pneumocystis screen or Legionella see Lab directory) Incubate plates in CO2 incubator or incubate chocolate plates in candle jar before transport to SJMC.
UPPER RESPIRATORY CULTURE RESP GS	Nares,nasal, nose,nasopharyngeal, sinus aspirate	BA/MAC, CHOC, Direct smear	Incubate plates in CO2 incubator or incubate chocolate plates in candle jar before transport to SJMC. Pertussis PCR: Collect NP on swab or ESwab. Can be held at room temp prior to testing
QUANT. BRONCHIAL WASHING QN BRWA	Bronchial washings	BA/MAC (P DISC) CHOC, Direct Smear	Use 0.01 disposable loop to inoculate media. (Streak once down center of media and then cross hatch as done for urine cultures). Prepare direct smear from sediment obtained after centrifugation at 3,200 RPM for 10 minutes. Incubate plates in CO2 incubator or incubate chocolate plates in candle jar before transport to SJMC.
QUANT. BRONCHIAL BRUSH QN BRBR	Bronchial brush	BA/MAC, CHOC, Direct smear	The specimen is submitted in 1 ml of sterile saline or thio. Vortex the sample and then use a 0.01 disposable loop to inoculate media. (Streak once down center of media and then cross hatch as done for urine cultures).

THROAT CULTURE THR	Throat	BA (A disc) at end of the shift, put inoculated plates into an anaerobic jar for overnight incubation.	If request is to R/O Haemophilus/all pathogens add CHOC plate, incubate in CO2 or candle jar. If GC screen add a Martin-Lewis-media plate If diphtheria screen- use PAI media from state lab
THROAT RAPID STREP SCREEN	Throat	BA(back-up culture)	Rapid Strep screen is ordered in LIS. A backup culture is inoculated before the rapid Strep is done. (see THR procedure). If the screen is negative, a Throat culture is ordered. Will reflex by LIS.
SCREEN FOR METHICILLIN RESISTANT S. AUREUS CMRSA	Usually nasal or axilla Can be most any source	MRSA chromagar Incubate plate in non CO2 incubator	No need to prepare direct smear. **Keep plate away from direct light**
AFBC/SM MTB PCR	PCR: Sputum, BAL and bronch wash	AFB culture/smears are sent to reference lab PCR done at SJMC	Specimen must be in a leak proof container and placed in t a bag before leaving the dept. Take specimen to sendout bench and place in refrigerator if there will be a delay . Send fresh specimen to SJMC for testing
GCCULTURE GC/GS GENGS	Cervix, urethra, penis rectal, throat, and other sources for N. gonorrheae	For GC cultures: Transport swab to SJMC For Genital cultures: use BA/MAC, CHOC, smear	Culturette swabs are an acceptable specimen to R/O GC. Plate at SJMC onto Martin-Lewis. Incubate plates in CO2 incubator Swabs are good for 24 hours. Incubate plates in CO2 incubator or incubate chocolate plates in candle jar before transport to SJMC
IUD FOR ACTINOMYCES WDGS, ANC	IUD	Thio, BA, CHOC, BRUC, BBE/LKB, SAB DEX, CNA, direct smear	Pour the contents of a thio broth into the sterile container with the IUD. Close tightly and vortex 30 sec. Remove fluid to a sterile centrifuge tube. Centrifuge 10 min. Use sediment to inoculate media used for body fluid samples. In addition, add an anaerobic biplate and 2 Sabouraud dextrose plates (at SJMC, incubate one in CO2 incubator and other in fungal incubator.) Add R/O Actinomyces to Micro comment in LIS.
DRAINAGE FROM IUD FOR ACTINOMYCES	Swab of IUD	Thio, BA, CHOC, BRUC ANA,CNA	If Actinomyces is suspected as the cause of an IUD infection and the IUD is not removed for culture, an anaerobic swab of the cervical drainage can be submitted

WDGS, ANC			with a comment "R/O Actinomyces". Process as you would for a wound or anaerobic culture and inoculate the media listed above for an IUD.
Group B Strep Culture GBS	Vagina, Vag/Rectal	Broth(LIM),BA	Inoculate a BA plate with the swabs rec'd., then put swabs into LIM broth. Incubate plate at 35˚ in CO2 LIM broth in non-CO2
TRICHOMONAS CULTURE TRICH	Urethra,Penis Female specimens perform Trichomonas Ag.	Trichomonas Trichosel	Submitted in Trichomonas transport media. After immediate reading, incubate and/or transport to SJMC for incubation. Keep tube upright during transport.
ST CX	Fresh stool or preserved in Cary-Blair or ETM	MAC, HE, GN Broth (Shiga toxin) ForCampylobacter Ag:fresh stool or stool in Cary-Blair (orange lid) or ETM is acceptable. Inoculate Cary Blair for Shiga Toxin assay at other sites. Direct Smear for fecal leukocytes	(Please use whole MAC if available) **Campylobacter specimens** hold fresh stool or Cary-Blair/ETM in refrigerator in labeled bucket. Other sites send fresh or preserved specimen in transport box to SJMC **Shiga Toxin**: other sites inoculate a Cary-Blair vial to the appropriate line on the vial, transport to SJMC. Request for fecal leukocytes - done on gram stain. Request for Vibrio, Aeromonas, Plesiomonas, or Staph aureus (add micro comment in LIS),set-up a BA plate Stool culture transported in Cary Blair liquid: Gram stains for fecal leukocytes are unreliable from transport. Recommend a swab of the sample submitted along with the transport. Kit with instructions available. If specimen recd in transport w/out swab or prepared smear, add comment in LIS micro comment area.
YERS	Fresh stool, stool in Cary-Blair or ETM	CIN AGAR PLATE	Incubate plate in fungal incubator. SFH, SCH, SAH, SEH and HCH will send stool specimen to SJMC for setup.
C DIFF PCR (Cl. difficile)	Fresh stool (loose, watery, soft spec) Hard, formed stools will be rejected		Fresh stool only. Test or refrigerate specimen in Micro. Other sites send stool specimens in Micro box to SJMC. Only one specimen performed every 7 days.
RECTAL SWAB	Culturette swab	If Campylobacter also requested, set up a Campy plate. Swab is not	If submitted for enteric pathogens – see Stool culture above. Other sites send swab to SJMC for Campylobacter If GC screen - submit on swab, plate on Martin-Lewis

		acceptable for Campy antigen.	If R/O beta Strep, plate on BA also.
SCREEN FOR VANCOMYCIN RESISTANT ENTEROCOCCI VRE	Can be any source, usually stool or urine	CNA BEAA w/ vancomycin (whole plate)	When a patient is in isolation for VRE, screening specimens may be submitted to see if the organism has been eradicated. Sources may be feces or rectal swab or other sources. For SFH/SCH/SAH/SEH or HCH send swab to SJMC for setup.
OVA & PARASITE EXAM OP	Stool, bronch wash, sputum, duodenal drainage or colon washes	Ecofix, Unifix or fresh specimen Direct smear on watery, loose specimens	One vial transport, Ecofix,(Formalin and PVA acceptable) Leave vials at room temperature. If sample is submitted fresh, add to vials as follows: using the spoon attached to the lid, add enough sample to bring the volume to the red line. Select areas that are bloody, slimy or watery. Mix well with the spoon and close tightly. Shake vigorously. Formalin or fresh stool specimen with formalin added can be used for Giardia antigen. Ecofix **cannot** be used for Giardia antigen or Cryptosporidium.
URINE CULTURE UC		BA/MAC	If routine, use 0.001 loop. If cath urine, bladder, suprapubic aspirate, or kidney urine, use 0.01 loop. Indicate on plate if 0.01 loop is used Streak down the middle of plate, then cross over streak to isolate organisms. If gram stain is requested, it is performed on unspun urine.
HERPES PCR	For suspected Herpes Lesions. For CSF order PCR	Collect in M6/VTM media. Hold in refrigerator after collection	Cannot be collected using wooden shafts. Use Dacron or rayon swabs. Processed in Microbiology at SJMC.
FUNGUS CULTURE FUNCSM			Processed by Microbiology at SJMC
Scrapings: skin, hair, nail FUNSKSM		IMA, Mycosel, KOH inoculate directly	If request for Malassezia, Pityrosporum, M. furfur, Tinea versicolor, pachydermatis, add SABDEX with 2-3 drops of oil over the inoculum. Oil in dropper bottle above sink.
Sterile body fluids		IMA, Mycosel, Gram stain	If viscous, use direct. If liquid, centrifuge and inoculate 0.5 ml per tube.

Sterile body sites (tissue)		IMA, Mycosel, Gram stain	Grind tissue with sterile saline and inoculate 0.5 ml per tube.
Sputum, ET		IMA, Mycosel, KOH	Plate directly onto media
Bronchial brush, Bronchial washing		IMA, Mycosel, KOH	Vortex brushes, centrifuge and directly inoculate 0.5 ml of sediment per tube.
Swabs		IMA, Mycosel Gram stain	Inoculate tubes directly.
CSF		IMA, Mycosel India Ink	Centrifuge if > 2 ml. Inoculate 0.5 ml per tube.
Blood		Aerobic bottle (green)	Run on BacTAlert, change to 30 day protocol
Urine		IMA, Mycosel Gram stain	Centrifuge 15 minutes, plate sediment (0.5 ml per tube).