KAISER PERMANENTE®
COLORADO LABORATORY

MICROBIOLOGY SPECIMEN PROCESSING

PURPOSE:

Once a specimen arrives in the laboratory, it is the responsibility of laboratory personnel to ensure that all pertinent information has been provided, the specimen is properly labeled, that the specimen has been collected in the proper transport device, and that all other conditions for an acceptable specimen have been met. Specimens should not be processed if they are received in inappropriate containers, improper transport medium, or after a prolonged delay. If a second specimen cannot be conveniently obtained, the final report should clearly indicate that the specimen was inadequate and the results may or may not be valid or complete. The goal of this section is to provide instructions for the proper processing and transport of specimens to be sent to RRL Microbiology. This will allow the Microbiology lab to provide accurate culture results for the provider and ensure the best quality care for our patients.

SCOPE: Medical Office Laboratory staff, Regional Reference Laboratory TLA staff

MATERIALS:	
Culture Media	Frosted end glass slides
Incinerator	Inoculation loops
Anaerobic transport system (CO2 Transport Pouch)	Urine loop (0.001 µl)
	BD ESwab Collection & Transport System

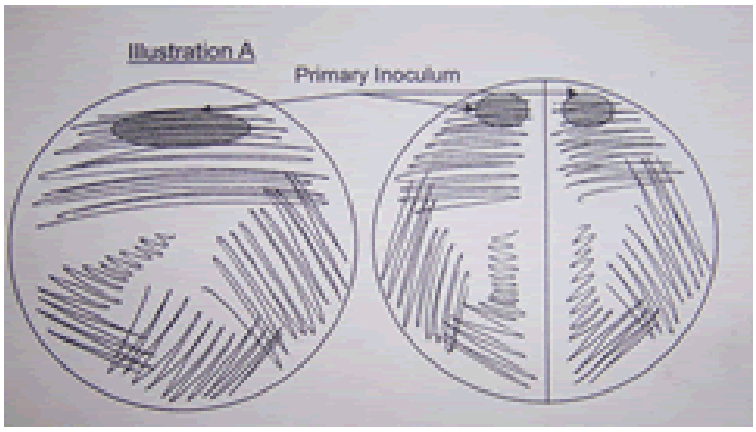
PROCEDURE:	
1	Specimen must be properly labeled.
2	The specimen must be submitted in the proper transport container *NOTE: Specimens received on dry swabs (other than Strep A screens) are not appropriate for culture. In the event that an adequate specimen cannot be recollected, the swab may be plated with a disclaimer comment added "Specimen received on dry swab. Culture results may be compromised."*
3	The specimen volume must be adequate to perform all tests requested. If an inadequate amount has been received, contact the provider to prioritize multiple tests ordered or for repeat specimen.
4	Specimens must be received in a timely manner. *NOTE: Refer to Microbiology Specimen Collection manual for further information regarding timely receipt of specimens.* *NOTE: If provider requests that a urine culture be added on to a UA order, the culture must be set up **within one hour of collection** or patient must collect a new specimen.*

5	Anaerobic cultures will be processed **ONLY** on appropriate specimens (refer to Microbiology Specimen Collection manual) and must be submitted in an anaerobic transport tube or sterile red top tube (fluid) which has been collected at bedside. **NOTE:** *RRL TLAs set up all cultures sent in anaerobic transport media on aerobic and anaerobic media.*
6	CSF and other sterile body fluid samples must be processed **immediately** on receipt. Bacterial meningitis is a critical condition and CSF specimens require immediate attention.
7	When stool specimens are returned to the laboratory, check in ORV to be sure that the appropriate labels for **all** tests ordered are properly attached to the transport media. Then proceed to setup the culture and/or smear and send the Cary-Blair transport vial to RRL Microbiology for any other testing that has been ordered.

MEDIA SELECTION AND LABELING:	
1	Select appropriate media for the test ordered. *NOTE: Refer to the Microbiology Setup Chart for specific information.*
2	Examine all media prior to inoculation for expiration date and contamination.
3	Warm media to room temperature prior to inoculation.
4	Specimens for **GC culture** (not PCR testing) must be inoculated onto pre-warmed selective GC media at the bedside and sent **immediately** to the laboratory to be incubated in CO2 Gas Pak Pouch. NOTE: *SoCo specimens will be sent on a special swab which will be inoculated onto a warm Thayer-Martin plate and incubated at 35ºC in CO2 incubator.*
5	Individually label all media with patient accession number using barcode labels. Place the long barcode label on at least one plate from each culture, avoiding the blood side of the media plate. For tube media, place the barcode label on the tube **vertically and straight** (similar to blood specimens). *NOTE: Use the small barcode label on all tubes.*
6	If a Gram Stain is indicated, **using a pencil**, label two slides on the frosted area with: ✓ Patient Name ✓ Accession Number ✓ Source ✓ Date Label the envelope containing the slides with a barcode label. *Note:* **DO NOT** *use small barcode labels on slides.* **NOTE:** *RRL TLAs place prepared slides for wounds and fluids on Desk 3, slides for sputums and fecal WBCs on Desk 1.*
7	Send extra barcode labels with the following culture/specimens only (additional labels are required for processing at RRL): ✓ AFB ✓ GBS ✓ Herpes ✓ OAP ✓ Pertussis ✓ Tissue specimens ✓ Stool

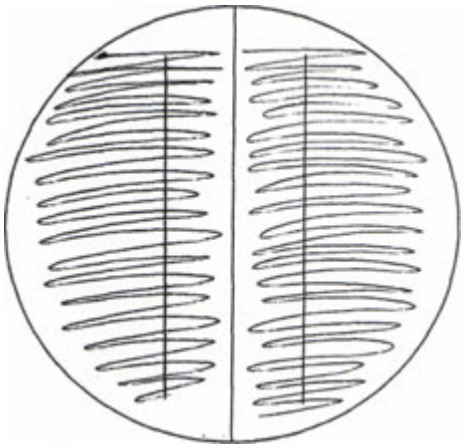
MEDIA INOCULATION:	
1	**IMPORTANT NOTE**: Delay of inoculation and processing of certain specimens can affect the quality of the culture and the ability to isolate pathogens! Therefore, specimens from surgery or normally sterile sites must be inoculated to media and incubated ASAP.
2	Process all specimens except urine cultures in the biological safety cabinet.
3	Fluids received with more than 1 ml of specimen must be centrifuged at 3000 rpm for 15 minutes. Use the sediment to inoculate media and slides.
4	Select the purulent part of the specimen for culture and smears.
5	Inoculate Thioglycollate broth first (if applicable), then proceed to solid media in order of least inhibitory to most inhibitory. This prevents the carryover of any inhibitory substance to another medium. *For example, Stool screen/culture is inoculated in the following order: BAP, MAC, HE, GN Broth, Campy Thio.*
6	For specimens requiring smears, refer to "Gram Stains of Direct Specimens procedure." **NOTE:** *Smears are always prepared **after** media inoculation*.
7	For prosthesis specimens, catheter tips and other foreign object specimens with no visible tissue or purulent fluid, add Thioglycollate broth to the sterile specimen container and incubate at 35°C.
8	Tissue specimens that have been received in an anaerobic transport tube or sterile container are sent directly to RRL Microbiology for processing.
9	**For Eswab transport:** Vortex the vial containing the swab for 5 seconds.
10	Using a sterile, plastic pipette, place one drop of vortexed specimen onto the first quadrant of each plate and slides.

STREAKING FOR ISOLATION (All specimens except urine):	
1	Inoculate culture plate by touching specimen/swab to one quadrant and proceed as follows: ✓ Sterilize inoculating loop by placing in the Bacti-cinerator for 5-10 seconds. Allow to cool. ✓ Using sterile loop, streak with gentle pressure onto one-fourth to one-third of the culture plate with back and forth motion several times. Avoid touching sides of the Petri dish. ✓ Turn plate a quarter turn. ✓ Pass the loop into the edge of the first quadrant approximately four times while streaking into the second quadrant. Continue streaking in the second quadrant without going back into the first quadrant. ✓ Rotate plate another quarter turn and repeat the above procedure until one or two additional quadrants are streaked. ✓ Refer to illustration A below.



MEDIA INOCULATION (Urine specimens):	
1	Using a 1 ul (.001 ml) calibrated loop, dip loop vertically into the well-mixed urine just far enough to cover the loop. Spread loopful of urine over the surface of the agar by streaking from top to bottom in a vertical line and again from top to bottom perpendicular to this line in a back and forth fashion as shown in Illustration B below. ***NOTE:*** *Prior to plate inoculation, ensure that a film of urine fills the loop with no bubbles to alter the calibrated volume.*

Illustration B



GROUP B STREP SCREEN (GBS) PROCESSING:	
1	NOTE: A BD ESwab should be received for GBS processing
2	Warm LIM broth to room temperature.
3	Attach one small barcode label to the LIM broth, leaving the remaining portion of the label to be sent with the specimen to RRL.
4	Vortex for 5 seconds and pipette 1 drop of vortexed specimen to LIM broth. Replace the cap tightly.
5	Transport LIM broth to RRL after overnight incubation except on Friday if your clinic

	is closed on Saturday. In that instance, broths must be sent to RRL on Friday evening. **NOTE:** *RRL TLAs place LIM broth (with cap slightly loosened) in a rack on top shelf of CO2 incubator.*

CULTURE INCUBATION:	
1	Place inoculated plates and Thioglycollate broth in the incubator at 35^{o}C until transport to RRL.
2	Place Campy Thio, GN Broth and corresponding Cary-Blair transport in refrigerator at 2-6^{o}C until transport to RRL.

TRANSPORT TO RRL MICROBIOLOGY:
DO NOT AFFIX LABELS TO THE OUTSIDE OF THE BAG UNLESS SPECIFICALLY DIRECTED TO DO SO IN THIS PROCEDURE.
AFB SPECIMENS Tightly seal AFB specimens, place in ziploc bag and transport to RRL. Transport the remaining portion of the barcode label(s) in a separate bag or separate compartment of the same bag to ensure that leakage does not contaminate the label.
ANAEROBIC TRANSPORT (PAC) **Tissue specimens**: Transport in anaerobic transport system or sterile container at room temperature to RRL for processing. **All other specimens**: Franklin, Rock Creek and RRL laboratory: Set up anaerobic cultures and transport in anaerobic bags to RRL. *RRL TLAs place anaerobe bags on top shelf of the CO2 incubator. Label the bags with the date of setup.* All other MOLs: Hold in anaerobic transport system at **room temperature** until transport to RRL. Transport using anaerobic transport system.
BLOOD CULTURE BOTTLES Place blood culture bottles carefully in ziploc bags, seal tightly and transport to RRL.
LIM & THIO LIM and Thioglycollate broths, seal cap tightly and bag separately from plated media. Transport the remaining portion of the GBS barcode label(s) in a separate bag or separate compartment of the same bag to ensure that leakage does not contaminate the label.
GN BROTH & CAMPY THIO Seal caps tightly and place GN and Campy thio broths into ziploc bag with the corresponding Cary-Blair transport.
FUNGUS TUBES Place a small barcode label on each tube of fungal media, secure lid loosely and place in separate bag for transport to RRL.

GENITAL, GC CULTURES

Place genital and GC culture in CO2 Gas Pak Pouch for incubation until transport to RRL. Transport using CO2 transport system.

GRAM STAINS, FECAL WBC

Place Gram stain and Fecal WBC slides in coin envelopes with the corresponding label clearly visible on the outside of the envelope for transport to RRL.

HERPES

Place Herpes cultures together in a separate bag for transport to RRL.

PERTUSSIS CULTURES

Place Pertussis cultures (Universal Viral Transport Medium) together in a separate bag for transport to RRL. (Nasal washes must be sent on ice.)

MALARIA SMEARS

Place air dried thick and thin smears in coin envelope or slide box and transport to the RRL Microbiology Department for processing. If EDTA tube was drawn, send this with slides as well.

PLATED MEDIA

Stack plated media for stool and urine cultures upside down (lid on bottom, media on top) and place in ziploc bag(s). Seal carefully to assure plates do not fall out and become contaminated. Plates that have been placed in CO2 Gas Pak Pouches (wound, sputum, genital, etc.) should be transported to RRL in those bags. Also transport remaining BD ESwab transport media to RRL for **wound** cultures only.

STREP SCREENS (BSS)

Place all strep screens together in a separate ziploc bag for transport to RRL.

DELAY IN TRANSPORT:

Incubate the following at 35°C:

- ✓ Plated media
- ✓ LIM broth
- ✓ Thioglycollate broth

Refrigerate the following at 2-8°C:

- ✓ AFB specimens (**except** heparin tubes)
- ✓ Campy thio broth
- ✓ Cary-Blair transport media
- ✓ C. difficile stool specimens
- ✓ GN Broth
- ✓ Herpes specimens
- ✓ Pertussis cultures (UTM and nasal washes)

Store the following at **Room Temperature:**

- ✓ AFB Blood Culture tubes (heparin)
- ✓ Anaerobic transport tubes (PAC)
- ✓ Blood culture bottles

- ✓ BSS swabs
- ✓ Fungal cultures
- ✓ O&P Para-Pak transport vials
- ✓ Malarial smears

REFERENCES:

1. Clinical Microbiology Procedures Handbook, American Society for Microbiology, Washington, D.C. 2004

2. Murray, P., Baron, E., Jorgensen, J.M., Landry, M., Pfaller, M.: Manual of Clinical Microbiology, 9th Edition, American Society for Microbiology, Washington, D.C. 2007.