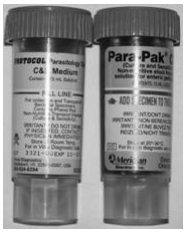
Specimen Collection – Clinically Relevant Microbiology Starts at the Source

Mike Costello, PhD, MT(ASCP)
ACL Laboratories
847.349.7403
mike.costello@advocatehealth.com

Mary Dikeman, MT (ASCP)
Affinity Health System
920.738.2138
mdikeman@affinityhealth.org



Program Objectives

- Emphasize that obtaining sensitive and specific microbiology results begins with the patient and not at the door of the microbiology laboratory.
- Accentuate the importance of proper collection and transport of specimens in both local and referral environments
- Stress the importance of timely communication between the Microbiology laboratory and those collecting specimens
- Describe common pitfalls in specimen collection and transport
- Discuss What rules or principles must be followed in order to collect microbiology specimens which will accurately reflect the pathogenesis of the microbiological agent. (Church D. The Seven Principles of Accurate Microbiology Specimen Collection. . Calgary Laboratory Services Microbiology Newsletter. Volume 6, 2005)

Introduction

The practice of sensitive, specific and cost effective clinical microbiology is intimately tied to the submission and proper handling of optimal specimens for analysis. Unfortunately, these aspects of clinical microbiology are not as critically controlled as our laboratory assays. It is our responsibility to educate and notify our healthcare colleagues when specimens arrive at the laboratory that will yield inferior results.

Quality assurance of specimen collection and transport is a never ending battle and requires long term commitment of your time and resources, but the end results are better patient care and a more rewarding experience for those of us who work in the microbiology laboratory.

Principle #1: The specimen must be collected with a minimum of contamination as close to site of infection as possible

Specimen	Source of Contamination	Storage and Transport	Solution/Monitor	Education
Urine Culture	All non surgical samples become contaminated with urogenital flora during collection. Contaminating bacteria will replicate if specimen is not quickly transferred to a preservative tube or stored (4°C).	Transfer urine to a Urine Preservative tube within 10 minutes of collection (good for 48 hrs. at ambient temp. Less optimal: store/transport urines at 4° C for up to 24 hrs.	Patients must be instructed to properly cleanse the peri-urethral genital skin area prior to collection of the mid-stream portion of the urine stream in order to get an accurate urine culture result. Use of urine preservative tubes.	Prompt feedback to individuals or sites who collected urine for culture. Urine preservative tubes should be used when appropriate.
Blood Culture, bacterial, mycobacterial, fungal	Improper cleaning of skin or catheter prior to drawing specimen. Transfer from SPS tube to blood culture vial. Collection from catheter.	Ambient. Must be incubated in automated system within 12 hours.	Ongoing education program. Monitoring contamination rates. Limit use SPS tubes. Do not draw from catheter unless specifically requested (protocol; discard 5X cath. volume); then one culture set from catheter and one from peripheral.	Timely feedback to individuals who collected specimen.

Urine Culture Contamination Rates

- **Urine Culture contamination rates (≥2 bacteria at >100,000 CFU) should be <20%**
  - CAP Q-Probe study (Valenstein P Meier F. Urine culture contamination: a College of American Pathologists Q-Probes study of contaminated urine cultures in 906 institutions. Arch Pathol Lab Med. 1998;122:123-129)..
    - 630 participants collected information of 155,037 urine culture specimens; 20.1% were considered contaminated (≥2 organisms at $>10^5$ CFU)
    - **The top 10% of institutions reported a rate of 5.6%. Bottom 10% of institutions reported a contamination rate of 36.8%**
    - **Males have a lower contamination rate than females (11.2% Vs. 22.8%)**
    - ER departments had a contamination rate of 17.8%, sites adjacent to lab had rates of 19.5%, and other sites had rates of 22.1%

Blood Culture

- Two sets of blood cultures should be drawn. Number of sets positive correlates with true sepsis (except for coagulase negative Staph?) (Clin Microbiol. Rev 19:788-802, 2006)
- Catheter drawn blood cultures
  - Catheter drawn blood cultures are equally likely to be truly positive (associated with sepsis), but more likely to be colonized (J Clin Microbiol 38:3393, 2001.)
    - One drawn through catheter and other though vein PPV 0f 96%
    - Both drawn from catheter PPV 0f **50%**
    - Both drawn through vein PPV of 98%
  - Study of positive coagulase negative Staphylococcus cultures and sepsis (Clin Infect Dis. 39:333, 2004.)

What is an "Acceptable" Blood Culture Contamination Rate for Your Lab??

Blood Culture Contamination Rate By Service Drawing Culture

Blood Cultures Collected By Indicated Staff Type (%)	No. of Labs	Mean Blood Culture Contamination Rate (%)
Dedicated phlebotomy staff		
0-25	94	3.27
26-75	127	3.02
76-100	120	**2.84**
Medical technologists or technicians		
0	165	3.25
1-10	113	2.95
11-100	60	**2.69**
Nonlaboratory staff		
0	36	2.17
1-50	253	3.00
51-90	36	3.40
91-100	17	**4.21**

Berkeris LG, JA Toworek, MK Walsh, PN Valenstein. Trends in Blood Culture Contamination. Arch Pathol Lab Med 129:1222-1294, 2005

What is an "Acceptable" Blood Culture Contamination Rate for Your Lab??

Blood Culture Contamination in Pediatric Patients Young Children and Young Doctors

Variable	True Positive	False Positive	Predicative Value of a Positive Result
Experienced physician-older child	**82**	**74**	**0.53**
Experienced physician-younger child	**165**	**151**	**0.52**
Inexperienced physician-older child	**92**	**158**	**0.37**
Inexperienced physician-young child	**221**	**385**	**0.37**
Total	**560**	**768**	

Young Children = 1-35 months
Older Children = $\geq$36 months
Inexperienced Physicians = Interns and residents in 1st half of training
Experience Physicians = Residents in 2nd half of training and senior physicians

Ped Infect Dis. 2006, 25:611-614.

What is an "acceptable" blood culture contamination rate*?

	Contamination Rate			
Population	No. of Labs	25th Percentile	50th Percentile (Median)	75th Percentile
Adults	326	2.23	2.92	3.8
Neonates	254	0.75	2.08	4.27
All Patients	356	2.15	2.89	3.67

* Blood culture is considered contaminated if 1 or more of the following organisms were identified in only one of a series of blood culture specimens; coagulase negative Staphylococcus, *Propionibacterium acnes*, Micrococcus spp., Viridans group Streptococcus, Corynebacterium spp., or Bacillus spp. (not *B. anthracis*)

CAP Q-Tracks (1999-2003) Median contamination rate of 2.92%

What should your blood culture contamination rate be?
1. Static model. Set a contamination rate (<3%. Range 2.23%-3.8% Adults; 0.75%-4.27% Neonates)). Define an "acceptable rate" and institute correct measures when rate drifts above critical value
2. Continuous Quality Improvement Model. Set a rate that at which 2/3 can achieve, <2.5%). Once 95% of units achieve this rate lower it to 2.0%. Strive to be in the top 10 percentile

Berkeris LG, JA Toworek, MK Walsh, PN Valenstein. Trends in Blood Culture Contamination. Arch Pathol Lab Med 129:1222-1294, 2005

Principle #1: The specimen must be collected with a minimum of contamination as close to site of infection as possible (cont.)

Specimen	Source of Contamination	Storage and Transport	Solution/Monitor	Education
Respiratory Culture	Improper mouth care prior to collection of specimen. Lack of deep cough to obtain lower respiratory material.	Ambient for 8 hours. Refrigerated 24 hours. Some organisms, such as *Haemophilus influenzae* are susceptible to drying or low temperature.	Monitor % rejected sputum. % with oral contamination (epithelial cells; multiple Strep species, usually in clumps on gram stain and culture results). Sputum culture Vs. blood culture results?	All sputum samples are contaminated to varying degrees with oropharyngeal flora. Rinsemouth with sterile saline/water immediately before expectoration reduces number of contaminating bacteria. Timely feedback to individuals who collected specimen. Sputum samples of <2 mL should not be processed unless obviously purulent.
Wound Culture	Improper cleaning of wound site prior or collection.	In transport container. Ambient for no longer that 24 hours. Maximize transport time.	Number of squamous epithelial cells Vs. PMNs seen on Gram stain. Presence of squamous epithelial cells associated with a superficial specimen. The representative specimen is taken from the advancing margin of the wound (ASCP Teleconference. 8130, 2007)	Superficial: cleanse with 70% alcohol; aspirate or swab fluid Deep: cleanse with 70% alcohol, use syringe, surgical procedure,\. Tissue: aspirate or 5-10 mm piece of tissue.
Mycobacteria Culture	Sputum: Improper mouth care prior to collection of specimen.	Ambient for 8 hours. Refrigerated 24 hours.	Contamination rate; track % NAOH required for decontamination. Culture redigests.	Timely feedback to individuals who collected specimen.

Respiratory Cultures

- Community Acquired Pneumonia – Sputum rejection rate and culture correlation with gram stain
  - 54% of all samples were judged to be of good quality.
  - Presence of a (predominant morphology) PM on Gram stain was predictive of whether the sputum culture could demonstrate a pathologic organism. In the presence of a positive PM, 86% of cultures yielded a pathologic organism, while a positive culture was obtained in 19.5% of Gram stains without a predominant organism. *S. pneumoniae* was the most common infection, growing in 55.7% of positive sputum cultures.
  - The sensitivity and specificity of finding Gram-positive diplococci for a positive culture of *S. pneumoniae* were 60% and 97.6%, respectively (*Arch Intern Med*. 2004;164:1725-1727, 1807-1811)
- Ventilator associated pneumonia (VAP) – appropriate specimen
  - Blood cultures highly specific but not sensitive (positive in <10% of VAP)
  - Quantitative cultures of lower respiratory tract specimens show a closer clinical correlation than sputum subcultures (Clinical Microbiol. Rev. 19:637-657, 2006.)

Viral Respiratory Cultures – Collect Sample From Site of Infection

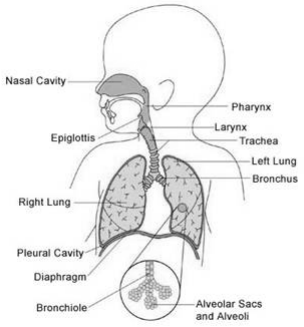
Comparison of nasal swab, nasopharyngeal swab, and nasopharyngeal wash specimens with an expanded gold standard in the Quidel QuickVue influenza test

	No. of positive samples/no. of samples (%)			
Specimen Type	Sensitivity	Specificity	Positive Predictive Value	Negative Predicative Value
Nasal Swab	46/59 (78)	46/48 (96)	61/74 (82)	107/122 (88)
Nasopharyngeal Swab	**50/59 (85%)**	**50/51 (98**	**62/71 (87)**	**112/122 (92)**
Nasopharyngeal Wash	41/59 (69)	41/42 (98)	62/80 (78)	103/122 (84)

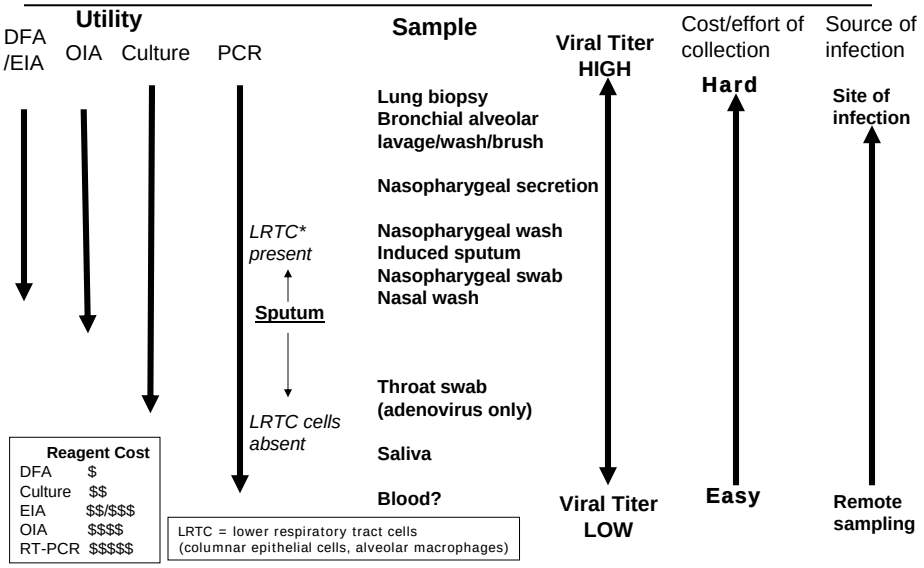
J Clin Microbiol. 2006: 44;2638-2641

Throat swabs are even worse!

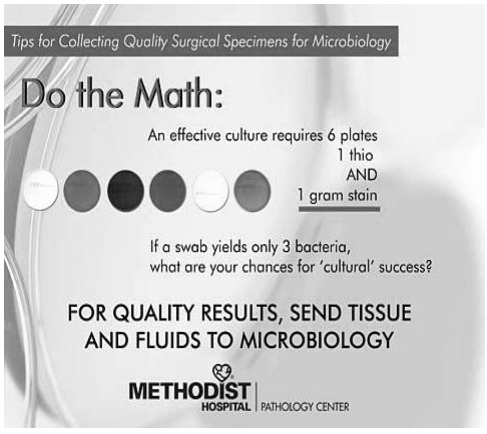
How do you know that an adequate Specimen was submitted for rapid EIA assays???



Samples for Diagnosis of Viral Respiratory Infections



Submit Specimens …Not Swabs



Nancy Cornish MD
www.cap.org

Skin and Soft Tissue (Wound) Cultures

- Collect with steel (needle aspirate or scalpel)
- Discourage the use of swabs
- If infection NOT suspected, DON'T culture
- Get infected tissue or body fluid [discourage swabs!]
- -use something sharp (syringe, scalpel, etc)
- -close doesn't count
- ***Don't** culture the surface / get deep infected sample*
- Remove needles / send capped syringe with aspirate
- Share specimen: Microbiology-Surgical Path-Cytology
- ** Label specimen and site accurately
- ** Give appropriate history

(Matkoski C. Sharp SE, Kiska DL. Evaluation of the Q Score and Q234 Systems for cost-effective and clinically relevant interpretation of wound cultures. J Clin Microbiol 2006;44:1869-1872)

Principle #2: A specimen must be collected at the optimal time(s) in order to recover the pathogen(s) of interest

Specimen	Optimal Time	Comments
Urine	First morning specimen preferred.	Or not have urinated in several hours
Blood Culture	Collect prior to administration of antibiotics. Collect 2-3 sets of blood cultures from different sites. If suspect bacterial endocarditis and initial cultures are negative at 48 hours then collect 2-3 additional cultures from different sites. Suspected bacteremia or fungemia with persistently negative blood cultures	Interpretation of one positive culture problematic, especially if isolate is coagulase negative Staphylococcus. Consider laternative blood culture methods dsigned to enhance recovery of mycobacteria, fungi, and other rare and fastidious microorganisms
AFB Culture	Three consecutive specimens collected 8-24 hours apart, with at least one being an early AM specimen	Sputum not saliva
GC/Chlamydia, urine	First voided urine of day. First stream of urine optimal. Less sensitive: Patient should not have urinated for at least 1 hour. Do not use NAT methods as "proof of cure".	Not midstream urine. Place sample in transport tube per manufacturer's instructions Lingering DNA may still be present.

Principle #2: A specimen must be collected at the optimal time(s) in order to recover the pathogen(s) of interest (cont)

Specimen	Optimal Time	Comments
Ova and Parasites	Wait 10 days if barium or oil present. For multiple samples, collect every other day.	Place stool in preservative (10% formalin, PVA, SAF, Ecofix) within one hour of collection. Instruct patient.
Stool Cultures	Recommend 2 samples on consecutive days. Prior to 3 days post admission.	Place in enteric preservative (Cary-Blair) immediately. Stool specimens that are obtained 3 days after admission are not usually helpful for the diagnosis of hospital acquired diarrhea
Blood Parasites	Collect during a febrile episode or every 6 hours for a 24 hour period.	Submit finger stick Thick & Thin slides or peripheral blood in an EDTA tube within 24 hours. Store at ambient temperature.
Viral Culture	Collect as soon after onset of symptoms as possible.	The first 3 days is best.

Principle #3: A sufficient quantity of the specimen must be obtained to perform the requested tests

Culture	Minimum Requirements	Comment
Blood Culture	10 ml of aerobic; 10 ml for anaerobic bottle	Sensitivity of a blood culture is directly related to the volume of blood submitted. Two blood culture sets (10 mL in both aerobic and anerobic bottles) before administration of antibiotics is 98% sensitive (J. Clin. Microbiol. 1998 36: 657-661).
One swab for multiple cultures	A separate swab(s) for each culture	Enough material must be submitted for gram stain, if required.
CSF Culture	2 mL from tube 2,3, or 4	Submit most turbid tube. At least 0.5 mL of CSF is required for cytospin gram stain.
Surgical and Shared Specimens	See chart	Cooperation with other departments (laboratory and non laboratory) is key.
Anaerobic Cultures	See Table	

Blood Cultures

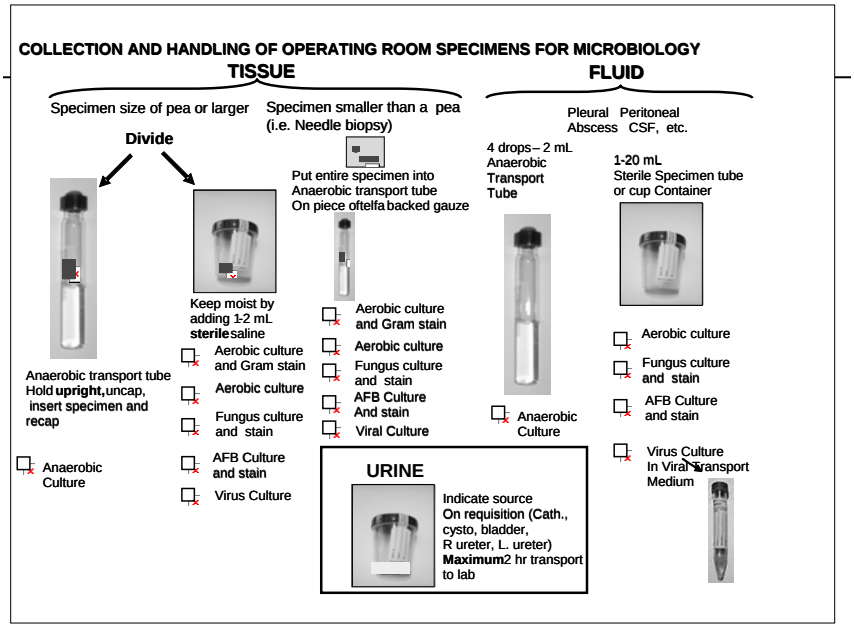
- **Volume of blood drawn is the single most important factor influencing sensitivity.** A single set for an adult blood culture consists of one aerobic and one anaerobic bottle. Optimally **10 mL of blood should be inoculated into each bottle**. Volume of blood for a pediatric culture can be related to the infants weight
  - Solitary blood cultures should be less than 5% (Arch Pathol Lab Med. 2001 125:1290-1294)
  - If only enough blood can be drawn for one bottle, inoculate the aerobic bottle.
    - 644 positive blood cultures, 59.8% from both bottles, 29.8% from aerobic bottle only and 10.4% from anaerobic bottle only (J Infect Chemother 9:227, 2003).

Pediatric Blood Cultures - Volume

Recommended Pediatric Blood Culture Volumes By Patient Weight

Weight (KG) of Patient	Weight (LB) of Patient	Minimum Volume (mL)	One Pediatric Bottle	Two Adult Bottles (aerobic and anaerobic)
<1.0 Kg	2.2 Lb.	1.0 mL	Yes	No
1.5-3.9 Kg	2.3-8.6 Lb.	1.5 mL	Yes	No
4.0-13.9 Kg	8.7-30.6 Lb	3.0 mL	Yes	No
14.0-24.9 Kg	30.7-54.9 Lb	10.0 mL	No	Yes (5 mL in each)
>25.0 Kg	>55 Lb.	20.0 Ml	No	Yes (10 mL in each)

Surgical Specimens (Other Shared Specimens)



Acceptable Specimens For Anaerobic Culture

Site	Acceptable Specimens	Unacceptable Specimens
Abdomen	Peritoneal fluid obtained by needle and syringe Abscess aspirate obtained by needle and syringe Bile Biopsy material surgically obtained Anaerobic swab surgically obtained	Aerobic swabs
Body Fluids	Ascitic fluid, bile, blood, bone marrow, CSF, pericardial, pleural, seminal, synovial fluid, throacentesis, transudates	
Bone and joint	Aspirate obtained by needle and syringe Biopsy material surgically obtained Anaerobic swap surgically obtained	Superficial material collected with swabs
Central nervous system	Abscess aspirate obtained by needle and syringe Biopsy material surgically obtained Anaerobic swab surgically obtained	Aerobic swabs
Female genital tract	Culdoscopy specimens Endometrial aspirate obtained by suction or protected collector Abscess aspirate obtained by needle and syringe Biopsy material surgically obtained Anaerobic swabs surgically obtained IUD for *Actinomyces* species	Vaginal or cervical swabs

Acceptable Specimens For Anaerobic Culture

Site	Acceptable Specimens	Unacceptable Specimens
Head and neck	Abscess aspirate obtained by needle and syringe after surface decontamination Biopsy material surgically obtained Anaerobic swab surgically obtained when aspiration is not feasible	Throat or nasopharyngeal swabs Gingival swabs Superficial material collected with swabs
Lungs	Transtracheal aspirate Material from percutaneous lung puncture Biopsy material surgically obtained Bronchoscopic specimen obtained by protected brush Thoracatomy specimen Anaerobic swab surgically obtained	Expectorated sputum Induced sputum Endotracheal aspirate Bronchoscopic specimens not specially collected
Soft tissue	Aspirate obtained by needle and syringe Biopsy material surgically obtained Aspirate from sinus tract obtained by needle and small plastic catheter Deep aspirate of open-wound obtained through decontaminated skin Deep aspirate of surface ulcer obtained through decontaminated skin	Superficial material collected from skin surfaces or edges of wound
Urinary tract	Suprapubic aspirate	Voided urine Catheterized urine

Principle #4: Appropriate collection devices and specimen containers must be used to ensure recovery of all organisms

Culture/Situation	Comments
Anaerobic Culture	Anaerobic cultures are best collected with metal (needle aspiration or with a scalpel). Aspirates of pus or fluids could be left in syringe if not a long distance transport. A large piece of tissue 5-10 mm will protect anaerobes in center. Specimen received in aerobic transport media. Stuart's and Amies media will allow for isolation of facultative anaerobes (Amies giving slightly better yields). Use of true anaerobic transport media will result in the best yields of all anaerobes. Consider rejection of swabs not in anaerobic transport.
Chlamydia or GC Culture	Specimen received in NAT transport tube can not be cultured. Collect Chlamydia in M-4, UTM. Collect GC culture in Amies + charcoal and or transport immediately. to lab at ambient temperature for immediate plating.
Tissue sent in preservative	Bacterial culture ordered on tissue placed in formalin. Culture is not an option. Request another specimen.
Bacterial Culture sent in viral transport media	Bacterial cultures sent in Viral Transport Media. Request another specimen, most VTM contain antibiotics
Dry Swab	Swab not placed in transport media. Specimen not viable, request another specimen.

How Does Transport Time Affect Yields?

Recovery of Anaerobic Bacteria Placed in in Aerobic/Anaerobic Transport Media

TABLE 1. Recovery of anaerobic gram-positive organisms

Swab system	Organism	No. (%) of evaluated organisms recovered at:			
		0 h	6 h	24 h	48 h
CVP	*C. perfringens*	1×10^5 (100)	8×10^4 (80)	4×10^4 (40)	1×10^4 (10)
	E. lentum	1×10^6 (100)	6×10^5 (60)	2×10^5 (20)	6×10^4 (6)
	P. acnes	5×10^5 (100)	2×10^5 (40)	2×10^5 (40)	3×10^5 (60)
	P. anaerobius	3×10^6 (100)	6×10^3 (20)	200 (0.7)	10 (0.03)
SSS	*C. perfringens*	1×10^5 (100)	8×10^4 (80)	2×10^2 (2)	0 (0)
	E. lentum	2×10^6 (100)	5×10^5 (25)	3×10^5 (15)	1×10^4 (0.5)
	P. acnes	4×10^5 (100)	4×10^5 (100)	1×10^5 (25)	2×10^5 (50)
	P. anaerobius	2×10^5 (100)	10 (0.01)	0 (0)	0 (0)
PAC	*C. perfringens*	2×10^5 (100)	6×10^3 (3)	1×10^2 (0.5)	0 (0)
	E. lentum	8×10^5 (100)	5×10^5 (63)	2×10^5 (25)	7×10^4 (9)
	P. acnes	4×10^5 (100)	4×10^4 (10)	1×10^4 (2.5)	1×10^4 (2.5)
	P. anaerobius	9×10^6 (100)	20 (0.02)	0 (0)	0 (0)

CVP = Copan Vi-Pak Amies Agar Gel collection and transport swabs
SSS = Starplex StarSwab II,
PAC = BBL Port-A-Cult

How Does Transport Time Affect Yields?

Recovery of Anaerobic Bacteria Placed in in Aerobic/Anaerobic Transport Media (Cont)

TABLE 2. Recovery of anaerobic gram-negative organisms

Swab system	Organism	No. (%) of evaluated organisms recovered at:			
		0 h	6 h	24 h	48 h
CVP	*P. bivia*	3×10^5 (100)	2×10^5 (67)	7×10^3 (2)	0 (0)
	P. melaninogenica	6×10^5 (100)	2×10^5 (33)	2×10^4 (3)	55 (0.01)
	F. nucleatum	3×10^6 (100)	1×10^6 (33)	0 (0)	0 (0)
	F. necrophorum	2×10^5 (100)	8×10^3 (4)	1×10^3 (0.5)	100 (0.05)
	B. fragilis	4×10^5 (100)	5×10^5 (125)	2×10^5 (40)	2×10^5 (50)
SSS	*P. bivia*	2×10^5 (100)	0 (0)	0 (0)	0 (0)
	P. melaninogenica	3×10^5 (100)	3×10^3 (1)	0 (0)	0 (0)
	F. nucleatum	1×10^5 (100)	1×10^5 (100)	0 (0)	0 (0)
	F. necrophorum	5×10^4 (100)	4×10^3 (8)	0 (0)	0 (0)
	B. fragilis	3×10^6 (100)	4×10^6 (133)	5×10^5 (17)	5×10^5 (17)
PAC	*P. bivia*	2×10^5 (100)	5×10^3 (3)	500 (0.3)	500 (0.3)
	P. melaninogenica	5×10^5 (100)	1×10^4 (2)	1×10^4 (2)	430 (0.1)
	F. nucleatum	2×10^5 (100)	1×10^5 (50)	0 (0)	0 (0)
	F. necrophorum	2×10^5 (100)	5×10^4 (20)	200 (0.1)	0 (0)
	B. fragilis	3×10^5 (100)	1×10^5 (33)	1×10^5 (33)	4×10^5 (133)

How Does Transport Time Affect Yields?

Recovery of Anaerobic Bacteria Placed in in Aerobic/Anaerobic Transport Media (Cont)

TABLE 3. Recovery of aerobic and facultative anaerobic organisms

Swab system	Organism	No. (%) of evaluated organisms recovered at:			
		0 h	6 h	24 h	48 h
CVP	*H. influenzae*	1×10^6 (100)	5×10^5 (50)	4×10^5 (40)	2×10^5 (20)
	N. gonorrhoeae	2×10^5 (100)	2×10^5 (100)	1×10^4 (5)	150 (0.1)
	S. pneumoniae	1×10^5 (100)	6×10^4 (60)	2×10^4 (20)	4×10^3 (4)
	"*S. milleri*" group	4×10^5 (100)	2×10^5 (50)	2×10^5 (50)	1×10^5 (25)
SSS	*H. influenzae*	4×10^5 (100)	3×10^4 (8)	200 (0.01)	0 (0)
	N. gonorrhoeae	5×10^4 (100)	4×10^3 (8)	40 (0.1)	0 (0)
	S. pneumoniae	2×10^5 (100)	2×10^4 (10)	2×10^4 (10)	5×10^2 (3)
	"*S. milleri*" group	3×10^5 (100)	2×10^5 (67)	2×10^4 (6)	3×10^4 (10)
PAC	*H. influenzae*	1×10^6 (100)	2×10^4 (2)	1×10^4 (1)	2×10^3 (0.2)
	N. gonorrhoeae	5×10^4 (100)	1×10^3 (2)	600 (1)	0 (0)
	S. pneumoniae	1×10^5 (100)	9×10^3 (9)	800 (1)	2×10^2 (2)
	"*S. milleri*" group	2×10^5 (100)	8×10^4 (40)	2×10^5 (100)	2×10^5 (100)

J Clin Microbiol. 2001:39 377-380

Principle #4: Appropriate collection devices and specimen containers must be used to ensure recovery of all organisms

Culture/Situation	Comments
Viral culture sent in bacterial transport media	Viral culture sent in Bacterial transport media. Request another specimen. Probably OK for adenoviruses and enteroviruses if cultured within 24 hours.
Catheter Tip Cultures	Submit 2-4 cm of the distal tip or entire catheter if small catheter. Transport ASAP to lab at ambient temperature.
Gastric Lavage Fluid Specimens for Mycobacteria	Fasting early morning preferred. Gastric fluid requires the addition of 1.5 mL of 7.5% sodium bicarbonate (or 100 mg of powder) within 4 hours of collection for neutralization
Mycoplasma/Ureaplasma culture sent in bacterial transport media	Sample should come in a multipurpose transport media (M-4, UTM). Acceptable samples include urine, urethral or cervical swab, semen, biopsy tissue, body fluid, CSF, tracheal, or nasopharyngeal aspirate. Urine can be transported at 4°C if transport time does not exceed 24 hours.
Duodenal/Gastric aspirates	Place aspirate in O&P fixative (PVA, SAF) within 30 minutes of collection. Transport at ambient temperature

Principle #4: Appropriate collection devices and specimen containers must be used to ensure recovery of all organisms (Cont)

Culture/Situation	Comments
Pneumocystis jaroveci (carinii)	Bronchoscopy or induced sputum preferred. Place sample in a sterile, tightly capped container and store/transport refrigerated within 24 hours
Skin parasites	Place skin scraping in a clean dry container, cap tightly and transport to lab within 24 hours at ambient temperature
Mycoplasma pneumoniae Culture	Respiratory sample or CSF in sterile cup. Transfer specimen into M-4 or UTM. Store/transport at 4°C. Transport time should not exceed 24 hours. Consider amplified nucleic acid assay
Blood culture from Heparin or EDTA tubes	Heparin is toxic to many organisms. Increased risk of contamination during transfer.

Suggested Transport Media – General Comments

Medium	Utility	Comments
Stuart's Medium	Most aerobic and some facultative anaerobes.	Good general purpose media. Dual swabs most convenient
Amies Medium	Most anaerobic and facultative anaerobes	Good general purpose media. Yield for facultative anaerobes may be higher than from Stuart's.
Amies with Charcoal	GC	Best media for GC
Cary-Blair	General purpose medium for transport of stool pathogens (Salmonella, Shigella, Vibro, Campylobacter, Yersinia, (C. difficile toxin A/B – some assays).	All stool specimens that can not be setup within 1 hour should be placed in Cary-Blair media Cary-Blair media especially useful for Campylobacter.
Anaerobic Transport Media	Many Types.	Recommend media with oxygen indicator. General transport media are not good for strict anaerobes. Do not refrigerate.
Ova and Parasite media (PVA, SAF, 10% formalin, Alcohol based – Ecofix)	Protozoa quickly lost in unpreserved stool	Media that do not contain mercury or formalin are more environmentally friendly.
Viral Transport Media	Many types	Most contain antibiotics which renders then unusable for bacterial culture.

Principle #5: Collect all microbiology test samples prior to the institution of antibiotics

Specimen	Comments
Blood Culture	Collect two sets at same time from different sets. DO NOT collect both sets from the same site (assessment for contamination)
Hair, skin and nails Fungal Culture	Collect before antifungal therapy or discontinue treatment for at least 5 days.
Urine Culture	Antibiotics may cause a transient decrease in bacterial concentration resulting in a false negative report

Principle #6: The specimen container must be properly labeled and sealed prior to transport

Situation	Comments
Any unlabeled or improperly labeled specimen sent to the lab	May decide to have the individual who collected the specimen to label specimen. Label only on bag not allowed.
Any leaking container	Reject.
Each sample must have at least two unique identifiers	Unique identifies may be name, medical record number, age, patient ID number, etc. Upholds patient safety initiatives.
Slides transferred– recommend two identifiers	Name and specimen number.
Identify what is in the container	Swab from where? Body fluid or urine?

Principle #7: Minimize transport time or maximize transport media. There is always some loss of viability during transport

Specimen	Maximum Transport Time not in Transport Media	Maximum Transport time in Transport media
All Specimens	Process within one hour	Place in transport media. Store and transport as recommended
Stool Culture	2 hours	Cary-Blair 48 hours
GC Cultures	Immediately place swab in Amies with charcoal or other GC approved transport medium.	Not more than 24 hours in Amies with charcoal. Store/transport at ambient temperature.
Respiratory Viral Cultures	Nasopharyngeal secretions or aspirates, BAL; 24 hours at 4°C	Not more than 48 hours if specimen transferred to Viral Transport Media
Clostridium difficile Toxin Assay	2 hours at ambient temperature 72 hours at 4°C; 1 week, frozen	Cary-Blair one week (check with manufacturer)
Urine for CMV	24 hours. Store at 4°C	Not recommended

Minimize transport time and maximize use of transport media as much as possible

Environmentally Fragile Organisms

Organism	Most Likely Specimen	Comment
Shigella spp.	Stool	Immediate processing recommended
N. gonorrhoeae	Genital	Sensitive to cold. Need 5-10% CO_2. Immediate processing recommended
N. meningitidis	CSF	Sensitive to cold. Immediate processing recommended
H. influenzae	CSF, eye, ear, throat	Sensitive to cold. Immediate processing recommended

QA monitor??

Principle #8: Special handling/Collection instruction must be followed

Specimen	Special Instructions
Blood Culture	Beware of decentralized phlebotomy
Gastric Washing for AFB	Since Mycobacteria may die in gastric washings if processing is delayed more than 4 hours. Neutralize acid with 1.5 ml of 40% disodium phosphate for every 35-50 ml of gastric washing
Bone Marrow	The specimen is collected in a 2 ml yellow-stoppered SPS vacutainer tube (or heparin) and sent to the Microbiology Laboratory immediately. Collection in heparin is also acceptable.
Eye	Direct inoculation by physician is recommended for optimal yields
Stool specimens for AFB	Process only samples from immunosuppressed patients (bone marrow transplant) or AIDS patients
Sputum	Food should not have been ingested 2 hrs. prior to collection. Mouth should be rinsed with saline or water. Patient should breathe and cough deeply. Collect expectorate in a sterile container and immediately transport to the lab.
Wound Specimen	Surface lesions are unsuitable for anaerobic culture.

Principle #8: Special handling/Collection instruction must be followed (Cont.)

- First, communicate with those that are doing collections.
- Collection instructions are written and available.
- Get involved with nursing orientation/education days and ask to have the instructions given out; poster board learning; quiz or competencies.
- Talk to providers when there are problems with specimen collection; they sometimes do not know they could do it better.

Principle #9: Improper specimen Collected for Ordered Test

Specimen	Not acceptable Specimen	Acceptable Specimen	Comments
Fungal Blood Culture	Routine blood cultures for detection of H*istoplasma capsulatum, Blastomyces dermatitidis, Coccidioides immitis*, or *Malassezia furfur*	Fungal Blood Culture	Routine blood cultures will detect majority of patients with candidemia.
Fungal Respiratory Specimens	Sputum swabs for AFB or fungus	AFB: Sputum Fungus: Sputum, Cryptococcus only. Thrush??	Need tissue to make a diagnosis of fungal pneumonia. Diagnosis of thrush usually only requires gram stain.
Anaerobic Culture	Autopsy material, respiratory, decubitus, environmental, stool, urine (not aspirate), vaginal secretions, superficial wounds	See Anaerobic specimen table	Polymicrobial
Slide for gram stain	Gram stain for diagnosis of GC on females	Gram stain for diagnosis of GC on males	Intracellular non gonococcal Neisseria may be detected in female specimens.
Stool Culture	Stool received in preservative for Viral Culture	Stool culture in Cary-Blair Media	Most viral transport media contain antibiotics.
CMV Culture	Clot tube received for CMV Culture	EDTA tube	Yields for CMV are higher in unclotted samples.

Criteria For Rejection of Microbiological Specimens

- Criteria for rejection must be readily available and laboratory specific
- Unlabeled or improperly labeled specimen
- Prolonged storage or transport
- Improper or damaged container
- Specimen received in fixative
- Oropharyngeal contaminated sputum
- Duplicate specimens stools, sputum) within a 24 hour period. Exceptions cleared by the laboratory
- Specimens unsuitable for culture request (anaerobic culture from not acceptable source, urine from Foley catheter)
- Dry Swab
- 24-hr collection of urine or sputum for AFB or fungal culture
- Other criteria specific to your laboratory

Cultures That Should Include a Gram Stain

- CSF or sterile body fluid (cytospin)
- Eye
- Purulent discharge
- Sputum or transtracheal aspirate
- All surgical specimens
- Tissue
- Urethral exudates (male only, intracellular gonococcus))
- Vaginal specimens
- Wounds

Summary

- Publish specific rules for specimen collection
  - There will be exceptions!
    - Make physician or healthcare provider aware of implications of culturing suboptimal specimens
- Communicate, communicate, communicate!
  - Real time feedback
  - Contact the health care worker who collected the suboptimal specimen

References

- Clinical Microbiology Procedures Handbook. 2nd Edition. . HD Isenberg ed. ASM. Cumitechs. ASM Press. Wash. DC.
- Manual of Clinical Microbiology, 9th Edition. ASM Press. Wash. DC. 2007.Miller MJ.
- A Guide To Specimen Management in Clinical Microbiology. ASM Press. Wash. DC. 1999.