S1. Laboratory Processes and Associated Clinical Considerations

Step and Related Scenarios	Laboratory Considerations	Clinical Considerations and Impact	Non-ideal Example	Ideal Example	PETACS Finding
Decision made to obtain TA					
Reasons may include pneumonia evaluation, suspected tracheitis, fever, change in respiratory status, surveillance.	Order indication may help the laboratory triage specimens and screen for specimen quality.	Reason for ordering and collecting TA will influence the interpretation of culture results. This may influence pretest probability.	Specimen is obtained for "surveillance," fever without change in respiratory status or test of cure.	Patient meets clinical criteria for nosocomial pneumonia(1, 2) or tracheitis(3).	Not studied.
TA specimen collected					
A) Direct specimen was collected without the use of saline B) Saline was used to collect specimen. C) In line suction used to collect specimen.	It is impossible to know the true dilution factor when saline is used, which can make the interpretation of Gram stain and culture results challenging. In line suction catheters are more likely to result in contaminated specimens (4).	If the specimen is diluted during collection, the quantity resulted by the microbiologic laboratory will not be accurate.	Saline is instilled to aid in the collection of a TA specimen or specimen is collected using in-line suction.	A sterile catheter is used to collect a deeper sample. If a BAL or mini-BAL is collected, the lab is aware and can process the specimen appropriately.	Labs are generally unaware if the TA specimen has been diluted or collected via in-line catheter. Some labs report receiving TA cultures for surveillance.
Specimen sent to laboratory					
A) Specimen is sent immediately. B) There is a delay in specimen transport. C) Specimen is leaking upon receipt or is received in a non-sterile container.	Delay in transport and poor specimen container quality negatively affect specimen quality. If the specimen cannot be sent immediately to the lab, it should be refrigerated and sent as soon as possible. If acceptable transport conditions are not met, the specimen should be rejected(5).	The yield of microorganisms from fresh specimens decreases over time. Refrigeration decreases the rate of growth of contaminating organisms(6). The clinical relevance of a TA specimen that was not transported properly is low (7).	The specimen is collected and left at room temperature for several hours before being sent to the laboratory.	The specimen is sent to the laboratory immediately or placed in the refrigerator if a delay is anticipated.	Large variation among laboratories with respect to rejection criteria for delay in transport and container type.
Gram stain is read					
The stained slide is observed for the presence of white	The microscopic magnification used will determine the average number of cells seen per field. The	It is important to know which objective is being used by the laboratory to quantitate what is	There are no formal guidelines specifying specimen rejection	A study by Wilson et al suggests that TA specimens with >10	Large amount of variation among laboratories

blood cells, epithelial cells, and microorganisms.	presence of >10 epithelial cells per low power field (10X) and absence of organisms suggests that the sample quality is poor(8).	seen on the Gram stain. Knowing this information helps to provide context for the quantity of human cells and microorganisms reported. Gram stains that do not have organisms, and/or have significant amounts of epithelial cells suggest a poor-quality specimen(8). Culture results may not be clinically relevant(7).	criteria or screening using the Gram stain for TA cultures as there are for sputum(7, 9).	epithelial cells and no organisms per low power field (10X) are associated with clinically insignificant culture results and should be rejected(8).	rejecting TA specimens based on Gram stain results including differences in microscope objective use, minimum field review requirements, organism and cell quantification and actual rejection criteria.
Specimen is plated					
A) Semi-quantitatively B) Quantitatively	If specimen is diluted, interpretation of quantity of growth will be challenging since dilution factor cannot be known.	For semi-quantitative culture, results will be reported similarly to rare, few, moderate, heavy or 1+,2+,3+,4+. For quantitative culture, a number of colony forming units per mL (CFU/mL) will be reported. quantitative culture, due to lack of standardization, may be difficult to interpret or compare to published literature.	Not applicable	In a minimally contaminated TA specimen, the quantitative threshold value (for significance) is >=105 CFU/mL. Semi-quantitative values of moderate, heavy, 2+, 3+, 4+ are considered to correspond to this quantitative threshold(1).	Most laboratories report using semi-quantitative plating methods for TA specimens.
Culture is reviewed					
A) Organism is identified and reported The presence of commensal oropharyngeal and respiratory flora is considered and compared to pathogen growth.	The criteria for organism identification and reporting differs between laboratories. These decisions are likely based on perceived potential for organism virulence, predominance in culture or presence in corresponding Gram stain, and patient population.	The identification and reporting of an organism in culture may suggest that the organism is a pathogen and encourage antibiotic treatment.	A gram-negative rod is growing in culture in the same quantity as normal commensal flora but is fully identified and reported. Consequently, the clinical team starts the patient on cefepime.	Organism reporting based on guidelines that help microbiologists decide when to report organisms from TA specimens. *Additional evidence and clinical studies are needed for this to occur.	Large amount of variability among laboratory organism identification and reporting practices.

-Susceptibility testing is performed	The decision to perform susceptibility testing varies and may be based on known organism susceptibility patterns, local data, patient history and organism predominance in culture. The ability to produce rapid results is not sufficient in of itself to optimize therapy (10).	Performing and reporting antimicrobial susceptibilities may lead to the initiation of antimicrobials, which may not be appropriate.	Reporting organisms from culture that may by commensal and not causing infection. Treatment will be escalated/tailored to the organisms mentioned in culture.	Organism susceptibility testing based on guidelines that help microbiologists decide when to perform them on organisms growing from TA specimens. *Additional evidence and clinical studies are needed for this to occur.	Large amount of variability among laboratory susceptibility testing practices.

References

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