

Pitfalls in Clinical Microbiology

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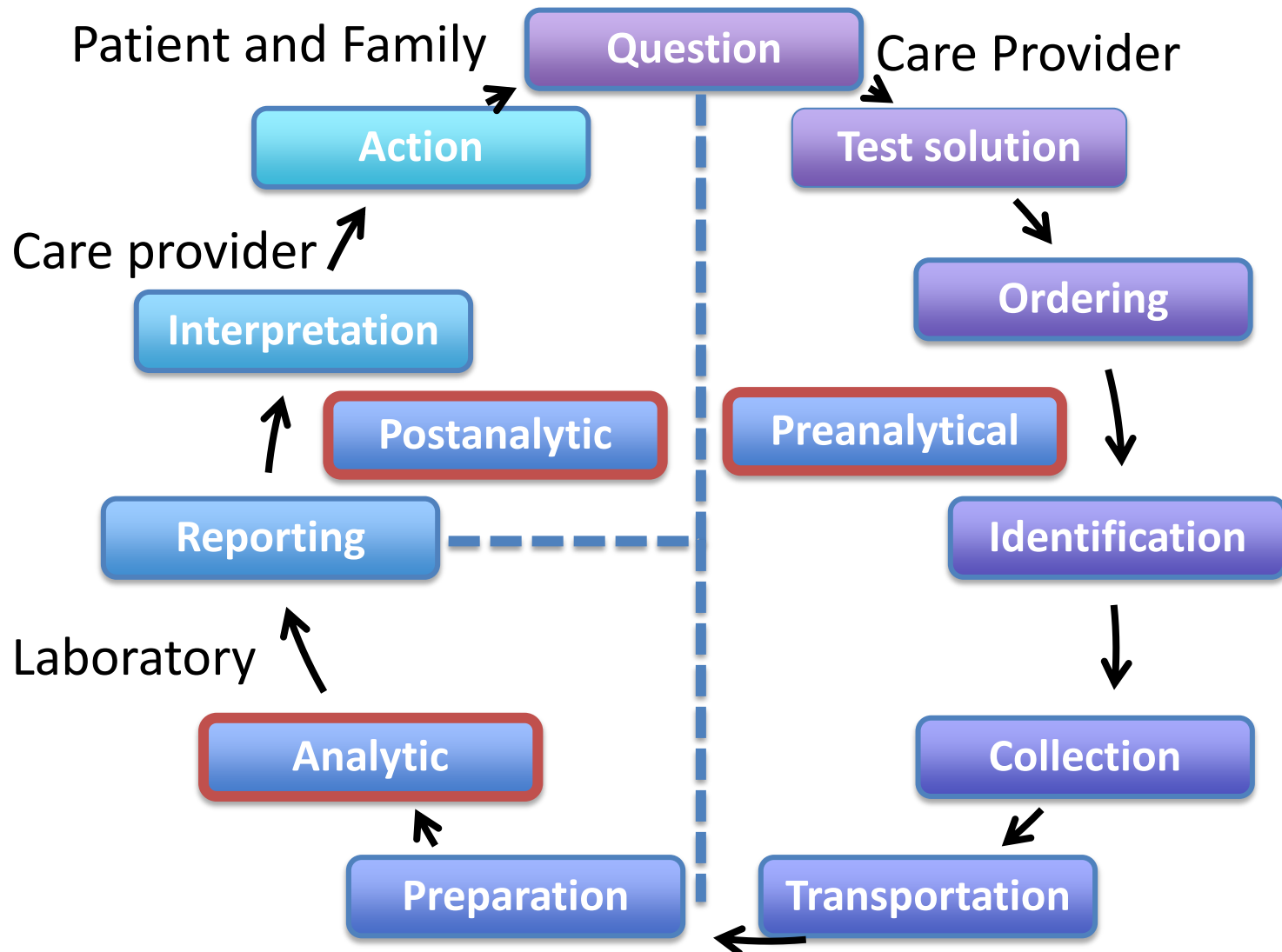
Disclosure

- **Pfizer, MSD, Siam Pharmaceutical, Thai Meiji Pharmaceutical, Q-Bioscience, Santen**

Outline

- **Total testing process & medical errors**
- **Pitfalls regarding utilization of the microbiology for diagnosis of infectious diseases**
 - **Preanalytical: Specimen collection and handling**
 - **Analytical: Direct microscopy and identification**
 - **Postanalytical: Antimicrobial susceptibility**
- **Diagnostic stewardship**

Total Testing Process



Medical Errors Involving Laboratory Testing

Preanalytic

46%-68.2%

- Inappropriate test request
 - **Ordering the wrong test**
 - **Ordering no test at all**
- Order entry errors
- Misidentification of patient
- Container inappropriate
- Sample collection and transportation inadequate
- Insufficient sample volume
- Sorting and routing errors
- Labeling errors

Analytical

7%-13%

- Equipment malfunction
- Sample mix-ups/interface
- Undetected failure in quality control
- Procedure not followed

Postanalytical

18.5%-47%

- **Misinterpreting of laboratory result**
- **Not looking at and acting upon test result in timely fashion**
- Failure in reporting
 - **Critical value**
- Erroneous validation of analytical data
- Improper data entry

**Specimen management is the
key to accurate diagnosis**

Garbage in, Garbage out

A Guide to Utilization of the Microbiology Laboratory for Diagnosis of Infectious Diseases: 2018 Update by the Infectious Diseases Society of America and the American Society for Microbiology^a

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Specimen selection, collection and transport

- The quantity material must be **adequate**
 - Example: Blood culture volume
- Specimens are selected **on the basis of signs and symptoms**, should be representative of the disease process
 - Example: Respiratory panels
- Contamination of the specimen must be avoided by using only sterile equipment and **aseptic precautions**
- The specimen must be **taken to the laboratory and examined promptly**
 - Example: CSF Urine
- Special **transport media** may be helpful
 - Example: anaerobic culture, body fluids in blood culture bottle
- Meaningful specimens to diagnose bacterial infections must be secured **before antimicrobial drugs** are administered



- **Send Blob, Not Swab**
- **Deep tissue, No superficial swab**

Blood cultures

- Principle

- Blood placed in enrichment broth contained in a bottle with a CO₂ sensor
- The sensor is constantly monitored for color/fluorescence changes
- When CO₂ is generated by microbial growth, a color change occurs in CO₂ sensor
- False positive when > 40,000 WBCs
- Incubated for five days before negative;
- most cultures are positive within 48 hours; a few organisms may take up to 4 or 5 days to grow including *HACEK, Brucella, Capnocytophaga, Campylobacter*
- Extended incubation may be needed for *Francisella, Burkholderia* and dimorphic fungi
- Blood volume, aseptic technique and prior ATB are important
- Can grow yeasts readily



Blood cultures

- The first **2 sets of blood cultures** (from two different “**needle sticks**”) are collected before antimicrobial treatment is started
- Depending on patient’s condition, additional blood cultures may be obtained
- Can be used for other type of specimen i.e. ascites (SBP), joint fluid **in addition to** direct specimen processing



Blood draw



1 set=1 aerobic + 1 anaerobic bottle



Place bottles in incubator/shaker; automatically monitors for growth



When “positive”:
Gram stain
Subculture for ID
& susceptibility

Keep at RT, Do not refrigerate

IE: typical pathogen- 2 separate blood culture, persistent culture: at least 2 positive drawn > 12 h or all 3 or a majority of >=4 separate (first and last at least 1 h)

Blood cultures

- Aseptic techniques and standard precautions to reduce contamination (<3%) and needle stick injury



- 2%Chlorhexidine in 70% alcohol
- Contact time 30 sec, maximum antiseptic effect, residual effect
 - Shelf life?
 - Irritation in children < 2 mo

Blood cultures

- **Blood volume** is the most important variable in recovering bacteria and fungi from patients with bloodstream infections
 - Adults: 20-30 mL of blood per culture set (depending on the manufacturer of the instrument)- 8-10 mL per bottle, 2-4 sets
 - Increasing the total volume from 20 to 40 mL increased the yield by 19%; increasing the volume form 40 to 60 mL increased the yield by and additional 10% (2 blood cultures cover 80-90%)

Table I-1a. Recommended Volumes of Blood for Culture in Pediatric Patients (Blood Culture Set May Use Only 1 Bottle)

Weight of Patient (kg)	Total Patient Blood Volume (mL)	Recommended Volume of Blood for Culture (mL)		Total Volume for Culture (mL)	% of Total Blood Volume
		Culture Set No. 1	Culture Set No. 2		
≤1	50–99	2	...	2	4
1.1–2	100–200	2	2	4	4
2.1–12.7	>200	4	2	6	3
12.8–36.3	>800	10	10	20	2.5
>36.3	>2200	20–30	20–30	40–60	1.8–2.7

When 10 mL of blood or less is collected, it should be inoculated into a single aerobic blood culture bottle.

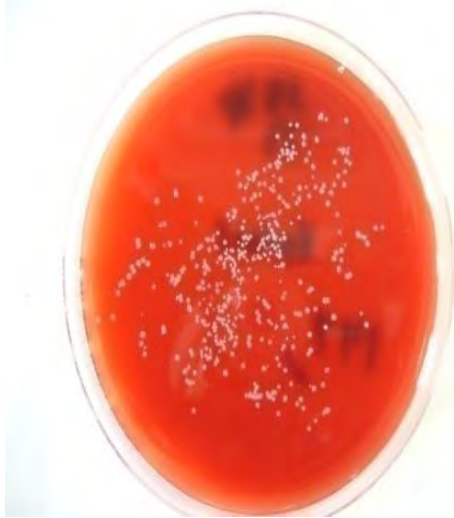
Blood culture for fungi

- **Yeasts** except for *M. furfur* are usually detected in **aerobic broth systems** but not for moulds and *Nocardia*; Serum cryptococcal antigen aids the diagnosis of cryptococcosis
- **Automated system with special fungal media** may enhance recovery of fungi (faster eg. *C. glabrata*, more extensive eg. **mould**)
 - ! False positive from bone marrow specimen
 - ! *Penicillium purpurescens* and *Blastomyces dermatitidis* were not detectable in the BD BACTEC Myco/F Lytic culture medium
 - ! *Hansenula anomala*, *Exophiala jeamselmei*, *Actinomyces bovis*, *Rhodotorula rubra*, and *Mucor ramosissimus* exhibited inconsistent results at low inoculum levels
- Lysis-centrifugation is more sensitive for recovery of molds and dimorphic fungi but high contamination rate, time-consuming

For *Candida* species

- **Adequate volume (40-60 mL) for adults, incubate at least 5 d**
- The overall sensitivity is roughly 50% (21-71%); Limit of detection ≤ 1 CFU/mL
- Median time to positivity 2-3 d (1 to ≥ 7 d)
- Negative: low candidemia, deep-seated candidiasis

Diagnosis of catheter-related blood stream infection



- **Simultaneous quantitative blood culture: $\geq 3X$**
- **Differential time to positivity: $\geq 2h$**
- **Roll plate method (Maki): >15 CFU/ 5-cm segment**
- **Quantitative (sonication) broth culture (Vortex): $>10^2$ CFU**

Multiple lumens?

Results from some retrospective studies

- **Sampling all the lumens of a multi-lumen catheter results in more positive blood cultures**
- **However, it is unclear whether blood culture samples should be obtained through one or multiple catheter lumens**

A 45-year-old male with a long-standing history of alcoholic cirrhosis and diabetes

- **The patient presented to the ED with fever, chills, headache and lethargy that developed over two days**
- **On physical examination, he was febrile to 39°C, was obtunded making neurological examination not possible but he had neck stiffness**

A 45-year-old male with a long-standing history of alcoholic cirrhosis and diabetes

- Laboratories were significant for a peripheral white blood cell count of 27,800 cells/ μ L with 95% neutrophils
- Lumbar puncture was performed
 - Opaque CSF containing 5,600 RBC/ μ L and 31,400 white blood cells/ μ L. With 95% neutrophils and 5% monocytes
 - Protein was 1,422 mg/dL and CSF glucose was 100 mg/dL (blood glucose of 483 mg/dL)



CSF Culture

- Gram stain & culture of CSF are standard of care
- Usually 3 or 4 tubes of CSF are collected
 - The first tube has the highest potential for contamination with skin flora
- A minimum of 0.5-1 mL in sterile container, larger volume (5-10 mL) increase the sensitivity and are required for optimal recovery of mycobacteria (a minimum of 3 mL) and fungi
 - Prioritize multiple test requests on small volume samples
- 2-4 blood cultures should also be obtained if bacterial meningitis is suspected
- Transport immediately, ≤ 15 min
- Do not refrigerate
- Anaerobic culture: shunt infection, hold 14 d for *C. (P.) acnes*