New Diagnostic Modalities for Infections

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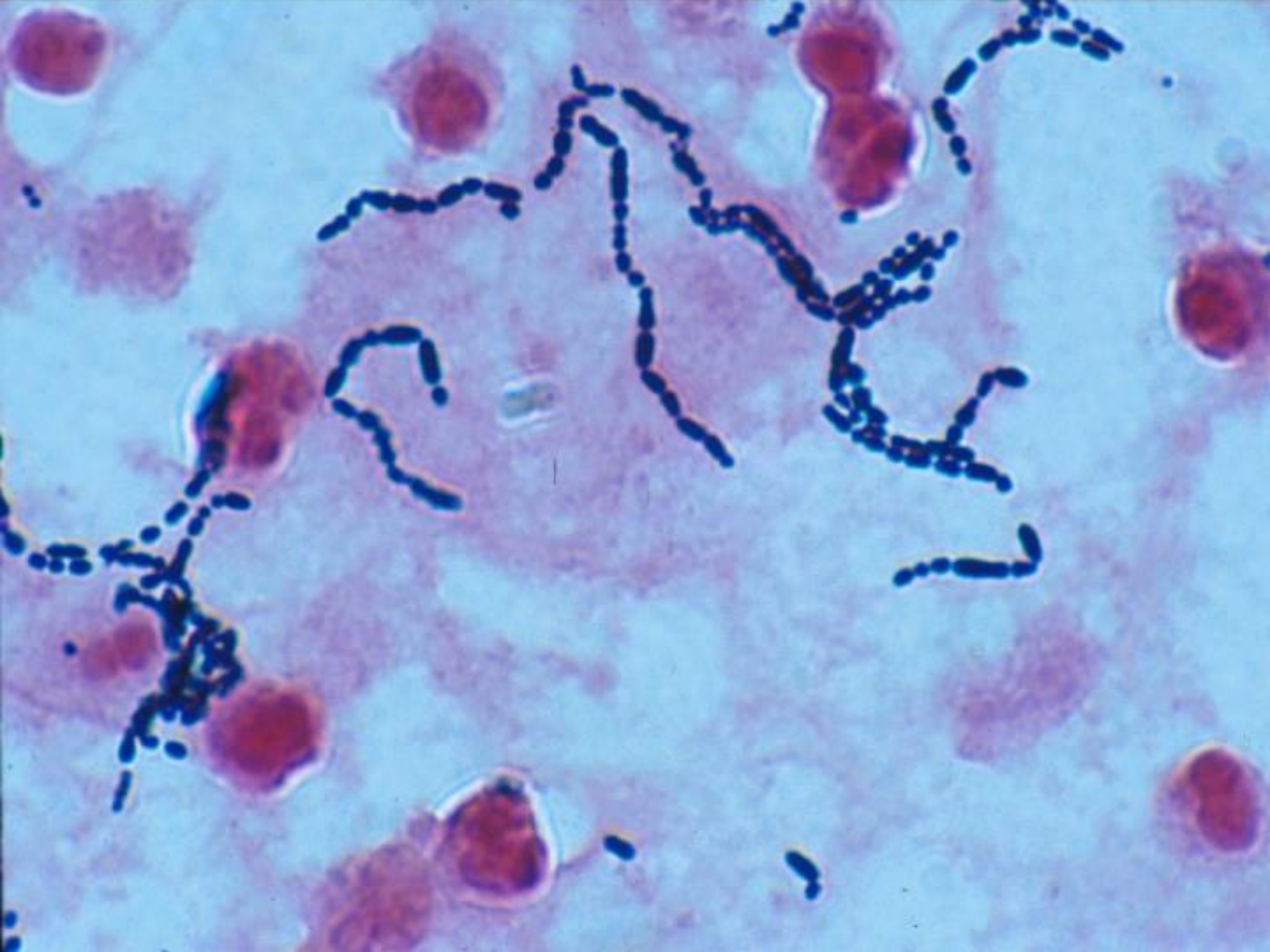
Outline

- Introduction
- Conventional methods
- Avoid contamination of blood culture
- Limitations of traditional tests
- Markers of infection: PCT
- Molecular methods
- MALDITOF

Introduction

- Lab diagnosis of infectious diseases is based on one or more of the following:
  - direct examination of specimens by microscopic or antigenic techniques,
  - isolation of microorganisms in culture & testing for antimicrobial drug susceptibility
  - serologic testing for development of antibodies
  - molecular detection of the pathogen's genome (DNA, RNA).
- Select the appropriate tests & specimens and, when possible, suggest the suspected etiologic agents to the microbiologist



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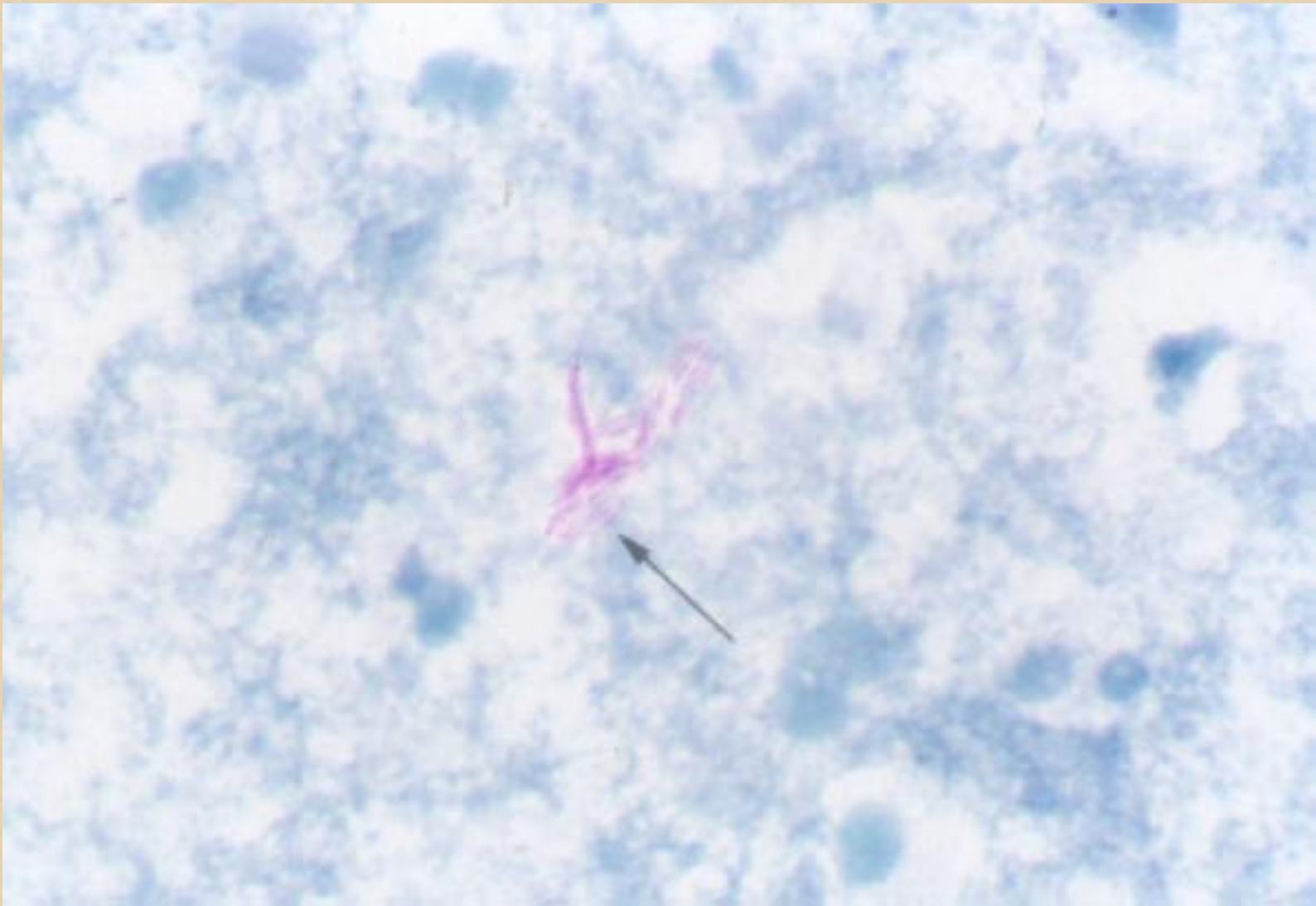

Traditional Diagnostic: Antigen Detection

- ***H.influenzae* type b, *S.pneumoniae,* group B streptococci, & *N.meningitidis* in CSF**
- Group A streptococci in the pharynx
- In blood & CSF: Ags of *Cryptococcus neoformans, Candida,* and *Aspergillus fumigatus.* Assays to detect galactomannan, a cell wall molecule in *Aspergillus*
- In urine: detecting pneumococcal antigen in the urine of patients with invasive pneumococcal disease
- In stool:*Campylobacter jejuni* Ag, *H.pylori* Ag*C.difficile* toxin A & toxin B by commercially available EIA kits.
  - The *C. difficile* toxins degrade rapidly at room temp, and thus specimens must be transported promptly or refrigerated at 4°C until testing is performed for optimal test results.

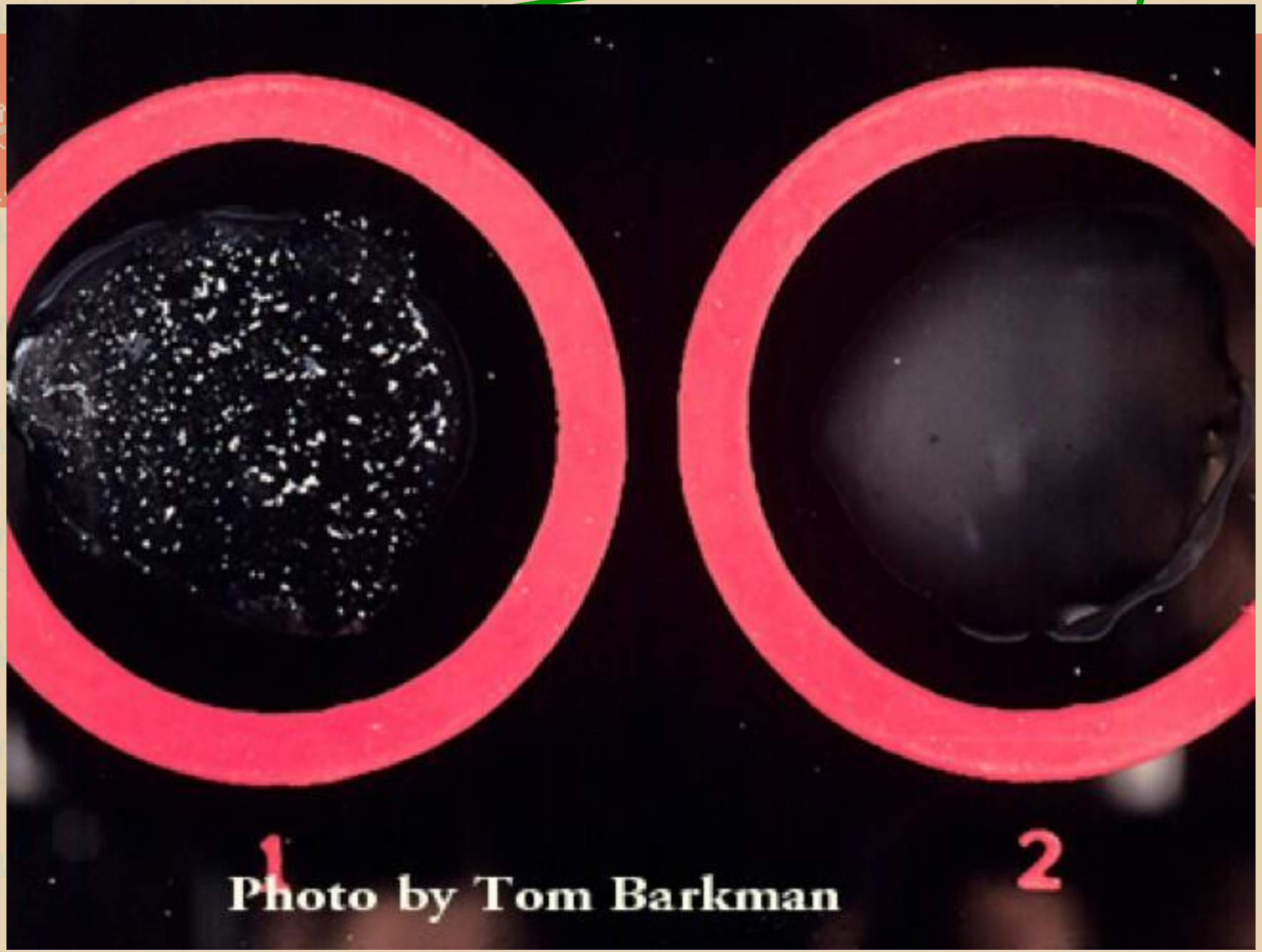
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Photo by Tom Barkman

Traditional Diagnostic Methods: culture

- the gold standard for the diagnosis of bacterial infection, but culturing blood, urine, CSF, or BAL specimens usually takes 24 to 48 hours for identification and AST; contamination may occur
- It's worthwhile to improve the yield of BI culture by increasing volume, collection before starting antibiotics, avoiding skin contamination & avoid drawing from CL
- Use of automated BI culture systems (BACTEC, BACTALERT) decrease TAT to an average of 10 hrs
- Use of automated ID & AST decrease TAT to 5- 18 hrs

Agar Plates

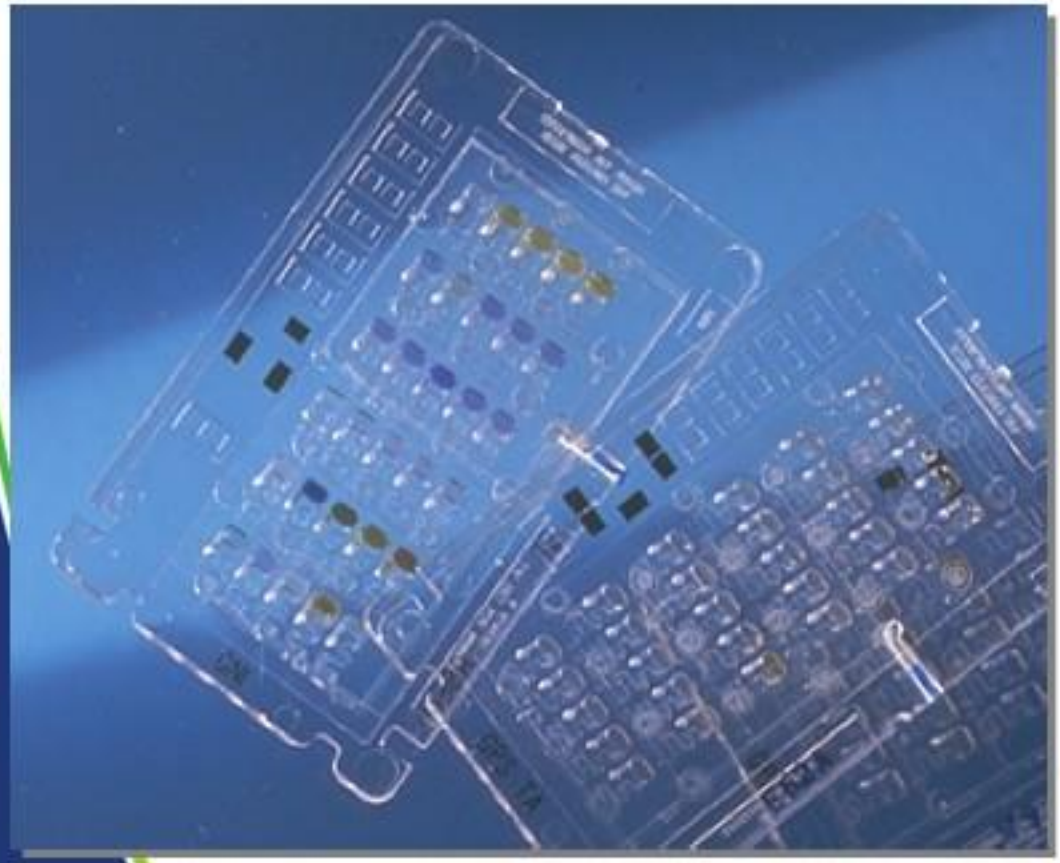
BIOMÉRIEUX

API

Kirby-Bauer

http://www.life.umd.edu/classroom/bsci424/LabMaterialsMethods/AntibioticDisk.htm

VITEK Card Convenience



- **Compact**
  - easy to handle
  - space saving
  - low waste volume
- **Sealed**
  - spill resistant
  - safe disposal
- **No reagents to add**

Obtaining Blood Cultures by Venipuncture Vs. From Central Lines: Impact on Blood Culture Contamination Rates & Potential Effect on CLABSI Reporting

- John M. Boyce, MD, Jacqueline Nadeau, M(ASCP), Diane Dumigan, RN, Debra Miller, RN, CMSRN, Cindy Dubowsky, MS, Lenore Reilly, RN, MS, Carla V. Hannon, RN, MS

- Infection Control and Hospital Epidemiology, 2013;34(10):1042-1047 .

Blood Cultures by Venipuncture Vs. From Central Lines: Impact on Blood Culture Contamination Rates: Abstract

- **Objective .**Reduce the frequency of contaminated blood cultures that meet NHSN definitions for a CLABSI.

Methods

- A new blood culture policy discouraged drawing blood samples from central lines.
- Phlebotomists were reeducated regarding aseptic technique when obtaining blood samples by venipuncture.
- The IV therapy team was taught how to draw blood samples by venipuncture & served as a backup when phlebotomists were unable to obtain blood samples.
- A 2-nurse protocol and a special supply kit for obtaining blood samples from catheters were developed. Rates of blood culture contamination were monitored by the microbiology laboratory

Blood Cultures by Venipuncture Vs. From Central Lines: Results

Results . Before Vs after the new blood culture policy:

- The proportion of blood samples obtained for culture from central lines decreased: 10.9% Vs 0.4% (p <0.001)
- Contamination rate decreased from 1.6% to 0.5% (p <0.001)
- The reduction in blood culture contaminants yielded an estimated annualized savings of 30%
  - 30% of 10 reported CLABSIs were suspected to represent blood culture contamination compared with none of 6
- **Conclusions .**Multiple interventions resulted in a reduction in blood culture contamination rates and substantial cost savings to the hospital, and they may have reduced the number of reportable CLABSIs.

LIMITATIONS OF CONVENTIONAL CLINICAL MICROBIOLOGY

- Culture
  - Inhibited by prior antibiotics
  - Some organisms require special media
  - Prolonged period of time to culture result
  - Some organisms are uncultivable on artificial media
  - Potential health hazards
- Antigen Detection
  - Negative tests in low microbe burden
- Serology
  - Unhelpful during early stage of infection & in endemic areas
  - Not quite useful in immunocompromised patients

General Markers of Infection

- The routine laboratory tests for sepsis, such as CRP or WBC count, lack diagnostic accuracy and are sometimes misleading.
- In severe infections, most classical pro-inflammatory cytokines, such as TNF-α, IL-1β, or IL-6, are increased only briefly or intermittently, if at all.

  - Carrigan SD, Scott G, Tabrizian M. Toward resolving the challenges of sepsis diagnosis. *Clin Chem*. 2004;50:1301–1314.
  - Lever A, Mackenzie I. Sepsis: Definition, epidemiology, and diagnosis. *BMJ*. 2007;335:879–883

Procalcitonin (PCT)

- PCT is a biomarker that exhibits greater specificity than other pro-inflammatory cytokines in identifying patients with sepsis and bacterial infections.
- PCT concentration is elevated in sepsis.
- The short half-life (25–30 hours in plasma) of PCT, coupled with its virtual absence in health and specificity for bacterial infections, gives it a clear advantage over the other markers in the rapid diagnosis of bacterial infection, especially for use in hospital ER and ICUs.
- persistently increased levels of PCT were always indicative of an unfavorable outcome.

Assicot M, Gendrel D, Garsin H, et al. High serum procalcitonin concentrations in patients with sepsis and infection. *Lancet*. 1993;341:515–518.

PCT following organ transplant

- Identify whether inflammation is due to bacterial infection or organ rejection.
- In liver transplant recipients, plasma PCT levels were significantly increased in infected pts Vs acute liver rejection that were similar to those of non-complicated pts (1)
- PCT levels following heart transplantation were useful marker of prognosis: were consistently low (<10 ng/mL) in pts with an uneventful course but increased in pts with postoperative complications and even associated with an increased mortality early postoperatively when values exceed 80 ng/mL. (2)

1. Mendonca et al. Is procalcitonin useful to differentiate rejection from bacterial infection in the early post-operative period of liver transplantation in children ?*Pediatr Transplant 2008*
2. Madershahian et al. Kinetic of procalcitonin in the early postoperative course following heart transplantation .*J Card Surg* . 2008

False high PCT levels

- There are also reports in the literature where elevations in PCT levels were not connected with bacterial infections:
- Addisonian crisis caused by adrenal failure
- In transplant pts receiving pan T-cell antibody therapy
- In pts scheduled for hematopoietic stem cell transplantation and receiving anti-thymocyte globulin during conditioning

- Schumm J, Pfeifer R, Ferrari M, et al. An unusual case of progressive shock and highly elevated procalcitonin level .*Am J Crit Care* 2009
- Sabat R et al. Massive elevation of procalcitonin plasma levels in the absence of infection in kidney transplant patients treated with pan-T-cell antibodies .*Intensive Care Med* . 2001
- Brodska H et al. Marked increase of procalcitonin after the administration of anti-thymocyte globulin in patients before hematopoietic stem cell transplantation does not indicate sepsis: A prospective study . *Crit Care* 2009

Applications of molecular diagnostic identification

- Molecular identification should be considered in three scenarios,

(a) for the identification of an organism already isolated in pure culture,

(b) for the rapid identification of an organism in a diagnostic setting from clinical specimens or

(c) for the identification of an organism from non-culturable specimens, e.g. culture-negative endocarditis.

Rapid Identification of Cultured Pathogens

- Accurate identification of pathogens is a primary driver of antimicrobial or antifungal selection.
- Delays in appropriate therapy affect pt outcomes in a negative fashion.
- Rapid identification of G+, G- & Candida to species level as critical to direct appropriate therapy .
  - Unnecessary Vanco for CONS that deemed a contaminant
  - Significant pharmaceutical cost savings due to de-escalation of therapy from an echinocandin to fluconazole in infections caused by fluconazole –susceptible *C. albicans* or *C. parapsilosis*
- Ibrahim EH et al. The influence of inadequate antimicrobial treatment of bloodstream infections on patient outcomes in the ICU setting .*Chest* 2000

Limitation of PNA-FISH

- A limitation of PNA-FISH is a requirement of an organism concentration of at least 10^5CFU/mL for detection.
- Limited number of PNA-FISH probes are available. However, additional specific PNA-FISH probes are now available for Group B *Streptococcus* , GNR Traffic Light (FDA-approved ,(*C. dubliniensis, C. parapsilosis* ,*K. pneumoniae* ,and *Acinetobacter*

Molecular identification techniques from clinical specimens

- When a culture would give comparable result, but several days later e.g
  - In critical patients (sepsis panel)
  - Need to isolate patient (TB)
  - In outbreak settings,
  - In potential bioterrorist attack.
  - Impact on Infection Prevention Programs

Identification from culture-negative specimens

Causes for failure to grow pathogen:

- prior antibiotic therapy, e.g. treatment of acute meningitis with i.v. benzylpenicillin,
- fastidious organism e.g.HACEK in endocarditis,
- slow growing, e.g. *Mycobacterium* spp.,
- specialized cell culture techniques are required, e.g. *Chlamydia* spp. and *Coxiella burnetti, viruses*

Nucleic Acid Amplification Techniques

- Most widely used is PCR
  - High sensitivity
  - High specificity
  - Automation

- Polymerase Chain Reaction
  - Specific PCR: Uses primers to known DNA targets.
  - Broad range PCR: uses complementary primers to conserved regions shared by a given taxonomic group

MOLECULAR DIAGNOSTICS

- Nucleic acid probes and PNA-FISH
  - Do not amplify DNA

MOLECULAR DIAGNOSTICS

- Multiplex PCR
  - Uses single clinical specimen to investigate several potential pathogens simultaneously
    - Encephalitis/meningitis panel: HSV,VZV, CMV ,HHV-6, EBV, Enteroviruses
    - Sepsis panel
    - Resp viral panel
- Real-time PCR
  - Utilizes a fluorescent labeled probe
  - Requires small volumes thus takes 30-60 minutes to complete

OTHER USES OF MOLECULAR DIAGNOSTICS

- Viral load monitoring
- Viral genotyping
- Bacterial resistance genes detection
- Bacterial genotyping: PFGE, MLST

Applications:

- Rapid Detection & Identification of BSI Pathogens
- Rapid Detection and Identification of TB
- Rapid Detection and Identification of GAS
- Rapid Group B Strep antepartum screening
- Rapid Detection of VRE, MRSA & MDRO
- Rapid Molecular Respiratory Panels & RSV
- Fast Detection of *M. tuberculosis*
- Detection of *C. difficile* Infection

LIMITATION OF PCR TECHNOLOGIES

- High Cost
- False positives caused by amplification of contaminants
- Only sample from normally sterile sites should be considered for broad-range PCR
- Specimen is required to be refrigerated before processing & frozen until amplification
- No antimicrobial sensitivity is available: Only certain resistance genes
- Needs the clinician to name the suspect

Use of MALDI-TOF

- Identify cultured bacteria by matrix-assisted laser desorption-ionization (MALDI) time-of-flight mass spectrometry (TOF/MS): within few minutes identifies proteins, DNA & RNA
- Other applications:
  - Direct Identification from Blood Cultures
  - Antimicrobial Susceptibility Testing
  - Direct Specimen Detection

How to improve the lab yield?

- The diagnostic value of lab tests depends on the quality assurance of pre- analytical, analytical and post-analytical factors, including collection and transport of microbial specimens, representative techniques and methods available for culture and diagnostic assays, diagnostic laboratory principles for specific types of microbial etiologies, antimicrobial sensitivity, quality control issues, and evaluation of reported findings.

Collaborate with the laboratory

- Recent tests are useful in a given setting and, if so, which test is most appropriate are questions that can be answered only through evaluations & collaboration between physician & laboratory
- Banoo et al., 2010 *Nature Reviews Microbiology* , S16-S28 | doi:10.1038/nrmicro1523

Thank You