LABORATORY MEDICINE COURSE
2004
CLINICAL MICROBIOLOGY LAB DX OF INFECTIOUS DISEASES
A BLEND OF ART & SCIENCE

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IT'S A GERM'S WORLD AFTER ALL

Microbes were the first & will be the last living forms on earth.

The human body harbors a 10- fold greater # microbial cells than human cells.

INFECTIOUS PATHOGENS

- ✓ 1st CAUSE OF DEATH WORLDWIDE
- ✓ TOP KILLERS GLOBALLY
  - RESPIRATORY DISEASES
    - TUBERCULOSIS
  - MALARIA
  - DIARRHEA
- ✓ 3rd CAUSE OF DEATH U.S.
- ✓ NEW INFECTIOUS DISEASES
  - ABOUT 30 IN LAST 20 YRS
    - WEST NILE, SARS, AVIAN FLU

THE SPECIMEN
GARBAGE IN GARBAGE OUT

SPECIMEN	PROBLEMS	SOLUTIONS
URINE	•>2-3 hr transit time •Overgrowth of commensal flora - False Positives	•Transport Tube with Boric Acid for inhibition
STOOL	•Raw Sewage- Loss of Pathogen Viability False Negatives	•PARA-PAK fixative for enterics
SURGICAL	•Swab - False Negatives •Tissues Sent Only to Pathology – No Pathogen Identification	•Sterile Container •Blood Culture Bottle

SEPTICEMIA

- MEDICAL EMERGENCY
- >200,000 CASES/YR
- MORTALITY 20-50%
- INTERPRETATON OF POSITIVE BLOOD CULTURES- TRUE POSITIVE OR CONTAMINANT?
  - ✓ CONSIDER
    - PROPORTION OF BLOOD CULTURE SETS POSITIVE TO NUMBER OF SETS OBTAINED
    - TIME IT TAKES FOR GROWTH DETECTION IN BLOOD CULTURE
    - IDENTITY OF MICROORGANISM

COLLECTION & TIMING
BLOOD CULTURES

- SKIN PREPARATION
  - ✓ CHLORHEXIDINE
  - ✓ 70% ALCOHOL + TINCTURE OF IODINE
  - ✓ DO NOT USE IODOPHORS (BETADINE)
    - Need 2 min exposure to iodophor compared to only 35 sec for 1% iodine for skin disinfection
- TIMING – SPECIMEN COLLECTION & RESULTS
  - ✓ COLLECT SPECIMEN ASAP AFTER FEVER SPIKE
  - ✓ BEFORE ADMINISTRATION OF ANTIBIOTICS
- THINKING MYCOBACTERIA OR FILAMENTOUS FUNGI?
  - ✓ INOCULATE ISOLATOR TUBE WITH LYTIC AGENT (SAPONIN) TO RELEASE INTRACELLULAR MICROBES

DOING IT RIGHT THE FIRST TIME
EVERY DROP COUNTS

- # BLOOD CULTURE SETS
  - ✓ 2-3 sets over 24 hr
    - 1 set = 1 aerobic & 1 anaerobic bottle
    - Each set drawn from separate venipuncture site
  - ✓ Pathogen Recovery
    - Second set gives 65% greater yield than first set
    - Third set gives 96% greater yield than first
- BLOOD VOLUME - MOST IMPORTANT VARIABLE
  - ✓ Septic Adults only 1-10 colonies/ml
  - ✓ 20 ml blood per culture set (10 ml per bottle)
  - ✓ CAP survey-mean culture vol/venipuncture-10 ml
    - 30 ml gives 47% greater yield than 10 ml

PEDIATRIC BLOOD CULTURES

- ONLY ONE BLOOD CULTURE BOTTLE NEEDED
  - ✓1 Peds Plus Bottle in Infants optimizes pathogen recovery
    - Bottle accepts up to 5 ml
    - Resins present to adsorb antibiotics
    - Only <0.1% bacteremia are due to anaerobes
  - ✓Anaerobes suspected?
    - Inoculate 1 anaerobic bottle + Peds Plus bottle
- BLOOD VOLUME
  - ✓0.5-2 ml Neonates
  - ✓2-3 ml 1 Mth to 2 Yr
  - ✓5 ml Older Children
  - ✓10-20 ml Adolescents

BACTERIAL LOAD HIGHER IN CHILDREN THAN ADULTS

BACTEREMIA OR CONTAMINATION?

FALSE POSITIVE BLOOD CULTURES

- Contamination
  - ✓ Disinfection optimal?
  - ✓ Line draw? Femoral stick?
  - ✓ Coag Neg staph, *Bacillus*, viridans strep, corynebacteria?
- Impact of Contamination
  - ✓ Increase LOS 4-5 days
  - ✓ Adds $5,000 to cost
- Multiple vs Single Blood Sets
  - ✓ 90% detection in blood culture instruments $\leq$ 2 days incubation
    - Check time to detection

FALSE NEGATIVE BLOOD CULTURES

- Insufficient
  - ✓ Blood volume per bottle
  - ✓ # sets
- Collection post antibiotic administration
  - ✓ Compromises pathogen viability & distorts Gram stain morphology
    - Determine antibiotic history
- Think mycobacterial, viral or other cause of febrile episode

BLOOD CULTURE METHODS

SYSTEMS	METHOD	DETECTION	TAT
BACTEC ADULTS: 8-10 ml/bottle 2 bottles/ set PEDS: 0.5-5 ml in 1 bottle	Continuous monitoring every 10 minutes Signals positives 24/7	CO_2 ↑ bacteria & yeast reacts with dye in sensor Fluorometrics Detect HACEK bacteria within 5 days	1-5 D
ISOLATOR ADULTS: 10 ml PEDS: 1-5 ml	Saponin lyses WBC Centrifugation & plating on media	Conventional growth media	1-2 D MAC 1- 8 WK

RESULTS FROM LAB

QUALITY SPUTUM

- GRAM STAIN
  - ✓>10-25 polys & <10 Epithelial Cells
  - ✓Polys are GRAM-NEG
- INTERPRETATION
  - ✓QUALITY SPUTUM

SALIVA

- GRAM STAIN
  - ✓<10-25 polys & >10 Epithelial Cells
- INTERPRETATION
  - ✓Spit, not sputum
  - ✓Specimen Rejected
- CONSEQUENCES
  - ✓Delay in Dx & Tx
  - ✓Repeat Specimens collected after Tx

THE TESTS

- MICROSCOPY
  - ✓GRAM, AFB, GIEMSA
- GROWTH DEPENDENT
  - ✓CULTURE & ANTIMICROBIC SUSCEPTIBILITY
- NON GROWTH DEPENDENT
  - ✓MOLECULAR DIAGNOSTICS
    - NUCLEIC ACID AMPLIFICATION TESTS
    - STRAIN FINGERPRINTING
  - ✓RAPID NON-MOLECULAR ASSAYS
    - ANTIGEN DETECTION
    - LATEX AGGLUTINATION

THE GRAM STAIN PITFALLS & ADVANTAGES

ADVANTAGES

- CLUE TO EMPIRIC TX
  - ✓GROUPS BACTERIA BY CELL WALL DIFFERENCES
- CLUE TO PATHOGEN IDENTITY
  - ✓CONSULT MICRO LAB FOR MORPHOTYPES
- INEXPENSIVE, FAST

PITFALLS

- INTERPRETIVE SKILL
- SENSITIVITY LIMITED TO HIGH BACTERIAL LOAD
  - ✓>10^4 PER ML
  - ✓FALSE NEGATIVES
- POOR SPECIFICITY
  - ✓NO DEFINITIVE ID
  - ✓FALSE POSITIVES

GRAM –NEGATIVE MORPHOTYPES

MORPHOTYPE	GROUP
SHORT RODS	ENTERIC *E. coli*
SHORT, PLUMP RODS BIPOLAR STAINING	ENTERIC *Klebsiella*
SLENDER, LONG FAINT STAINING	NON FERMENTER *Pseudomonas*
POINTED ENDS, FILAMENTOUS RODS FAINT STAINING	ANAEROBE *Fusobacterium* *Bacteroides*

THE BACTERIAL MASQUERADE
GRAM-STAIN IMPERSONATORS

BACTERIAL CLASSIFICATION		RESULT OFTEN APPEARS
Acinetobacter	GNR	GRAM POSITIVE Can mimic cocci
Bacillus	GPR	GRAM NEGATIVE
Moraxella	GNR	GRAM POSITIVE

OTHER STAINS
CLUE TO PATHOGEN IDENTITY

- ACID- FAST STAIN
  - ✓ MYCOBACTERIA SPPS
  - ✓ *CRYPTOSPORIDIUM*
  - ✓ *NOCARDIA* (PARTIALLY ACID- FAST)
- GIEMSA STAIN
  - ✓ MALARIA
  - ✓ OVA & PARASITE (i.e. *GIARDIA*)
- INDIA INK
  - ✓ *CRYPTOCOCCUS NEOFORMANS*
- IMMUNOFLUORESCENCE
  - ✓ VIRUSES

C. neoformans
TEST PITFALLS & STRENGTHS

LATEX AGGLUTINATION TEST
Target: Polysacch Antigen

STRENGTHS
- Sensitivity is ~94%
- Quantitative
  - ✓ Titer of >1:4= positive
  - ✓ Monitor Response to Tx

PITFALLS
- False Positives
  - ✓ Rheumatoid Factor
- False Negatives
  - ✓ Low numbers of organisms
  - ✓ Poorly or nonencapsulated
  - ✓ High titers (Prozone Effect)

INDIA INK
Target: Polysacch Antigen

STRENGTHS
- Rapid Results
- Technically simple to perform
  - ✓ Off hour lab shifts

PITFALLS
- Sensitivity is ~55%
- Need >10^4 yeast/ml
- Poorly or nonencapsuled strains

MEASURING QUALITY
ALL TESTS ARE NOT CREATED EQUAL

TEST RESULT	GOLD STANDARD	
	POSITIVE +	NEGATIVE -
POSITIVE +	TRUE POSITIVE (TP) +/ +	FALSE POSITIVE (FP) +/ -
NEGATIVE -	FALSE NEGATIVE (FN) -/ +	TRUE NEGATIVE (TN) -/-

TEST PERFORMANCE PARAMETERS

SENSITIVITY

$$\frac{TP}{TP+FN} \times 100$$

THE HIGHER THE TEST SENSITIVITY…. THE LOWER THE FALSE- NEGATIVES

SPECIFICITY

$$\frac{TN}{TN+FP} \times 100$$

THE HIGHER THE TEST SPECIFICITY…. THE LOWER THE FALSE-POSITIVES

TP = true positive
FP = false positive
TN = true negative
FN = false negative

TEST PERFORMANCE PARAMETERS

POSITIVE PREDICTIVE VALUE (PPV)

$$\frac{TP}{TP + FP} \times 100$$

INDICATES % THAT TEST WILL PREDICT A TRUE-POSITIVE RESULT

NEGATIVE PREDICTIVE VALUE (NPV)

$$\frac{TN}{TN + FN} \times 100$$

INDICATES % THAT TEST WILL PREDICT A TRUE-NEGATIVE RESULT

FINGERPRINTING THE CULPRITS

THE MICROBIOLOGY DETECTIVES

WHO DUNNIT

MOLECULAR DIAGNOSTICS

- DETECT VERY LOW # INFECTIOUS AGENTS
  - ✓ DIRECTLY FROM SPECIMENS
  - ✓ HIGH SENSITIVITY
- RAPID DETECTION
  - ✓ FASTIDIOUS PATHOGENS
    - TB, MALARIA
    - *Aspergillus fumigatus*
  - ✓ HIGHLY INFECTIOUS
    - INFLUENZA A
- QUANTITATION (VIRAL LOAD)
  - ✓ MONITORING RESPONSE TO DRUG THERAPY
  - ✓ HIV, CMV, HCV
  - ✓ TYPES OF SAMPLES
    - WHOLE BLOOD, SERUM, PLASMA
    - EDTA PRESERVATIVE REQUIRED
      - HEPARIN IS NOT ACCEPTABLE
  - ✓ DELIVERY REQUIREMENT
    - WITHIN 6 HOURS

MRSA DETECTION

- PCR – GOLD STANDARD
  - ✓ *mecA* & *nuc* genes – coamplification
  - ✓ Samples
    Blood culture bottles, nasal swabs, or pure culture
- SmartCycler
  - ✓ Amplification & Detection
    - Thermocycler & fluorimeter
  - ✓ Closed instrument system
    - Minimizes contamination
  - 1 ½ hour test
  - ✓ Expensive, technically challenging

MRSA DETECTION CULTURE VS PCR

CULTURE
Blood Bottle
Day 1

- ✓ GRAM STAIN-GPC clusters
- ✓ DAY 2 – Growth
- Rapid Ag test for *S. aureus* +
- PBP2a latex agglutination test for oxacillin- resistance +
- ✓ DAY 3 - MicroScan
- MIC ≥ 4 µg/ml by antibiotic susceptibility test
- Oxacillin Screen Plate 6 µg/ml
- ✓ DAY 4 – FINAL RESULT

MRSA

PCR
Blood Bottle
DAY 1

- ✓ GRAM STAIN- GPC clusters
- ✓ PCR TEST
- *Nuc* + = *S. aureus*
- *mec*A += oxacillin- resistant
- ✓ FINAL RESULT

MRSA

MRSA PROFILE

- NOSOCOMIAL MRSA 1970s
  - ✓ Resistance to the penicillins, cephalosporins, carbapenems & monobactams
  - ✓ Often multiply resistant to gentamicin, rifampin, clindamycin & T/S
  - ✓ Staph Chromosomal Cassette (SCC) mec 1-III
  - ✓ Multiple Clones
- COMMUNITY ACQUIRED MRSA 1990s
  - ✓ Usually susceptible to genta, clindamycin, T/S
  - ✓ SCC mec IV
  - ✓ +/- Panton-Valentine leukocidin
  - ✓ 2 Major Clones

WHY IS DNA FINGERPRINTING NEEDED?

- EPIDEMIOLOGY INVESTIGATION
  - ✓Which clinical isolates are the result of patient-to-patient transmission?
  - ✓Identify epidemic strain or index case
- INVESTIGATION AND CONTROL OF EPIDEMIC
  - ✓Nosocomial infections in long stay patients
  - ✓Contamination vs infection?
  - ✓Isolate interrelationships
    - >Sequential blood isolates from same patient

THE POWER OF PULSED FIELD GEL ELECTROPHORESIS

- GOLD STANDARD FOR MOST ORGANISMS
  - ✓Provides chromosomal overview
  - ✓Separates very large DNA fragments (40-800 kb)
- PFGE TECHNIQUE
  - ✓Microbe embedded in agarose & lysed
  - ✓Endonucleases cleave chromosome into fragment patterns
  - ✓Electrophoretic current "pulsed" in different directions for different lengths of time

INTERPRETING PFGE DATA

- CLONES
  - ✓GENETICALLY RELATED ISOLATES
- CATEGORIES OF DNA FRAGMENT RELATEDNESS
  - ✓INDISTINGUISHABLE (0)
  - ✓CLOSELY RELATED (2-3)
  - ✓POSSIBLY RELATED (4-6)
  - ✓UNRELATED (>6)