

The Laboratory Diagnosis of Urinary Tract Infection

**Gerri S. Hall, PhD.
Cleveland Clinic, Ohio
SCACM
September 4, 2010
hallg@ccf.org**

CLEVELAND CLINIC CLEVELAND, OHIO

Downtown
Cleveland

Cleveland
Clinic Complex



**CCF: ~ 1000 hospital beds
12 family health centers
Medical School
Reference Laboratory**

COULD BE VALUABLE



American Board of Medical Microbiology (ABMM)

American College of Microbiology

Certification in Medical and Public Health Microbiology, Doctoral-Level

- Documents your value and credibility with physicians
- Highest credential available to practicing medical laboratory microbiologists
- Benchmark your skills
- Enhance your credibility in the medical arena
- Recognized under CLIA '88

For more information please visit:

www.asm.org/college

Certify your worth.

Don't make people guess how valuable you are.

OBJECTIVES

- Provide an update on Laboratory Diagnosis of Urinary Tract infections
- Discuss the new Cumitech guidelines for laboratory diagnosis of UTI
- Describe the new CDC Guidelines for detection of GBS in urine cultures

Conflict of Interest

- **Speaker for Becton-Dickinson and bioMerieux**
- **Consultant: Opgen, Intelligent MDx**
- **Most recently involved in research with:**
 - Pocared
 - Advandx
 - bioMerieux
- **Committees/Boards**
 - CLIAC
 - CAP Microbiology Resource Committee
 - ASCP Teleconference Committee
 - ASMBL: ASM Branch Lectureship Program

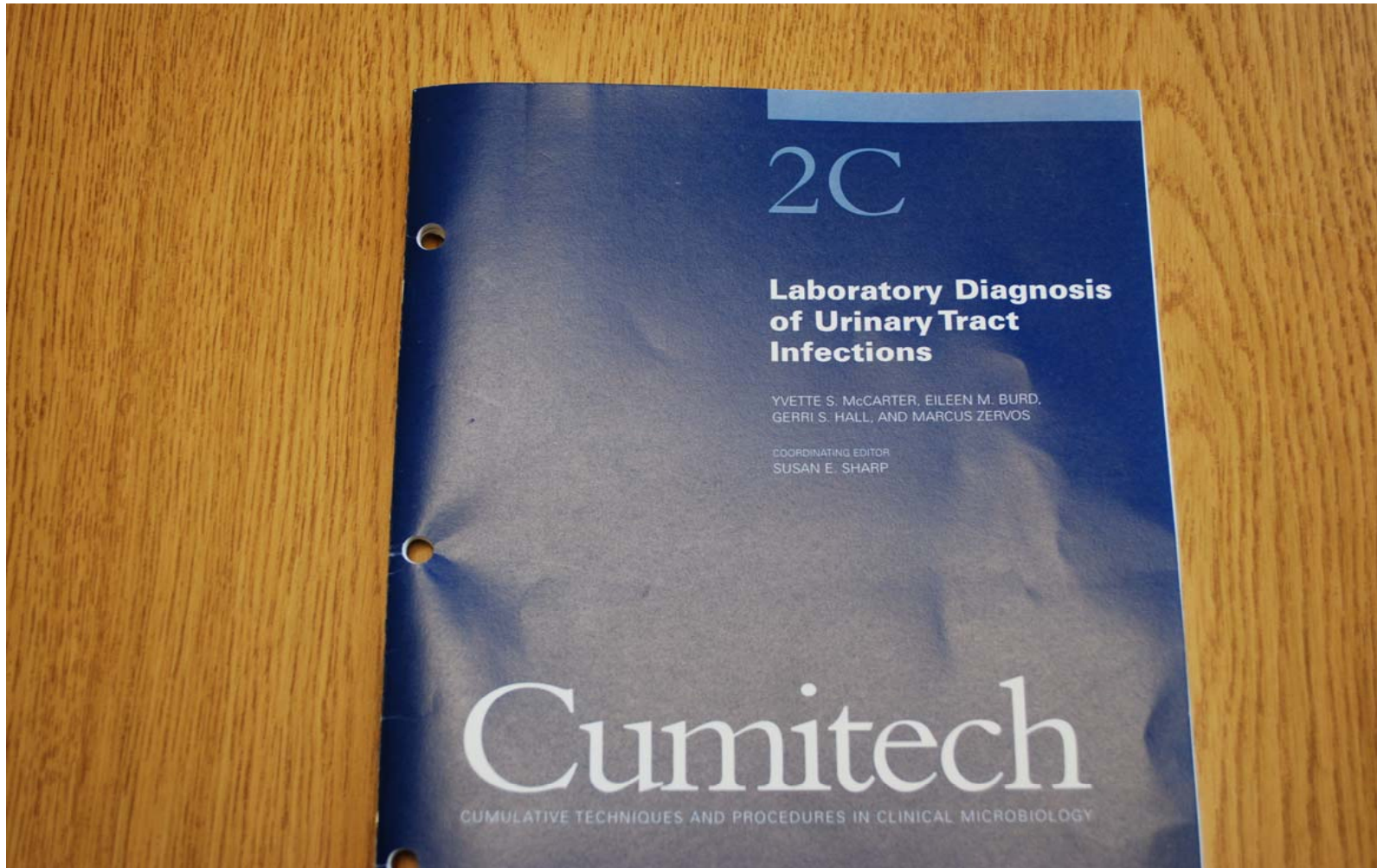
A lighter vein



© 1984 MLO/MEDICAL LABORATORY OBSERVER

“I think it happened when the 85th urine specimen arrived in an old mustard jar with the words ‘Spread a little sunshine’ on the lid.”

Urine Cultures... Where to get current information



Urinary Tract Infections

- **Most common bacterial infectious disease**
 - **Urines-- most common sample type for culture**
- **UTI: presence of microorganisms in urine**
 - **Symptomatic UTI: presence of clinical features along with critical numbers of potential uropathogens**
 - **Asymptomatic UTI: absence of symptoms in setting of critical numbers of potential uropathogens; local host response usually present**

Most commonly used Quantitative Criteria for UTI

- Symptomatic women
 - $\geq 10^3$ CFU bacteria/ml = most likely cause of the UTI
 - IDSA: 1000 CFU/ml: 80% sensitivity and 90% specificity
 - $>10^2$ CFU/ml = 95% sensitivity and 85% specificity for cystitis in women (Stamm, WE. 1982. NEJM 307: 463-8)
- Symptomatic men
 - $\geq 10^3$ CFU bacteria/mL
- Catheterized patients
 - $\geq 10^3$ CFU bacteria/mL
- Asymptomatic individuals
 - $\geq 10^5$ CFU bacteria/mL (IDSA recommends 2 urine samples)
- Usually any growth of a pathogen in a suprapubic aspirate or intraoperatively obtained sample is considered significant.
- ? Do we always know what category our patients' sample fits into?

Laboratory Issues to Discuss

- **Collection and transport of urine**
- **Screening vs. no screening of urine**
- **Cultures for urine**
 - **Media and methods**
 - **Incubation Time**
 - **Work-Up and Interpretation of results**
- **Discussion of a few less common uropathogens**

Urine Cultures: Indications

- Culture is not always necessary in women with dysuria, pyuria
 - Uncomplicated UTI may be treated with empiric therapy for “common pathogens”
 - Quality Issues
 - Better patient Care
 - Cost issues
 - In one study, Stuart et al (HMO Practice 1997; 11:150-7) demonstrated
 - a savings of \$367,000/year by not culturing 147,000 women (18-55 yo) with acute dysuria
 - no “harm” to the patients
 - UK Study (Fenwick, EA et al, Br J Gen Pract 50:612-3)
 - Empiric therapy was cost effective in uncomplicated UTI in women, however, they did comment that in this day of antibiotic resistance, a “dipstick strategy” might be considered

Urine Cultures: Indications

- Cultures are indicated in the following situations:
 - Complicated UTI including pyelonephritis
 - UTI in past 3 weeks indicating possible relapse or the presence of symptoms for > 7 days
 - Recent hospitalization or catheterization indicating possible nosocomial infection
 - Transplant patients
 - MS patients
 - Prostatitis patients
 - Pregnancy
 - Diabetes
 - Other

Urine: Collection

- **Midstream urine is “usual” manner of collection**
 - **Instruct patient**
 - No prior cleansing (data to support this, but Koneman, for ex., still recommends)
 - “mid” portion of urine; there are papers that support its discontinuation (Lifshitz, Prandoni, Lipsky)
- **Catheterized urine**
 - **Foley**
 - Prone to contamination
 - Do not accept catheter tips for culture
 - **Straight (in and out)cath**
- **Suprapubic aspirate: any quantity of growth should be considered significant**

UriSWAB

URINE COLLECTION SYSTEM

Copan:

http://www.copanswabs.com/html/uriswab_howto.html



Boric acid 6.7 mg/ml
Sodium formate 3.35 mg/ml
Homogeneous distributed and bonded
through out the sponge matrix



Sponge is saturated at 2.25ml fill
volume

Not available in US presently

Urines: to screen or not

Why screen a urine before culture?:

**Rapid answers of negative results
Reduced workloads for technologists
Treatment is often empiric**

Why not screen Urines before culture?:

**too costly;
“false positives”
Hospitalized patients: decreased PVN
Clinician acceptance
Culture is easy**

Urine Screens: If Yes, What are the Methods?

- **Gram stain of non-centrifuged urine**
 - **Quick, reliable, good correlations with $> 10^5$ CFU/ml**
 - **Can be part of “expanded urinalysis”**
- **Alternatives**
 - **Centrifuged urine examinations**
 - **Unstained urine examination**
- **Automated Microscopy**
 - **DiaSys R/S 2000.**
 - **Yellow IRIS (Chatsworth CA)**

Urine Screens:

If yes, what other possibilities?

- **DIPSTICKS**

- **Leucocyte esterase (LE)**

- 90% UTI = pyuria
- 75-96% sensitivity and 94-98% specificity

- **Nitrite test**

- ~ 95% Specific, but 35-85% sensitive

- **Combo Le + Nitrite**

- Rapid; may be negative with some bacteria like *Enterococcus*, *S. saprophyticus*
- 79-93% sensitive and 82-98% specific

Urine Screens: What is in the future?

UF 1000i Urine Screening Device



Sysmex analyzer; distributed by bioMerieux for urine screening

UF 1000*i*

UF-1000*i* is a fully automated, flow cytometer that stains cell components with a fluorescent dye and rapidly measures bacteria, leukocytes and RBCs in urine.

- Laser flow cytometry technology**
- Specific fluorescent dyes bind to the nucleic acid in bacteria and leukocytes**
- Results within minutes for bacteria ranges between 10^3 /mL to 10^4 /mL**

CultureStat (Microbionetics)

- **Tube contains dehydrated TSB + triphenyl tetrazolium chloride indicator**
- **System differentiates log vs. lag phase organisms**
- **Two readings in the spectrophotometer at 1 hr and 2 hr**
- **Detects changes in the indicator and bacterial concentration by measuring the light scatter**

CultureStat Procedure



Inoculate the CultureStat ampoule by inserting the ampoule into 7 ml urine and squeezing as if the ampoule and plastic housing was a syringe.



Place a bar code sticker on the glass ampoule . Scan the bar code with the scanner which identifies the specimen on the database program.



Following incubation, the ampoule is placed into the Reader. The test is run automatically and data is recorded on the CultureStat database.

Total time to label and identify sample, run test and record results: 20-30 seconds.

POCARED P-1000



Optical analyzer →

Specimen processor
→

Processing fluid &
Waste container↑



Carousel with
cuvettes

POCARED P-1000

Organisms identified	
<i>E. coli</i>	<i>P. aeruginosa</i>
<i>K. pneumoniae</i>	<i>Enterococcus spp.</i>
<i>E. cloacae</i>	<i>S. saprophyticus</i>
<i>P. mirabilis</i>	<i>S. agalactiae</i>
<i>C. freundii</i>	<i>Candida albicans</i>

Approach for use of a Urine Screen

- **Screen, followed by culture of positives:**
 - “Normal cath” and midvoid urine
 - suprapubic catheter;
 - VB1
 - Urine from ureterostomy and nephrostomy tubes
- **Urine culture without screen:**
 - Grossly bloody urine
 - catheterized urines from Urology;
 - all patients ≤ 12 years
 - MS pts
 - Transplant patients
 - reference clients
 - urines with specific “culture” request

Low colony count or “special”

- **catheter urines on pts \leq 12 years**
 - **VB2 and VB3**
 - **Midvoid from female with diagnosis of “acute urethral” or “dysuric syndrome”**
 - **a code that signifies urethritis (597)**
 - **urines obtained via surgical procedures**

Pyuria and relationship to UTI

- ≥ 10 leucocytes/mm³ occur in < 1% of asymptomatic non-bacteriuric patients and in > 96% symptomatic men and women with bacteriuria
 - Most symptomatic women with pyuria but without significant bacteriuria have UTI with bacterial uropathogens in colony counts < 10⁵/ml or have *C. trachomatis*
 - Women with asymptomatic bacteriuria are 2 groups:
 - True asymptomatic infection associated with pyuria
 - Transient self-limited bladder colonization
 - Most patients with catheter-associated bacteriuria also have pyuria and hence infection
- Pyuria should be determined in all cases in uncentrifuged urine and expressed as cells/mm³ (hemocytometer)

Urine Processing

- **Process urines within 2 hr of collection or refrigerate them**
 - **If delayed > 24 hr or unable to refrigerate, transport device, ie., B-D Urine Collection Kit or Sage Urine Culture Tubes**
 - **Has boric acid; supposedly good for up to 96 h**
 - **One study showed need for ≥ 3 ml (Nickander et al, 1982)**
 - **May change counts of some uropathogens (Enterococcus)**
 - **May inhibit small numbers of potential pathogens**

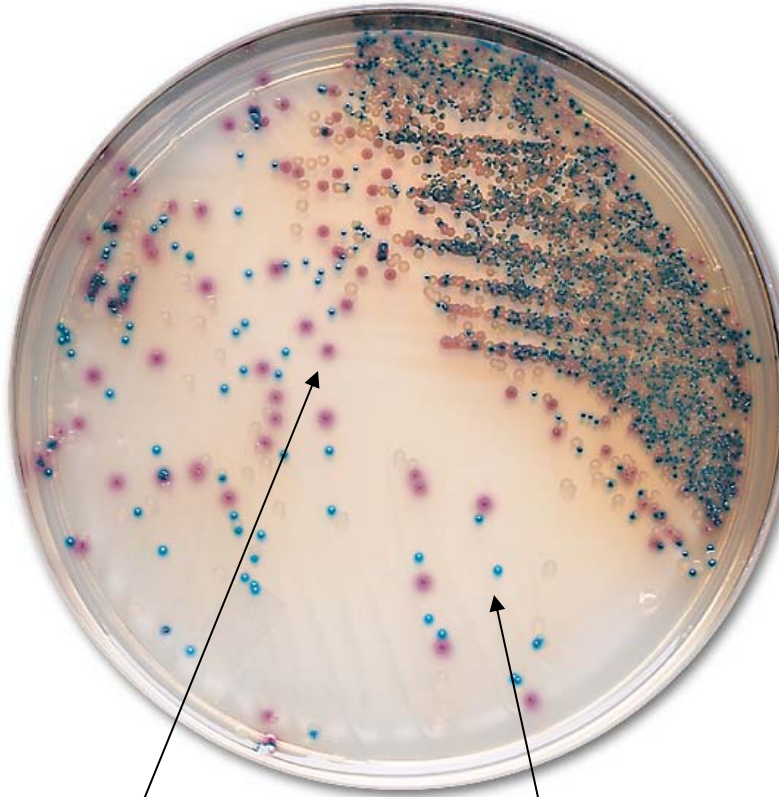
“Automated” Processing”

- **Copan WASP Specimen Processor**
- **Dynacon**
 - **InocuLab for Urine Processing**
 - **Innova Specimen Processor**
- **bioMerieux Specimen Processor**

Media for Culture

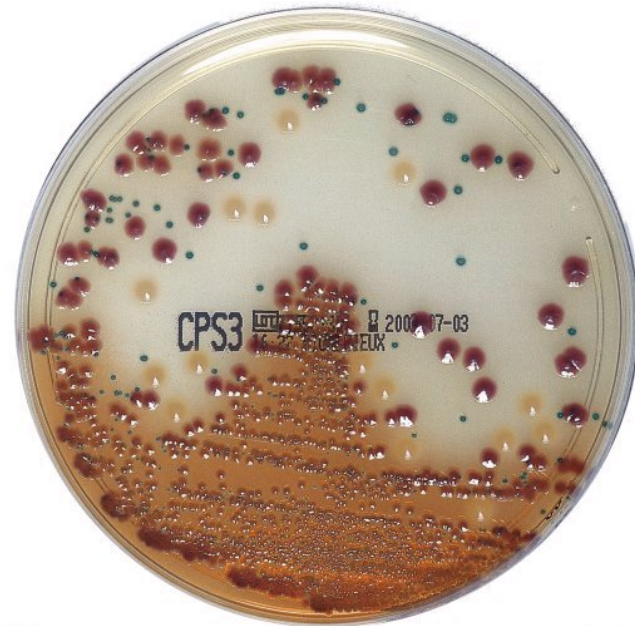
- **Conventional: 5 % sheep BAP and MAC**
 - **CLED: Cysteine lactose electrolyte deficient medium: inhibit Proteus swarming and accommodate common pathogens**
- **Paddles or “Dip” type devices**
 - **SOLAR-CULT(Solar Biologicals, NY)**
 - **OnSite™ (Trek Diagnostics, Cleveland, OHIO)**
 - **DipStreak (Novamed, Israel)**
 - **Other**
- **Chromogenic media**
 - **BBL CHROMAgar**
 - **bioMerieux CPS ID2**
 - **Remel Spectra UTI**
- **CCF: BAP and MAC is usual**

CHROMOGENIC MEDIA



E. coli

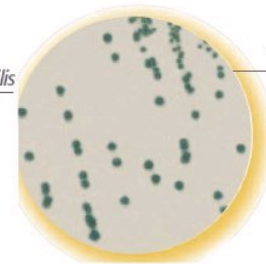
Enterococcus



E.coli



P.mirabilis



Enterococci

Always need to follow manufacturers' instructions for their use

Chromogenic Media: Potential Cost Savings

- CHROMagar + BAP/MAC (D'Souza, 2004 JCM 42: 60-64)
 - Reported on an annual savings overall of **\$35,680** based on processing of 20,000 urines
 - Differences would occur based on
 - Local cost of the media
 - Use of an up-front urine screen
 - Salaries and wages
- CHROMagar in urine (as well as pus and stool cultures) (Ohkusu, et al JCM. 2000; 38: 4586-92)
 - Gram Stain + centrifuged urines were planted onto chromogenic media
 - determined a 70% reduction in gnb “full ID” with good agreement to conventional methods
- Mixed Culture Work-UP: considerable time and labor may be saved with appropriate use of chromogenic media

Inoculation of Conventional Media: Cumitech Recommendations

- **Routine or non-invasive:** Clean-Catch mid-void; indwelling catheter or pediatric “bag”
 - 0.001 ml calibrated loop onto BAP and MAC
 - Streak down center; spread out from there
 - **> 16 hr incubation, 35 ° in an O₂ incubator before reading plates initially; most laboratories discard as no growth at ~ 24 hrs.**
- **Invasive collection methods:** straight catheter, suprapubic aspirate, cystoscopy, nephrostomy; “low colony count urine requests”
 - 0.01ml calibrated loop onto BAP and MAC
 - Streak down center and spread out from there
 - **Consider > 24 - 48 hr*** incubation, 35 °C in a O₂ incubator
 - May want to include the 0.001 ml inoculum as well for easier CFU determination
 - **We need to know what type of urine it is!**
 - *incubation time may vary if specific organisms, or requests are made

NOW THE REAL QUESTIONS

- **What to work-up**
- **How to Report & Interpret**

HOW TO REPORT URINE CULTURES: Cumitech Recommendations

- **Negative urines (no growth)**
 - 0.01 ml inoculum
 - Sterile or < 100 CFU/ml OR
 - No growth of ≥ 100 CFU/ml
 - 0.001 ml inoculum
 - Sterile or < 1000 CFU/ml OR
 - No growth of ≥ 1000 CFU/ml
- **Positive cultures: colony count reported along with ID (with or without AST)**
- **Mixed cultures: reported as such with note about calling for consultation and/or further work-up**
- **Unusual pathogens/isolates**
 - Bring it to attention of supervisors
 - Call clinician or other health care provider

What to do with MID-VOID URINE CULTURE

- Work up 1 or 2 pathogens each at $\geq 10,000$ CFU/ml: ID and AST*
- If 1 or 2 pathogens at $< 10,000$ CFU/ml: simplified ID including gram stain, spot tests, hemolysis, and rapid biochemical tests for both—full ID probably not necessary
- If 1 pathogen is $\geq 10,000$ CFU/ml and 1 $< 10,000$ CFU/ml:
 - Perform ID and AST on isolate $\geq 10,000$ and give simplified ID for isolate $< 10,000$ CFU/ml

What to do with MID-VOID URINE CULTURE

- If there are ≥ 3 organisms
 - If one organism is predominant and $\geq 100,000$ CFU/ml even with this mixed flora, perform ID and AST
 - If there are 2 isolates in this mixed flora that have counts 10,000 – 100,000 CFU/ml each, and others $< 10,000$, use the simplified identification only for both $> 10,000$ CFU/ml
 - If the 3 or more are in roughly same numbers report as mixed flora and/or give morphologic ID of components only.
 - Suggest recollection.
 - Sign out as mixed skin flora or mixed enteric flora or mixed skin flora
- Always exceptions; communicate your algorithms; call if unusual

What about the low colony count urine (0.01 inoculum)

- Similar to “routine” urine culture, but with ID of lesser CFU/ml since inocula is 10 fold less
- If both inocula are planted with special requests for low colony count, may use the 0.001 inoculum plates for quantitation if counts are > 1000.
- Mixed cultures are probably still “mixed” and may represent
 - Contamination
 - Improperly transported urine specimens
 - Need to take patient population into account as well
 - May consider need to treat suprapubic aspirates differently since any growth of potential pathogen is probably significant.

Commensal Flora and Potential Pathogens in Urinary Tract

- **Usual Commensals; most often non-pathogens**
 - α -hemolytic streptococci
 - *Bacillus* sp.
 - Coagulase negative staphylococci (CoNS)
 - Diphtheroids
 - *Lactobacillus* sp.

Common Pathogens

- Enterobacteriaceae
 - *E. coli*: 75-95% uncomplicated UTI
- *Enterococcus* sp.
- *S. saprophyticus* : can be 2nd to *E. coli* in some populations

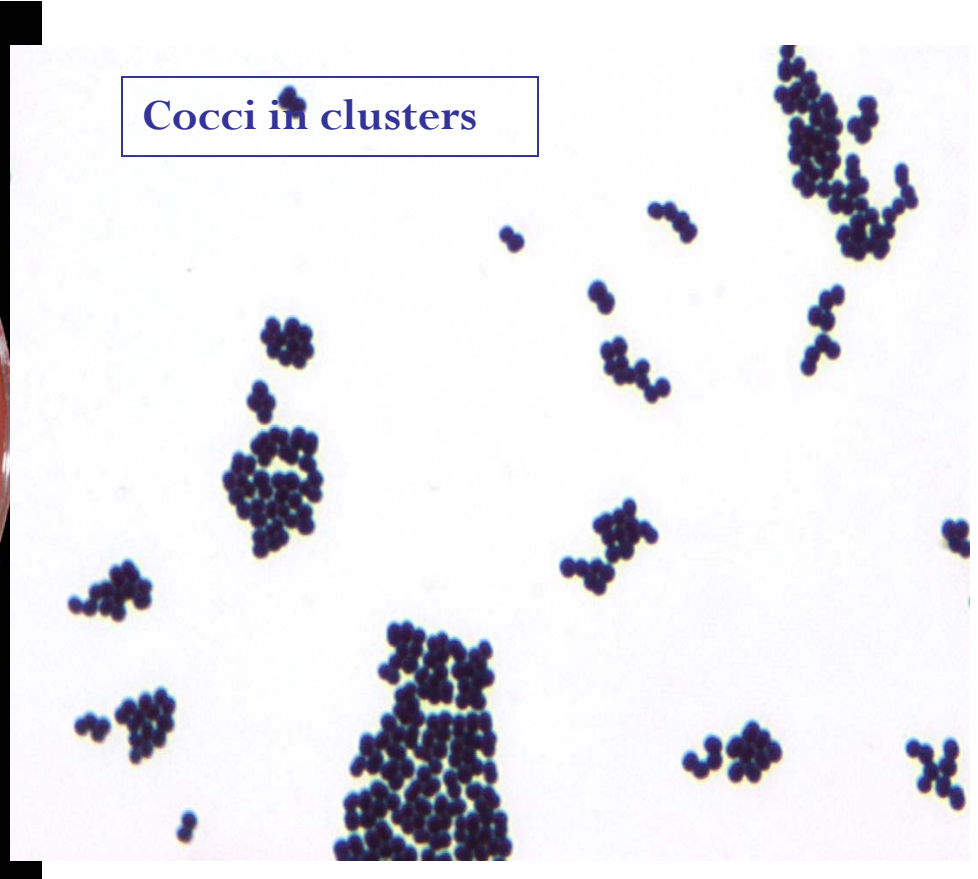
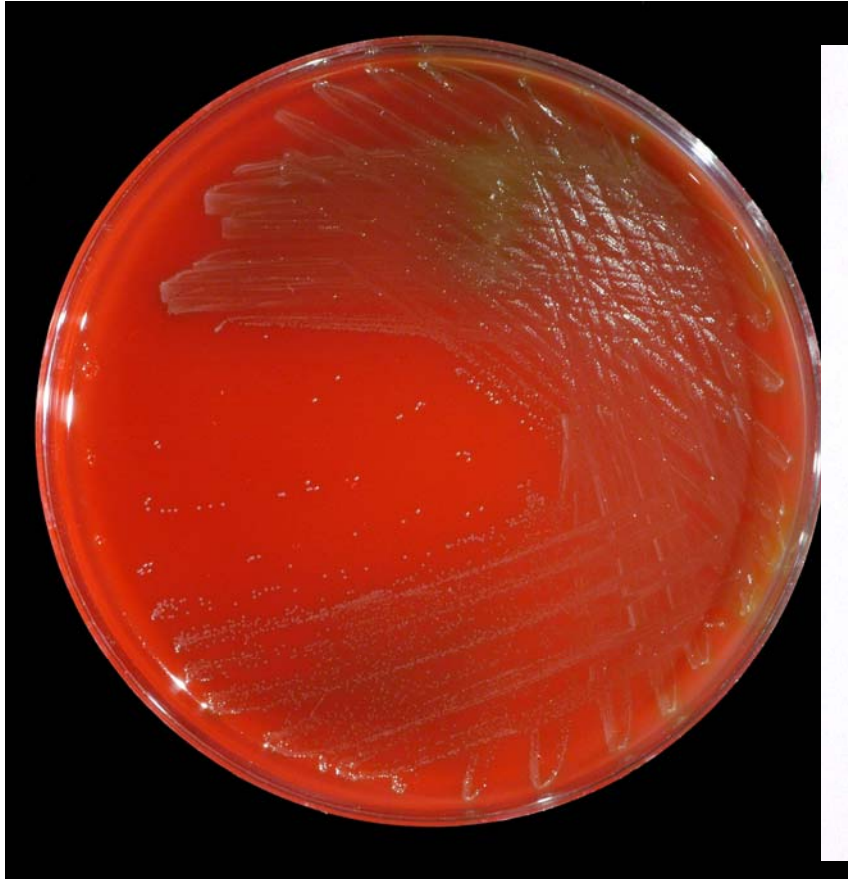
Less Common Pathogens (Incidence)

- *Coagulase negative staphylococci* (CoNS)
- *S. aureus*
- *Pseudomonas* spp.
- *Corynebacterium urealyticum*
- *Aerococcus urinae/A. sanguinicola*
- *Other*

“Unusual or Infrequent Pathogens: *Aerococcus* sp.”

- **GPC in clusters; catalase negative; α -heme; may resemble enterococcus or other viridans streptococci**
 - ***A. urinae*: PYR – and LAP +**
 - **Most commonly isolated from UTI; probable pathogen**
 - ***A. sanguinicola*: PYR + and LAP +**
 - **Not commonly isolated, but can be a UTI Pathogen**
 - ***A. viridans* = PYR +/-LAP –**
 - **Can be isolated from urine; ? If significant**

Aerococcus sp.



Infrequent Pathogens

- ***C. urealyticum* (Group D2)**
 - Associated with alkaline-encrusted cystitis and pyelitis in adults and children and urinary tract struvite calculi
 - Rare chronic inflammatory disease of the bladder
 - May occur in transplant patients
 - Skin flora of ~ 12% healthy individuals; and up to 30% hospitalized patients
- ***Haemophilus influenzae*/ *H. parainfluenzae***
 - Hansson (2007; Ped Nephrol 22:1321-5): in 24 yr timeframe, 36 children/ 5,000 UTI
 - Galan (Enferm Infect Microbiol Clin. 1996;14:483-5)
 - > 16, 000 urine cultures: 0.88% incidence in pediatrics
- ***Gardnerella vaginalis***
 - Normal vaginal flora; increased numbers in bacterial vaginosis;
 - unclear as to UTI significance
 - Gonzalez-Pedraza A et al (Inferm Infec Micro Clin 2003; 21:89-92) ~ 3% of 1507 urine samples but no pathogenicity determined**

Anaerobes: very low incidence

Group B Streptococcus

- Urine can be a ‘surrogate’ for the vaginal/rectal specimens when screening for GBS in the pregnant female
 - Asymptomatic
 - Any quantity **was** considered significant
 - Newer recommendations, $\geq 10,000$ CFU/ml
 - If in mixed flora: use laboratory guidelines
 - AST if allergic patient—information for labor/delivery
- GBS can cause a “true” urinary tract infection
 - Symptomatic and no other uropathogens isolated
 - AST probably not needed
- Consider comments about use of this as surrogate for vaginal/rectal specimen in pregnant woman when positive for GBS; if negative, will still need to perform the latter.

Box 1 in 2010 Guidelines

- Routine screening for asymptomatic bacteriuria is recommended in pregnant women, and laboratories should screen urine culture specimens for the presence of GBS in concentrations of 10^4 colonies or greater.
- Laboratories should identify GBS when present at $\geq 10^4$ in pure culture or mixed with a second microorganism.
- Identification of GBS from urine specimens from pregnant women with heavily mixed cultures (≥ 3 organisms, all $\geq 10^4$) may not be feasible. These patients should be screened with a vaginal-rectal swab at 35-37 weeks as recommended.

Empiric Therapy for “Simple” UTI

- **IDSA advocates SXT as initial therapy for females with acute uncomplicated bacterial cystitis in settings where prevalence of SXT resistance does not exceed 10-20%**
 - **3 day course recommended**
- **Alternatively, use fluoroquinolone, nitrofurantoin, fosfomycin**
- **Obtain information on and provide ongoing surveillance of resistance (Karlowsky et al 2002)**

When to do an AST on Urine Isolate?

- Follow laboratory guidelines for susceptibility testing of isolates in which a definitive ID and AST is recommended, according to Cumitech, CMPH, MCM, IDSA, other guidelines. That would include “significant CFU/ml” of:
 - Enterobacteriaceae
 - *P. aeruginosa*
 - *Enterococcus* sp.
 - *S. aureus*
 - Significant CoNS, except *S. saprophyticus*
 - Others, including yeast, upon request or if SOP’s have been set up in accordance with ID/Pharmacy/Institutional Guidelines of Best practices

Antimicrobial Susceptibility Testing

- If significant and an organism for which there is a method for AST, consider performing but reporting appropriate agents
 - Ampicillin, SXT and nitrofurantoin for *E. coli*
 - Increasing requests for fosfomycin
- for *E. coli* only in urine:
- $< 64 = S$; $64 = I$ and $\geq 128 = R$
- For *E. faecalis* only in urine:
- $\leq 64 = S$; $128 = I$; $\geq 256 = R$

CLSI Guidelines “For Urine Only”, group U, Table 1(M100-S15)

- **Enterobacteriaceae**

- Carbenicillin
- Quinolones
 - Gatifloxacin
 - Cinoxacin
 - Lomefloxacin or norfloxacin or ofloxacin
- Loracarbef
- Nitrofurantoin
- Sulfisoxazole;
Trimethoprim

- **Non-Enterobacteriaceae GNB**

- Carbenicillin
- Ceftizoxime
- Quinolones
 - Lomefloxacin or norfloxacin or ofloxacin
 - Sulfisoxazole
 - Tetracycline

CLSI Guidelines “For Urine Only”, group U, Table 1(M100-S15)

- ***Staphylococcus* spp**
 - Nitrofurantoin
 - Sulfisoxazole
 - Trimethoprim
 - Quinolones
 - Lomefloxacin or norfloxacin
- ***Enterococcus* spp.**
 - Quinolones
 - Ciprofloxacin
 - Levofloxacin
 - Norfloxacin
 - Nitrofurantoin
 - Tetracycline

Summary

- **Appropriate handling of the urine culture request in Clinical Microbiology is important and ever challenging**
- **Proper collection and transport is essential to quality performance of the urine culture**
- **To perform a urine “screen” is still controversial and an individual lab decision**
- **Interpretation of the urine culture results differs between laboratories, but guidelines should be determined and consistently utilized**
- **Communicate your procedures and guidelines for interpretation to clinicians and other health care personnel**

You can do it my way or
“urine”

**Quote by the late Frank Koontz, PhD
Department of Pathology
University of Iowa Hospitals**

**In article: Cost accountable Urine culture: an
opinion.**

Thanks

- **To Bob Fader and SWACM for inviting me**
- **To you the audience for listening today**
- **To Marie Pezzlo for being a wonderful microbiologist, pioneer in the world of Lab Diagnosis of UTI**
- **To the Medical Technologists at Cleveland Clinic for all they teach me everyday.**
- **To my family & friends & colleagues in Cleveland!**

