How do you store a urine sample if the collection site is far from the laboratory ? Can a urine sample collected 24h before testing at the lab give a good result?

Urine samples should be plated as quickly as possible to avoid overgrowth from contaminating urethral organisms. The suggested time for room temperature urines is within 2 hour of collection. Urine is stable to plate for up to 24 hrs if it is refrigerated. Preservatives like boric acid (gray-top tubes) can be used for 48 hr stability at room temperature. We use boric acid gray-tops at UCLA.

A urine held at room temperature for 24hrs before plating would typically not give a good result.

How has the introduction of molecular technologies (like the genomics and proteomics tools) impacted your routine culture workflow at UCLA?

Proteomics tools like MALDI-TOF have greatly impacted our workflow by increasing accuracy of speciation compared to biochemical identification and decreasing turn around time. Genomics or next gen sequencing has helped us be able to identify organism to the species, subspecies, and strain level that were not able to be identified by any other methods.

What existing bacteriological innovations do you have at UCLA that can be adopted for clinically efficient diagnosis in Sub-Saharan Africa?

MALDI-TOF identification of bacteria is probably the most impactful innovation. The challenge is that it is very expensive. I really think that the more labs that are able to do gram-stain and basic culture and identification would have the highest impact.

For Urine specimen, do you also need to culture on fungal culture media?

Fungus (including both molds and yeast) are a very rare cause of urinary tract, bladder, or kidney infections. Because of this, urines do not need to be routinely plated on fungal culture media.

Could you please speak briefly on enrichment media

Enrichment media (like chocolate) contain additional nutrients not available in blood agar plates that allow the growth of fastidious organisms like *Neisseria* and *Haemophilus*.

On Temperature differences, you talked about Campylobacter. If I am not mistaken, it requires 42 - 45°C. What can you do if you have no clue, even after doing a gram stain, of what kind of bacteria you are dealing with?

Campylobacter is a common cause of bacterial diarrhea. If possible, all stool culture set ups should include a culture plate in microaerophilic conditions at 42-45C to culture for campylobacter.

What is the incubation period for stool cultures?

We incubate stool cultures for 5 days before calling it negative.

Currently, we are incubating blood cultures for 7 days, which is somewhat different from today's presentation. How can this discrepancy be understood?

This depends on the blood culture system. We use the BD Bactec blood culture system that is approved for incubation to 5 days. Other systems may use other incubation criteria.

There are many gram negative bacterial that causes eye infection like conjunctivitis and other ophthalmic infection. As you mention the appropriate culture media are BAP and CAP only. why not use MAC?

MAC plates really serve the purpose of eliminating gram positive flora overgrowth or differentiating between multiple types of gram-negative species in a single specimen. Eye specimens don't fall into either of these categories so a MAC plate is unnecessary. Any gram-negative that is causing infection in the eye can be recovered and identified on the blood agar plate.

On slide 33, does the incubation you are referring to correspond to the time after seeding or before seeding?

This is the time after seeding and being placed in the incubator.

My question is for the different incubation durations. You have stated for the different samples, how do you account for the TAT? and what about AST results ? will they be early enough to inform doctors on prescribing antibiotics?

The incubation duration is for how long the culture will go before we call it negative. Any positive growth within the incubation time will be worked up immediately. For example, if a wound culture grows staph aureus 24 hrs after incubation, it will get species identification and AST results as quickly as possible. If nothing grows at 72 hrs, then it will get resulted as negative.

Is GC (Thayer Martin) media suitable for Chlamydia?

Chlamydia can not be cultured on agar plates. TM media is only good for Gonorrhea.

From the case study, why was it negative in direct smear of abscess case? How can we relate that with the whole step of analysis?

The abscess was negative by gram stain because a gram stain is not as sensitive as culture. Gram stain positivity requires at least 1x10^5 organisms per ml. A culture can find bacteria that are below this threshold. This means that a gram stain on primary specimen is important, but it cannot replace culture methods.

I work in a lab where we do a minimum of 30 gram stains on a daily basis. Would performing Gram stain IQC once per week be sufficient to address the risk of harm to the patient?

Weekly gram stain QC would be sufficient.

I wish to find out how can one verify the viability and purity of ATCC control strains over time?

We order new QC strains when we start seeing QC issues. We do not have a set date range. Upon receiving new ATCC QC strain, we create bacteria stock vials for long term storage. Depending on how frequently we need to use this QC, we may either sub directly from the -80 stock as needed or make additional mini stock bullets if we need to sub these frequently. This way we can prevent extensive subculture of the strain or prevent accidental freeze thaw of the primary stock. The organism keeps a very long time when stored at -80C without freeze thawing.

I have a concern. Why is that most Microbiology Labs don't do AST for Coagulase Negative Staphylococcus?

Coagulase negative staph can be a pathogen, but it can also be a contaminant. We will automatically perform AST when it is isolated from critical specimens (CSF, eye, surgical specimens . . .) but we only perform it on physician request from other specimen types where it may be a contaminant (blood culture). For specimens that have contact with skin flora (wound, urine) we discourage AST on coagulase negative staph because it is less likely to be a pathogen.

For QC e.g for McConkey, can you divide one plate into 3 other than using 3 plates

Yes.

which culture media are exempt from QC according to CLSI?

CLSI M22-A3 covers QC for Commercially Prepared Microbiological Culture Media – Table1B Exempt and NonExempt Categories for Media

Table 1B. Exempt and Nonexempt Categories for Media Included in CAP Surveys (1984, 1988, 2001)[a] (From Jones RN, Krisher K, Bird DS, and the College of American Pathologists Microbiology Resource Committee. Results of the survey of the quality assurance for commercially prepared microbiology media. *Arch Path Lab Med.* 2003;127(6):661-665. Reprinted with permission from the College of American Pathologists.)

CATEGORY	EXEMPT [c]	NONEXEMPT[d,e]
General bacteriologic media	Blood agar Chocolate agar[c] Thioglycolate broth Urease agar	Nutrient broth
Blood culture media[b]	Brain heart infusion (BHI) blood culture broth Biphasic blood culture bottle medium Centrifugation/isolation tubes (adult) Thiol blood culture broth Trypticase soy blood culture broth Peptone broth	
Media for gram-positive bacteria	Columbia (CNA) agar Selective media for enterococci with or without azide LIM broth Mannitol salt agar Phenylethyl alcohol (PEA) agar Selective agar for Group A Streptococcus Sheep blood agar with sulfamethoxazole/trimethoprim (SXT) *Enterococcus* (*Streptococcus*) *faecalis* broth	Todd-Hewitt broth Desoxycholate broth[e] Trans-vaginal broth[e] Chocolate agar with pyridoxal[e]
Media for gram-negative bacteria	Cefsulodin irgasan novobiocin (CIN) agar Citrate agar Cystine lactose electrolyte deficient (CLED) agar Eosin methylene blue (EMB) agar Gram-negative (GN) broth Hektoen (HEK) agar MacConkey agar *Salmonella-Shigella* (SS) agar Selenite broth Thiosulfate citrate bile salts sucrose (TCBS) agar Triple sugar iron (TSI) agar Trypticase soy agar with sheep blood with ampicillin Xylose lysine desoxycholate (XLD) agar	MacConkey sorbitol agar Chocolate agar with bacitracin[e]
***Neisseria gonorrhoeae* (GC) media**	Thayer-Martin agar (modified)[c] GC-Lect[c,d] ™	Martin-Lewis agar Chocolate agar with IsoVitaleX® New York City agar[c]

Table 1B. (Continued)

CATEGORY	EXEMPT [c]	NONEXEMPT [d,e]
Bordetella pertussis media		Reagan-Lowe agar Bordet Gengou agar[e]
Legionella media *Burkholderia cepacia* (PC) media	*Legionella* selective (CYE/BCYE) agar [c,d] *Pseudomonas cepacia* (PC) agar[c]	Selective *Legionella* agar with DGVP[d,e] OFPBL agar[d,e]
Campylobacter media	Charcoal selective agar with CVC[d]	*Campylobacter* blood agar (Blaser) *Campylobacter* agar with CVA[d]
Anaerobic media	Anaerobic blood agar Anaerobic phenylethyl alcohol (PEA) agar Bacteroides bile esculin (BBE) agar Brucella agar Brucella agar w/hemin/Vitamin K Brucella laked blood agar with KV[d] CDC anaerobe laked blood agar with KV[d] CDC anaerobic 5% sheep blood with KV[d] Egg yolk (modified) agar Kanamycin laked blood agar	CDC anaerobe 5% sheep blood agar with PEA
Mycobacteria (AFB) media[d]	AFB biphasic bottle medium[d] Middlebrook 7H9 broth Lowenstein-Jensen media Middlebrook agar Automated AFB bottle broths[b,d]	Middlebrook 7H10 agar Middlebrook 7H11 agar American Trudeau Society (ATS) agar[e] Mitchison's agar[e] Petragnani medium[e]
Fungal media	Cornmeal agar Inhibitory mould agar Inhibitory mould agar with gentamicin Soy peptone agar with CC without pH indicators[d] Potato dextrose agar Brain heart infusion agar with 5% sheep blood/CG[d] Sabouraud's dextrose agar Sabouraud's dextrose agar with CG[d]	Cornmeal agar with Tween Brain heart infusion agar with 5% sheep blood/CC[d] BIGGY agar[d,e] Birdseed agar[e] Brain heart infusion agar with 5% sheep blood/PS[d,e] Dermatophyte test medium[e] Potato flakes agar with or without CC[d,e]

[a] Exempt: Extrapolated Failure Rate of ≤ 0.5%; Nonexempt: Extrapolated Failure Rate of > 0.5%; media with insufficient data for categorization is considered nonexempt and QC is required.

[b] Represents formulations from BD Diagnostic Systems (Sparks, MD) or BioMérieux (Raleigh/Durham, NC). Refer to manufacturer's package insert for specific QC information.

[c] Quality control of exempt media used for fastidious organisms (in particular exempt media for recovery of *N. gonorrhoeae, H. influenzae, Campylobacter* sp., *Legionella* sp., and *B. cepacia* among others) strongly recommended to ensure optimum recovery of organisms. Refer to Table 3.

[d] Abbreviations: AFB (Acid Fast Bacilli); BIGGY (Bismuth sulfite Glucose Glycine Yeast); CC (Cycloheximide/Chloramphenicol); CG (Chloramphenicol/Gentamicin); CVA (Cefoperazone/Vancomycin/Amphotericin B); CVC (Cefoperazone/Vancomycin/Cycloheximide); CYE/BCYE (Buffered Charcoal Yeast Extract); DVGP (Dye,Vancomycin, Glycine, Polymyxin B); GC (Gonococcal); KV (Kanamycin/Vancomycin); OFPBL (Oxidative Fermatative Polymyxin B, Bacitracin and Lactose); PS (Penicillin/Streptomycin).

[e] Media deemed nonexempt because of insufficient data for calculation of extrapolated failure rate. See footnote a.

What is the standard guideline on break-pont for Cefriaxone- sulbactam? because many clinitians used for the management of patients in my country Zanzibar.

I am not familiar with ceftriaxone-sulbactam. There are CLSI breakpoints for Ampicillin-sulbactam and for sulbactam-durlobactam.

If you want to do csf culture how long do you incubate that plate since I saw on this presntation you can incubate all sterile cuture up to 5 days

We incubate CSF for 5 days before calling it negative.

I work in a veterinary clinical bacteriology laboratory and am trying to identify standardized methods for routine diagnostic testing. While there are numerous ISO methods for food microbiology, I am struggling to find equivalent standards for veterinary clinical bacteriology. Are there ISO standards, CLSI documents, WOAH guidelines, EUCAST recommendations, or other internationally recognized reference methods that cover specimen processing, bacterial isolation, identification, and quality assurance.

I am not familiar with culture standards for veterinary microbiology. That said, a lot of the same bacterial pathogens found in animals are the bacterial pathogens found in humans. I would think the relevant culture techniques for clinical bacteriology would apply directly to veterinary clinical bacteriology.

Is it ideal to do a direct gram stain from a blood culture sample immediately when the sample is received in the lab, or should it be incubated for even a day before doing a gram stain?

Gram stains on blood should only be done after the blood culture turns positive. It is not useful to do a gram stain on a direct blood sample.

In my laboratory, I routinely use API 20E for bacterial identification; however, I do not have access to the APIWEB software for result interpretation. Could you kindly advise on alternative approaches for interpreting API 20E results? Additionally, would it be possible to obtain an API 20E interpretation chart, guide, or any supporting resources that could assist with manual identification and interpretation of API profiles?

I don't know of alternative approaches to API20E identification.

What is the relevance and importance of AI initiatives in Microbiology culture and identification systems?

I think AI will start to be used in automated micro process to help with result interpretation, but this innovation is not yet available.