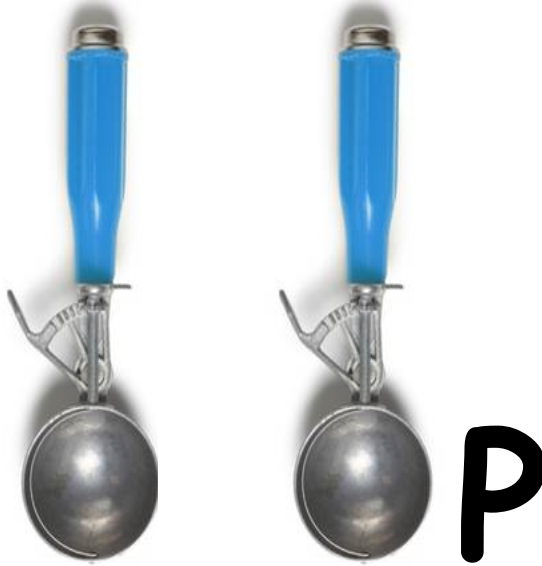


The Straight



SCOOP

on POOP

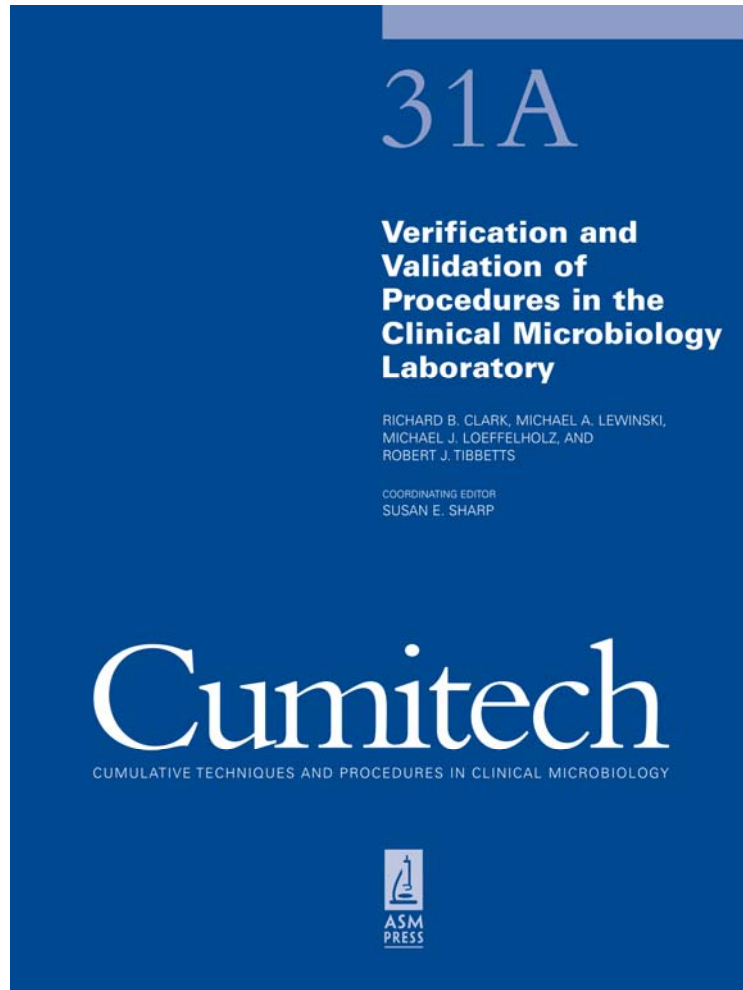


Susan E. Sharp, Ph.D., DABMM
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But FIRST - - Quiz time !



But FIRST - - Quiz time !



Here's the QUIZ

Here's the QUIZ

**What is
this called?**



Bonefish

Tarpon

Permit

One more hint...



2000 The US Open
The British Open,
The PGA

2001 The Masters



Tarpon
Permit
Bonefish

OK - one more hint...



2000 The US Open
The British Open,
The PGA

2001 The Masters

1988
Australian Open
French Open
Wimbledon
US Open



Tarpon
Permit
Bonefish

"ACM"

"ACM" Grand Slam ??

The 2010 "ACM" Grand SLAM Career Tour !

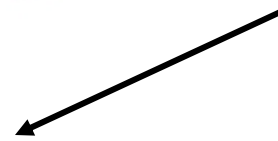


**March 20,
Louisville**

**south central association
for clinical microbiology**
www.scacm.org



**September 4,
San Antonio**



**November 3,
Charlotte**

Objectives

- Understand the limitations of *Campylobacter* EIA vs. stool culture.
- Discuss ways to diagnose STEC from stool specimens.
- Review methodologies for detection of *Clostridium difficile* infections.

Comparison of Campy selective media with Campy-antigen EIA (ProSpecT microplate assay)

631 specimens tested

	EIA	
	<u>Sens</u>	<u>Spec</u>
Campy-CVA media	93%	99%
CAT* media	93%	99%
Both media	89%	99%

*CAT: Blood-free campylobacter agar with cefoperazone, amphi B & teicoplanin

Comparison of Campy selective media with Campy-antigen EIA (ProSpecT microplate assay)

Conclusions:

This EIA is easy to perform; is amenable to testing in small laboratories.

The EIA is less sensitive than dual plate culture (89% sensitivity).

The high specificity of the assay (99%) allows a diagnosis to be made from a positive result.

The cost-effectiveness of this assay requires evaluation: direct cost of the EIA is higher than culture.

Campy-antigen EIA v. Culture

A. Sens: 89.1% Spec: 97.7%

- Rapid and easy to use

B. Sens: 96% Spec: 99%

- Rapid and acceptable for Campy detection

A. Dediste A, et al. 2003. Evaluation of the ProSpecT Microplate Assay for detection of *Campylobacter*: a routine laboratory perspective. *Clin Microbiol Infect.* 9(11):1085-90.

B. Tolcin R, et al. 2000. Evaluation of the Alexon-Trend ProSpecT *Campylobacter* Microplate Assay. *J Clin Microbiol* Vol. 38(10) 3853-3855.

Some Public Health concerns...

- Specificity of the test is reportedly high, but in settings where the prevalence of the disease is low the PPV could be quite low...
 - Laboratories should confirm positive results by culture.
- Test does not produce an isolate that can be used for molecular subtyping when clusters are identified.
- A positive EIA test alone is not sufficient to consider a case 'confirmed'; might affect surveillance numbers.
- Laboratories should clearly indicate that EIA was performed when reporting cases to public health.

Shiga toxin testing: Refresher...

- E. coli* that produce Shiga toxin (Stx) = STEC
- - 100 different STEC serotypes
 - O antigen= lipopolysaccharide
 - H antigen= flagellar
- - *E. coli* O157:H7 most commonly reported

Shiga toxins similar to toxin produced by
Shigella dysenteriae type 1

- Stx1 and Stx2

Epidemiology

- **STEC causes ~100,000 illnesses/yr**
 - 3200 hospitalizations, 91 deaths
- **Approximately 8% of people with O157 STEC develop hemolytic uremic syndrome (HUS)**
 - Thrombocytopenia
 - Hemolytic anemia
 - Kidney failure
- **Non-O157 STEC just as virulent**
 - Virulence linked to production of Stx2

Transmission

Occurs year round but especially summer

Occurs in persons of all ages, but most common in children <5 yr

- - risk for HUS highest in this age group

Occurs by consumption of undercooked ground beef, unpasteurized juice, raw milk and produce (spinach), contaminated water

Low infectious dose (<100 organisms)

Why Detect STEC Infection Early?

Diagnosis made in the laboratory

- Finding O157 STEC high predictive positive value for severe disease

Prompt treatment with parental volume expansion decrease renal injury and improves outcomes

Antibiotics should not be given for STEC

Early recognition of public health problem

CDC RECOMMENDATIONS:

MMWR:

October 16, 2009 / Vol. 58

"Recommendations for Diagnosis of
Shiga Toxin-Producing *Escherichia coli*
Infections by Clinical Laboratories."

104 references to support recommendations

Information made available by CDC; Roberta Carey, Ph.D.

Current Lab Approaches to STEC

1. Screen all stools with STEC-EIA as part of the profile to detect bacterial stool pathogens, refer positive broths to PHL
2. O157 Culture and STEC-EIA only stools ordered by healthcare providers
3. O157 Culture all stools but do STEC-EIA only on request

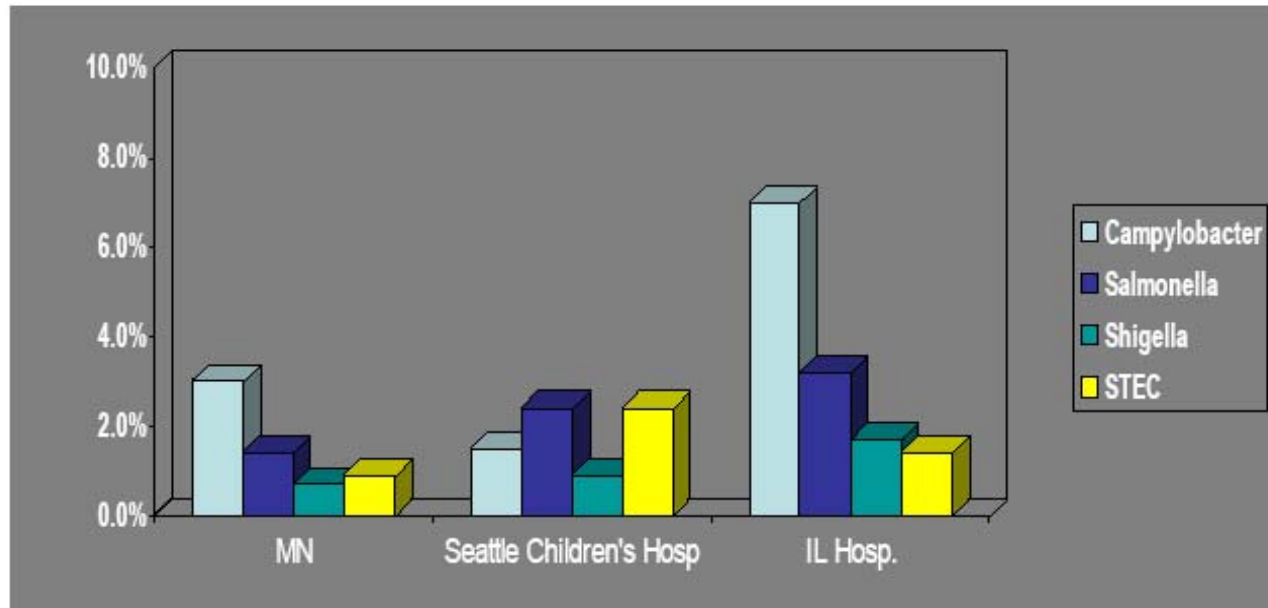
Test all stools with SMAC and STEC-EIA:

- Manning, EID Feb. 2007 - Michigan
 - Detected additional 66 cases in 5 yr, 47% non-O157

Best Practice Recommendation

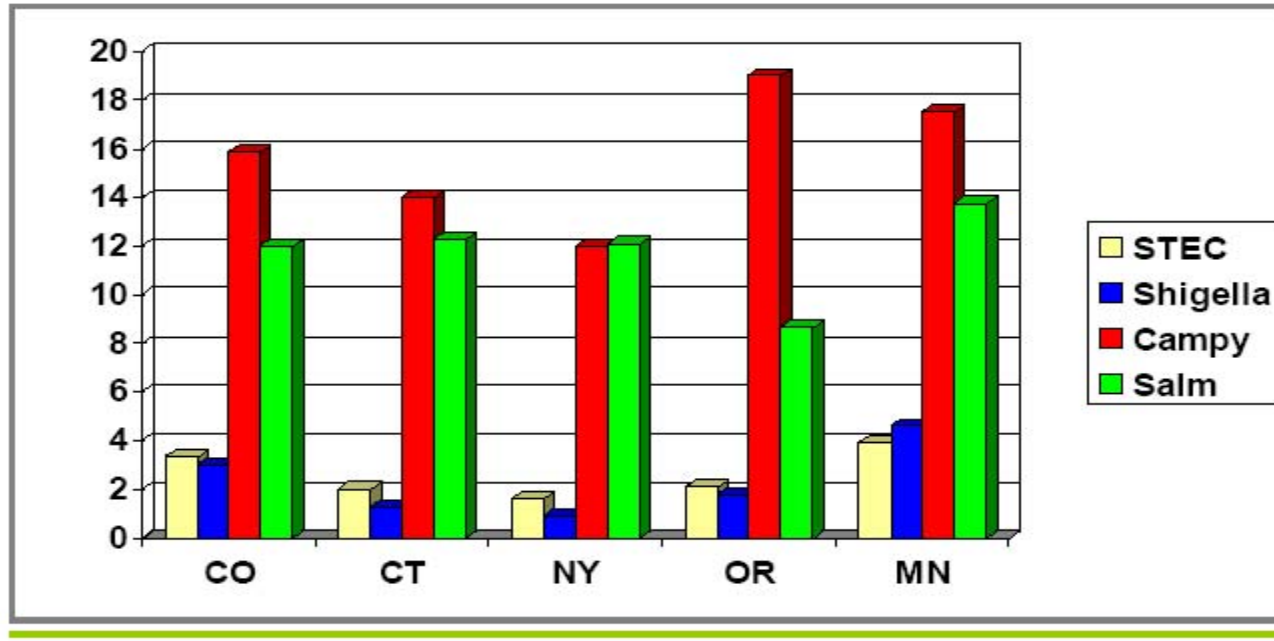
- Simultaneous culture for O157 STEC and toxin assay for STEC
- All stools submitted for diagnosis of community acquired diarrhea (Salmonella, Shigella, Campylobacter) should also include culture for *E. coli* O157:H7 and toxin assay for non-O157 STEC
- Prompt reporting of STEC to physician
- Forward all positives to public health lab ASAP

STEC Infections Are Not Rare Events



Besser et al, unpublished data (1995-1999 study)
Klein et al, CID 43: 807, 2006 (1998-2001 study)
Gavin et al, JCM 42:1652, 2004 (2001-2002 study)

Incidence of Laboratory-Confirmed Infections at Selected FoodNet Sites, 2007



Evidence to Support Recommendations

Culture on selective and differential media plus EIA testing for Shiga toxin yield more positive infections than either alone.

- Cohen, J. Ped. 141: 155-156, 2002
- Klein, J. Ped. 141: 172-177, 2002
- Gavin, J. Clin. Micro. 42:1652-1656, 2004
- Kehl, J. Clin. Micro. 35:2051-2054, 1997

Evidence to Support Recommendations

Absence of blood in stool does not rule out STEC:

- In some studies most STEC regardless of serotype were in patients with non-bloody diarrhea

STEC more frequent in children but almost half of all isolates are from persons >12 yr

Infections more common in summer but outbreaks occur year-round

WBCs not always observed in stool of patients with STEC

Evidence to Support Recommendations

Prompt diagnosis and submission to PHL allows for timely public health action

- Prevent secondary transmission in daycare or nursing home facilities, food service, home
- Detect STEC serogroup and PFGE pattern to trace outbreaks
- Detect multi-state outbreaks related to widely distributed food products

Criteria for Specimen Selection

- Collect specimen as early as possible in course of the disease (hard to find >7d)
- 3 day rule: diarrhea starts after 3 days in hospital, think *C. difficile*
- STEC an unlikely cause of chronic diarrhea
- Multiple stool samples unnecessary
- Don't retest stools once positive detected
 - Follow-up may be required for food handler, daycare, institution to show no longer shedding, send to PHL

Non-O157 STEC Infections

In non-outbreaks studies of STEC cases:

- 46% non-O157 STEC
- 54% O157 STEC
- 36% of patients with HUS lacked blood in stool

Ref: Johnson et al. *CID* 43: 1587, 2006

Clinical Presentation of Patients with *E. coli* non- O157

Longer duration of diarrhea (9.1 d vs. 5.7 d)

- Bloody diarrhea less common (42% vs. 97%)
- Abdominal pain, vomiting, fever $>38^{\circ}\text{C}$

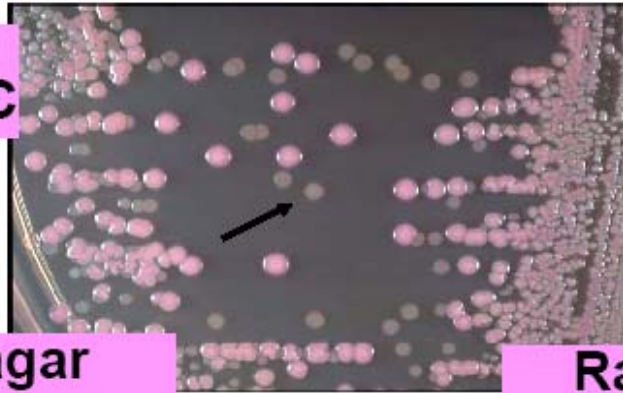
Rates equal to *E. coli* O157

- Less likely to progress to HUS
 - 7-15% O157 cases vs. 1% non-O157 cases

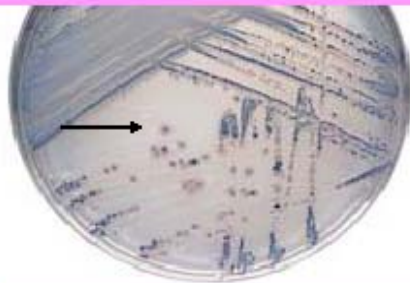
Some serogroups (O103, O111) account for significant # HUS cases in the U.S.

Isolation Media to Recover *E. coli* O157

SMAC
CT-SMAC



CHROMagar
O157



Rainbow Agar
O157



Rapid Tests to Detect Stx 1 and 2

- Premier EHEC- stool, broth, isolate
- ProSpecT Shiga Toxin- stool, broth
- Duopath Verotoxin GLISA -colony sweeps
- ImmunoCard STAT! EHEC - broth
- BioStar OIA SHIGATOX - stools, colony sweeps, broth

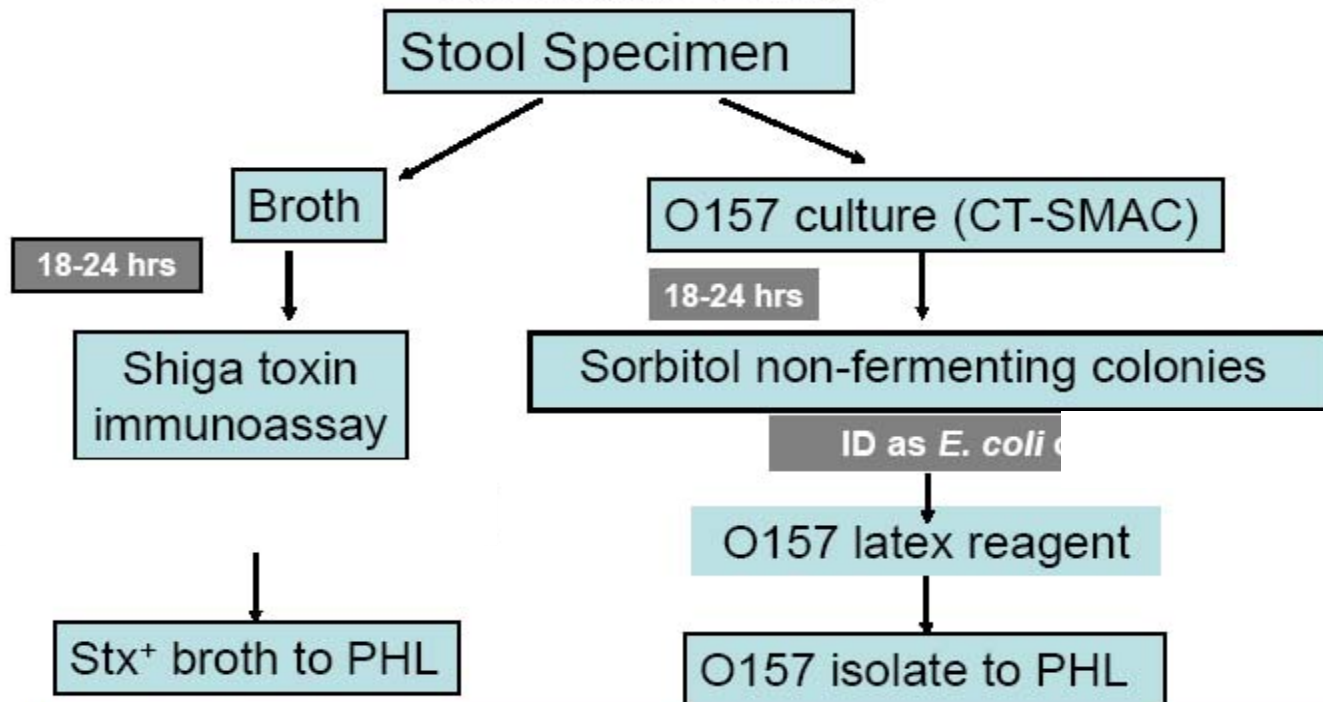


Manufacturers' Stated Sensitivity

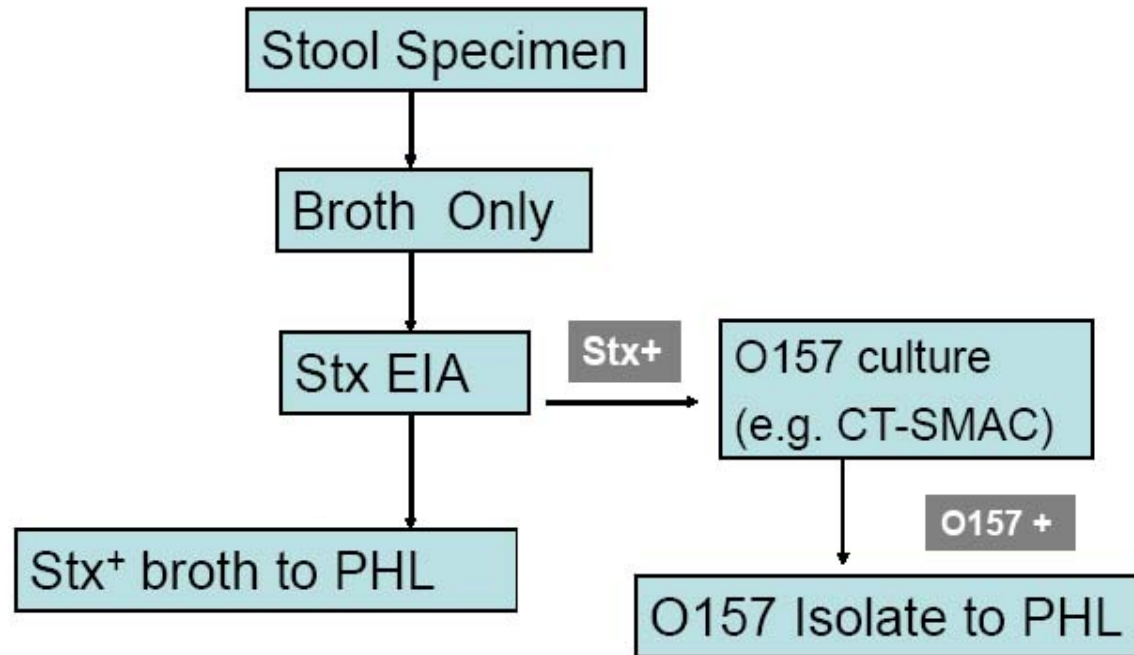
Sample	Premier EHEC (cytotoxin assay standard)	Prospect STEC (cytotoxin assay standard)	Immunocard STAT! EHEC <small>(Premier EHEC assay standard)</small>	Duopath Verotoxin <small>(Premier EHEC assay standard)</small>
Stool	79%	92%	NA	NA
Cary Blair	Not stated	Not stated	NA	NA
Stool Broth Enrichments	100%	99%	NA	NA
Sweeps of Agar Growth	93%	NA	100%	100%

Can use for Broth Enrichments

Algorithm for STEC Detection: Clinical Labs



Alternate Algorithm for Clinical Labs



What Clinical Microbiology Laboratories Need to Know

SMAC is not enough

EIA has false positives and false negatives

Importance of promptly communicating positive results to the physician

Participate in PT program (API, CAP)

Importance of submitting an organism / toxin positive broth to Public Health Lab

Clostridium difficile: Refresher

- *Clostridium difficile* is a bacterium that can cause intestinal symptoms ranging from diarrhea to life-threatening inflammation of the colon.
- Illness from *C. difficile* most commonly affects older adults in hospitals or in long term care facilities and typically occurs after use of antibiotic medications.

Clostridium difficile: Refresher

- *C. difficile* infections:
 - Becoming more frequent, more severe, and more difficult to treat.
- Incidence:
 - 10,000s of people in the United States
 - Responsible for 15-20% of antibiotic related diarrhea cases; & almost 100% of pseudo-membranous colitis
- Treatment:
 - Mild diarrhea (definition = 3x/day, > 1 day) w/ mild abdominal cramping and tenderness = d/c antibiotics, flagyl
 - More severe diarrhea →

Clostridium difficile: Refresher

Severe cases:

- Colon becomes inflamed (colitis)
- Patches of raw tissue form that bleed/pus → pseudomembranous colitis

Severe symptoms:

- Severe abdominal cramping and pain
- Watery diarrhea 10-15x/day
- Fever
- Blood and/or pus in the stool
- Nausea
- Dehydration
- Loss of appetite, weight loss

Severe treatment:

- Vancomycin, fecal transplant

Clostridium difficile: Refresher

- **Mortality/Morbidity:**
 - Leading cause of infectious diarrhea in hospitalized patients
 - ~\$4000-7000 per episode; ~ 3-5.5 additional days in hospital
 - US Mortality has increasing significantly
 - Projections for management/direct costs: 3.4 bill/yr
- **Major risk factors include antimicrobial exposure, but...**
 - Starting to include otherwise healthy people who aren't hospitalized or taking antibiotics (no risk factors) including children and pregnant women

Clostridium difficile: Refresher

- Epidemic strain
 - NAP1/027/BI = produces significantly larger amounts of toxin
 - Associated with hospital outbreaks (fecal-oral)
 - Significant increase in the morbidity and mortality
 - Incidence of infection increased from 0.5% in 2004-2005 to 1.6% in 2008.

Increase in incidence/mortality rates + Pts w/unknown risk factors + Changes in the virulence of *C. difficile*

= A new strategy for laboratory testing

Clostridium difficile: Refresher

Epidemic strain

- Uncommon strain which produces higher amount of toxins A and B, is becoming more common
- These strains are often associated with outbreaks of infections (“epidemic strain”)
 - Epidemic strain produces 16-23x more toxin
 - Epidemic strain carries binary toxin in addition to toxin B.
- Epidemic strains are 'R' to fluoroquinolones
- Transmission is mainly via fecal-oral route
 - Contaminated environment
 - Hands of healthcare personnel

Clostridium difficile: Detection

- **Enzyme Immunoassay (EIA) for Toxins:**
 - Common rapid assay for toxin A/B detection
- **Glutamate Dehydrogenase (GDH):**
 - Common antigen for *C. difficile* & a few other anaerobes
 - Used as a screening test - requires confirmation
- **Direct Cell Culture Cytotoxin Neutralization**
 - Old "*Gold Standard*"
- **Toxigenic Culture (Culture + Cytotoxin Assay)**
 - New/Current "*Gold Standard*"
- **PCR**

Clostridium difficile: Toxin Detection

EIA Toxin Product	Sensitivity	Specificity	Gold Standard Used	Publication
Premier Toxin A/B (Meridian)	99%	97%	Cytotoxin Assay	Musher et al, JCM, 2007
ImmunoCard Toxin A/B (Meridian)	91%	97%	Cytotoxin Assay	Berg et al, JCM 2005
TechLab C.diff TOX A/B II	87%	97%	Cytotoxin Assay	Novak et al, CVI 2008
Premier Toxin A/B (Meridian)	48%	98%	Toxigenic Culture	Sloan et al, JCM 2008
ImmunoCard Toxin A/B (Meridian)	67%	95%	Toxigenic Culture	Alcala et al, JCM 2008
TechLab C.diff TOX A/B II	80%	100%	Toxigenic Culture	Alcala et al, JCM 2008
Wampole Tox A/B Quick Check	55%	96%	Toxigenic Culture	Alcala et al, JCM 2008
Remel X/pect C.difficile toxin A/B	49%	96%	Toxigenic Culture	Alcala et al, JCM 2008

Clostridium difficile: Detection

Glutamate Dehydrogenase (GDH) =

- > sens = ~87% to >99% (GDH result
neg = neg CDIFF)

Toxigenic and non-toxigenic *C. difficile* produce GDH

- Detection of GDH therefore requires a test to detect toxin production

Clostridium difficile: Detection

- The detection of *C. difficile* disease evaluating:
 - New CDIFF Complete assay
 - a combination *lateral flow* assay for glutamate dehydrogenase antigen and A/B toxins (results in ~30 minutes)



Ag = GDH
Tox = Toxin B

Clostridium difficile: Detection

Algorithm	Publication
GDH + EIA + Cytotoxin Assay	Snell et al, JCM 2004
GDH + Cytotoxin Assay	Tichurst et al, JCM 2006
GDH + EIA + Cytotoxin Assay	Reyes et al, DMID 2007
GDH + Cytotoxin Assay + Toxigenic Culture if needed	Reller et al, JCM 2007
GDH + Cytotoxin Assay	Gilligan, JCM 2008
GDH + EIA + broth-Toxigenic Culture if needed	Sharp, DMID 2009

Clostridium difficile: Detection

- PCR assay FDA approved
 - BD GeneOhm™ Cdiff Assay
 - Prodesse proGASTRO™
 - Cepheid Xpert® *C. difficile*

Clostridium difficile: Detection

- **BD GeneOhm: (batch test; sample prep required)**
 - Package Insert, compared to cytotoxin assay
 - Sens 93.8%, Spec 95.5%
 - Stamper et al., 2009, JCM; assay compared to **toxigenic culture**:
 - Sens = 83.6%, Spec = 98.2%
- **Prodesse: (batch test; sample prep required)**
 - Package Insert, compared to cytotoxin assay
 - Sens 91.7%, Spec 94.7%
 - Gluck et al., 2009 CVS Poster; assay compared to **toxigenic culture**:
 - Sens = 73.3%, Spec = 99.2%
- **Cepheid-GeneXpert: (random access; no sample prep)**

Xpert C.difficile



1. Insert swab into Elution reagent vial and break at score



2. Vortex and dispense Sample into port S



3. Dispense Reagent 1 into port 1



4. Dispense Reagent 2 into port 2



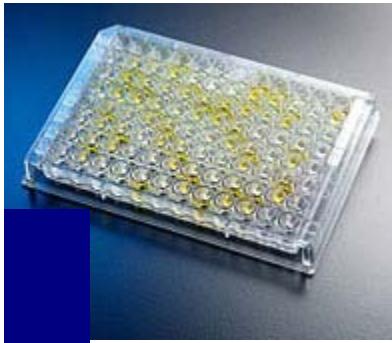
Total hands-on time = 2 minutes

Results ready in ~ 2 hours.



5. Insert cartridge and start assay

Clostridium difficile: KP-NW Study



GDH EIA



LF TOX A/B



T.CULTURE



GDH/TOX FL combo



Xpert ToxB gene

Clostridium difficile: KP-NW Study:

[Sharp, SE et al. 2010 J Clin Microbiol 48(6):2082-6.]

- 284 samples submitted for *C. difficile* toxin testing
 - All tested by:
 - CDIFF COMPLETE LF assay (2 tests: GDH + TOX A/B)
 - GDH EIA assay (96 well plate)
 - CDIFF A/B toxin LF assay
 - Xpert *C. difficile* PCR
 - If positive for GDH & toxin & PCR = Positive for *C. difficile* toxin
 - If all negative for all 5 tests = Negative for *C. difficile* toxin
 - If discrepant:
 - ↓
 - **GOLD STANDARD:** Toxigenic culture

Clostridium difficile: KP-NW Study

- 27 specimens tested + for GDH and toxin and PCR assay → True Positives
- 224 were negative by all five tests → True Negatives
- 33 had discrepant results
 - ↓
 - Toxigenic culture

Clostridium difficile: KP-NW Study

Results of initial 33 discrepant specimens.

GDH	GDH-comp	TOX	TOX-comp	PCR	# with Initial Results	# + by Toxig. Culture	# of True Pos
+	+	-	-	+	15 ^a	15	15
+	+	-	-	-	10	0	0
+	-	-	-	-	3	0	0
-	+	-	-	-	2	0	0
-	+	+	+	-	1	0	0
-	-	+	+	-	1	0	0
-	-	-	-	+	1 ^a	0	0

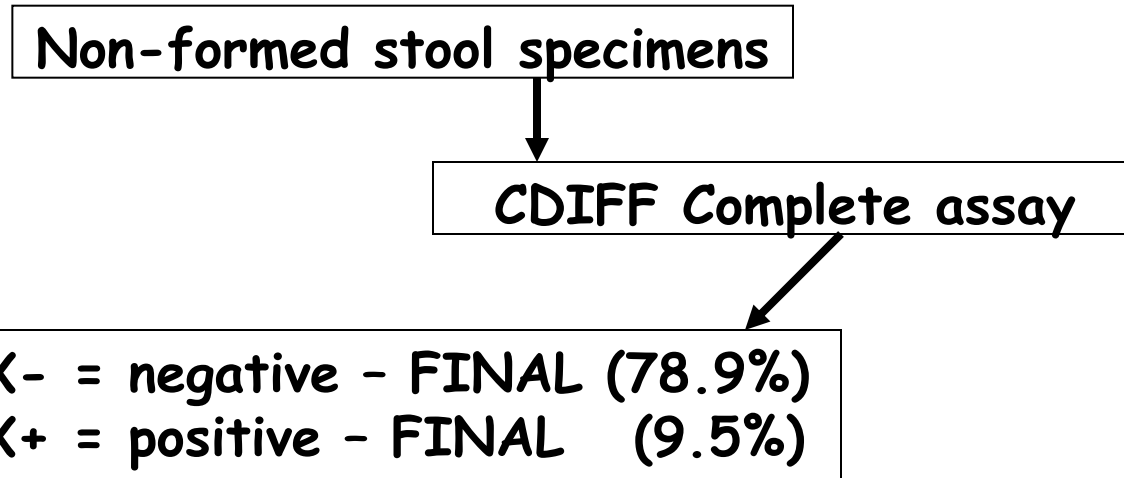
Clostridium difficile: KP-NW Study

Sensitivity & Specificity of study assays.

ASSAY	Sensitivity (%)	Specificity (%)
PCR	100	99.6
GDH	100*	94.2
GDH-comp	97.6*	94.6
TOX-A/B	59.5	99.2
TOX-comp	61.9	99.2
CDIFF Complete	60.0	99.6

*GDH sens: other studies compared to tox. cult: 87-100%

Clostridium difficile: Our algorithm



Clostridium difficile: Our algorithm

Non-formed stool specimens

CDIFF Complete assay

COMP GDH-/TOX- = negative - FINAL (78.9%)
COMP GDH+/TOX+ = positive - FINAL (9.5%)

**88% of samples
resulted
w/in 30 minutes**

Clostridium difficile: Our algorithm

Non-formed stool specimens

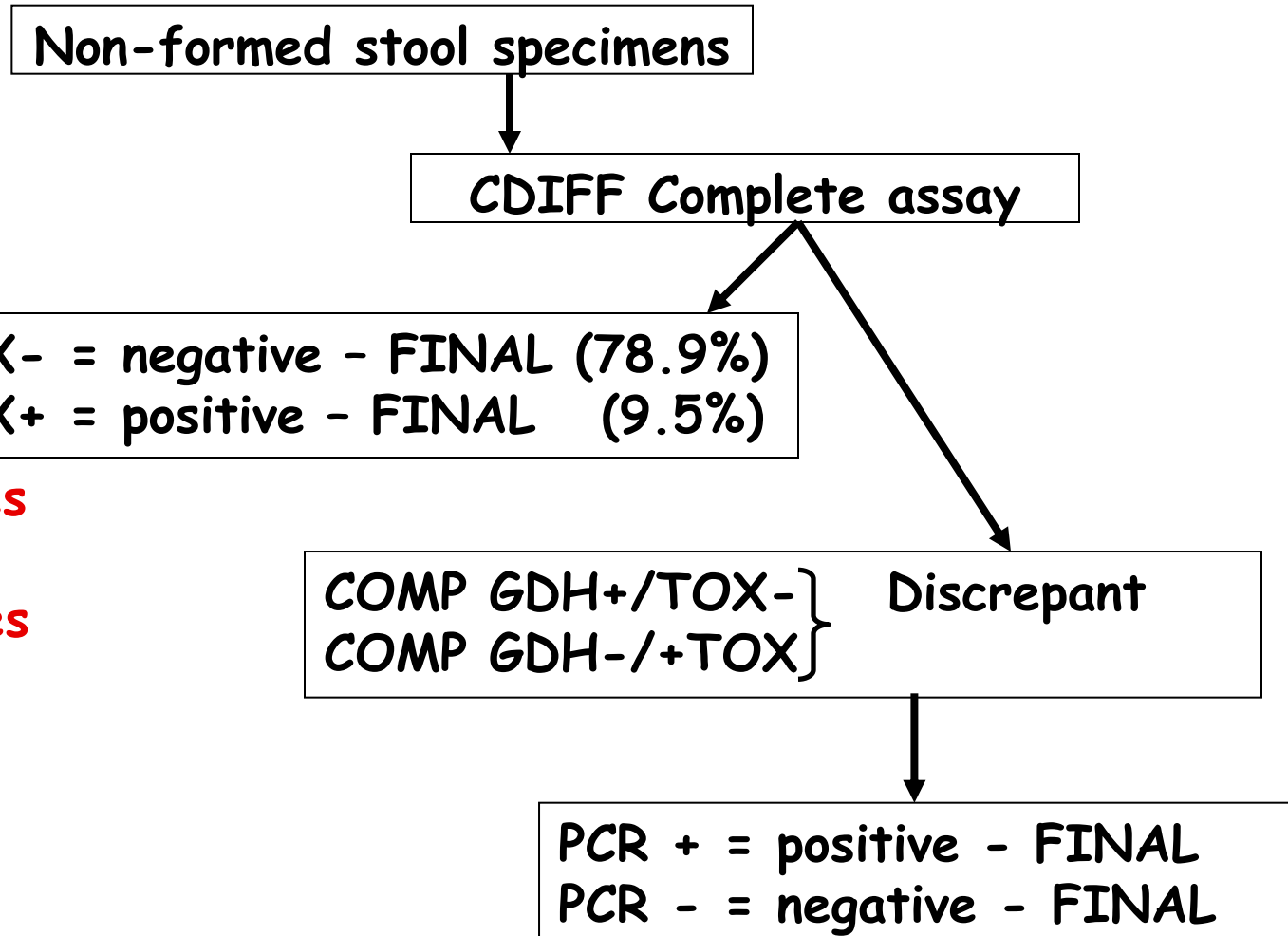
CDIFF Complete assay

COMP GDH-/TOX- = negative - FINAL (78.9%)
COMP GDH+/TOX+ = positive - FINAL (9.5%)

**88% of samples
resulted
w/in 30 minutes**

COMP GDH+/TOX- } Discrepant
COMP GDH-/TOX+ }

Clostridium difficile: Our algorithm



**88% of samples
resulted
w/in 30 minutes**

Clostridium difficile: Our algorithm

Non-formed stool specimens

CDIFF Complete assay

COMP GDH-/TOX- = negative - FINAL (78.9%)
COMP GDH+/TOX+ = positive - FINAL (9.5%)

88% of samples
resulted
w/in 30 minutes

COMP GDH+/TOX- } Discrepant
COMP GDH-/TOX+ }

100% of samples
resulted
w/in 3 hours

PCR + = positive - FINAL
PCR - = negative - FINAL

Clostridium difficile

- **GDH assays proved to be highly sensitive in our study with sensitivities of 97.6-100%.**
 - This agrees with other studies concluding that the GDH is an excellent screening test (Reller JCM '07, Gilligan JCM '08, Sharp DMID '09),
- **Contrast to others who have reported lower sensitivities of GDH assays**
 - Between 70-88% (Alcala JCM '08; Sloan JCM '08; Peterson & Robisek AIM '09, Eastwood JCM '09, Kvach JCM09; Novak, personal communication).
- **These differences might possibly be due to:**
 - Regional/geographical differences in ribotype strains affecting the GDH assays (indicating the possibility of a limited number of strains in our area),

OR

 - Freezing and thawing of stool samples prior to testing in the GDH assay.

Clostridium difficile

- Freeze-thaw cycle: differences in GDH sensitivity
 - Tested 27 frozen & then thawed + GDH stool samples:
 - GDH EIA assay
 - 26 of the 27 specimens again tested positive
 - loss of 3.7% positivity
 - COMP-GDH assay
 - 24 of the 27 specimens tested positive
 - loss of 11.1% positivity
 - Although based on a small number of repeat samples:
 - if using fresh stool specimens either assay can be used
 - if samples are frozen prior to testing, GDH-EIA assay is preferred

Clostridium difficile

- The improved sensitivity provided by this algorithm might preclude the need for repeating patient testing. *
- A recent study found little value in repeat testing of specimens within 7 days when utilizing Xpert *C. difficile* PCR. **
- We found if repeat testing were not incorporated for up to 3 wks, approximately 25% of testing could be eliminated.
 - The time exclusion of repeat patient testing should be determined by the laboratory in consultation with Infectious Diseases and Infection Control.
- **References:**
 - *Petersen & Robisek, *Annals Internal Med* 151:176-179 - 2009.
 - **Aichinger, et al; *JCM* 46:3795-3797- 2008.

Clostridium difficile

Contact Isolation protocol (prior to study)

- Isolate patients upon test order
- TAT: 1-3 days for results
- Batch screen w/GDH 1x/day → LF Toxin → broth culture
- **New Contact Isolation protocol**
- Isolate patients upon a call of a + test result (within 3 hrs)
 - Nursing costs saved
 - Material costs of contact isolation saved
 - = Total annual cost savings
- **Adverse affects of isolation...**

SUMMARY:

- **CDIFF**

- Testing:
 - Screen first: GDH or GDH+Toxin AB EIA/LF
 - Confirm: PCR for timely confirmatory testing
 - **Accuracy: Compare algorithms to toxigenic culture**
- IP/OP diarrhea
- For cost-effective, accurate, timely diagnosis of CDIFF disease

- **STEC**

- Do SMAC & Shiga toxin testing per CDC

- **Campy**

- Look at possible EIA for work flow; speak to your state laboratory colleagues.



THE "ACM" GRAND SLAM

Wish me luck!

