Diagnostics for Group B *Streptococcus*: The role of the Clinical Microbiology Laboratory in Prenatal Screening

Gerald A. Capraro, PhD, D(ABMM)
Carolinas Pathology
Medical Director, Clinical Microbiology Laboratory
Atrium Health
Charlotte, NC
July 18, 2019

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Atrium Health

69,800+
Teammates

50
Hospitals*

44
Urgent Care Locations

45
EDs

25
Cancer Care Locations

3,705+
Physicians

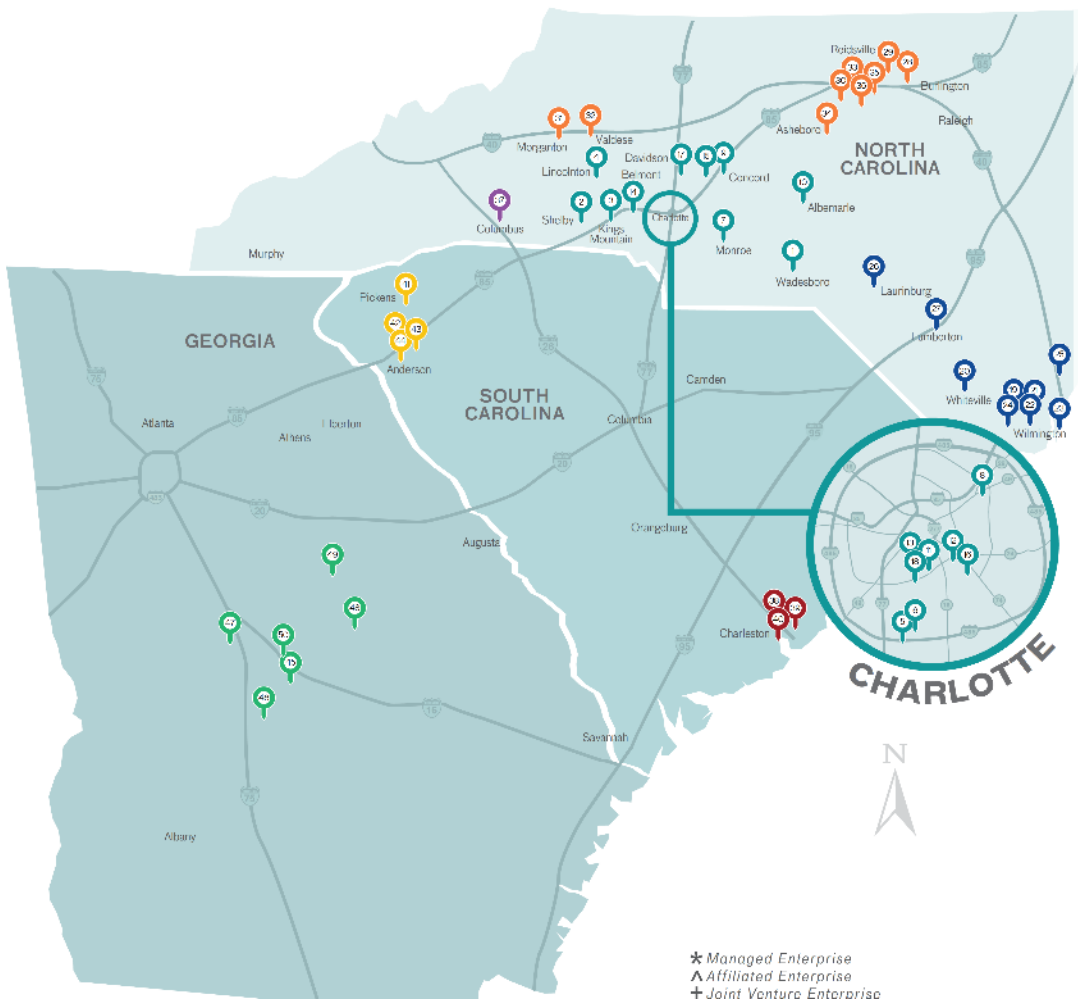
17,000+
Nurses

$11.1 Billion
Net Operating Revenue

$2.9 billion
In last 5 years

Invested into renovations, new care locations, equipment upgrades and other capital projects

2019 Facilities & Locations



* Managed Enterprise
^ Affiliated Enterprise
+ Joint Venture Enterprise

CHARLOTTE
1. Atrium Health Anson
2. Atrium Health Cleveland
3. Atrium Health Kings Mountain
4. Atrium Health Lincoln
5. Atrium Health Pineville
6. Atrium Health Pineville Rehabilitation Hospital
7. Atrium Health Union
8. Atrium Health University City
9. Carolinas HealthCare System NorthEast
10. Carolinas HealthCare System Stanly
11. Atrium Health's Carolinas Medical Center
12. Carolinas Medical Center-Mercy
13. Carolinas Rehabilitation
14. Carolinas Rehabilitation-Mt. Holly
15. Carolinas Rehabilitation-NorthEast
16. CHS Behavioral Health-Charlotte
17. CHS Behavioral Health-Davidson
18. Levine Children's Hospital

COASTAL (NORTH CAROLINA)
19. Betty H. Cameron Women's and Children's Hospital^
20. Columbus Regional Healthcare System*
21. New Hanover Regional Medical Center^
22. New Hanover Regional Medical Center Behavioral Health^
23. New Hanover Regional Orthopedic Hospital^
24. New Hanover Regional Rehabilitation Hospital^
25. Pender Memorial Hospital^
26. Scotland Memorial Hospital*
27. Southeastern Regional Medical Center^

TRIAD
28. Alamance Regional Medical Center (Cone Health)*
29. Annie Penn Hospital*
30. Behavioral Health Hospital (Cone Health)*
31. CHS Blue Ridge-Morganton^
32. CHS Blue Ridge-Valdese*
33. Moses H. Cone Memorial Hospital (Cone Health)^
34. Randolph Hospital*
35. Wesley Long Hospital*
36. Women's Hospital (Cone Health)*

WESTERN
37. St. Luke's Hospital*

LOW COUNTRY (SOUTH CAROLINA)
38. Bon Secours/St. Francis Hospital*
39. Mount Pleasant Hospital*
40. Roper Hospital*

UPSTATE
41. AnMed Health Cannon^
42. AnMed Health Medical Center*
43. AnMed Health Rehabilitation Hospital*
44. AnMed Health Women's and Children's Hospital*

CENTRAL (GEORGIA)
45. The Medical Center, Navicent Health
46. Medical Center of Peach County (Navicent Health)
47. Monroe County Hospital (Navicent Health)*
48. Navicent Health Baldwin
49. Putnam General Hospital (Navicent Health)*
50. Rehabilitation Hospital, Navicent Health

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Laboratory Scope of Services

Atrium Health Laboratory provides testing services to Acute Care Facilities (metro), Physician Office Practices (outreach), and free-standing Emergency Departments.

Testing locations

- 12 Acute Care Hospitals
- 6 Free Standing ED Laboratories

Phlebotomy Services

- 19 Patient Services Centers (Locations for outpatient blood draws)
- 16 physician practices
- 21 skilled nursing facilities

Reference Laboratory Testing

- 2,978 providers located in 938 medical practices

Laboratory Departments

1) Hematology
2) Chemistry/Toxicology
3) Microbiology
4) Histology/Cytology
5) Blood Bank
6) Cytogenetics
7) Molecular Diagnostics
8) HLA Transplant
9) Coagulation

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Centralized Microbiology Laboratory

Full Service Lab

- Bacteriology
- Mycobacteriology
- Mycology
- Virology
- Molecular Micro: *C. difficile*, positive blood cultures
- Minimal parasitology: EIA, WORM, INSECT

Core Lab

- Levine Cancer Institute
- Levine Children's Hospital
- Reference Lab for non-system hospitals in the region

- Teaching: CLS students, Peds ID residents/MSIV, Pharmacy residents
- Frequent site for method comparison and workflow studies
- High sample volume allows utilization of continuous flow

TEST	Annual Volume
Urine Cultures	300,000
GAS Cultures	82,000
Exudate/Wound Cultures	31,000
Respiratory Cultures	29,000
GBS Screens	21,000
Positive Blood Cultures	17,000
Sterile Fluid Cultures	16,000
Stool Cultures	14,000
C. difficile Testing	14,000
Fungal Cultures	13,000
Anaerobic Cultures	10,000
Virology Cultures	9,000
AFB Cultures	7,000
Parasitology Testing	6,000
MRSA Screens	4,000

Microbiology Testing Schedule

TEST	1st SHIFT	2nd SHIFT	3rd SHIFT
Hood Processing	X	X	X
Processing of Positive Blood Cultures	X	X	X
Gram Stains	X	X	X
Cdiff EIA	X	X	X
Respiratory Bench	X		
Anaerobe Bench	X		
Blood Bench	X	X	(x)
Virology	X		
AFB	X		
Mycology	X		
Urine Bench	X	X	
Exudate Bench*	X	X	
Fungal Smears	X	X	
Stool Bench		X	
Cdiff PCR		X	
AFB Processing/Smears		X	X
Fecal Lactoferrin	X		X
Rotavirus Antigen	X		X
Trichomonas Antigen	X		X
Giardia/Cryptosporidium EIA	X		X

Microbiology Staffing

- We staff 24/7 and modify based on volume demand

Technologists per shift per day of the week:

Shift	M – T	W – F	Sat	Sun
1st	10	12	8	7
2nd	8	10	4	4
3rd	4	4	2	2

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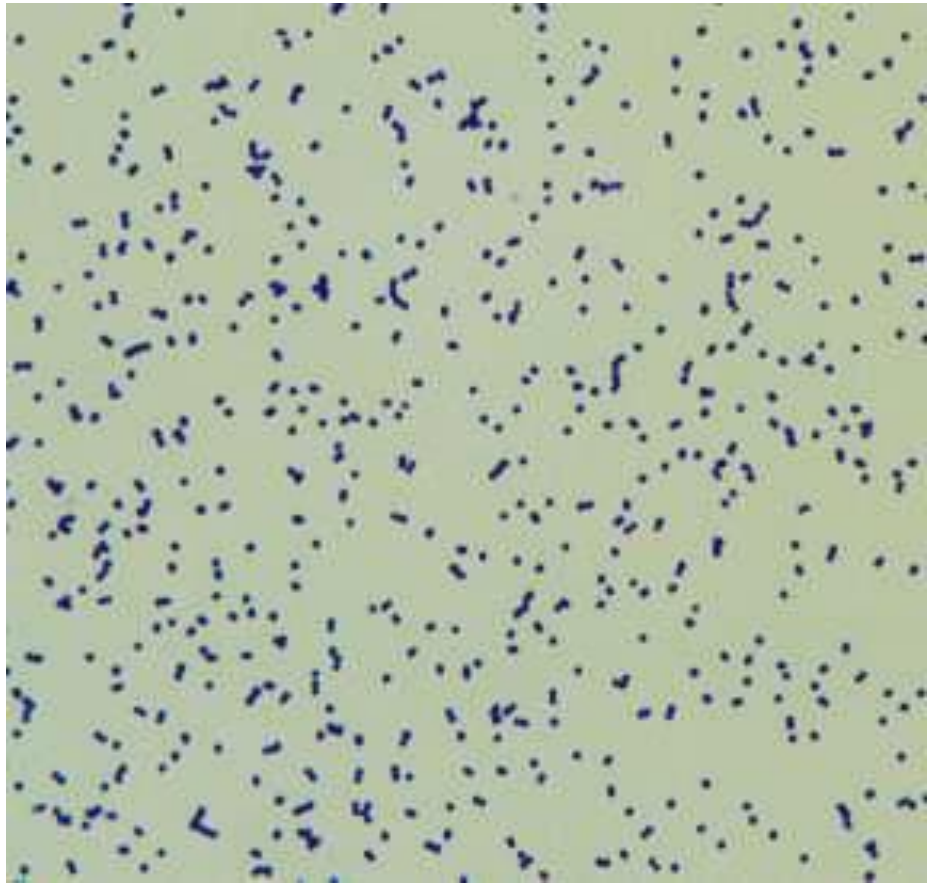
Group B *Streptococcus*

- *Streptococcus agalactiae*
- Virulence factors
  - Capsule, C’ inactivation factors, hemolysins
- Normal habitat – GI Tract, Vagina, Upper Respiratory Tract
- Females – 10 – 40% vaginal and/or rectal carriers – duration unpredictable
- Clinical Relevance
  - Serious infections in newborns and adults (esp., > 65 yo)
  - Bacteremia, pneumonia, SSTI, bone/joint infections
- Risk factors for infection:
  - Diabetes mellitus
  - HIV infection
  - Cancer
  - Advanced age



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Group B *Streptococcus*

Neonatal Infections

- Leading cause of neonatal pneumonia, meningitis, and sepsis
- Early- and Late-onset disease

Early-onset Disease

- Within 6 days of birth
- 0.1 – 0.6 cases/1000 births in 2015
- Bacteremia (60%), pneumonia (30%), meningitis (10%)
- Mortality rate: 4 – 6%
    - Down from 50% in 1970s
    - Rate inversely proportional to birth weight

	Early-Onset		Late-Onset	
Race	**No.**	**(Rate*)**	**No.**	**(Rate*)**
White	49	(0.17)	73	(0.25)
Black	41	(0.43)	54	(0.56)
Other	11	(0.24)	6	(0.13)
	101	**(0.23)**	**133**	**(0.31)**

* Per 1,000 live birth for ABCs areas

CDC. 2017. Active Bacterial Core Surveillance Report, Emerging Infections Program Network, GBS 2017.

Late-onset Disease

- 7 days – 3 months of age (mean 24 days)
- 0.2 – 0.7 cases/1000 live births in 2015
- Occult bacteremia or meningitis
- Source of infection – vertical transmission, non-maternal sources, nosocomial
- Predisposing factors – prematurity (< 34 wks)
- Mortality rate – 3%

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Group B *Streptococcus*

Early-onset Disease

- Factors associated with increased vertical transmission:
  - Heavy colonization at term (≥50%)
  - Prolonged rupture of membranes (≥ 18 hrs)*
  - Prematurity (< 37 wks)*
  - Intrapartum fever (≥ 38ºC)*
  - *Maternal bacteriuria w/ GBS during pregnancy***
  - *Previous delivery of GBS-infected infant***

* Key elements of risk-based prevention approach

** Absolute indication for intrapartum antibiotics

Prevention of Neonatal Disease

- Prenatal vaginal/rectal cultures @ 35-37 wks for **ALL** pregnant women (screening approach)
- If no prenatal culture results available at term – prophylaxis for those at high risk (risk-based approach)

CDC. Prevention of perinatal GBS disease: revised guidelines. MMWR 2010; 59 (No. RR-10): 1 – 32.

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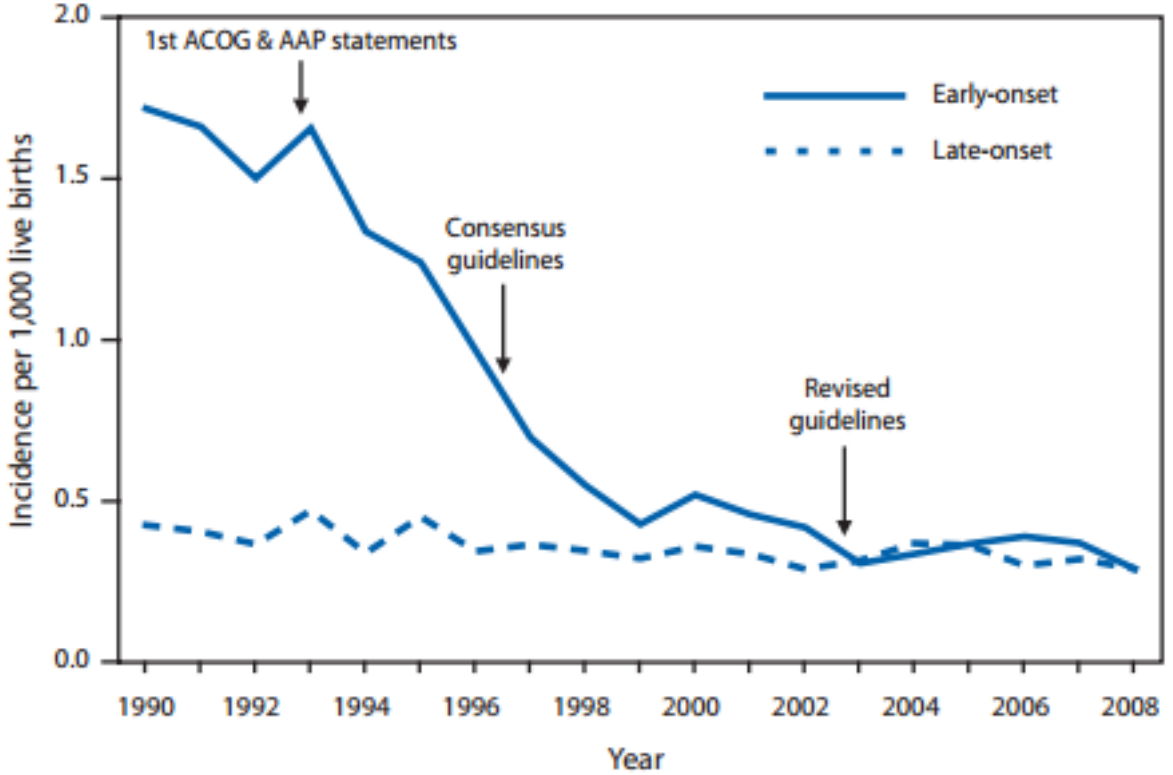
Surveillance for GBS Colonization

- GBS is normal flora of GIT
  - Sexual activity increases risk of vaginal colonization
  - Ethnicity (Blacks > Whites > Hispanics)
  - Higher income, education level
  - High BMI
  - Healthcare occupation
  - Use of tampons or IUDs
  - Absence of lactobacilli in GIT

- Screening of pregnant women at 35 – 37 weeks gestation

FIGURE 1. Incidence of early- and late-onset invasive group B streptococcal (GBS) disease — Active Bacterial Core surveillance areas, 1990–2008, and activities for prevention of GBS disease



Abbreviations: ACOG = American College of Obstetricians and Gynecologists and AAP = American Academy of Pediatrics.

Source: Adapted from Jordan HT, Farley MM, Craig A, et al. Revisiting the need for vaccine prevention of late-onset neonatal group B streptococcal disease. Pediatr Infect Dis J 2008;27:1057–64.

* Incidence rates for 2008 are preliminary because the live birth denominator has not been finalized.

November 2010

Oddie & Embleton. 2002. BMJ 325: 308 – 312.
Stapleton *et al*. 2005. Obstet & Gynecol. 106: 1246 – 1252

CDC. Prevention of perinatal GBS disease: revised guidelines. MMWR 2010; 59 (No. RR-10): 1 – 32.

Risk Based Prevention

Intrapartum GBS Prophylaxis Indicated:

- Previous infant with invasive GBS disease
- GBS bacteriuria during any trimester of the current pregnancy
- Positive GBS vaginal-rectal screening culture in late gestation (35 – 37 weeks) during current pregnancy
- Unknown GBS status at the onset of labor (culture not done, incomplete, or results unknown) and any of the following:
  - Delivery at <37 weeks gestation
  - Amniotic membrane rupture >= 18 hours
  - Intrapartum temperature >=100.4°F (>=38.0°C)
  - Intrapartum NAAT positive for GBS
  - Known GBS positive status in a previous pregnancy

CDC. Prevention of perinatal GBS disease: revised guidelines. MMWR 2010; 59 (No. RR-10): 1 – 32.

ACOG Committee Opinion. 2019. Obstet & Gynecol 134: e19 – 40.

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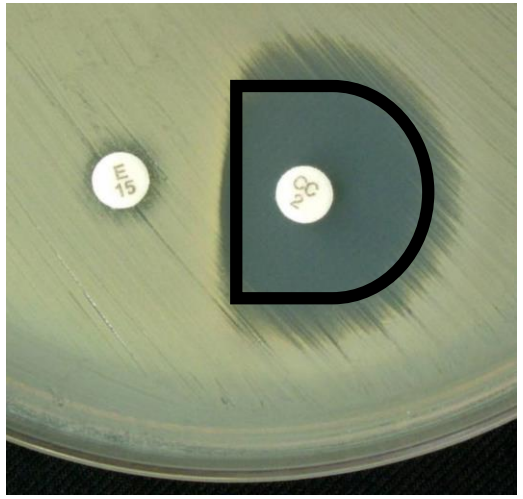
Laboratory Surveillance for GBS

ACOG Committee Opinion. 2019. Obstet & Gynecol 134: e19 – 40.

New ACOG Guidelines:

- Collect vaginal-rectal swab at 36 – 38 weeks
- Communicate beta-lactam allergy for AST
- Culture in enrichment broth
- Subculture to agar, followed by ID using:
  - Latex agglutination with group B antisera
  - Chromogenic agar
  - DNA probes
  - NAAT
- D-test for inducible clindamycin resistance testing



Box 2. Transport and Laboratory Processing of Vaginal–Rectal Swab Specimen for Group B Streptococcus During Pregnancy

Place the swab(s) into a nonnutritive transport medium (eg, Stuart or Amies medium with or without charcoal). Group B streptococcus (GBS) isolates can remain viable in transport media for several days at room temperature; however, the recovery of isolates declines within 1–4 days, especially at elevated temperatures, which can lead to false-negative test results.

- Specimen requisitions should clearly indicate that specimens are for GBS culture obtained from a pregnant woman. If the woman reports an allergy to penicillin, the laboratory requisition that accompanies the screening GBS culture should be marked for the laboratory to ensure that appropriate testing of any GBS isolates for susceptibility is performed. If a woman is determined to be at high risk of anaphylaxis to penicillin, susceptibility testing for clindamycin should be ordered.
- Laboratories will process sample swabs identified as intended for GBS culture by incubating first in appropriate selective enrichment broth to optimize sensitivity of subsequent culture results.
- After incubation in enrichment broth, a subculture is made onto blood agar plates, followed by identification of any bacterial colonies as GBS using latex agglutination with group B antisera, chromogenic agars, DNA probes, or nucleic acid amplification tests.
- Inducible resistance to clindamycin is detected by the D-zone test, which tests the isolate for resistance to clindamycin.*

*Determination of susceptibility to clindamycin typically also includes analysis by the D-zone test which indicates the presence of inducible resistance from macrolides including erythromycin. This macrolide-induced resistance is produced through an induced enzyme that alters the common ribosomal binding site for macrolides and clindamycin, resulting in clindamycin failure (Woods CR. Macrolide-inducible resistance to clindamycin and the D-test. Pediatr Infect Dis J 2009;28:1115–8.) Therefore, in vitro susceptibility or resistance to erythromycin may be reported as a laboratory adjunct to clindamycin testing. If reported, it does not change the fact that erythromycin is no longer a recommendation drug for GBS prophylaxis.

Modified from Verani JR, McGee L, Schrag SJ. Prevention of perinatal group B streptococcal disease: revised guidelines from CDC, 2010. Division of Bacterial Diseases, National Center for Immunization and Respiratory Diseases, Centers for Disease Control and Prevention (CDC). MMWR Recomm Rep 2010;59(RR-10):1–36. (This Committee Opinion, including Table 1, Box 2, and Figures 1–3, updates and replaces the obstetric components of the CDC 2010 guidelines, "Prevention of Perinatal Group B Streptococcal Disease: Revised Guidelines From CDC, 2010.")

Group B *Streptococcus*

- Lab diagnosis
  - Enrichment Culture: Broth (Carrot, LIM, T-H) + BAP or chromogenic agar
  - ß-hemolysis, serologic grouping
  - Prenatal screening
    - Culture – rectal/vaginal 35-37 weeks
    - Molecular – faster, increased sensitivity and specificity
- Treatment
  - Penicillin, ampicillin, cephalosporins, vancomycin
  - Clindamycin (30 – 40% R)
  - Erythromycin (40 – 50% R)

Table 1. Antibiotic Susceptibilities of GBS Isolates—ABCs, 2010. (n=1427)

Antibiotic	Susceptible	Intermediate	Resistant
Penicillin	1427 (100%)	0	0
Clindamycin	1018 (71%)*	13 (0.9%)	396 (28%)
Erythromycin	724 (51%)	7 (0.5%)	696 (49%)
Vancomycin	1427 (100%)	0	0
Cefotaxime	1427 (100%)	0	0

†Based on 2010 CLSI susceptibility definitions.

CDC. 2012. Antimicrobial Susceptibilities among Group B Streptococcus Isolates (GBS) - Active Bacterial Core Surveillance, 2010.

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Conventional Culture Methods

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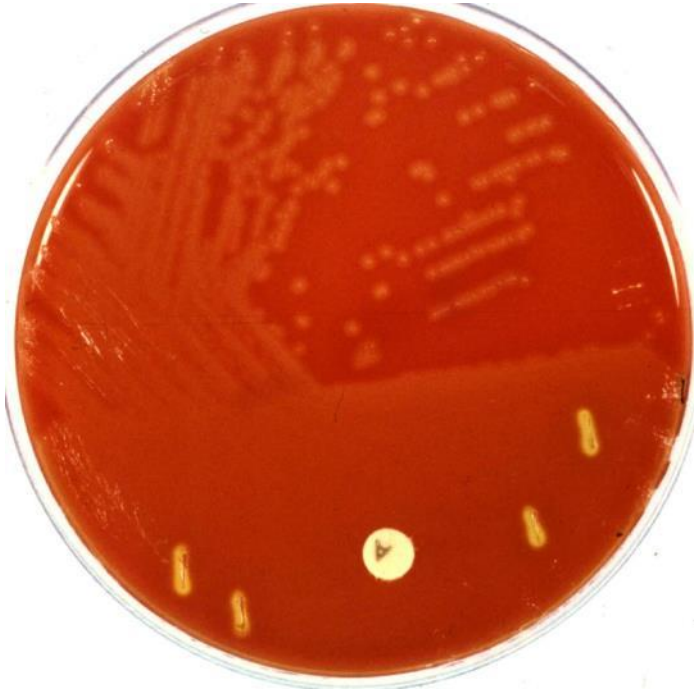
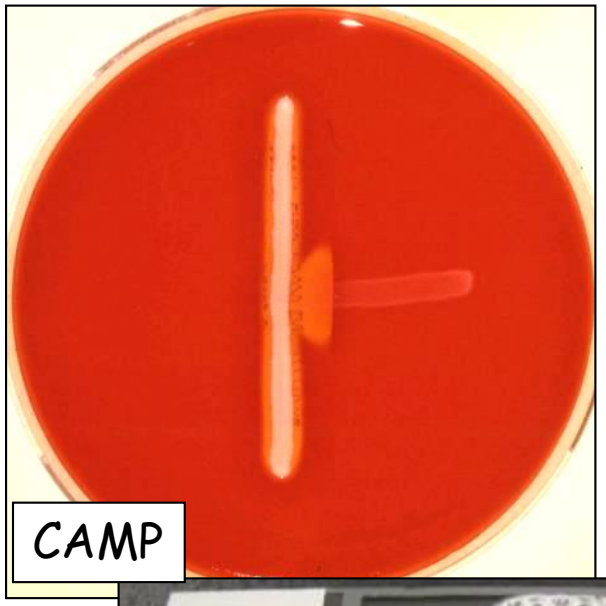
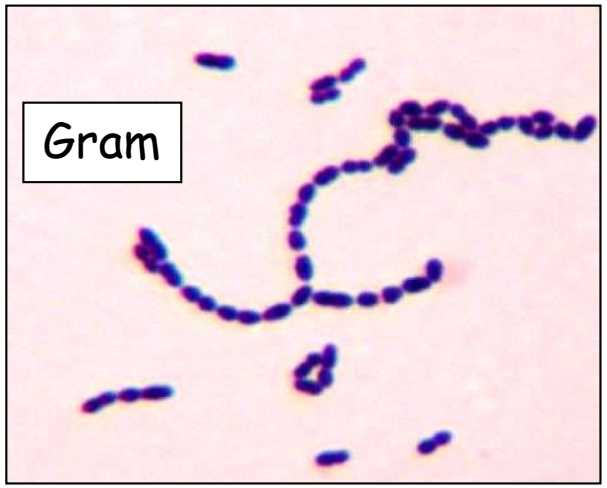
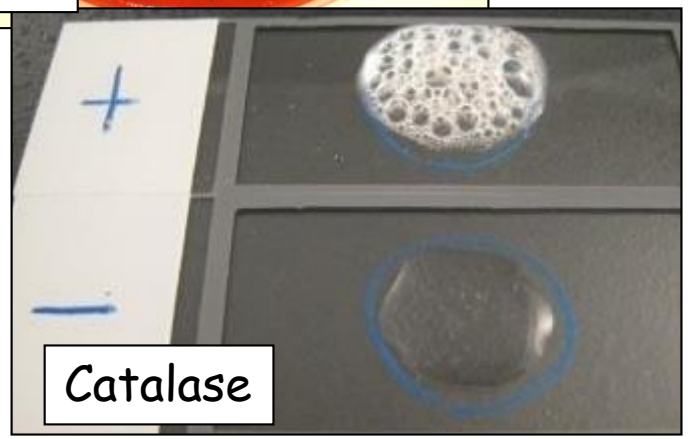








Granada Medium (de la Rosa *et al*. 1992. J Clin Micro 30: 1019 – 1021)

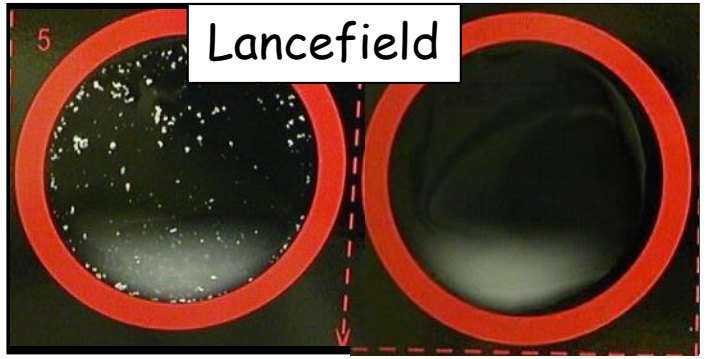


TABLE 1 Overview and characteristics of laboratory methods for identification of group B streptococcus

Laboratory method	Special equipment requirements	Sample type	Time to results	Relative sensitivity[a]	Relative specificity[a]	Relative costs[b]	Advantages	Disadvantages	Comments
Beta-hemolysis	None	Clinical samples; isolated colonies required	Overnight incubation to 2 days	++	++	$	Traditional, inexpensive, very easy to perform	Failure to detect nonhemolytic GBS strains	Beta-hemolysis may be difficult to observe
Granada-type media	None	Clinical samples; isolated colonies in Granada agar required	Overnight incubation	++++	+++++	$	Very easy to observe, 100% specific for GBS	Anaerobic incubation, failure to detect nonhemolytic GBS strains	Highly discriminatory; anaerobiosis not required for Granada broths; frequently negative results for nonhuman GBS strains
Chromogenic media	None	Clinical samples; isolated colonies required	1–2 days	++++	++	$	Anaerobic incubation not necessary	GBS-like colonies require confirmatory tests	Sensitivity, specificity, and colony aspects vary among suppliers
CAMP test	None	Isolated colonies; previous culture isolation required	Overnight incubation	++++	+++	$	Very easy to perform, easy to read	Requires 1 additional day to confirm GBS	Also positive for *Streptococcus porcinus* and some group A streptococci
PYR test	None	Isolated colonies	Minutes	Always negative for GBS	Always negative for GBS	$	Easy to perform, commercially available from many suppliers		Always positive for group A streptococci; used to differentiate GBS from group A streptococci, enterococci, and *S. porcinus*
Hippurate	None	Isolated colonies	Minutes	++	+	$	Easy to perform	Too unspecific for identification or confirmation of GBS	Positive results for most GBS but also other streptococci
Biochemical profiling	Manual kits or automated systems	Isolated colonies	Minutes to overnight	+++	++	Test, $$; platform, $$$	Commercially available from many suppliers	Costs	Not widely used for identification of GBS; better to reserve kits for identification of other streptococcal species
MALDI-TOF MS	MALDI-TOF MS instrument	Isolated colonies	Minutes	++++	++++	Test, $; instrument, $$$$	Easy and simple to perform	Requires specialized equipment, with high initial investment costs	Displacing phenotypic identification techniques in laboratories
Latex agglutination/ coagglutination	None	Isolated colonies	Minutes	++++	+++	$	Easy and simple to perform, available from many manufacturers	*S. pseudoporcinus* and atypical enterococci (hemolytic) may cross-react with GBS grouping antisera	
Direct antigen detection	None	Clinical samples	Minutes	+	++	$	Easy to perform, point-of-care test	Sensitivity insufficient for direct detection of GBS in clinical samples	Can help in diagnosis of neonatal GBS meningitis
PCR, clinical sample	Automated PCR platform	Clinical samples	hours	+++	++++	Test, $$; platform, $$$	Can be performed at delivery	No antibiotic susceptibility data	Not CDC recommended for GBS screening purposes
PCR, enrichment broth	Automated PCR platform	Enrichment broth	Hours to overnight	++++	++++	Test, $$; platform, $$$		No clear advantage over culture-based methods	Complies with CDC recommendation
DNA sequencing	Diagnostic molecular biology	Isolated colonies	Days	+++++	+++++	$$$	Gold standard for taxonomic purposes	Requires molecular biology expertise	Very rarely required to identify GBS

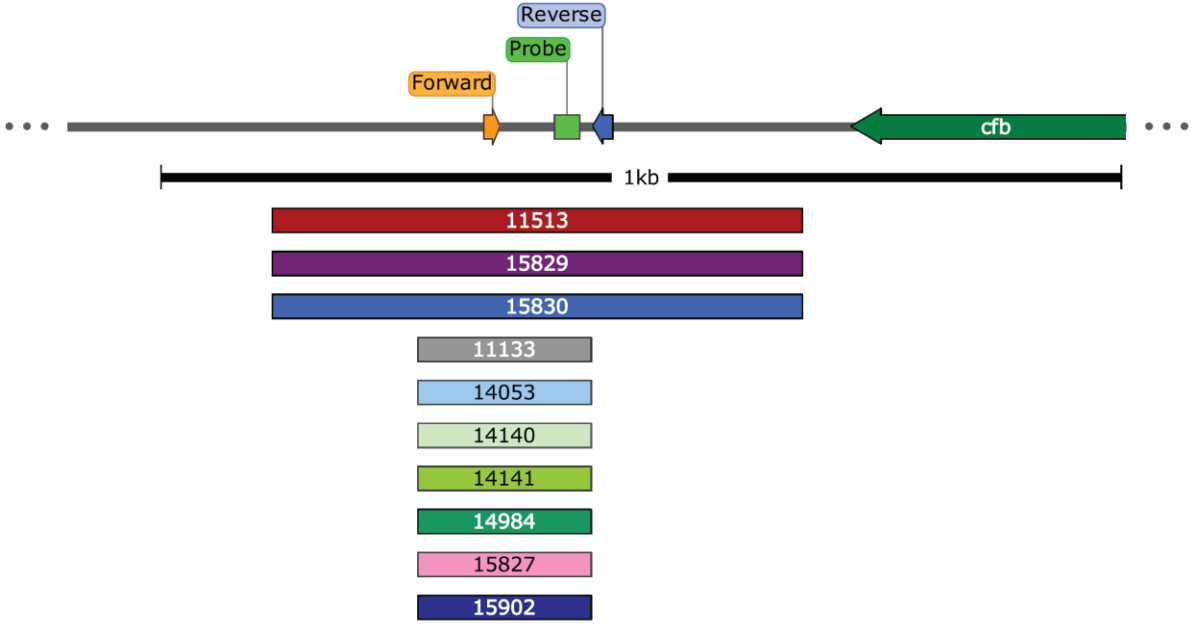
[a]Values for sensitivity and specificity estimates may be misleading because they depend on the test used for comparison. Thus, relative sensitivity and specificity are shown (from + to +++++).
[b]Costs can vary according to the location and laboratory testing volume.

Rosa-Fraile & Spellerberg. 2017. J Clin Micro 55: 2590 – 2598.

Molecular Testing

Faster TAT & accurate detection

- Increased sensitivity leads to superior detection
- Decrease length of stay
- Stop unnecessary ordering of additional tests
- Improved sensitivity and specificity plays an important role in antimicrobial stewardship
- Limitations:
  - Costs
  - Expertise
  - Rare false negative results due to mutations/deletions in targeted genes



Tickler *et al*. 2019. J Clin Micro 57: e02040 – 18.

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- Limitations:

Molecular Testing

Faster TAT & accurate detection

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Table 2
Performance characteristics of the GBS molecular assays evaluated in this study.

Assay	GBS-positive samples (%)	PPA[a] (95% CI)	NPA[b] (95% CI)	HoT[c] (h:min:s)			Total TAT[d] (h:min:s)		
				1 Specimen	6 Specimens	12 Specimens	1 Specimen	6 Specimens	12 Specimens
ARIES® GBS	151/299 (50.5%)	98.7% (94.9%–99.7%)	98.0% (94.1%–99.3%)	00:01:55	00:04:45	00:08:42	01:56:43	01:59:49	02:04:06
BD MAX™ GBS[e]	150/299 (50.2%)	n/a	n/a	00:03:05	00:10:22	00:19:07	01:52:16	02:00:31	02:10:26
Illumigene® GBS	149/299 (49.6%)	98.0% (94.1%–99.3%)	97.3% (93.2%–99.0%)	00:03:32	00:11:41	00:18:25	00:53:32	01:01:41	02:03:23
Xpert® GBS LB	151/299 (50.5%)	99.3% (95.5%–99.9%)	98.7% (94.9%–99.7%)	00:01:55	00:07:25	00:14:00	00:56:59	01:02:44	01:02:44

[a] Positive percent agreement.
[b] Negative percent agreement.
[c] Hands-on time.
[d] Turnaround time.
[e] Standard of care method, used as comparator for this study.

Relich *et al*. 2018. Diag Micro & Infect Dis 91: 305 – 308.

Clinical Trial – Molecular GBS Assay

- “Real-time” isothermal platform
  - Helicase Dependent Amplification (HDA) technology, fluorescent probe based detection
  - Amplification and detection occur simultaneously
- No sample extraction
- Throughput: 12 results in ~35 minutes
- Minimal hands on time
- LIS Interface and data management capabilities
  - Virena

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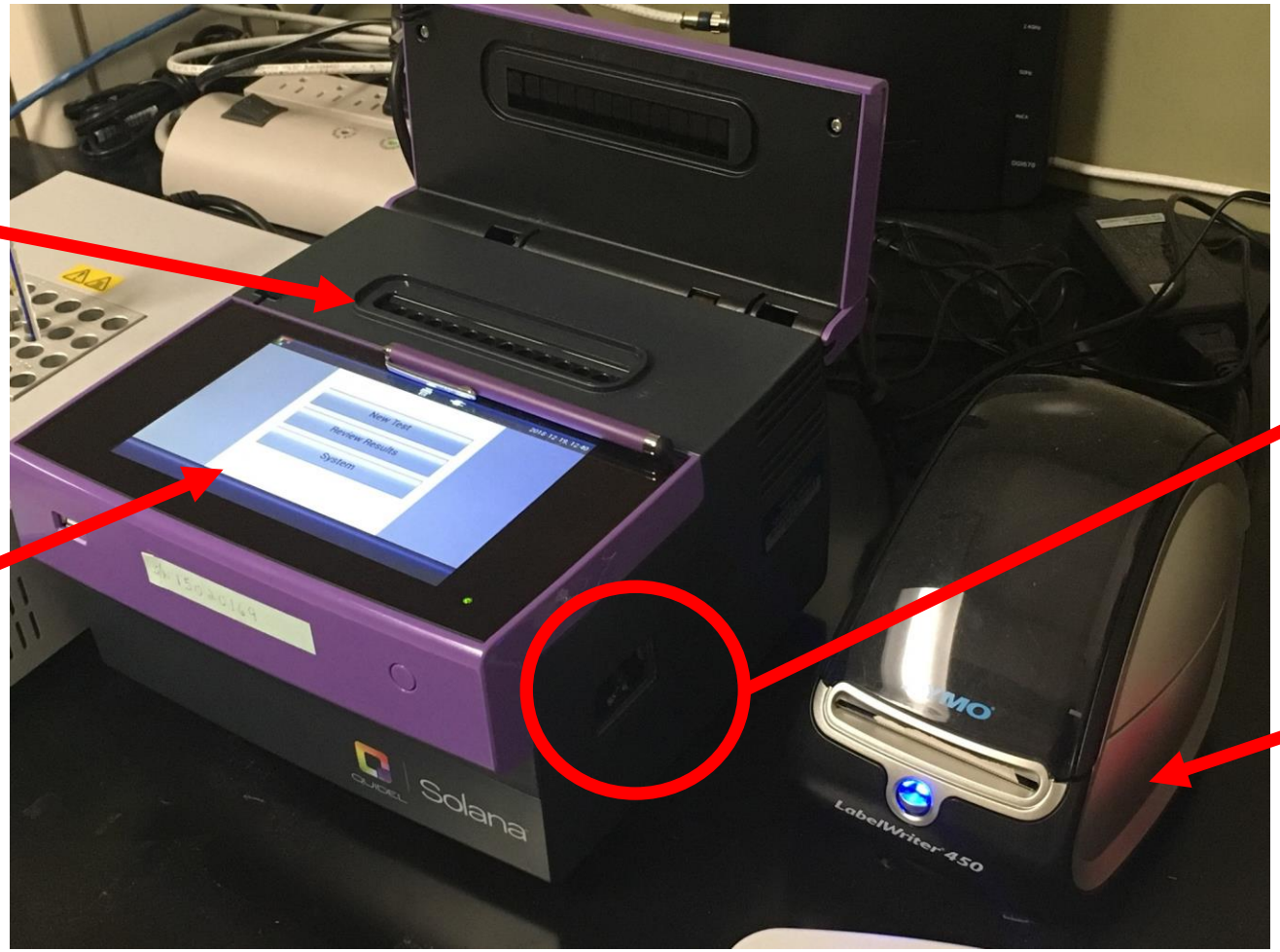
Clinical Trial – Molecular GBS Assay



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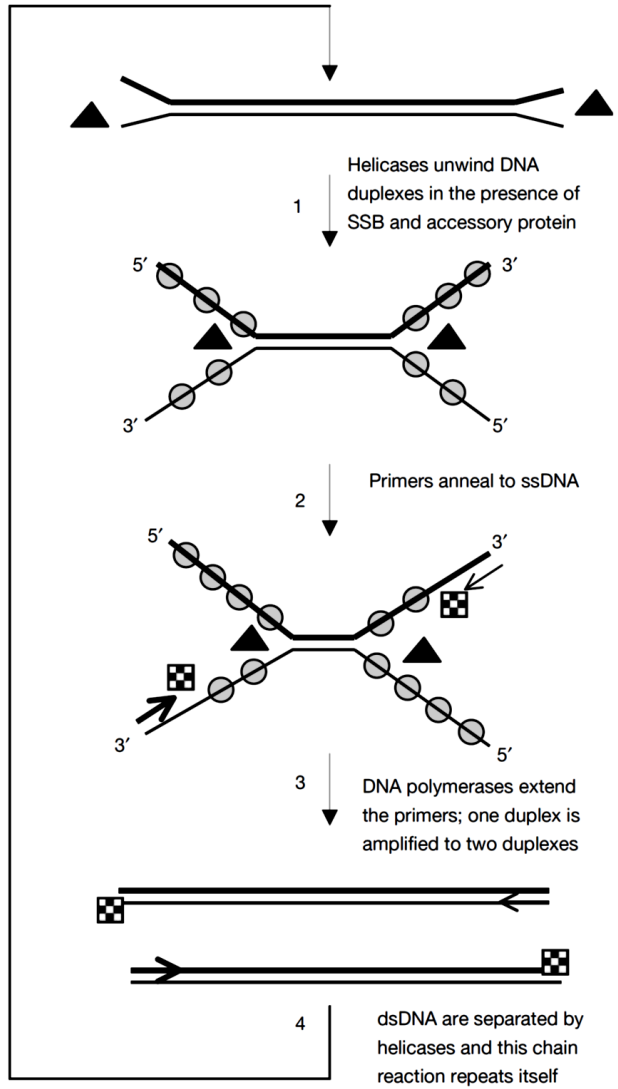
Helicase-Dependent Amplification

- Uses two sequence specific primers targeting the thiolase (*atoB*) gene
- A helicase enzyme separates the strands of DNA/RNA
- Strands are copied by the polymerase
- Detection mediated by probes bound to a fluorophore and a quencher



Vincent *et al*. 2004. EMBO Reports 5: 795 – 800.

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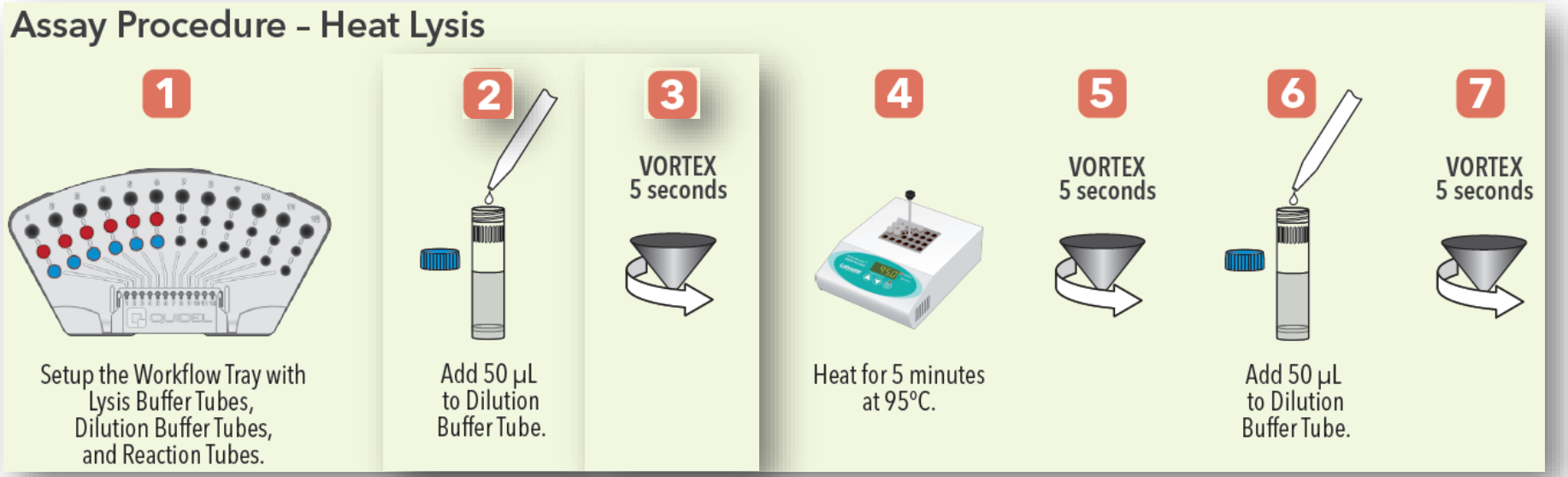
Solana GBS Workflow



- Maximum of 12 samples per run
- Average hands-on time:
  - 1 minute of setup time per sample
  - 15 seconds of instrument time per sample

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Solana GBS Workflow

Amplification and Detection

1

Transfer 50 µL to each Reaction Tube.

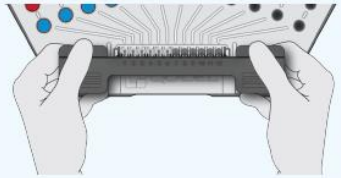
2

Rehydrate by pipetting up and down a minimum of 3 times.

3

Close lid tightly and proceed to next step.

4



Use the Transfer Rack to lift Reaction Tubes from the Workflow Tray.

5

Lower the Reaction Tubes into Solana.

6

Release the Reaction Tubes from the Transfer Rack by pulling the Transfer Rack toward your body.

7

Close the lid and select appropriate assay protocol. Run complete in 30 minutes.

8

Review results.

PCM307000EN00 (03/17)

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Solana GBS Performance

- We tested a total of 200 specimens from positive and negative Carrot Broth
- Compared Solana GBS to conventional culture

	Carrot Broth/Culture		
Solana GBS		POS	NEG
	POS	51	4
	NEG	1	144

Picked up 15 positives from negative Carrot Broth (would have saved ~24 hours in TAT)

Sensitivity: 98.1%
Specificity: 97.3%
PPV: 92.7%
NPV: 99.3%

Percent Agreement: 97.5%
GBS Prevalence: 26%

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Summary

- GBS remains a significant infectious pathogen in infants and adults
- GBS surveillance of pregnant patients, with appropriate antimicrobial prophylaxis for colonized patients, has been the leading factor in reducing invasive neonatal GBS disease
- Microbiology laboratories have an important role to play in accurate detection and identification of GBS in antenatal surveillance cultures
- Molecular diagnostics for GBS are widely available and provide an improved performance over conventional, culture-based methods

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Questions?

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