Laboratory Detection and Reporting of *Streptococcus agalactiae*

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The presenter states no conflict of interest and has no financial relationship to disclose relevant to the content of this presentation.

OUTLINE

I. Importance of prenatal screening strategies

II. Past approaches

III. Current guidelines

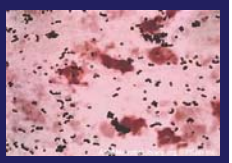
- A. General indications for prophylaxis
- B. Laboratory methods and reporting
- C. Adaptations of molecular approaches
- D. Antimicrobial susceptibility testing

Importance of Prenatal Screening

Streptococcus agalactiae

- Colonizes 15-40% of pregnant women

J. Infect. Dis. **143**: 761-766; 1981
Am. J. Obstet. Gynecol. **142**: 617-620; 1982
J. Infect. Dis. **145**: 794-799; 1982
J. Infect. Dis. **148**: 802-809; 1983
Obstet. Gynecol. **88**: 811-815; 1996
Obstet. Gynecol. **96**: 498-503; 2000



- Vertical transmission

Streptococcus agalactiae

- Neonatal incidence rate per 1000 live births:

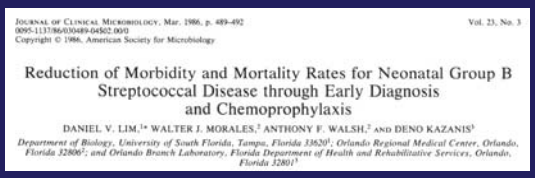
Infection	3.0
Septicemia	2.0
Case fatality	1.0

J. Pediatr. **82:** 707-718; 1973

- Group B streptococcal disease

Early onset	0-72 hours; pneumonia ± bacteremia
Late onset	1-3 months; meningitis

INTERVENTION



JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 1986, p. 489-492 Vol. 23, No. 3
0095-1137/86/030489-04$02.00/0
Copyright © 1986, American Society for Microbiology

Reduction of Morbidity and Mortality Rates for Neonatal Group B Streptococcal Disease through Early Diagnosis and Chemoprophylaxis

DANIEL V. LIM,[1]* WALTER J. MORALES,[2] ANTHONY F. WALSH,[2] AND DENO KAZANIS[3]

Department of Biology, University of South Florida, Tampa, Florida 33620[1]; Orlando Regional Medical Center, Orlando, Florida 32806[2]; and Orlando Branch Laboratory, Florida Department of Health and Rehabilitative Services, Orlando, Florida 32801[3]

803 women screened at 36 weeks gestation

173 (21.5%) positive for *S. agalactiae*

80 received intrapartum ampicillin
93 did not receive antimicrobials

J. Clin. Microbiol. **23**: 489-492; 1986

INTERVENTION

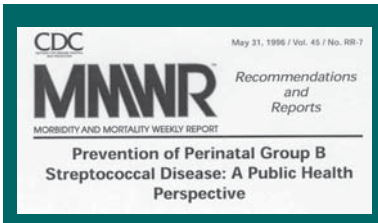
Intrapartum Ampicillin Treatment	Number of Colonized Moms	Number (%) of Colonized Babies
Yes	80	0 (0.0)
No	93	43 (46.2)

J. Clin. Microbiol. **23**: 489-492; 1986

INTERVENTION

Demographic	Number of Moms	Number of Births	GBS Sepsis/ 1000 Births	
			Incidence	Resultant Fatality
GBS screen + and treated; GBS screen -	710	710	0.00	0.00
GBS screen + and not treated; Not screened for GBS	1269	1274	5.49	2.35
Not treated; Not screened for GBS	3095	3110	2.25	0.32

J. Clin. Microbiol. **23**: 489-492; 1986


CDC

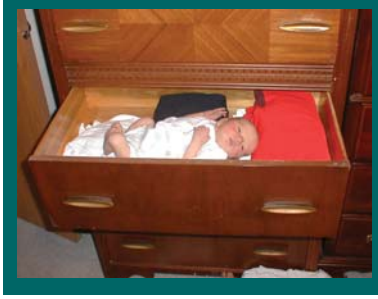
May 31, 1996 / Vol. 45 / No. RR-7

MMWR

Recommendations and Reports

MORBIDITY AND MORTALITY WEEKLY REPORT

Prevention of Perinatal Group B Streptococcal Disease: A Public Health Perspective



Second trimester assessment

Screening- or risk-based

DISEASE REDUCTION

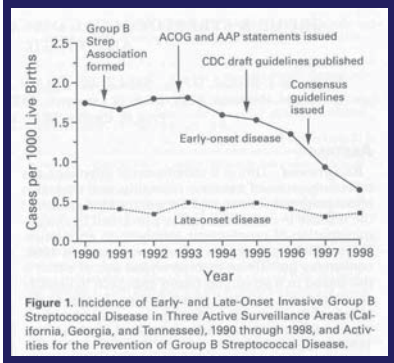


Figure 1. Incidence of Early- and Late-Onset Invasive Group B Streptococcal Disease in Three Active Surveillance Areas (California, Georgia, and Tennessee), 1990 through 1998, and Activities for the Prevention of Group B Streptococcal Disease.

65% reduction in early-onset disease prevalence from 1993-1998

N. Engl. J. Med. **342**: 15-20; 2000


The New England Journal of Medicine

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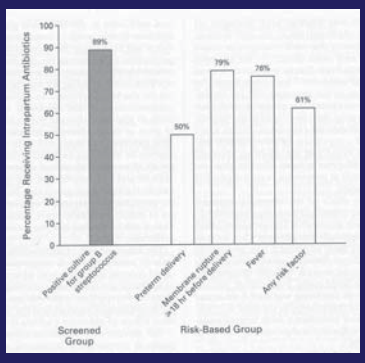
VOLUME 347 JULY 25, 2002 NUMBER 4

A POPULATION-BASED COMPARISON OF STRATEGIES TO PREVENT EARLY-ONSET GROUP B STREPTOCOCCAL DISEASE IN NEONATES

STEPHANIE J. SCHRAG, D.PHIL., ELIZABETH R. ZELL, M.STAT., RUTH LYNFIELD, M.D., AARON ROOME, PH.D., KATHRYN E. ARNOLD, M.D., ALLEN S. CRAIG, M.D., LEE H. HARRISON, M.D., ARTHUR REINGOLD, M.D., KAREN STEFONEK, R.N., M.P.H., GLENDA SMITH, B.S., MELANIE GAMBLE, M.P.H., AND ANNE SCHUCHAT, M.D., FOR THE ACTIVE BACTERIAL CORE SURVEILLANCE TEAM

SCREENING- VERSUS RISK-BASED

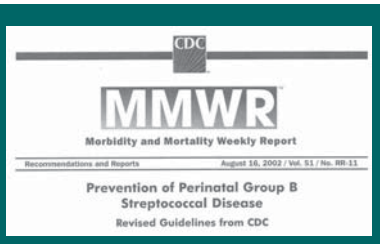


Adjusted relative risk for early-onset GBS disease associated with screening approach was 0.48

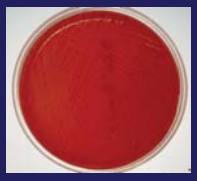
N. Engl. J. Med. **347**: 233-239; 2002

35- to 37-week assessment

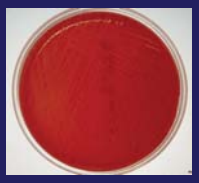
Screening-based



SCREENING-BASED METHODS



Blood agar plate



Blood agar plate

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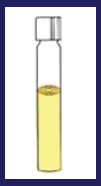

Todd Hewitt (LIM) broth plus subculture

Increases yield 20-35%

J. Matern. Fet. Med. **7**: 172-176; 1998
Arch. Pathol. Lab. Med. **127**: 718-720; 2003

ADDITIONAL (RECTAL) SAMPLING

Study	Patients	Carriage Rate (%)	Recovery Only by Rectal Sampling (%)
Badri *et al*. 1977	789	20.5	50.0
Dillon *et al*. 1982	2540	35.0	51.4
Philipson *et al*. 1995†	383	20.4	31.1
Platt *et al*. 1995*	651	16.9	26.4
Quinlan *et al*. 2000	222	24.3	18.5
Kovavisarach *et al*. 2007	320	41.9	24.6

J. Infect. Dis. **135**: 308-312; 1977
J. Infect. Dis. **145**: 794-799; 1982
†Obstet. Gynecol. **85**: 437-439; 1995
*Diagn. Microbiol. Infect. Dis. **21**: 65-68; 1995
J. Fam. Pract. **49**: 447-448; 2000
J. Med. Assoc. Thai. **90**: 1710-1714; 2007

WHO'S SAMPLING??

Investigation	Location	*S. agalactiae* Culture Sensitivity (%)	
		Patient Collection	Provider Collection
Mercer *et al*. 1995	Tennessee	91.7†	70.8
Molnar *et al*. 1997	Ontario	97.4	82.1
Price *et al*. 2006	Ontario	87.5*	96.9
Arya *et al*. 2008	Ireland	84.3	94.3

†$P < 0.05$
*$P = 0.11$

Am. J. Obstet. Gynecol. **173**: 1325-1328; 1995
Fam. Pract. **14**: 403-406; 1997
J. Obstet. Gynaecol. Can. **28**: 1083-1088; 2006
Eur. J. Obstet. Gynecol. Reprod. Biol. **139**: 43-45; 2008

SCREENING-BASED METHODS

Blood agar plate

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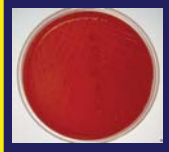
Todd Hewitt (LIM) broth plus subculture

87.0% sensitivity



Blood agar plate

\+

Carrot broth plus subculture

96.3% sensitivity

Wheaton Franciscan Laboratory in-house data

CARROT BROTH (observed at 24h)

Negative for *S. agalactiae*

Positive for *S. agalactiae*

SCREENING-BASED METHODS

Blood agar plate

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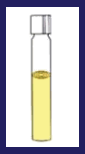

Todd Hewitt (LIM) broth plus subculture

38.3% resulted on day 1

Blood agar plate

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Carrot broth plus subculture

80.8% resulted on day 1 ($P < 0.0002$)

Wheaton Franciscan Laboratory in-house data

Is This Working?

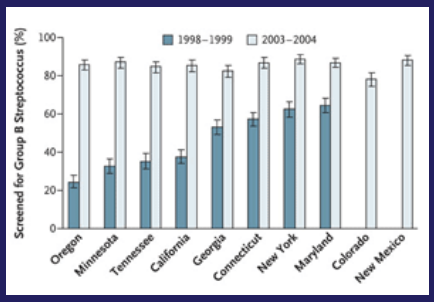
THE NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Evaluation of Universal Antenatal Screening for Group B Streptococcus

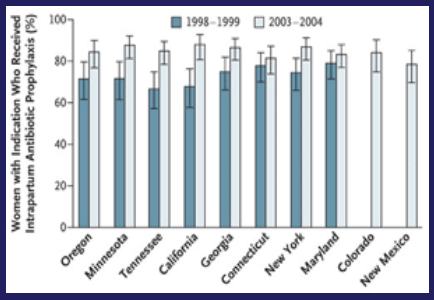
Melissa K. Van Dyke, Ph.D., Christina R. Phares, Ph.D., Ruth Lynfield, M.D., Ann R. Thomas, M.D., Kathryn E. Arnold, M.D., Allen S. Craig, M.D., Janet Mohle-Boetani, M.D., Ken Gershman, M.D., William Schaffner, M.D., Susan Petit, M.P.H., Shelley M. Zansky, Ph.D., Craig A. Morin, M.P.H., Nancy L. Spina, M.P.H., Kathryn Wymore, M.P.H., Lee H. Harrison, M.D., Kathleen A. Shutt, M.S., Joseph Bareta, M.P.H., Sandra N. Bulens, M.P.H., Elizabeth R. Zell, M.Stat., Anne Schuchat, M.D., and Stephanie J. Schrag, D.Phil.

"SUCCESS" IN SCREENING

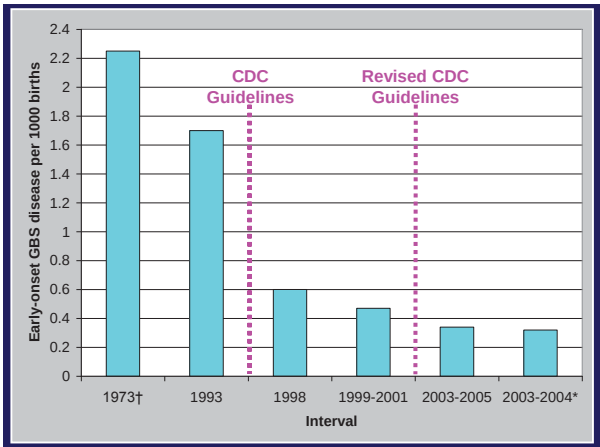


N. Engl. J. Med. **360:** 2626-2636; 2009

"SUCCESS" IN PROPHYLAXIS



N. Engl. J. Med. **360:** 2626-2636; 2009



† Estimate

* Ten-state surveillance

J. Pediatr. **82:** 707-718; 1973
N. Engl. J. Med. **342:** 15-20; 2000
JAMA **299:** 2056-2065; 2008
N. Engl. J. Med. **360:** 2626-2636; 2009

DISAPPOINTMENT???

Table 3. Implementation of 2002 Recommendations Regarding Intrapartum Chemoprophylaxis, According to Term Status, 2003–2004.*

Group B Streptococcus Status	Preterm Delivery† (N=962)	Term Delivery (N=6727)
	% (95% CI)	
Positive prenatal screening test before delivery‡		
Total	29.7 (23.9–36.3)	23.9 (22.6–25.2)
Received intrapartum antibiotics		
Overall	84.5 (72.9–91.7)	87.0 (84.9–88.9)
<4 hr between admission and delivery	79.6 (54.8–92.6)	62.7 (56.2–68.8)
≥4 hr between admission and delivery	85.8 (71.7–93.5)	94.0 (92.2–95.5)

N. Engl. J. Med. **360:** 2626-2636; 2009

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DISAPPOINTMENT???

Table 3. Implementation of 2002 Recommendations Regarding Intrapartum Chemoprophylaxis, According to Term Status, 2003–2004.*

Group B Streptococcus Status	Preterm Delivery† (N=962)	Term Delivery (N=6727)
	% (95% CI)	
Unknown colonization status§		
Total	54.2 (49.3–59.0)	0.7 (0.5–1.0)
Received intrapartum antibiotics		
Overall	63.4 (57.0–69.4)	78.5 (63.7–88.4)
<4 hr between admission and delivery	34.0 (24.3–45.3)	38.9 (8.4–81.5)
≥4 hr between admission and delivery	74.1 (66.7–80.4)	84.3 (69.3–92.7)

N. Engl. J. Med. **360:** 2626-2636; 2009

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DISAPPOINTMENT???

Table 3. Implementation of 2002 Recommendations Regarding Intrapartum Chemoprophylaxis, According to Term Status, 2003–2004.*

Group B Streptococcus Status	Preterm Delivery† (N=962)	Term Delivery (N=6727)
	% (95% CI)	
History of group B streptococcus bacteriuria or previous infant with group B streptococcus disease		
Total	6.2 (4.3–8.7)	6.7 (6.1–7.5)
Received intrapartum antibiotics		
Overall	73.5 (53.9–86.8)	80.7 (76.0–84.7)
<4 hr between admission and delivery	59.9 (28.7–84.7)	55.6 (44.5–66.1)
≥4 hr between admission and delivery	74.9 (51.6–89.3)	89.7 (85.0–93.1)

N. Engl. J. Med. **360:** 2626-2636; 2009

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BIG DISAPPOINTMENT???

Expected 44 to 86 cases of group B streptococcal disease among term infants

→ Observed 116 cases ←

Table 4. Characteristics of Mothers Who Delivered at Term and Whose Infants Had Group B Streptococcal Disease, 2003–2004.

Characteristic	Mothers Who Delivered at Term and Whose Infants Had Group B Streptococcal Disease (N=189)
	no. (%)
Screened	155 (82.0)
Positive for group B streptococcus	37 (19.6)
Negative for group B streptococcus	116 (61.4)
Unknown colonization status	2 (1.1)

N. Engl. J. Med. **360:** 2626-2636; 2009

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Need improved diagnostics

At same time, demographics may benefit from rapid & accurate diagnostics

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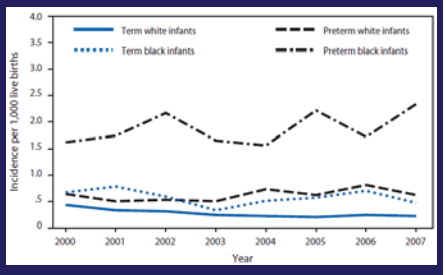
BENEFIT FROM A RAPID RESULT

- Increased attack rates and mortality in low birth weight neonates

TABLE 1. NUMBER OF CASES OF EARLY-ONSET NEONATAL INVASIVE GROUP B STREPTOCOCCAL DISEASE AND CASE FATALITY RATES ACCORDING TO GESTATIONAL AGE IN SELECTED COUNTIES IN THE UNITED STATES, 1993 TO 1998.

Gestational Age	No. (% of Early-Onset Cases)	Case Fatality Rate (%)*
≤33 wk	137 (9)	30
34–36 wk	116 (7)	10
≥37 wk	1247 (83)	2



N. Engl. J. Med. **342:** 15-20; 2000

MMWR. **59 (RR-10):** 1-32; 2010

30

BENEFIT FROM A RAPID RESULT

- Increased attack rates and mortality in low birth weight neonates
- Inadequate/no prenatal care

  Higher probability in African Americans
  Increased disease in those with inadequate care
  Increased disease in African American neonates

Obstet. Gynecol. **87:** 575-580; 1996
Obstet. Gynecol. **89:** 28-32; 1997

BENEFIT FROM A RAPID RESULT

- Increased attack rates and mortality in low birth weight neonates
- Inadequate/no prenatal care
- Moms who screen negative at 35-37 weeks, but are colonized at parturition (estimated 4-9%)

Pediatrics **115:** 1240-1246; 2005
J. Infect. Dis. **148:** 802-809; 2005

COMMERCIAL PCR

- Rapid detection of *S. agalactiae* DNA in vaginal/ rectal specimens from prepartum or intrapartum women (direct swab)
- 86-94% sensitivity (LIM broth reference)

Clin. Infect. Dis. **39:** 1129-1135; 2004

PERFORMANCE INDICES

Carrot broth-enhanced PCR 33.0% detection
LIM broth-enhanced PCR 30.5% detection
Carrot broth culture 29.6% detection

Parameter	Carrot Broth PCR	LIM Broth PCR
Sensitivity (%)	100.0	92.5
Negative predictive value (%)	100.0	96.4
Unresolved rate (%)	0.0	0.5
Processing time/specimen (min)	5.1	5.1

J. Clin. Microbiol. **46:** 3615-3620; 2008

PCR-POSITIVE SPECIMENS; n = 67

Parameter	Timepoint of Carrot Broth Culture Observation	
	Overnight Incubation	Final Subculture Result
Positive culture	34	60
Sensitivity (%)	50.7	89.6
Negative predictive value (%)	80.5	95.1

J. Clin. Microbiol. **46:** 3615-3620; 2008

COMMERCIAL PCR

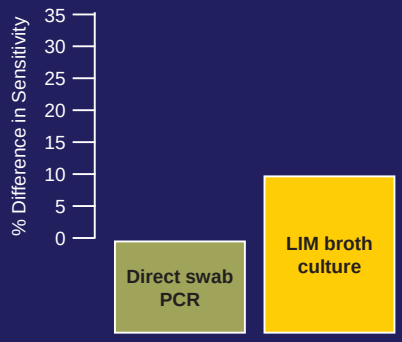
- Rapid detection of *S. agalactiae* DNA in vaginal/ rectal specimens from prepartum or intrapartum women (direct swab)
- 86-94% sensitivity (LIM broth reference)

Clin. Infect. Dis. **39:** 1129-1135; 2004

- 56-59% sensitivity (carrot broth reference)

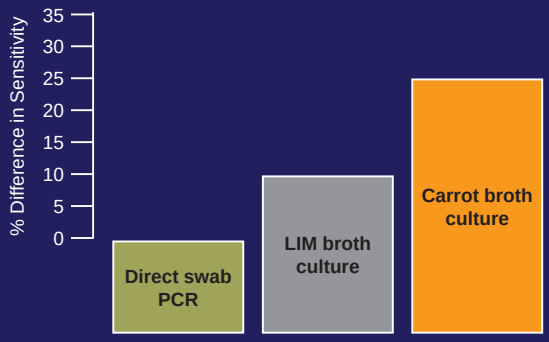
J. Clin. Microbiol. **46:** 3615-3620; 2008
J. Clin. Microbiol. **48:** 4495-4500; 2010

WHY??



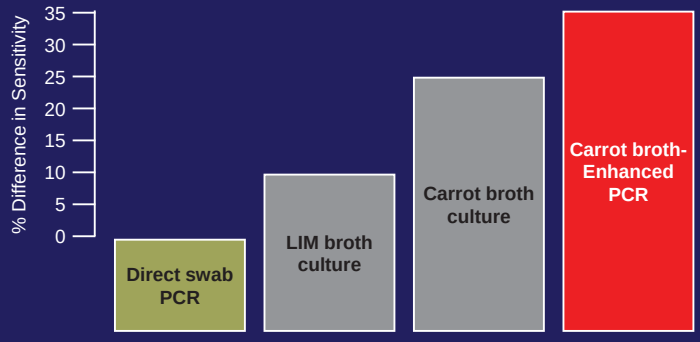
Clin. Infect. Dis. **39:** 1129-1135; 2004

WHY??

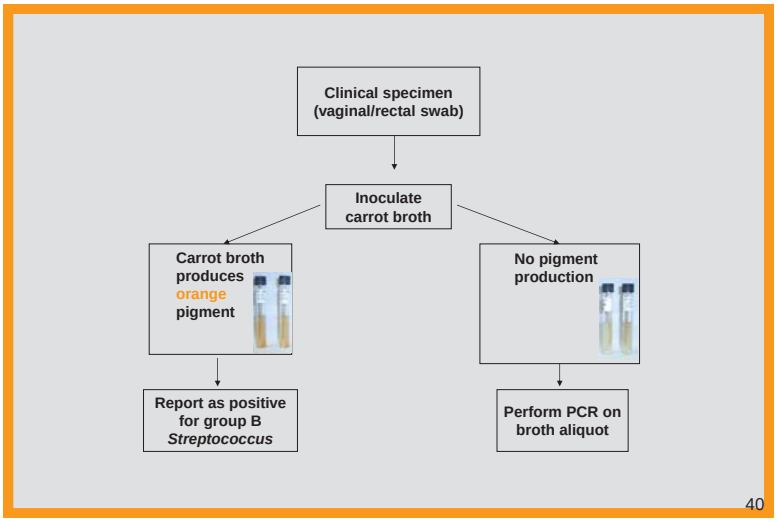


Wheaton Franciscan Laboratory in-house data

WHY??



J. Clin. Microbiol. **46:** 3615-3620; 2008



CAN THIS BECOME MORE RAPID??

IN VITRO EXPERIMENTATION

- Inoculate carrot broth tubes with 10^3, 10^2, 10^1 *S. agalactiae*
- Mock inoculation with 10^9 flora; simulating…
  - Anaerobic flora
  - Gastrointestinal flora
  - Urogenital flora
  - Pathogenic flora
- Collect 500-µL aliquots at specified intervals for carrot broth-enhanced PCR

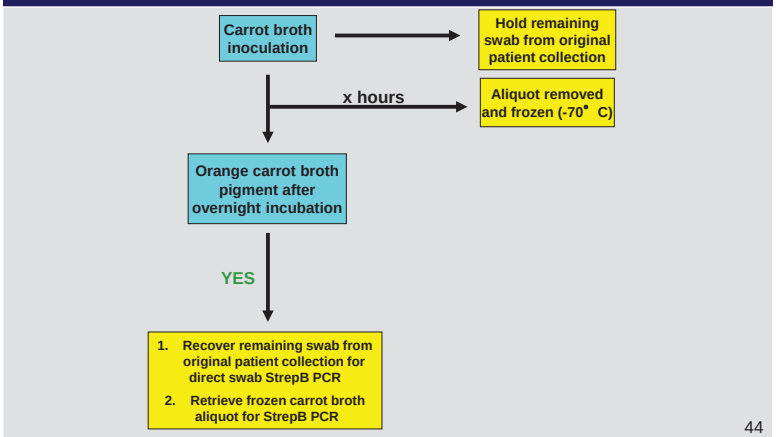
CARROT BROTH-ENHANCED PCR

S. agalactiae Inoculum	Percentage Positive				
	Time of aliquot collection (hours)				
	2	*4*	*6*	*12*	*24*
10^1	0.0	33.3	41.7	25.0	33.3
10^2	58.3	66.7	91.7	ND	ND
10^3	100.0	100.0	100.0	ND	ND

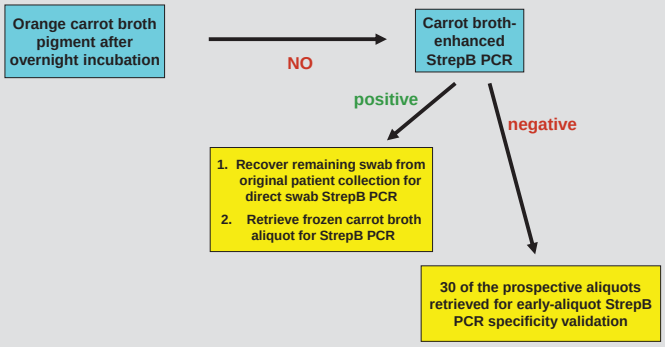
ND; not determined

J. Clin. Microbiol. **46:** 4495-4500; 2010

CLINICAL EXPERIMENTATION



CLINICAL EXPERIMENTATION



CLINICAL EXPERIMENTATION

Number of Specimens	Early-aliquot Carrot Broth-enhanced PCR		% Positive from Remnant Direct Swab PCR	*P* value
	Collection Interval (h)	% Positive		
33	< 3.00	54.5	66.7	0.31
35	3.00-3.99	40.0	54.3	0.23
35	4.00-4.99	51.4	48.6	0.81
41	5.00-5.99	73.2	65.9	0.47
39	6.00-6.99	82.1	46.2	0.0009
44	> 7.00	86.3	56.8	0.002
Total (227)		66.1	56.4	0.03

J. Clin. Microbiol. **46:** 4495-4500; 2010

POSITIVE CARROT BROTH CULTURE

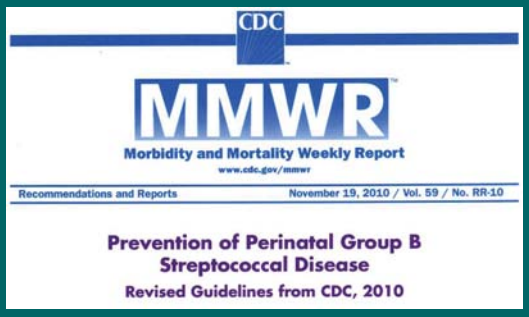
Number of Specimens	Early-aliquot Carrot Broth-enhanced PCR		% Positive from Remnant Direct Swab PCR	*P* value
	Collection Interval (h)	% Positive		
12	< 3.00	83.3	91.7	ND
12	3.00-3.99	50.0	75.0	ND
10	4.00-4.99	80.0	80.0	ND
19	5.00-5.99	94.7	89.5	ND
13	6.00-6.99	100.0	69.2	ND
10	> 7.00	100.0	70.0	ND
Total (76)		85.5	80.3	0.39

J. Clin. Microbiol. **46:** 4495-4500; 2010

NEGATIVE CARROT BROTH CULTURE

Number of Specimens	Early-aliquot Carrot Broth-enhanced PCR		% Positive from Remnant Direct Swab PCR	*P* value
	Collection Interval (h)	% Positive		
21	< 3.00	38.1	52.4	0.35
23	3.00-3.99	34.7	43.5	0.55
25	4.00-4.99	40.0	36.0	0.77
22	5.00-5.99	54.5	45.5	0.55
26	6.00-6.99	73.1	34.6	0.005
34	> 7.00	82.4	52.9	0.01
Total (151)		56.2	44.4	0.04

J. Clin. Microbiol. **46:** 4495-4500; 2010



35- to 37-week assessment

Screening-based

INDICATIONS FOR PROPHYLAXIS

- Previous infant with invasive early-onset disease
- *S. agalactiae* bacteriuria during any trimester of current pregnancy
- Positive *S. agalactiae* vaginal/rectal screening culture in late gestation during current pregnancy
- Unknown *S. agalactiae* status at labor PLUS one:
  - Delivery at < 37 weeks' gestation
  - Amniotic membrane rupture ≥ 18 hours
  - Intrapartum temperature ≥ 100.4° F
  - Positive intrapartum nucleic acid amplification test

MMWR. **59 (RR-10):** 1-32; 2010

SPECIMEN COLLECTION/TRANSIT

- Lower vaginal, then rectal collection
  - 35-37 weeks' gestation; can be self-collected
  - Cervical, perianal, perirectal not acceptable
- Swabs placed into non-nutritive transport medium
  - Recovery decreases over 1-4 days (room temp)
  - Refrigerate swabs, if feasible
- Clinicians indicate if patient possesses allergy to penicillin or cephem agent

MMWR. **59 (RR-10):** 1-32; 2010

SPECIMEN PROCESSING

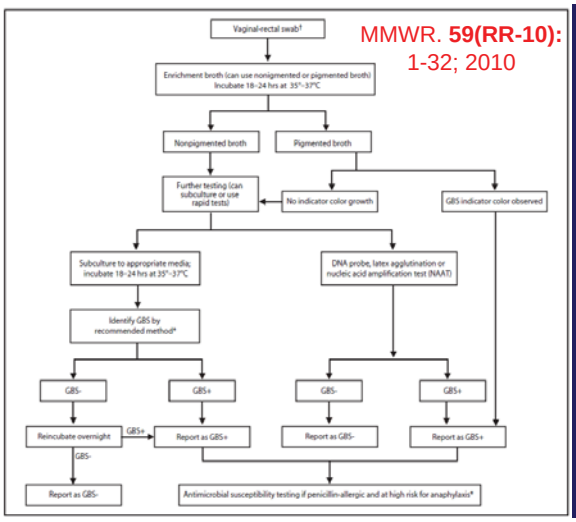
- Selective broth medium (Todd-Hewitt base)
  - LIM broth
  - Transvag broth
- Alternative selective media can be chromogenic
  - Carrot broth
  - Granada biphasic broth
- 18-24 hours in 35-37C ambient air or 5% CO_2
- Direct plating may be included
  - Lower sensitivity than broth enrichment
  - Should not be used as sole means of recovery

MMWR. **59 (RR-10):** 1-32; 2010

RESULTS AND INTERPRETATION

- Selective broths subcultured to appropriate agar(s)
- Non-pigmented chromogenic broths subcultured to appropriate agar(s)
- Positive identification may be derived from:
  - Biochemical or probe testing of isolated growth
  - Pigmented broth (β-hemolytic *S. agalactiae*)
  - Probe testing of selective broth
  - Nucleic acid amplification testing of selective broth
  - Latex agglutination of selective broth

MMWR. **59 (RR-10):** 1-32; 2010



MMWR. **59(RR-10):** 1-32; 2010

DIRECT MOLECULAR DETECTION?

"Accurate results are more important than rapid turnaround time for antenatal screening."

MMWR. **59 (RR-10):** 1-32; 2010

College of American Pathologists MIC.64817

"A pre-enrichment step using a selective broth enrichment culture is performed for antepartum (35-37 weeks gestation) vaginal/rectal swab screening for Group B streptococci (GBS) colonization by nucleic acid amplification testing (NAAT)."

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ANTIMICROBIAL SUSCEPTIBILITY

- Disk diffusion or broth microdilution performed on antenatal *S. agalactiae* isolates from women at risk for anaphylaxis (related to penicillin or cephem)

Anaphylaxis	Respiratory distress
Angiodedema	Urticaria

- Inducible clindamycin testing on erythromycin-resistant *S. agalactiae*
- CLSI M100 document recommends suppression of erythromycin susceptibility testing data

MMWR. **59 (RR-10):** 1-32; 2010

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INDUCIBLE CLINDAMYCIN RESISTANCE

- CDC recommends "D-test" on erythromycin-R/ clindamycin-S isolates of *S. agalactiae*; allows for performance on other validated AST systems
- 2 μg clindamycin disk
  15 μg erythromycin disk
  12 millimeters apart

  Mueller-Hinton w/blood
  35C; 5% CO_2
  20-24 hours

MMWR. **59 (RR-10):** 1-32; 2010

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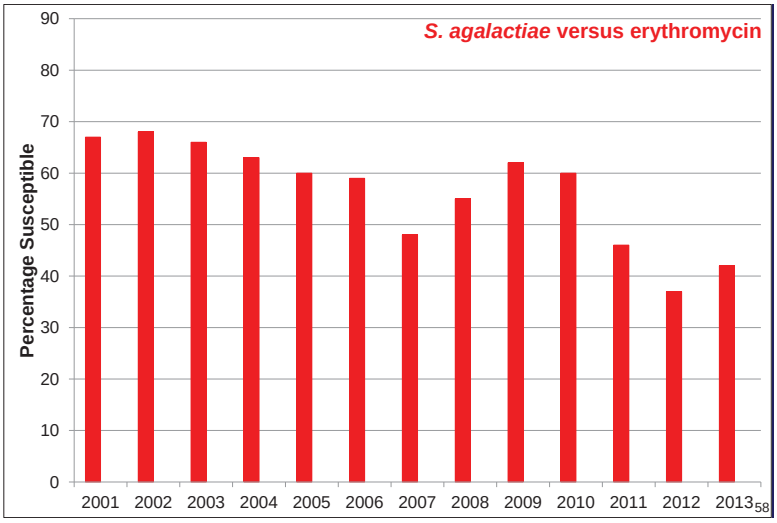

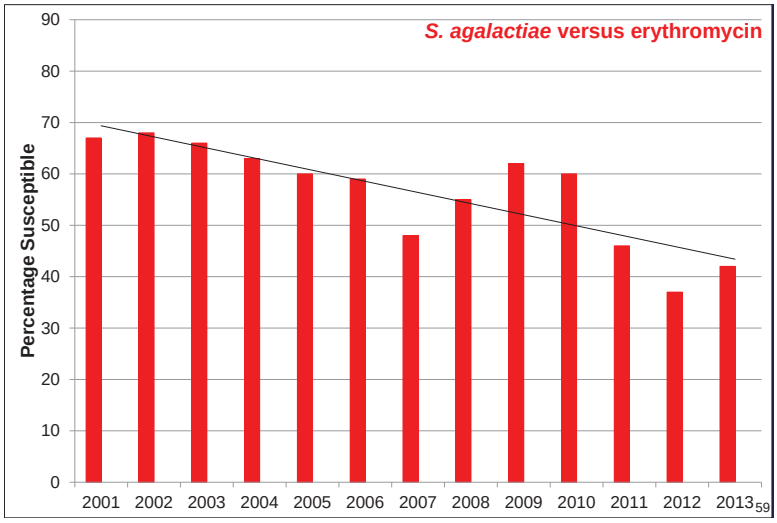

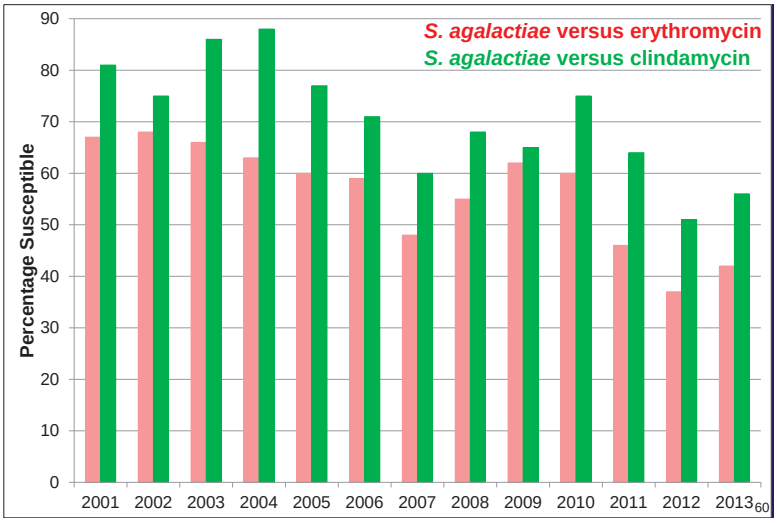

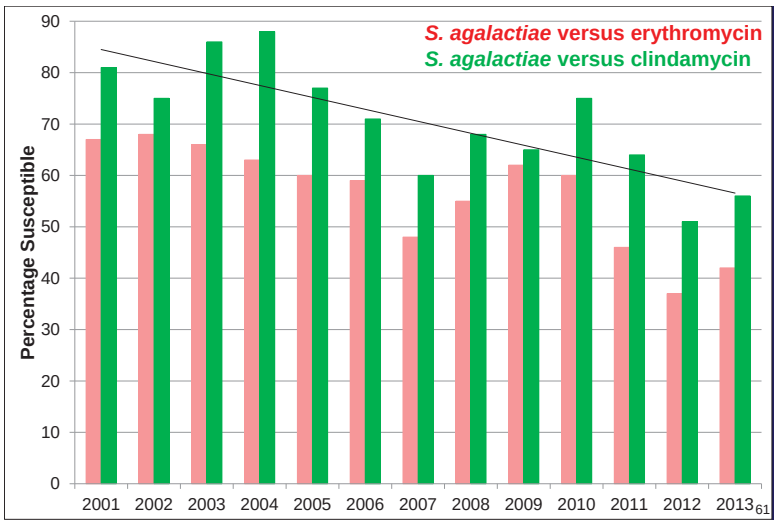

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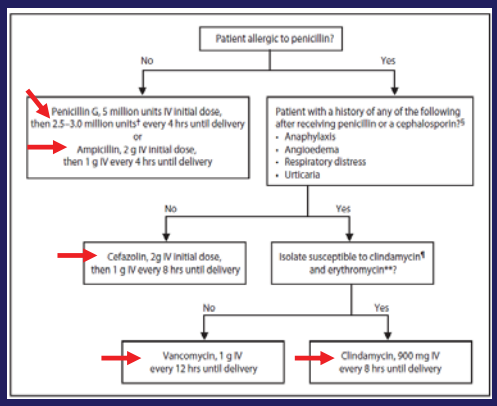
INTRAPARTUM PROPHYLAXIS



MMWR. **59 (RR-10):** 1-32; 2010

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THE TIMES…

First Molecular Characterization of Group B Streptococci with Reduced Penicillin Susceptibility

- Fourteen non-invasive *S. agalactiae* isolates between 1995-2005 had alterations in PBP2X
- Clinical significance unclear

Antimicrobial. Agents Chemother. **52:** 2890-2897; 2008

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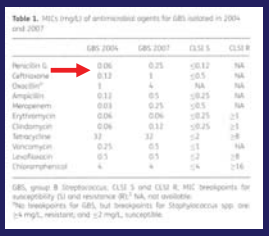
…THEY ARE A CHANGIN'

Prosthetic hip joint infection with a *Streptococcus agalactiae* isolate not susceptible to penicillin G and ceftriaxone



J. Antimicrob. Chemother. **65:** 594-595; 2010

Novel Mutations in a Patient Isolate of *Streptococcus agalactiae* with Reduced Penicillin Susceptibility Emerging after Long-Term Oral Suppressive Therapy

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S. agalactiae BACTERIURIA

- Marker for heavy genital tract colonization; risk factor for early-onset GBS disease

Scand. J. Infect. Dis. **17:** 195-199; 1985

- 1996 guidelines — no threshold specification
  2002 guidelines — report any concentration
  2010 guidelines — 10^4 colony forming units/mL
- Few data available on risk for early-onset GBS in context of low-count bacteriuria

MMWR. **45 (RR-7):** 1-24; 1996
MMWR. **51 (RR-11):** 1-24; 2002
MMWR. **59 (RR-10):** 1-32; 2010

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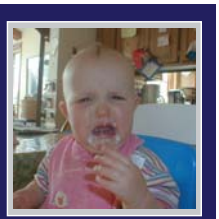
THE END



- Identification of candidates for intrapartum chemoprophylaxis is essential for prevention of early-onset group B streptococcal disease
- Much of this has fallen into the hands of laboratory
- Situation has improved since the 1970s; more work to be done
- Molecular diagnostics and antimicrobial susceptibility testing, when applied appropriately, play major role

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