Early-onset Group B Streptococcal Disease Prevention: Procedures for Laboratories

Overview of CDC prevention guidelines 2010

National Center for Immunization and Respiratory Diseases
Division of Bacterial Diseases

November 19, 2010

Group B Streptococcus Disease

- Gram positive, beta-hemolytic cocci
- Causes invasive disease in young infants, pregnant women and older adults
- In 1970s, emerged as most common cause of sepsis and meningitis in infants <3 months in U.S.

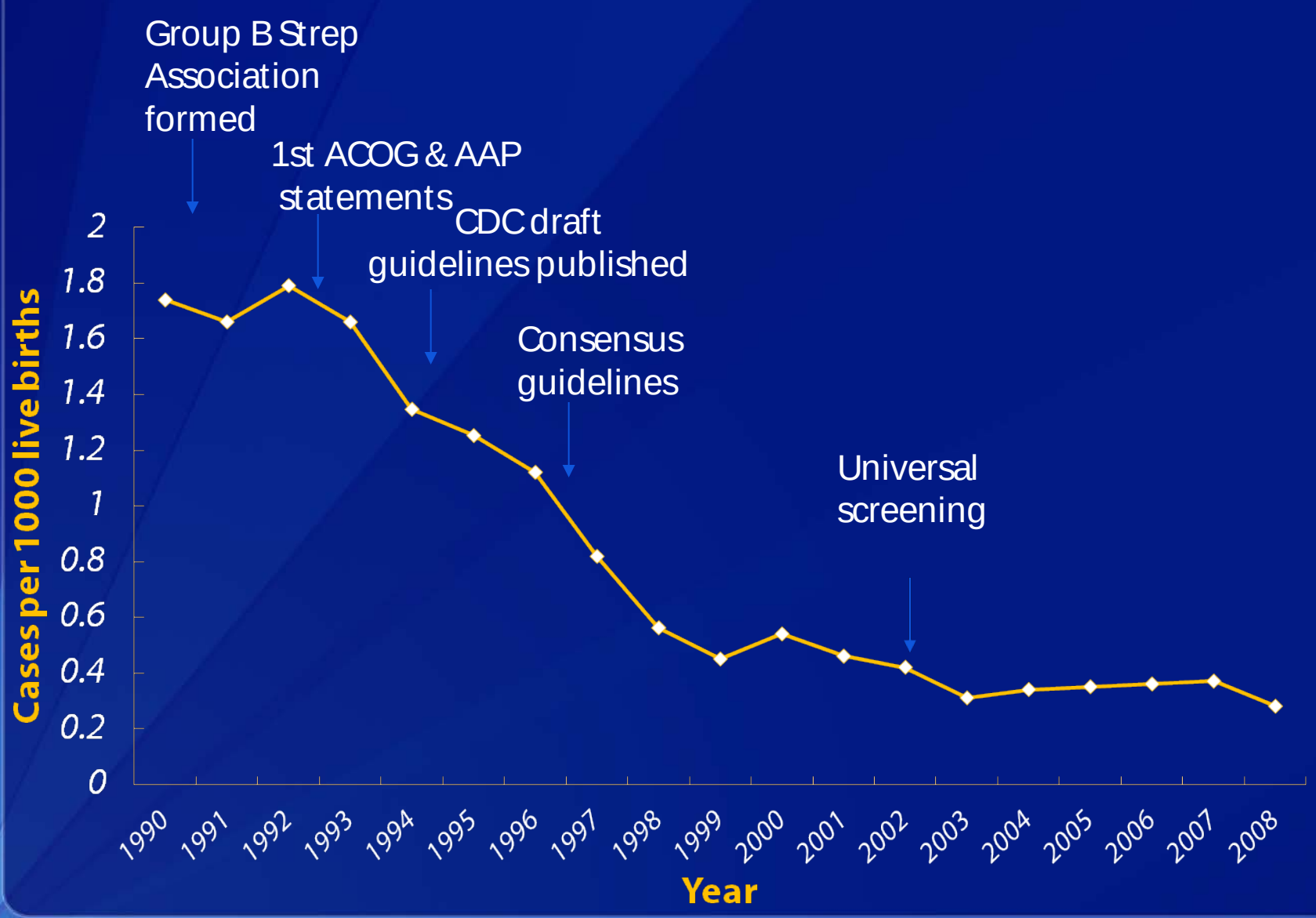
GBS Maternal Colonization

- GBS is a common colonizer of genital, GI tracts in women
  - 10-30% of all women colonized
  - Rates higher in African-American women
- Colonization difficult to detect clinically
  - Asymptomatic
  - Dynamic condition
  - Cannot determine from history or physical
- GBS maternal colonization is a strong risk factor for early onset GBS disease in infants

GBS Prevention: Intrapartum Antibiotic Prophylaxis (IAP)

- Clinical trials in 1980s of IAP to prevent GBS disease
  - IV penicillin or ampicillin
  - Given to GBS-colonized women (+/- additional risk factors)
  - Reduction in infant colonization & GBS disease
- Efficacy against GBS disease: 100% (Boyer and Gotoff, NEJM 1986;314:1665-9)
- Effectiveness in observational studies: 86-89%

Early-onset GBS Disease in the U.S.



CDC 2010 GBS Guidelines:

Late-antenatal screening for GBS colonization remains the cornerstone of recommendations

Labs Play a Critical Role in Success of Universal Screening

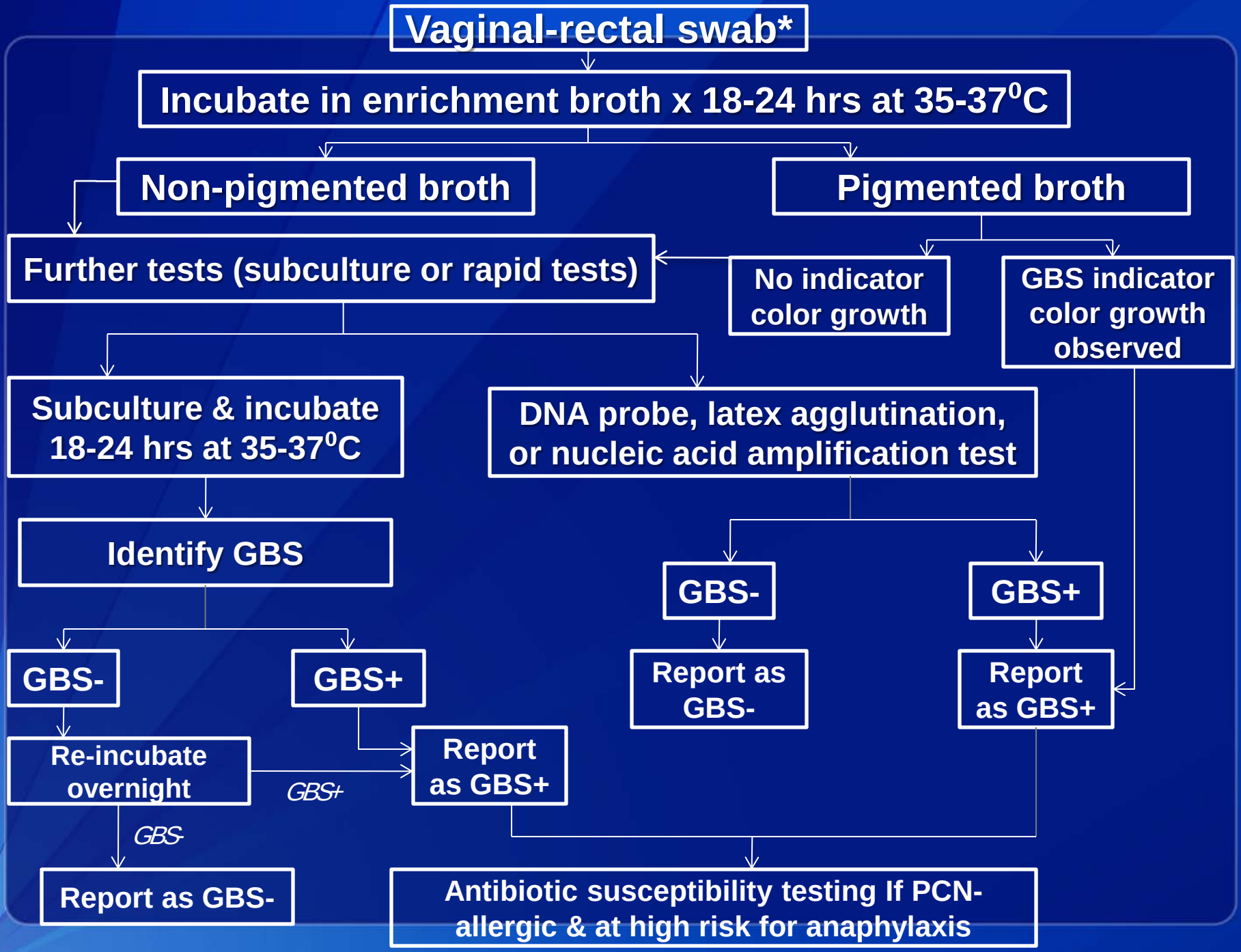
- Correct laboratory processing of specimens critical to the success of universal screening
- Most cases of early onset GBS disease now occur in infants born to women who screened negative (Van Dyke 2009; NEJM; *360:2626-36*)
  - *Due in part to false negative prenatal screening results*
- To prevent as many cases as possible, it is important to optimize specimen collection and processing procedures

Organizations Endorsing the 2010 GBS Guidelines

- American College of Obstetricians and Gynecologists
- American Academy of Pediatrics
- American College of Nurse-Midwives
- American Academy of Family Physicians
- American Society for Microbiology

The 2010 CDC Early Onset GBS Disease Prevention Guidelines:

Prenatal Specimen Collection & Processing Recommendations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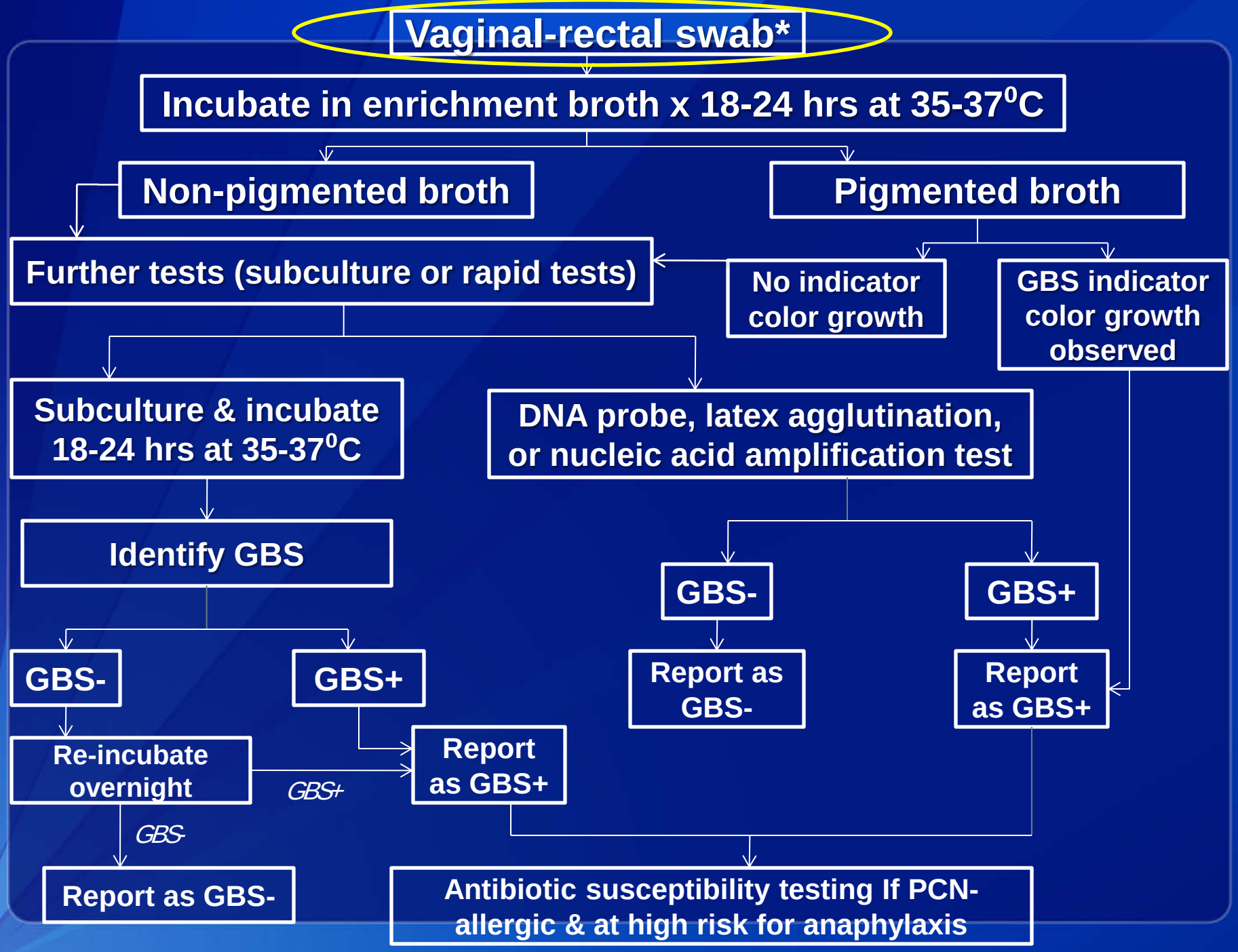
Antibiotic susceptibility testing If PCN-allergic & at high risk for anaphylaxis

2010 GBS Prevention Guidelines: Key Methods for Laboratories

- Enrichment step critical
  - ALL prenatal samples must be enriched in broth media for 18-24 hours
  - Enrichment greatly enhances sensitivity of culture
- 48 total hours of incubation for subculture plate important for negative samples
  - Higher likelihood of false negatives without 48 hours incubation
- Clindamycin susceptibility testing for PCN-allergic women at high risk of anaphylaxis
  - Studies have shown that samples from PCN-allergic women at high risk of anaphylaxis rarely undergo susceptibility testing
  - Increasing rates of clindamycin resistance make testing extremely important for disease prevention

2010 GBS Prevention Guidelines: Changes in Laboratory Methods

- Pigmented media to detect GBS
  - Color change in presence of beta-hemolytic GBS
  - Available for enrichment broth and agar
- Nucleic acid amplification tests (NAAT) such as PCR
  - Adequate sensitivity with broth enrichment step; suboptimal without enrichment
  - Option for prenatal testing, **with enrichment**
  - Option for intrapartum testing for GBS unknown without risk factors
- Revised cut-off for reporting GBS bacteriuria ($\geq 10^4$ cfu/mL)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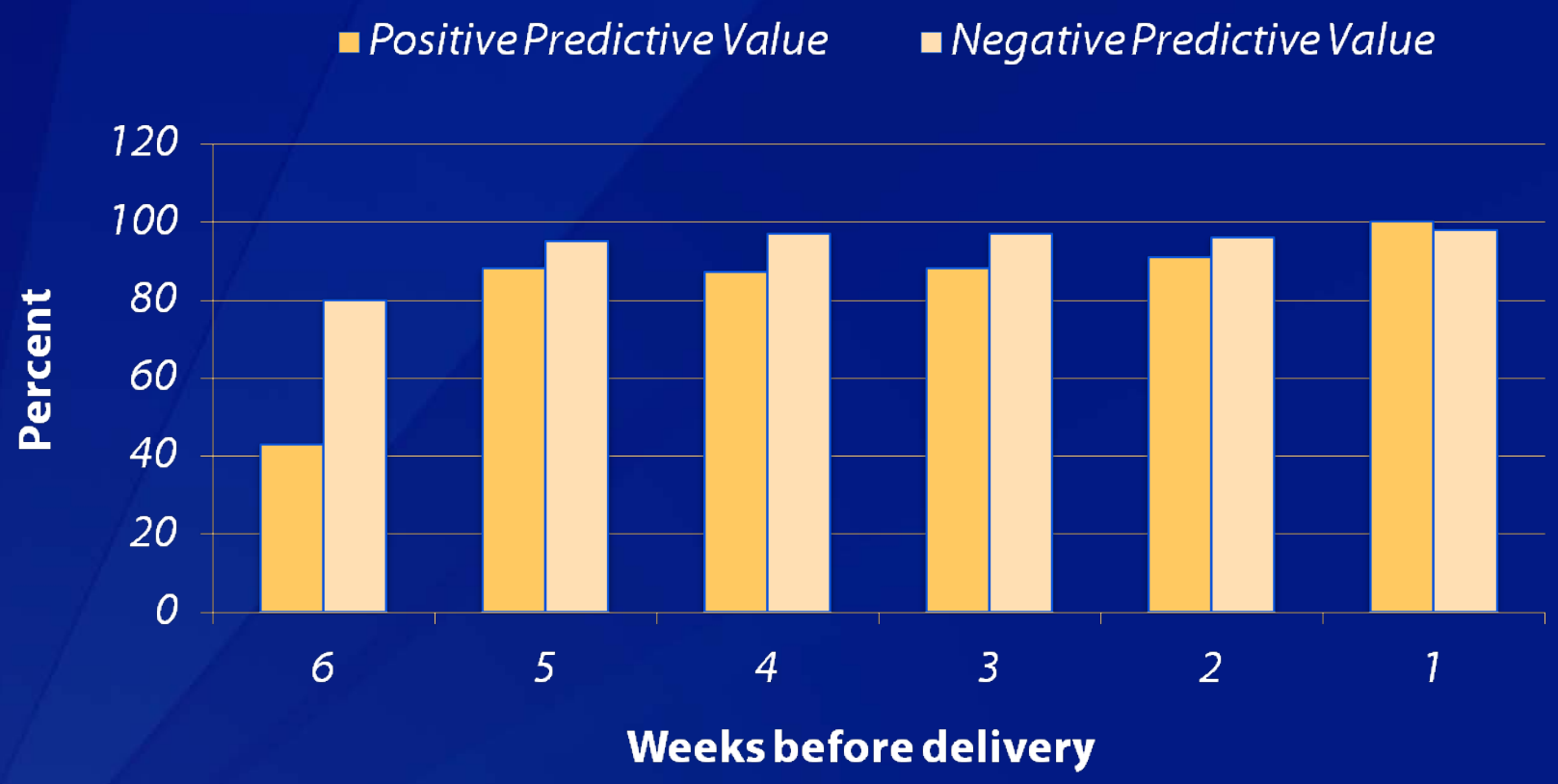
Prenatal Specimen Collection

- Lab feedback to clinicians can help optimize specimen collection
- Vaginal-rectal swabs
  - Swab lower third of vaginal
  - Insert through anal sphincter
  - Single swab or two swabs can be used
  - **Do NOT collect by speculum**
  - **Self collection an option**
- Timing: 35 to 37 weeks gestation

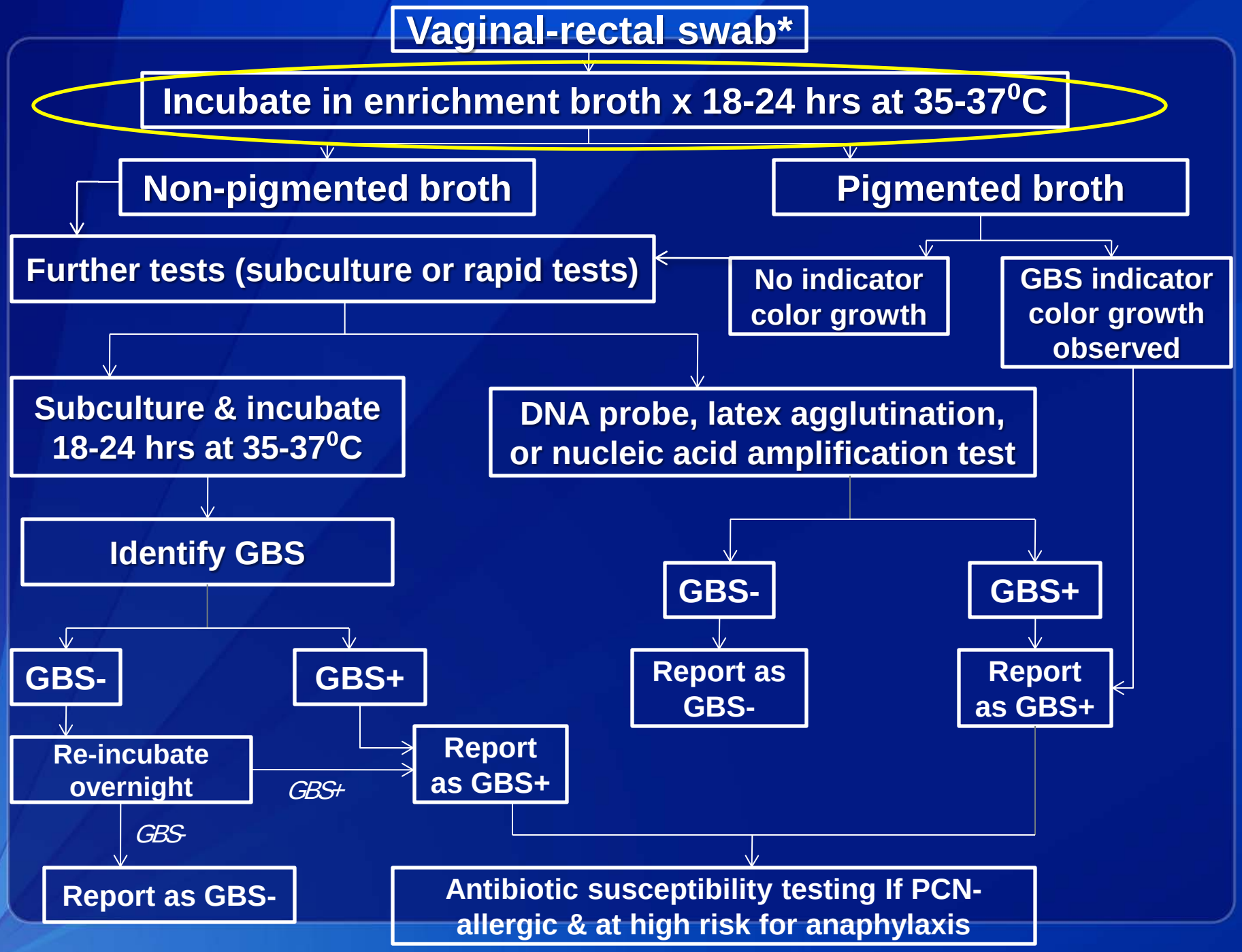
When to Culture?



N=826
Yancey et al., OB GYN 1996;88:811-5.

Prenatal Specimen Transport

- Inoculate swabs into nonnutritive transport medium
  - Appropriate transport systems are commercially available (Amies' or Stuarts')
  - Specimens in transport media may remain viable at room temperature for up to 4 days, *however*:
    - **Results most sensitive when processed within 24 hours**
    - **Results most sensitive when refrigerated prior to processing**
  - Specimens should be labeled clearly
    - **GBS specimen, penicillin allergy status**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Enrichment

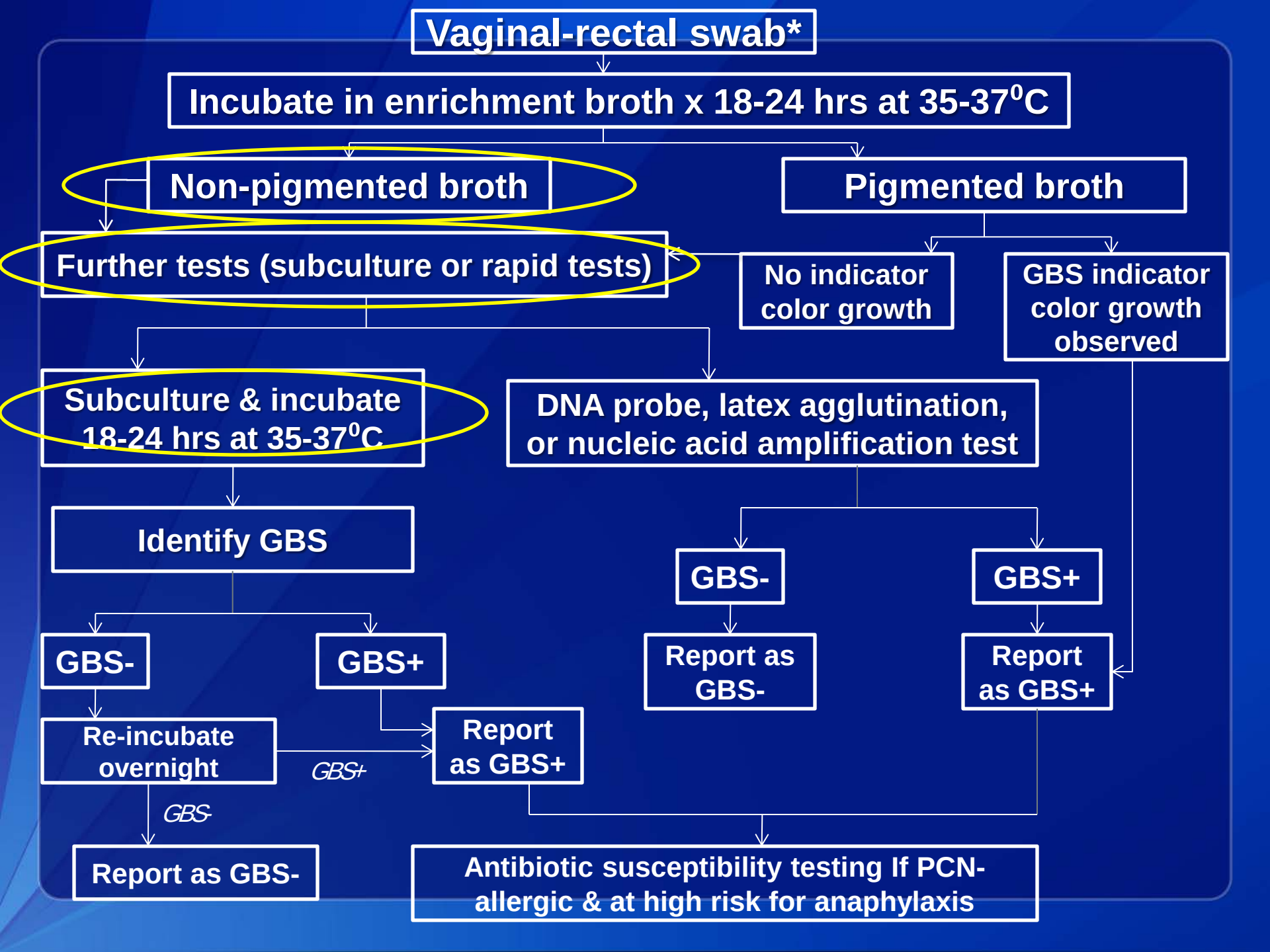
- Remove swabs from transport medium
- Inoculate into enrichment broth
  - **Non-pigmented broth**
    - **Lim broth, TransVag broth**
    - **TransVag broth should be supplemented with 5% defibrinated sheep blood**

<u>OR</u>

  - **Pigmented broth**
    - **StrepB carrot broth, Granada biphasic broth**
- Incubate inoculated broth for 18-24 hr at 35-37°C ambient air or 5% CO_2

Why Enrich?

- Enrichment in appropriate broth media for 18-24 hours critical step for recovery GBS cultures
- ~50% of women will have a false negative result if sample is not incubated for 18-24 hours in enrichment broth
- Direct plating can be done, but only as an additional step to broth enrichment
- If the direct plate is negative for GBS then the enrichment broth should be processed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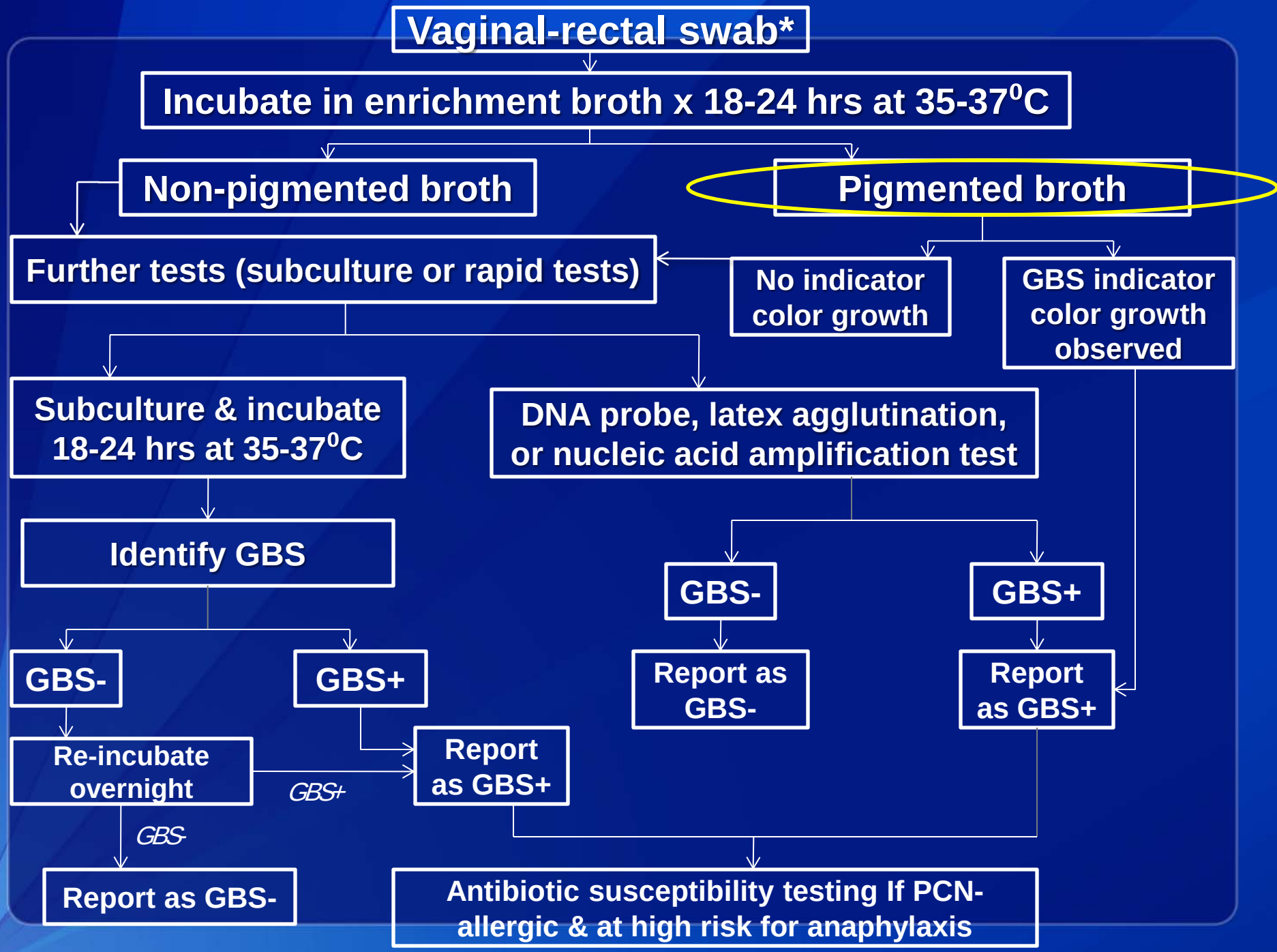
Testing—Non-Pigmented Broth

- Remember: for prenatal samples, *accurate* results much more important than *rapid* results
- Options for further testing after incubation in broth:
  1. **Direct test of broth – DNA probe, latex agglutination or NAAT testing**
  2. **Subculture to appropriate agar plate (e.g., sheep blood agar plate, CNA, commercial chromogenic agar) and incubate for 18-24 hours***

* If subculture negative, and GBS is not identified after 18-24h on BAP, re-incubate and inspect at 48 hrs; if still negative, report as GBS negative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Testing — Pigmented Broth

Check incubated inoculated broth for color change as per product instructions

- If color change present, report as GBS positive
- If color change NOT present:
  - **Important to realize that chromogenic methods may only identify beta-hemolytic GBS**
  - ***Must* try to identify non-beta hemolytic GBS by either:**
    - **Subculturing broth onto appropriate agar plate, incubating 18-24 hours, and identifying organisms suggestive of GBS***
    - **Direct testing of broth using appropriate methods**

* If subculture negative, and GBS is not identified after 18-24h on blood agar plate, re-incubate and inspect at 48 hrs; if still negative, report as GBS negative

Pigmented Broth — Positive Result

Positive color change

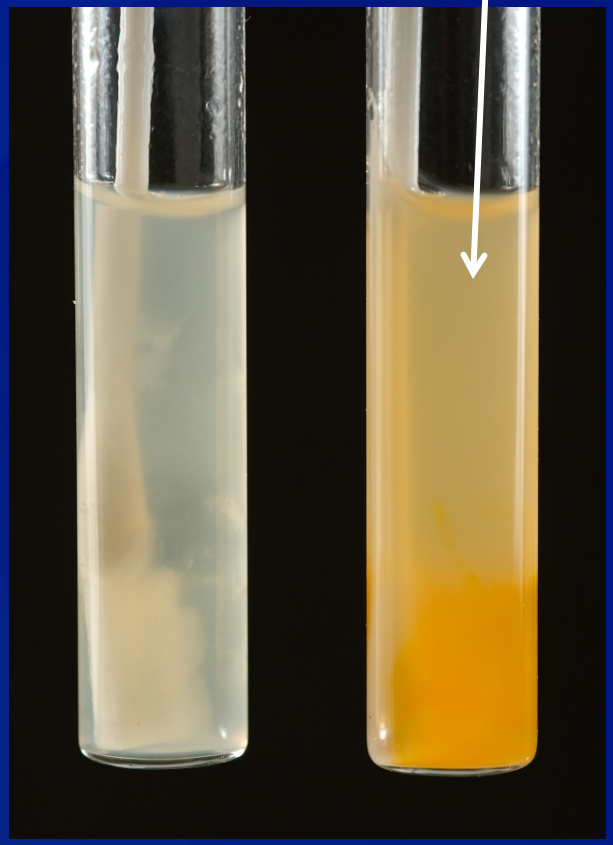
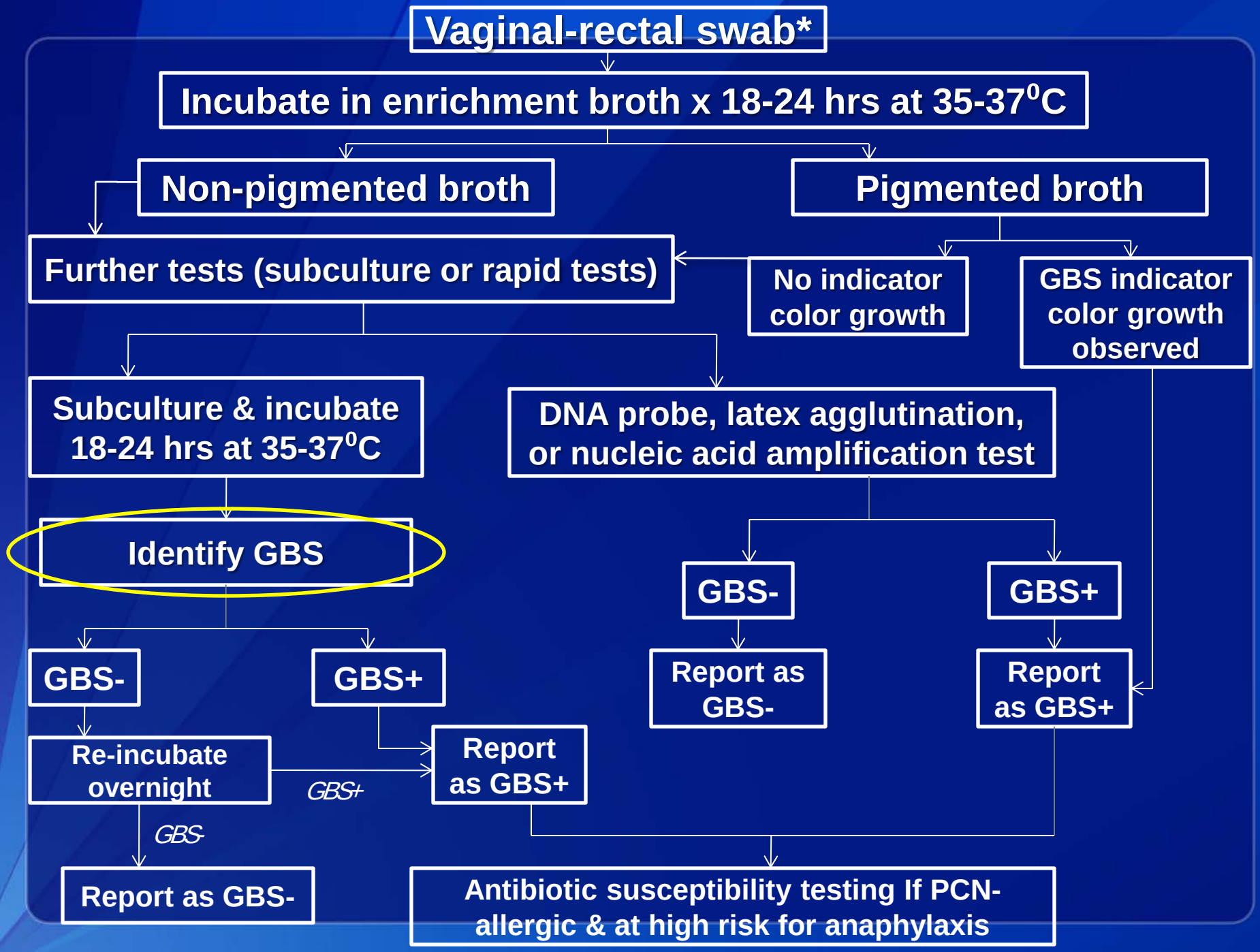


Photo courtesy of Dr. Lesley McGee, CDC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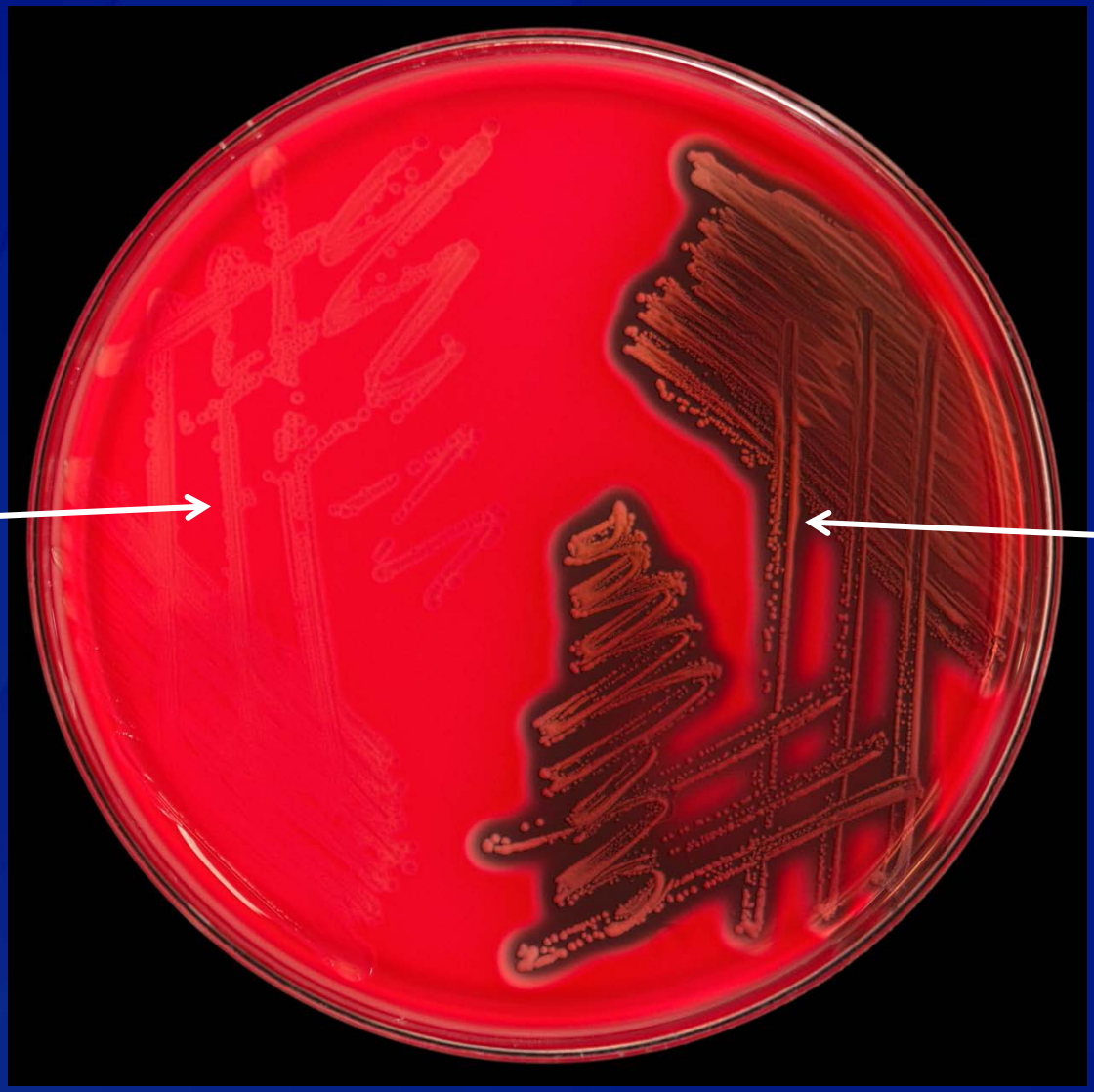
Tests to identify GBS from subculture

- Inspect subculture plates for hemolysis
  - **Generally beta-hemolytic on blood agar but non-hemolytic GBS are also possible**
- Additional tests to perform on selected likely colonies
  - **Gram stain yielding gram positive cocci**
  - **Catalase negative**
  - **Presumptive identification: CAMP positive (also hippurate positive)**
  - **Confirmatory identification: streptococcal grouping latex agglutination or other antigen detection tests (e.g., GBS Accuprobe)**

Comparison of GBS & GAS Hemolysis



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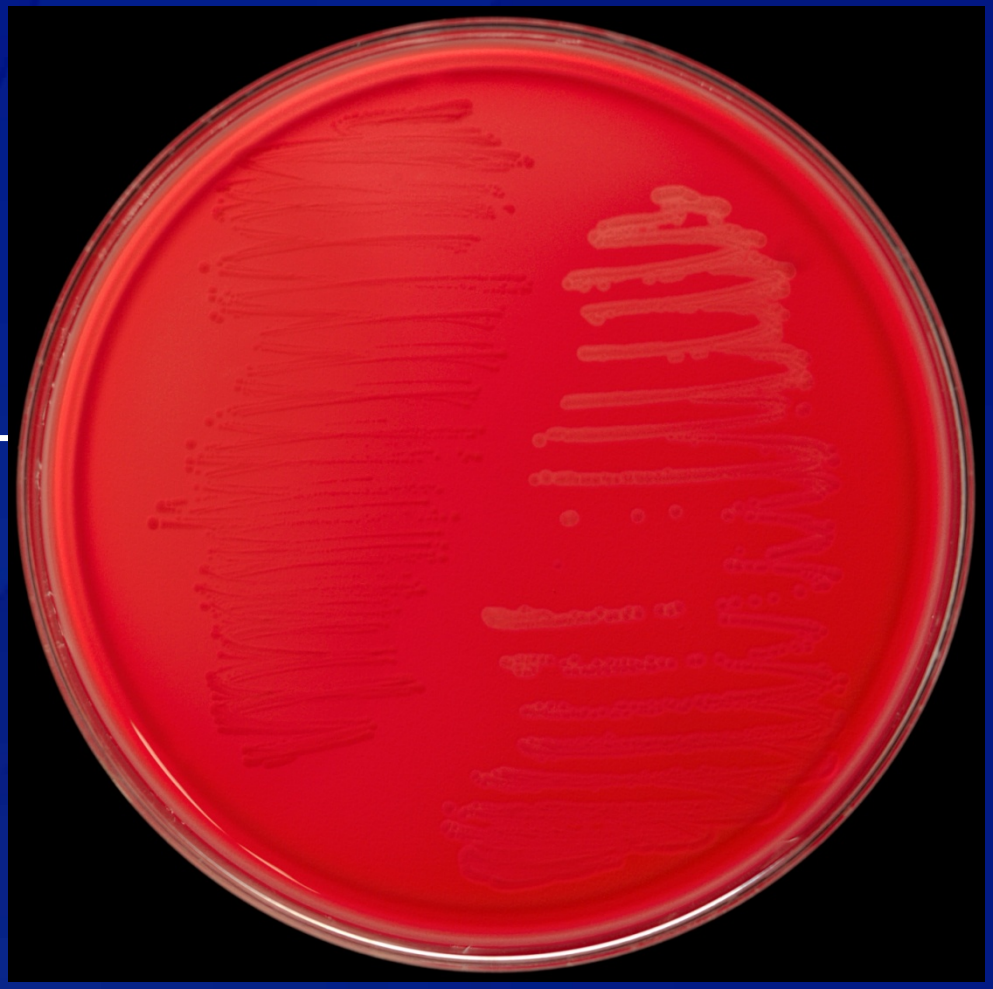
Non-hemolytic and Hemolytic GBS

Non-hemolytic GBS



Hemolytic GBS

Photo courtesy of Dr. Lesley McGee, CDC

Gram Stain

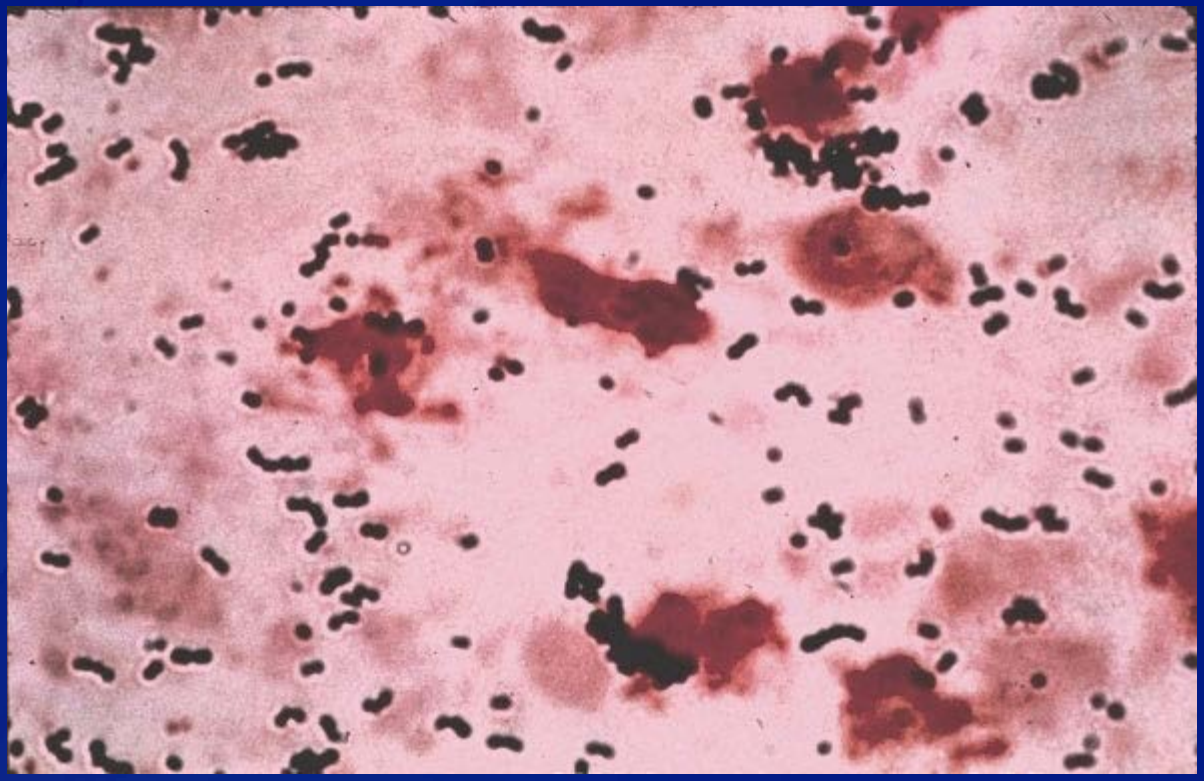
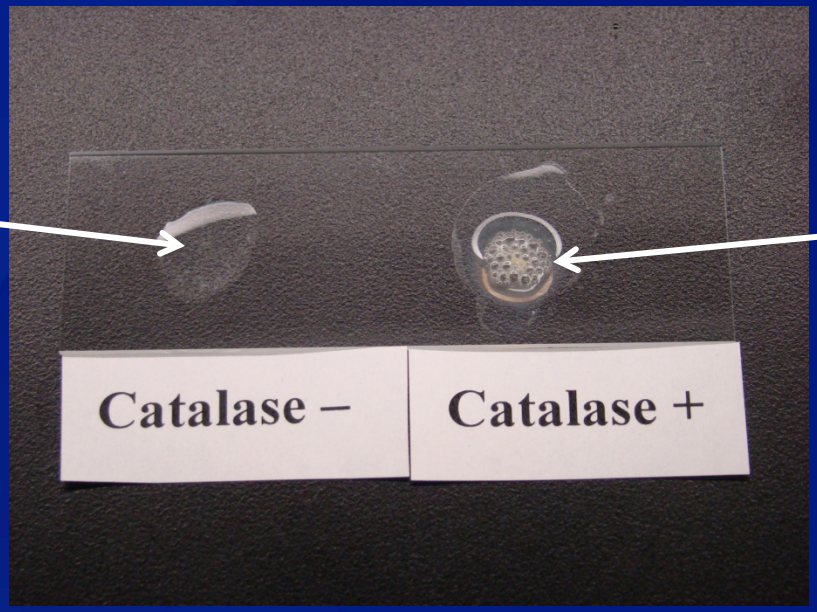


Photo courtesy of ASM MicrobeLibrary

Catalase

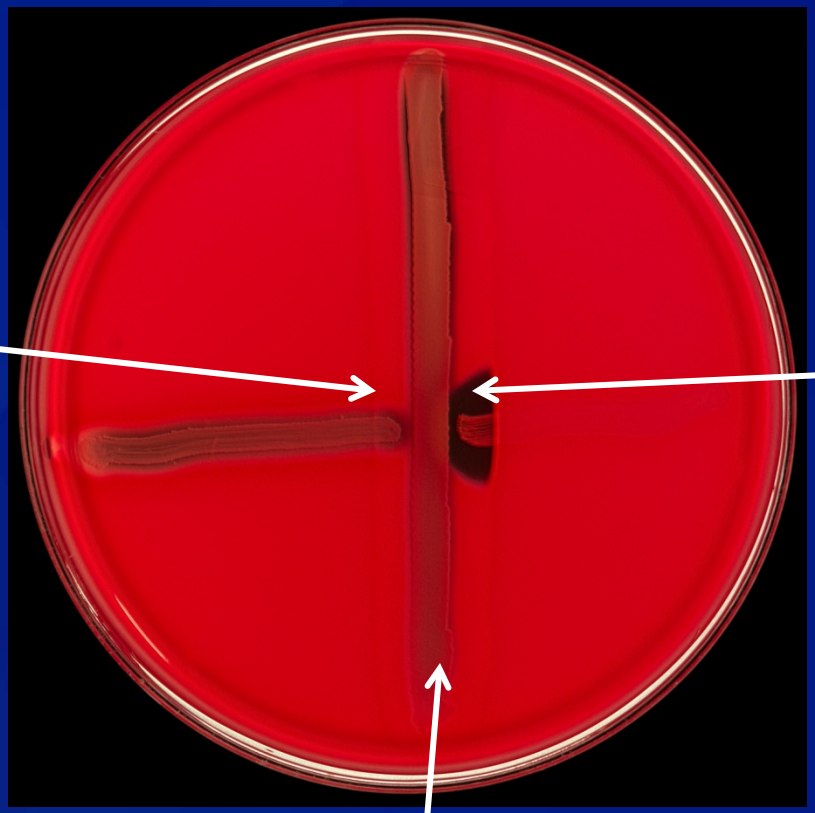
GBS – catalase negtaive



S.aureus – catalase positive

Photos courtesy of Dr. Lesley McGee, CDC

CAMP Test



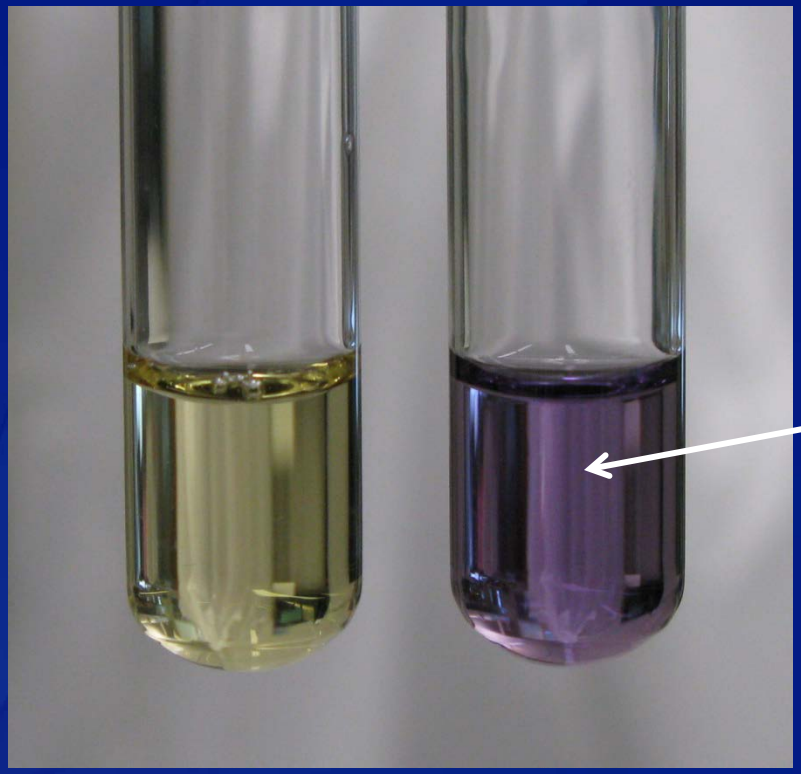
Negative reaction for GAS

Positive zone of enhanced hemolytic activity (GBS)

S. aureus

Photo courtesy of Dr. Lesley McGee, CDC

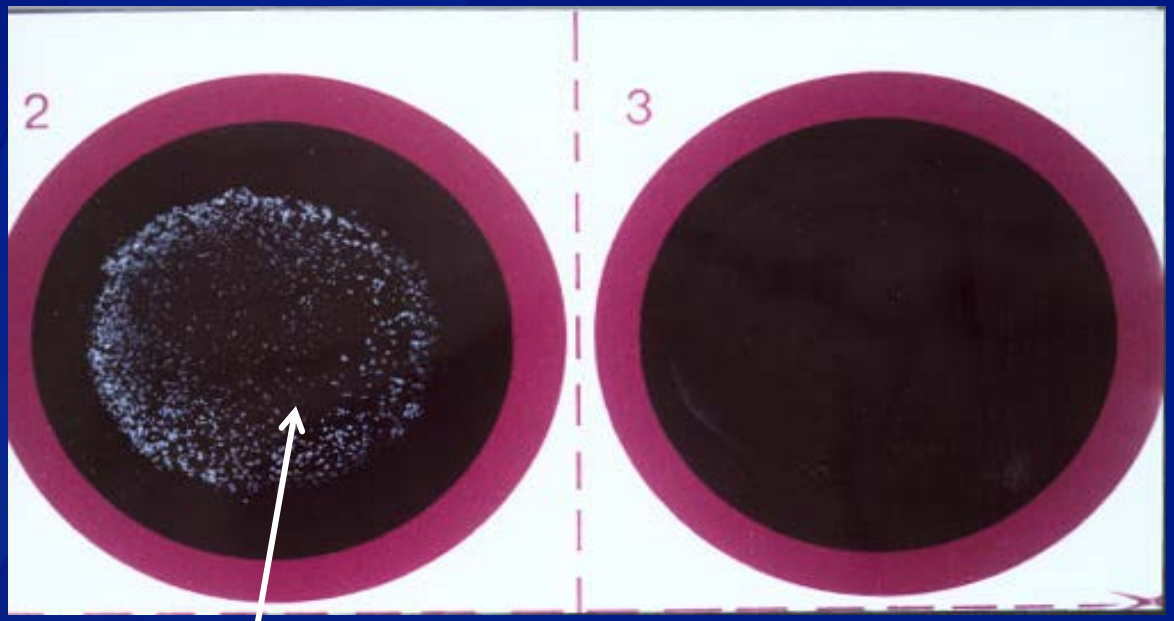
Rapid Hippurate Test



Purple color is positive for hippurate hydrolysis

Photo courtesy of Dr. Lesley McGee, CDC

Commercial Agglutination Tests



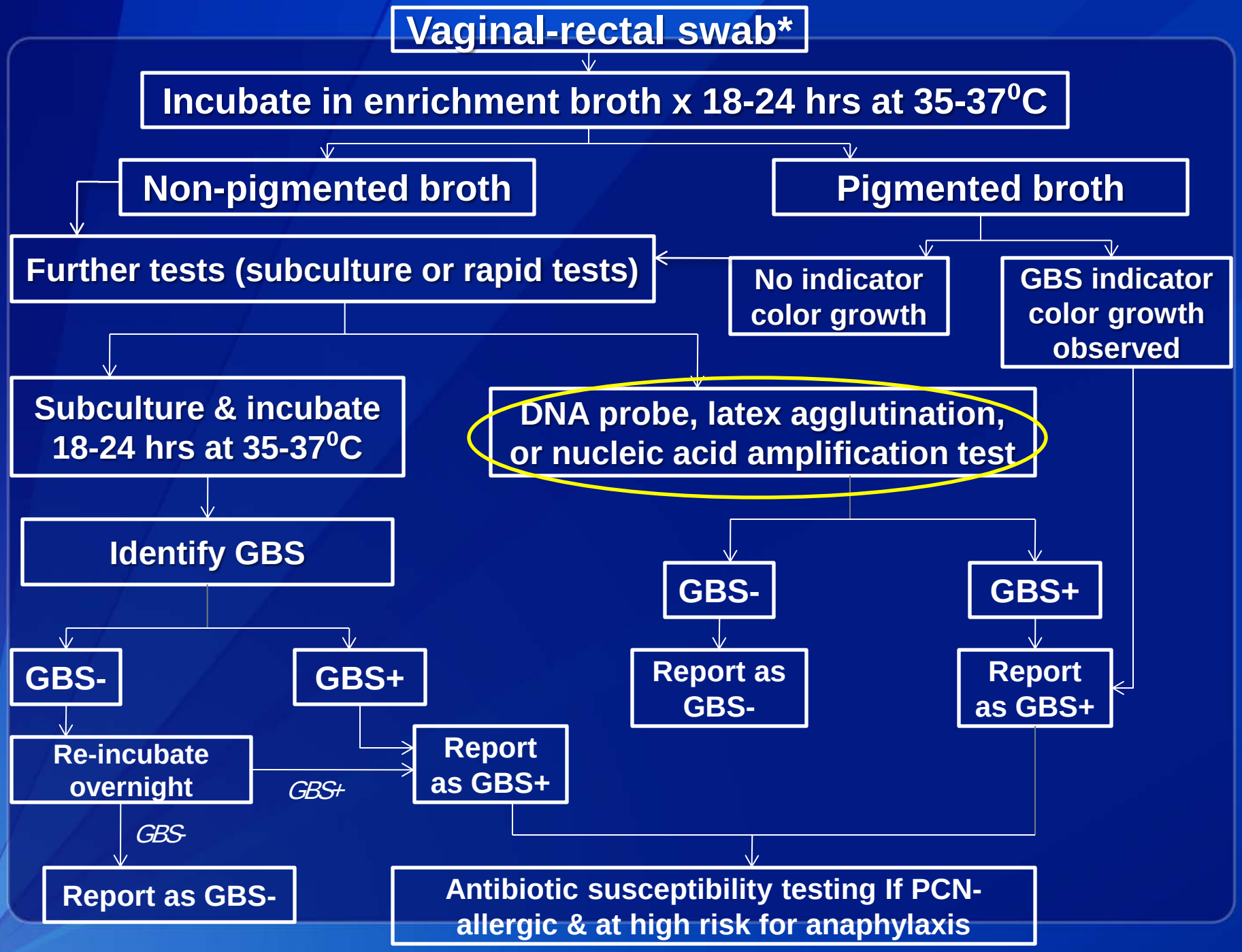
Positive agglutination

GBS is present

Negative agglutination

GBS is not present

Photo courtesy of Dr. Richard Facklam, CDC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Identification Tests from Enrichment Broth

- Direct testing for GBS from broth can occur AFTER incubation in enrichment broth
- The following methods are supported for direct testing of the enrichment broth:
  - DNA probe
  - Latex agglutination test
  - Nucleic acid amplification testing (NAAT)

Prenatal PCR Testing with Nucleic Acid Amplification (NAAT)

- New addition to 2010 guidelines
- Can be done *only* after enrichment in broth
  - **Very important for accuracy of result**
  - **Enrichment lengthens time to final result, but accuracy is much more important for antenatal testing**
- Use only FDA-approved/cleared tests
- NAAT should only be performed if laboratory has:
  - **validated NAAT performance**
  - **instituted appropriate quality controls**

Antimicrobial Susceptibility Testing

Antibiotic Recommendations — Intrapartum Prophylaxis

Standard: Penicillin (PCN) or ampicillin

Alternatives:

- **PCN-allergic and low risk for anaphylaxis: cefazolin**
- **PCN-allergic but high risk for anaphylaxis→ depends on susceptibility to clindamycin & erythromycin**
  - **If susceptible to clindamycin (including lack of inducible resistance) →clindamycin**
  - **If unknown or not susceptible→vancomycin**

Susceptibility of GBS: ABC Isolates, 2006-8

- 2479 invasive isolates collected from 5 sites*
- All susceptible to penicillin, ampicillin, cefotaxime, and vancomycin, however:
  - **Erythromycin resistance: 46%**
  - **Clindamycin resistance: 24%**

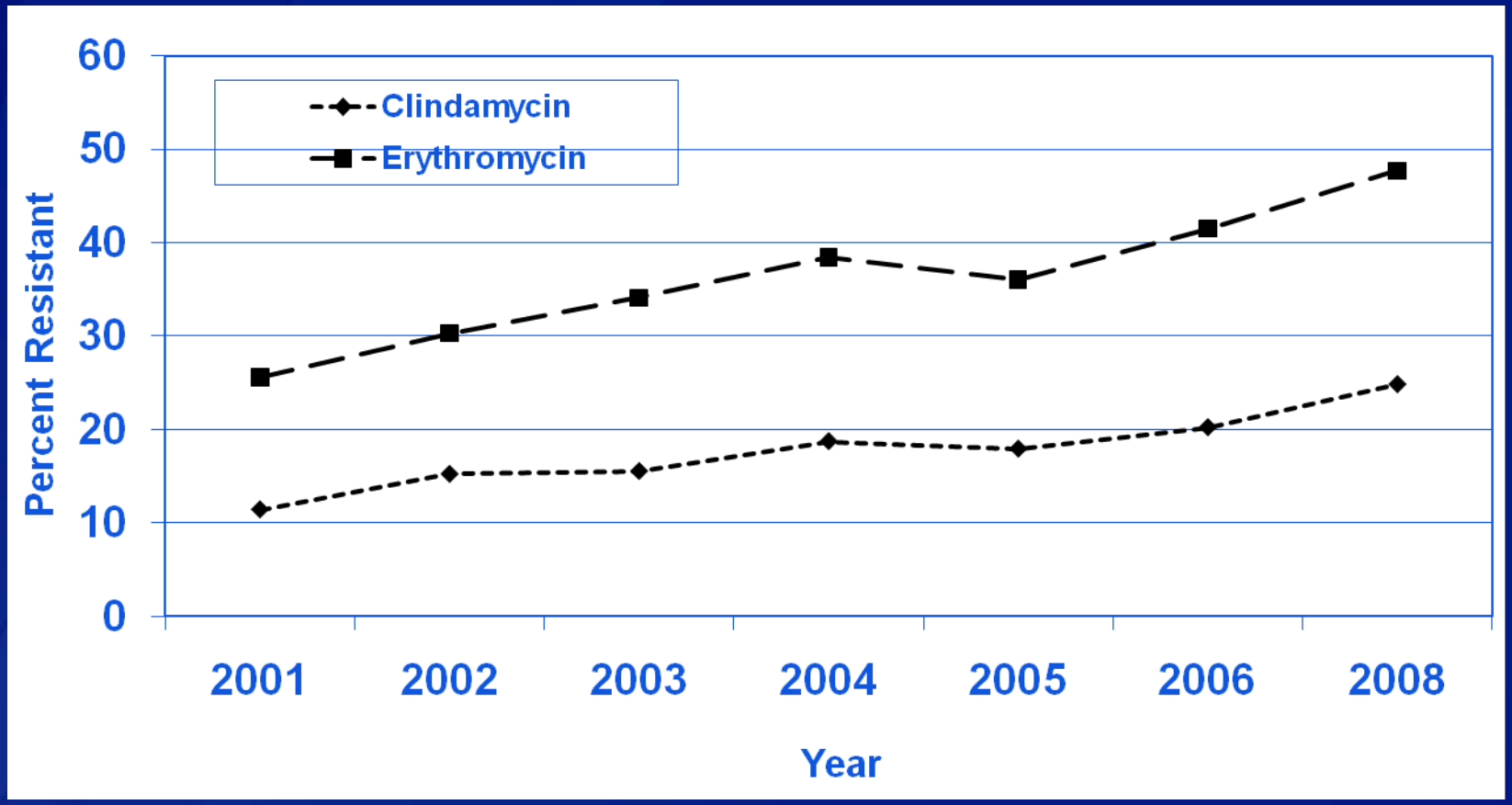
*For 2007, only early-onset isolates were tested

What about Resistance to Penicillin?

- Emergence of elevated MICs to β-lactams
  - 12 ABCs isolates from different states and years (1999-2006)
    - Isolates are just at the susceptibility threshold; clinical significance is unclear
  - 1 Japanese study, non-invasive adult isolates
    - Evidence of modified penicillin binding proteins

References: Dahesh et al. 2008. AAC 52: 2915; Kimura et al. 2008 AAC 52: 2890

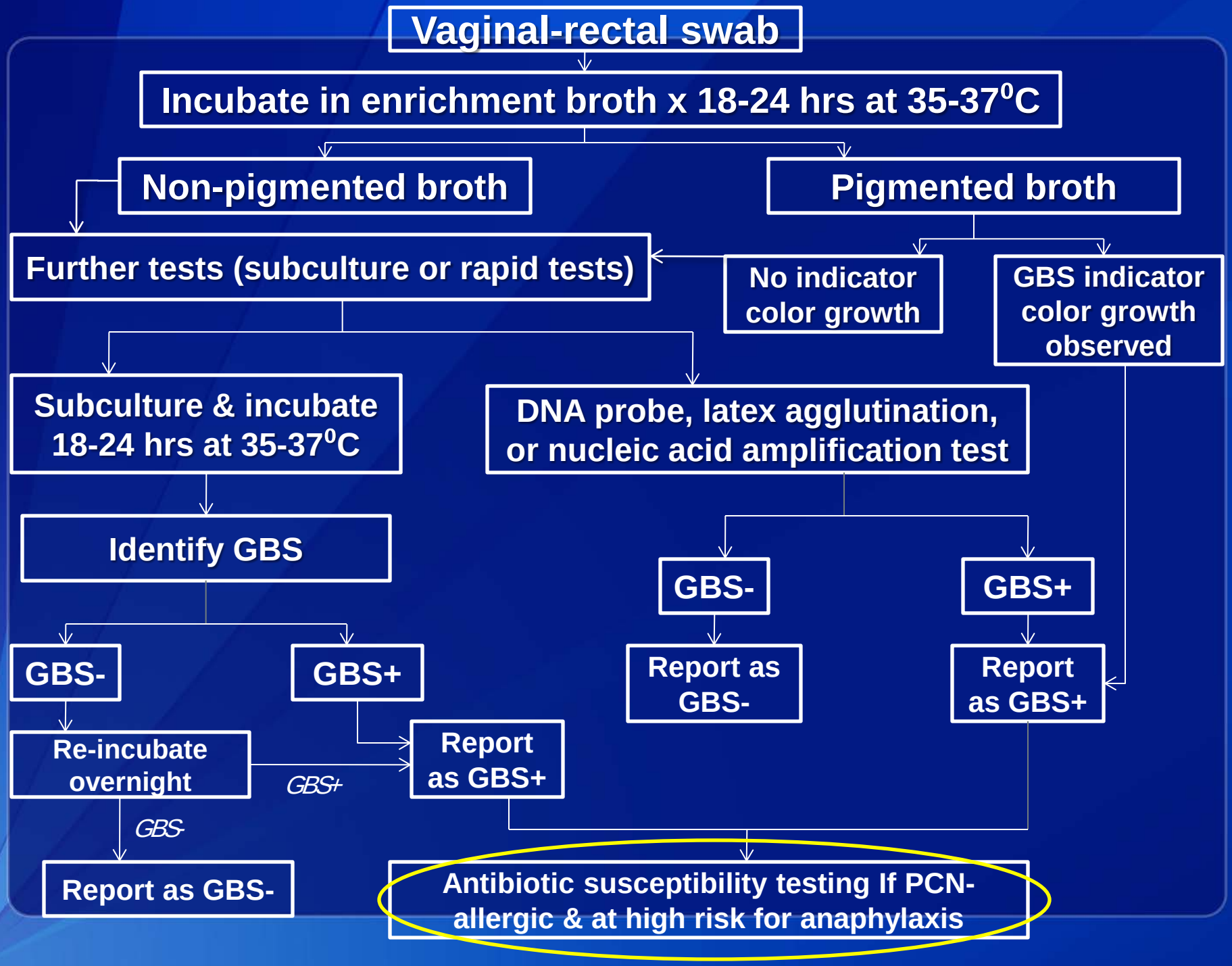
Clindamycin & Erythromycin Resistance among GBS isolates, ABCs sites, 2000-2008



*Isolates are from CO, GA, MD, MN, NY, and OR. 2007 data excluded

Clindamycin/Erythromycin Susceptibility Testing for GBS

- Testing only required for women with PCN allergy who are at high risk for anaphylaxis
- Room for improved implementation
  - **In a review of U.S. births in 2003-4, 84% of mothers at high risk for penicillin anaphylaxis received clindamycin**
  - **Only 18% had documented susceptibility testing (Van Dyke 2009; NEJM;** 360:2626-36)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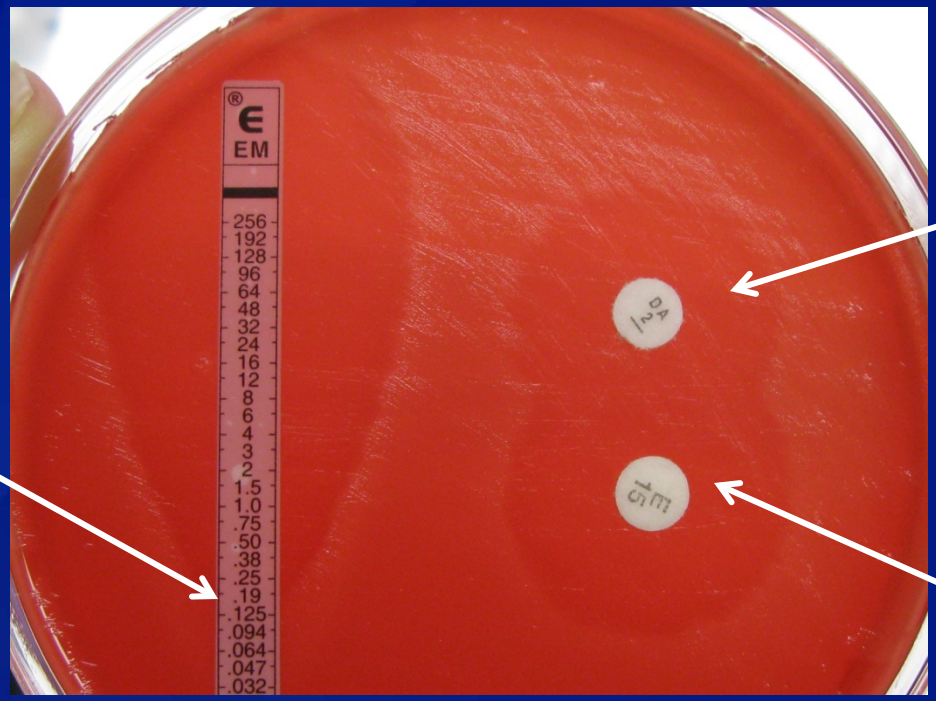
Antimicrobial Susceptibility Testing

- CLSI recommends using either:
  - Disk diffusion
  - Broth microdilution
    - FDA-cleared/approved commercial system may also be used
  - Testing for inducible clindamycin resistance
    - D-zone or other validated test

Antimicrobial Susceptibility: Etest & Disk Diffusion

Erythromycin
MIC = 0.19µg/ml



Etest

Disk Diffusion

Zone of inhibition of growth for clindamycin is ≥19 mm (susceptible)

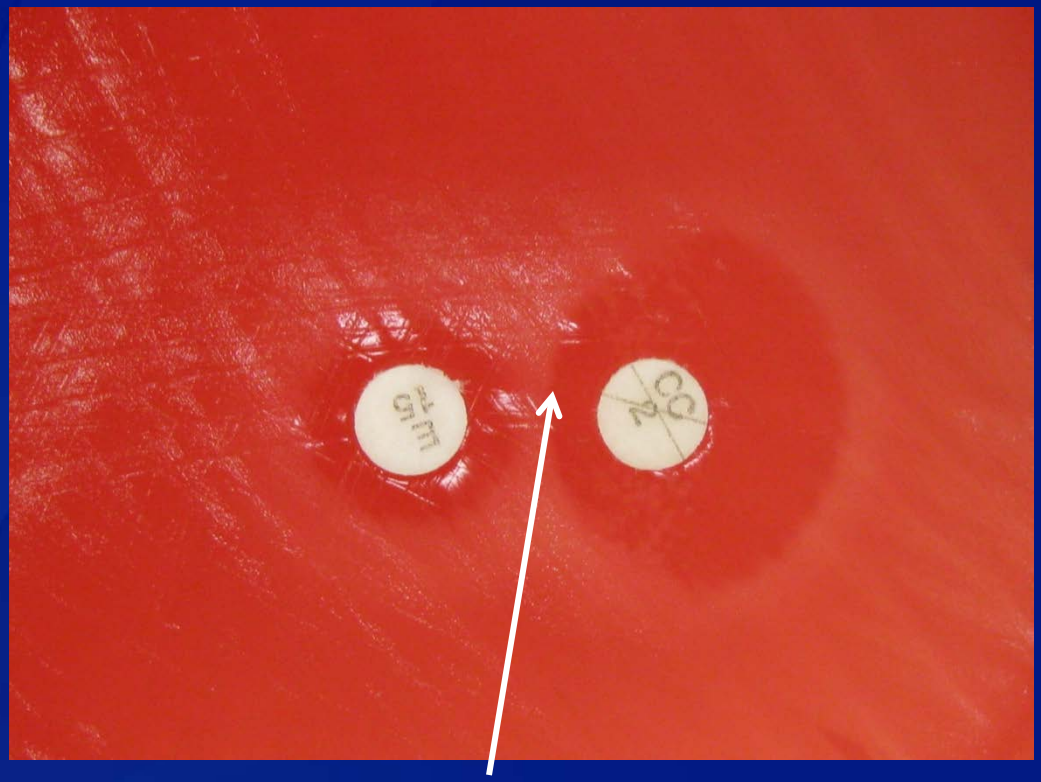
Zone of inhibition of growth for erythromycin is ≥21 mm (susceptible)

Photo courtesy of Dr. Lesley McGee, CDC

Procedure for D-zone Testing to Detect Inducible Clindamycin Resistance

- Use swab to make suspension from 18-24 hr growth of GBS in saline or Mueller-Hinton broth
- Dip sterile swab in adjusted suspension
- Inoculate entire surface of Mueller-Hinton sheep blood agar plate
- Place erythromycin (15µg) disk & clindamycin (2µg) disks 12 mm apart
  - **Incubate for 20-24 hrs at 35°C in 5% CO_2**
- Blunting of inhibition zone around clindamycin disk adjacent to erythromycin disk are considered D-zone positive
  - **If D-zone positive, report as clindamycin resistant**

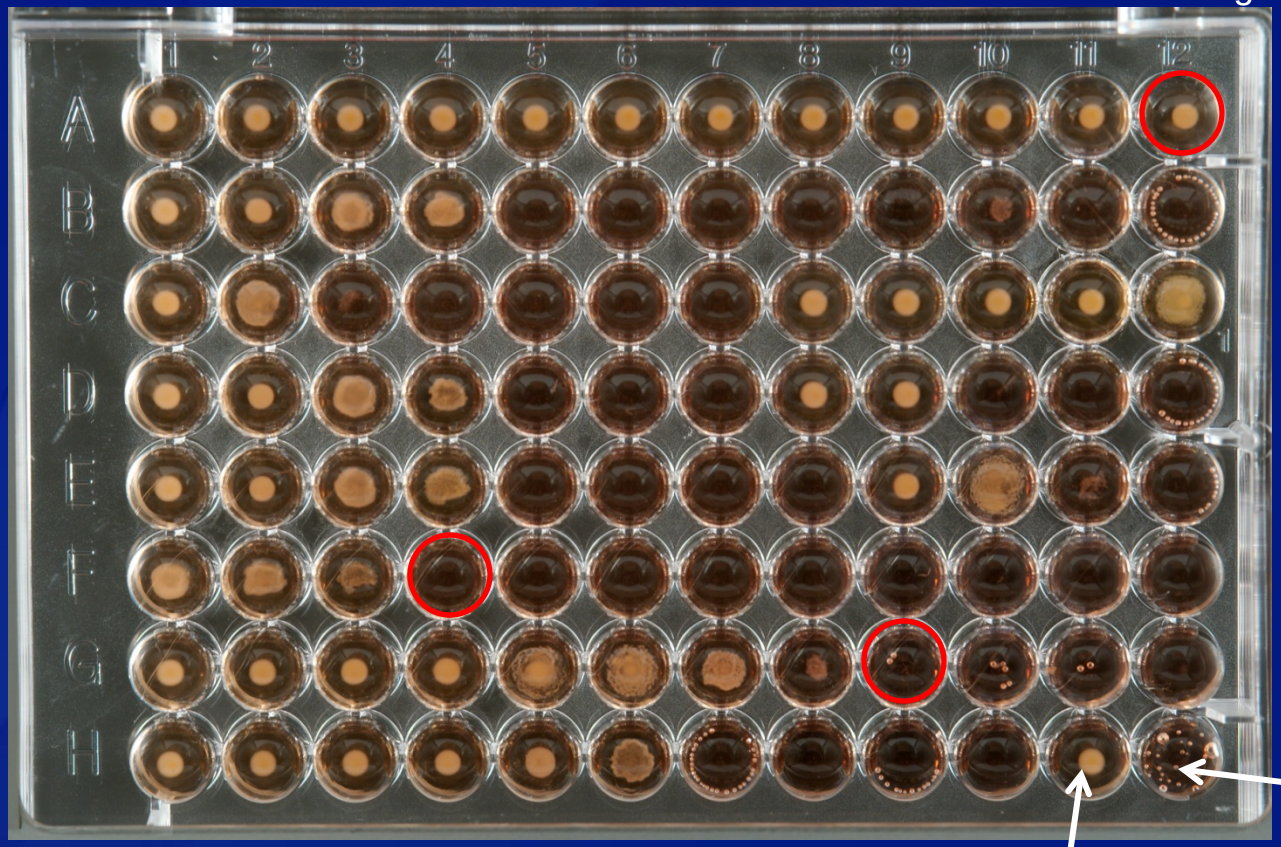
D-zone Test Result for GBS



Blunting of the inhibition zone indicating inducible clindamycin resistance

Photo courtesy of Dr. Lesley McGee, CDC

Antimicrobial Susceptibility Test
Broth Microdilution Dilution



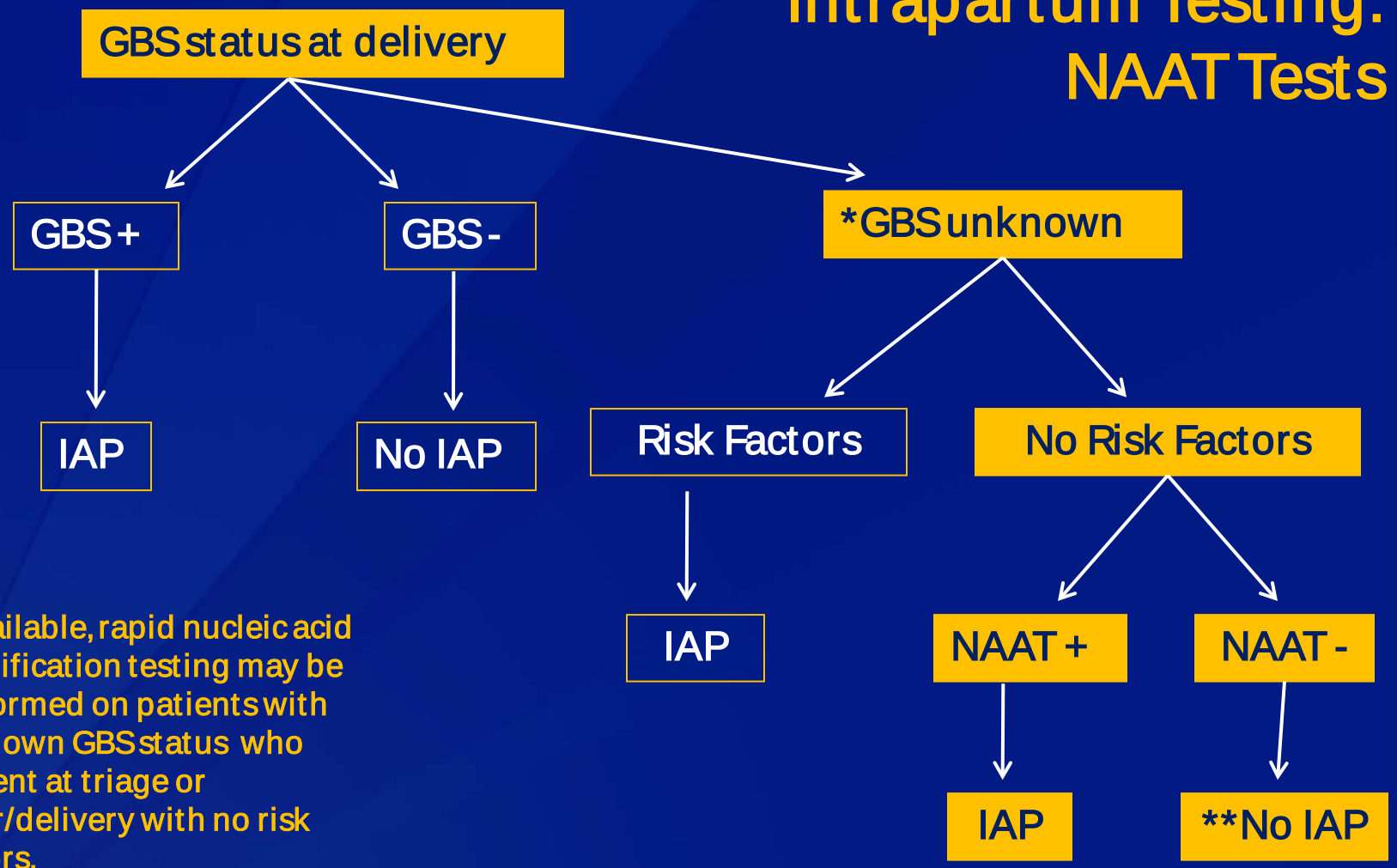
(MIC) Minimum Inhibitory Concentration

Photo courtesy of Dr. Lesley McGee, CDC

Intrapartum Testing

- Some centers can provide intrapartum PCR for GBS
- Enrichment step is not practical intrapartum because of the delay in results
- Decreased sensitivity of test result without broth enrichment step
- Can do intrapartum PCR testing directly on swabs (no-enrichment) for women who are GBS unknown at time of delivery who have no risk factors:
  - **Positive result: intrapartum antibiotic prophylaxis**
  - **Negative result: No intrapartum antibiotic prophylaxis, unless risk factors develop**

Intrapartum Testing: NAAT Tests



- If available, rapid nucleic acid amplification testing may be performed on patients with unknown GBS status who present at triage or labor/delivery with no risk factors.
- **Unless risk factors develop

Other changes: Bacteriuria

- 2002 guidelines required labs to report ANY quantity of GBS found in urine cultures
- Required great deal of lab time
- Studies of bacteriuria as evidence of 'heavy' colonization have used $\geq 10^4$ CFU/mL as cutoff
- Difficult with available data to determine significance of lower colony counts
- 2010 recommendation is to report positive urine cultures with $\geq 10^4$ cfu/mL of GBS

Summary: GBS Guidelines for Laboratories

– Key changes in 2010:

- Specimen transport options clarified
- GBS identification options for prenatal specimens expanded to include chromogenic media and identification directly from enriched broth (including use NAAT after broth enrichment)
- A direct plating option can be included but only in addition to (not in place of) enriched culture
- Laboratories should report GBS in urine culture specimens when present at concentrations of $\geq 10^4$ cfu/mL
- Testing for inducible clindamycin resistance should be performed on isolates sensitive to clindamycin, resistant to erythromycin, and from PCN-allergic women at high risk for anaphylaxis
- Guidance for use of NAAT testing in intrapartum setting for GBS unknown status at delivery with no risk factors

Key GBS Resources

- CDC's GBS Internet page
  - http://www.cdc.gov/groupbstrep
- MMWR: http://www.cdc.gov/mmwr/
- GBS Association home page
  - http://www.groupbstrep.org
- American Society for Microbiology
  - http://www.asm.org
- FDA website (includes GBS molecular diagnostic tests)
  - http://www.amp.org/FDATable/FDATable.doc
- Further tools (online and paper-based) coming soon for labs

For more information please contact Centers for Disease Control and Prevention

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E-mail: cdcinfo@cdc.gov Web: www.cdc.gov

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.