

***GBS  
Screening,  
diagnosis and  
clinically relevant  
resistance***

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- ***Background***
- **Evolution of culture methods**
- **Rapid non-cultural GBS screening**
- **Antimicrobial resistance**



# Required RF for GBS EOD

## *Vaginal (rectal) GBS colonization at delivery*

- **GBS carriers**
  - **GI tract = natural reservoir**
  - **10 - 35 % of women (vagina/rectum)**
  - **Clinical signs not predictive**
  - **Dynamic condition (transient – chronic – intermittent)**
  - **Prenatal cultures late in pregnancy can predict delivery status**

# Background Prevention for neonatal GBS EOD

- **Prevention for neonatal early onset disease**
  - **Antibioprophylaxis**
  - **Universal **GBS** screening-based strategy**
  - **Successful but cases continue occurring**

## **Goal of GBS screening**

*To predict GBS vaginal (rectal) colonization at the time of delivery*

**How could you  
know if my mom  
is GBS-colonized?**



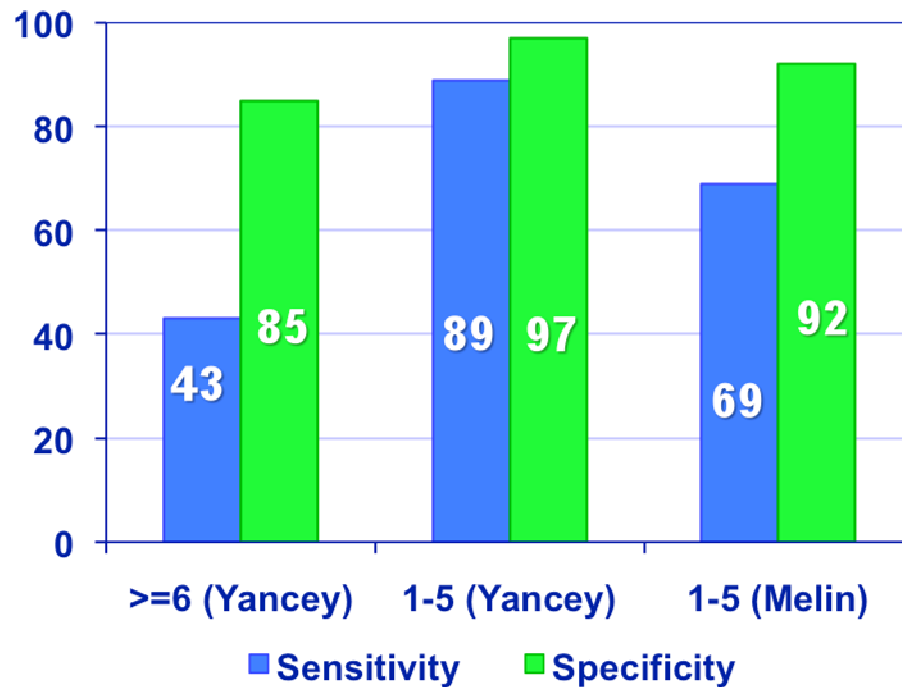
# Background GBS Screening

- **Critical factors influencing accuracy**
  - **Screening methods**
    - Culture
    - Non-culture
  - **Timing of sampling**
  - **Swabbed anatomic sites**
  - **Culture media**

Background  
Culture  
Non-culture  
Resistance

# Optimal time for screening 35-37 weeks gestation

**Culture-based screening done 1 to 5 or  $\geq 6$  weeks before delivery (Yancey, 860 cases; Melin, 531 cases)**



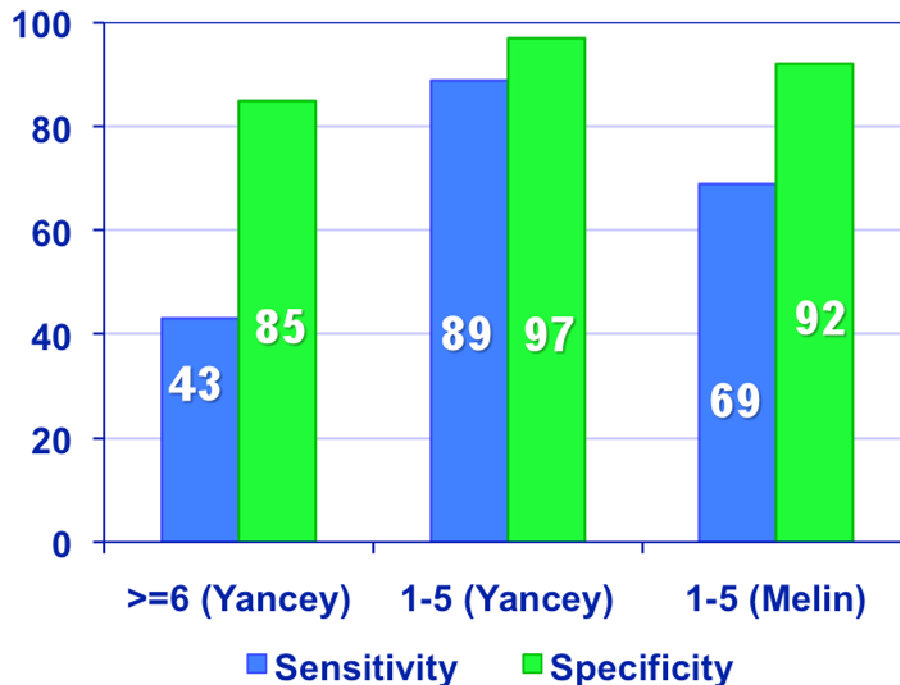
*Yancey MK et al. Obstet Gynecol 1996;88:811-5*

*Melin et al. ICAAC 2000*

Background  
Culture  
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Resistance

# Optimal time for screening 35-37 weeks gestation

**Culture-based screening done 1 to 5 or  $\geq 6$  weeks before delivery (Yancey, 860 cases; Melin, 531 cases)**



**Melin, 13-16% GBS Pos**

**PPV= 56%**

**NPV= 95%**

**or 5% False negative**

**or 30% of GBS pos in labor not detected with prenatal screening !**

*Yancey MK et al. Obstet Gynecol 1996;88:811-5*

*Melin et al. ICAAC 2000*



# Choice of the anatomic sites

## Vagina + rectum

**Vagina & rectum > vagina or rectum > cervix**

*Badri et al., J Infect Dis 1977;135:308-12*

- **Rectum**
  - = reservoir, source of vaginal colonization
- **Rectum GBS positive and vagina negative**
  - 15 to 20% of GBS positive pregnant women
- **Lower vaginal area**
  - To exclude use of speculum for collection

# Evolution of culture methods

## Use of selective enrichment broth

- **To maximize the isolation of GBS**
- **To avoid overgrowth of other organisms**

<b>Nb women, medium</b>	<b>Direct culture 48hrs GBS+</b>	<b>Sub- culture from SEB % GBS+</b>	<b>Authors</b>
<b>200, Granada</b>	<b>88 %</b>	<b>100 %</b>	<b>Tazi A et al, 2008</b>
<b>500, Granada</b>	<b>72 %</b>	<b>99 %</b>	<b>Melin P et al, 2008</b>
<b>StrepB select</b>	<b>74 %</b>	<b>96 %</b>	
<b>288, Blood /Lim</b>	<b>52 %</b>	<b>82 %</b>	<b>Shibuya R, 2009</b>
<b>New Granada</b>	<b>52 %</b>	<b>100 %</b>	

# Evolution of culture methods

## Use of selective enrichment broth

- **Todd Hewitt broth**  
**+ colistin + nalidixic acid**  
**= LIM broth**
- **Todd Hewitt broth**  
**+ gentamicin + nalidixic acid (+ 5% sheep blood)**  
*(C.Baker, 1973 Applied Microbiology )*  
**= « Trans-Vag™**  
**broth »**
- **Granada biphasic broth**

# Evolution of culture methods

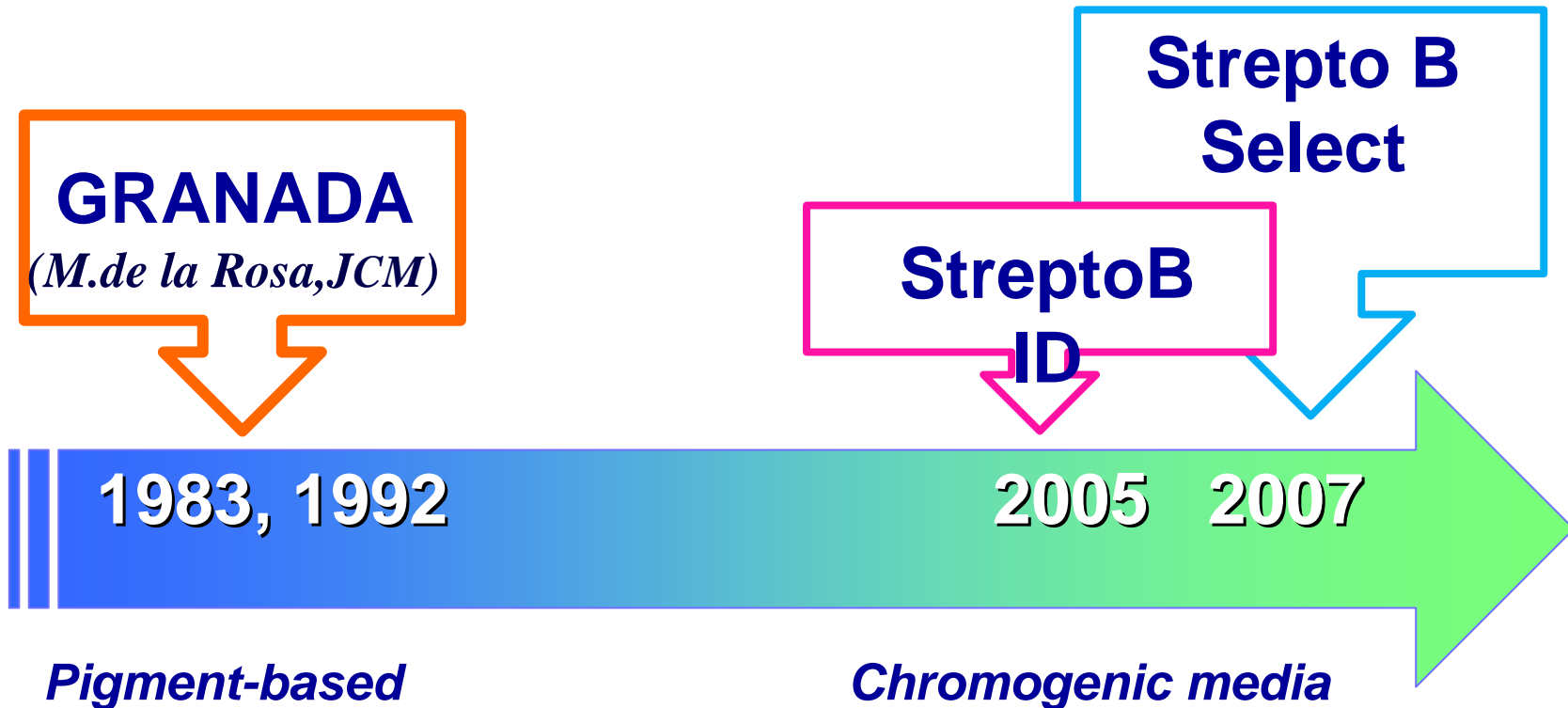
## Revised guidelines from CDC (2002)

- **Sub-culture < selective enrichment broth**
  - **Blood agar**
    - **Advantage**
      - Growth of all GBS Isolates beta-hemolytic or not
    - **Disadvantage**
      - Difficulty in seeing rare GBS colonies within mixed flora
      - Difficulty in recognizing non-hemolytic GBS in mixed flora

# Evolution of culture methods

## Use of differential agar media

Recommended by some European guidelines



# Granada medium agar

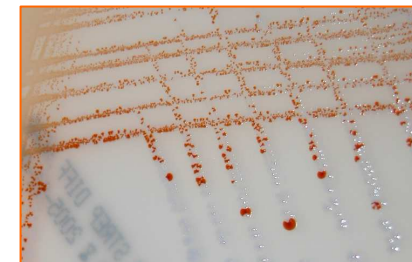
*M de la Rosa Fraile, JCM 1983 & 1992*



- **Orange color: GBS pigment, Granadaene**

- **100% specific for GBS //  $\beta$ -hemolysis**

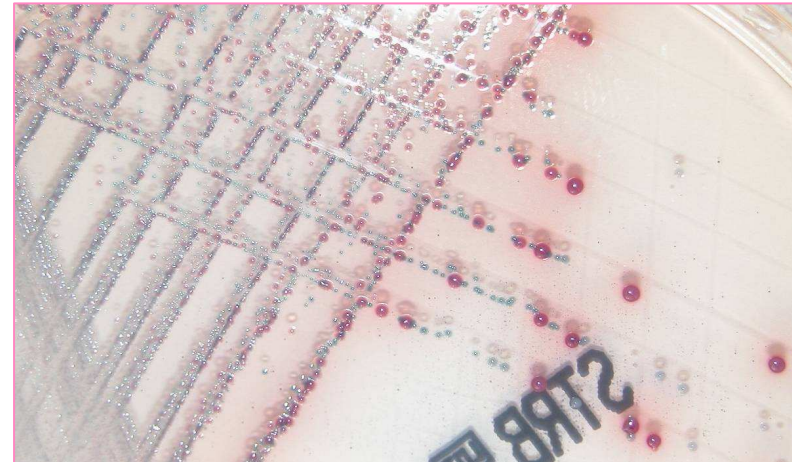
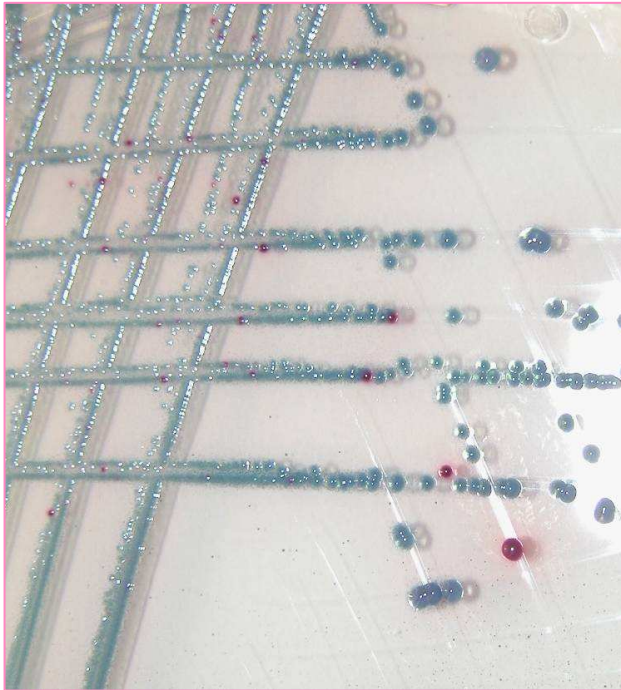
- **Group B Streptococcus Differential Modified Granada Medium™ (BD)**
- **Carrot Medium (Hardy)**



**Does not show non-hemolytic strain !**  
**(<5 % of invasive isolates)**

Background  
**Culture**  
Non-culture  
Resistance

# Strepto B ID agar (BioMérieux)

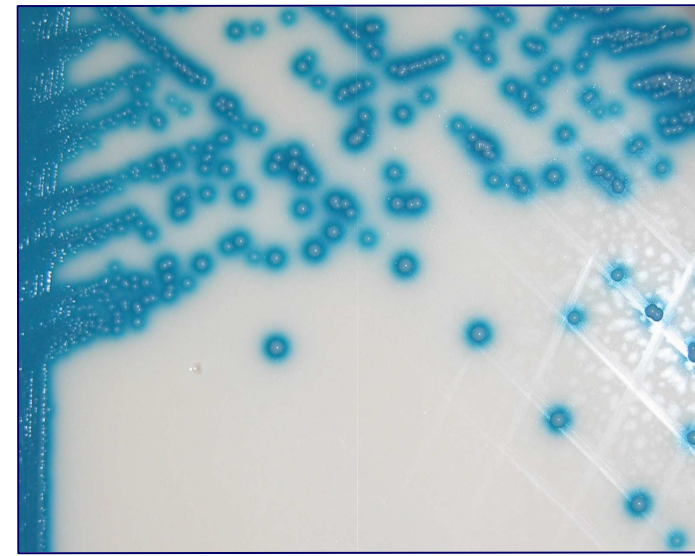
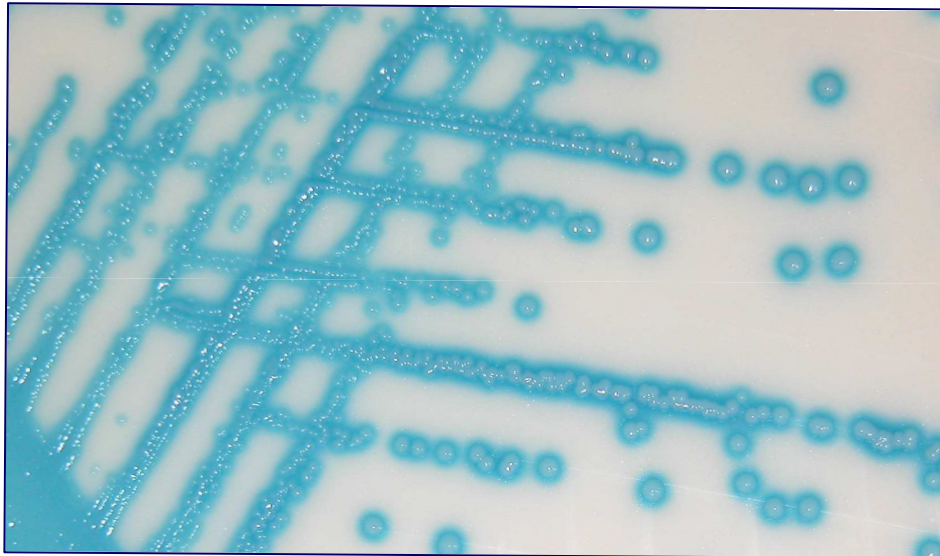


**High sensitivity  
for growth of GBS  
GBS = pink to red colonies**

**Chromogenic media  
Not 100 % specific for GBS: Id to confirm (latex)**

Background  
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# Strep B Select agar (BioRad)



**GBS = pale to dark blue-turquoise colonies**

**Chromogenic media**

**Not 100 % specific for GBS: Id to confirm (latex)**



# Granada (BD) - StreptoB ID - StrepB Select versus Blood agar +/- CNA

500 genital swabs (29.4 % GBS Positive)

	Number of GBS Positive culture (%)		
	Direct culture	Lim sub-culture	Total
<b>Strep B Select</b> (BioRad)	103 (70.1)	134 (91.1)	139 (94,6)*
« <b>Granada</b> » (BD)	90 (61.2)	123 (83.7)	124 (84.4)
<b>Strep B ID</b> (bioMérieux)	93 (63.2)	124 (84.3)	128 (87.1)
<b>BA ± CNA</b>	76 (51.7)	113 (76.9)	120 (80.6)
<b>&gt;=1 Medium</b>			147 (100)

\* StrepB Select > BA (p<0,5)

# Granada (BD) - StreptoB ID - StrepB Select *versus* Blood agar +/- CNA

## « False-Positive »

= Characteristic colonies not confirmed as GBS

	Identified as
<b>Strep B Select</b>	GAS, GCS, GDS-enterococci, Staphylococci, <i>S.bovis</i> , $\alpha$ -hemolytic colonies, (yeasts, Gram negative bacilli)
<b>Granada</b>	/
<b>Strep B ID</b>	GCS, Staphylococci, $\alpha$ -hemolytic colonies, (Gram negative bacilli)
<b>BA +/- CNA</b>	GAS, GCS, GFS, Staphylococci, GDS-enterococci, (Gram negative bacilli)

# Positive predictive value Granada (BD) - StreptoB ID - StrepB Select versus Blood agar +/- CNA

	PPV Primoculture	PPV Lim sub-culture
Strep B Select	71,5 %	77,9 %
Granada	100 %	100 %
Strep B ID	80,9 %	87,9 %
BA +/- CNA	62,8 %	65,7 %

## Sensitivity

Strep B Select > Granada - Strep B ID > CNA

## Specificity

Granada > Strep B ID > Strep B Select > CNA

# Which agar or which combination?

+/- Blood agar



**Workload – costs – extra-testing to be considered**



Vagino-rectal swab or  
Vaginal & rectal swabs



Inoculate swab(s) in 1 Lim broth



LIM broth

Overnight  
And subculture  
following  
at 35-37°C  
to one of the  
media



Granada  
agar

Anaero



StrepB  
Select

Ambient air  
48 h at 35-37°C



ID  
StreptoB

Ambient air



<b>POSITIVE GBS Screening if</b>	Orange colonies - GBS	Blue-turquoise colonies - suggestive GBS id. to confirm	Pink colonies - suggestive GBS id. to confirm
<b>Negative GBS Screening if</b>	No orange colonies	No blue-turquoise colonies	No pink colonies

# Crucial conditions to optimize SCREENING

- ❖ **WHEN** 35-37 weeks
- ❖ **WHO** ALL the pregnant women
- ❖ **Specimen** Vaginal + rectal swab(s)
- ❖ **Collection** WITHOUT speculum
- ❖ **Transport** Transport/collection device  
(non nutritive medium: Amies/Stuart)
- ❖ **Request form** To specify prenatal « **GBS** »  
screening + *expected address  
for delivery*
- ❖ **Laboratory procedure**



**Adhesion to a common protocol is a key for success**  
**Multidisciplinary collaboration is mandatory**

# Prenatal culture-based screening

- **Limiting factors**
  - **Positive and negative predictive values**
    - **False-negative results**
      - Failure of GBS culture (oral ATB, feminine hygiene) or new acquisition
      - Up to 1/3 of GBS women at time of delivery
      - Continuing occurrence of EO GBS cases
    - **False-positive**
      - Unnecessary IAP
  - **Need for more accurate predictor of intrapartum GBS vaginal colonization**



# Alternative to prenatal GBS screening: intrapartum screening

Turnaround time

Collect specimen at admission

Optimal  
management  
of patient



Specimen  
analysis

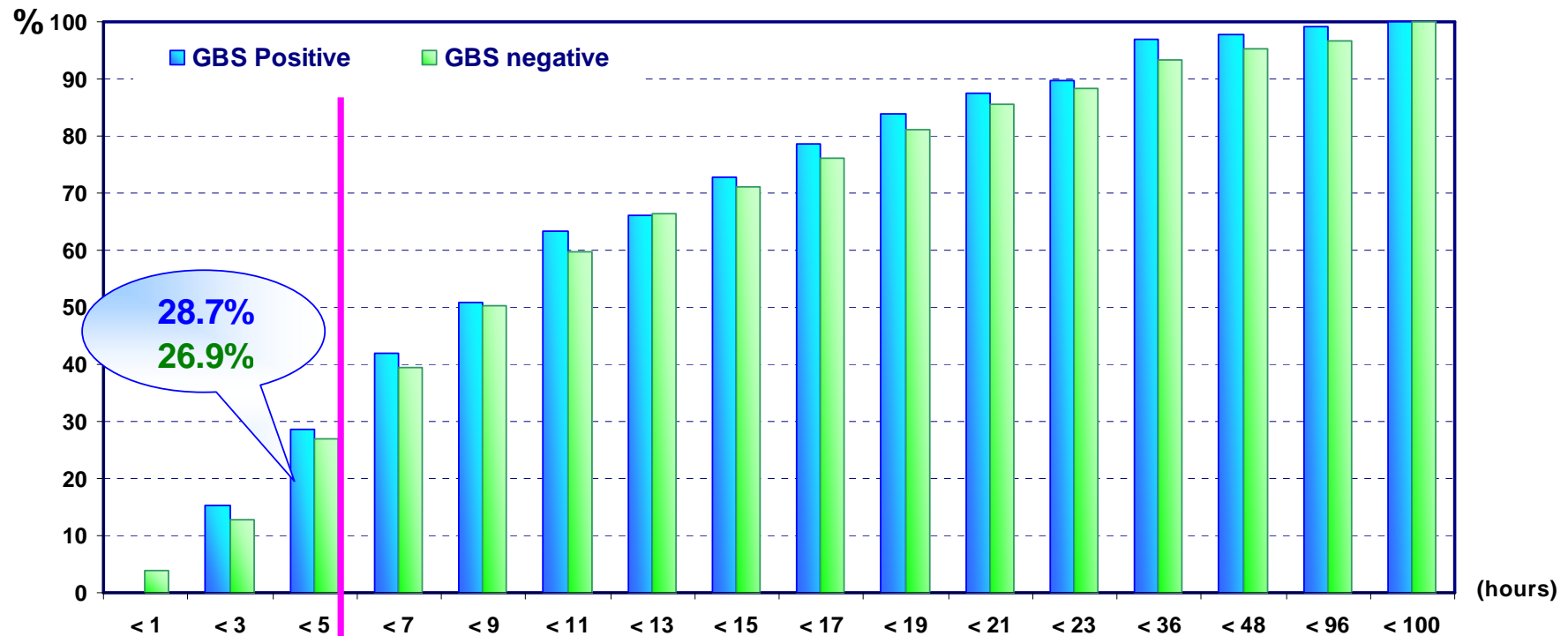
Results

**30-45 minutes, 24/24 hrs and 7/7 d, robust**  
*Benitz et al. 1999, Pediatrics, Vol 183 (6)*

# Time between admission and delivery

Optimal time for IAP efficiency  $\geq 4$  hour

Cumulative histogram (% of patients) of time elapsed between admission to labor room and delivery for 532 women (sites CHR & CHBA)



# Rapid non-cultural GBS screening

- **Available antigenic tests**
  - **Variety of Immuno-assays**
  - **Lack of sensitivity**
    - **Announced  $5 \cdot 10^5$  CFU, but not confirmed**
- **Hybridization tests**
  - **Not enough rapid**
  - **Lack of sensitivity if no enrichment step**

# Real Time PCR for intrapartum screening

- **Advance in PCR techniques & development of platforms**
  - **BD GeneOhm™ Strep B Assay (+/- 1 hr) (in laboratory)**
  - **Xpert GBS, Cepheid (+/- 75 min) (can be performed as a POC)**



*(Gen Expert)*

# Rapid non-cultural GBS screening Real-time PCR

- **IDI Strep B (BD GeneOhm)**
  - **Sensitivity : 94 %**
  - **Specificity : 96 %**
  - **PPV : 84 % and NPV : 98.6 %**

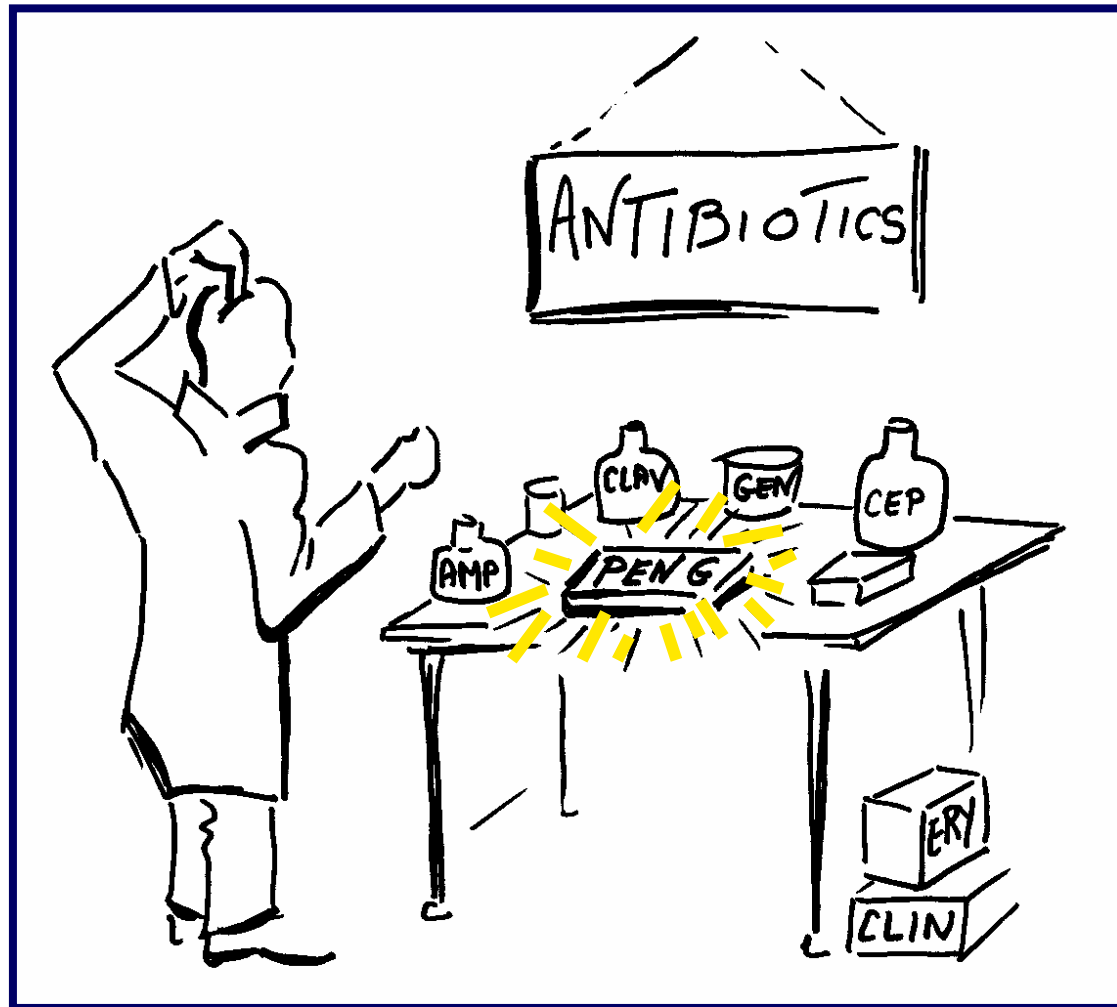
*HD Davies et al., CID 2004*

- **Xpert™ GBS**
  - **Sensitivity : 92 %**
  - **Specificity : 95.6 %**
  - **PPV : 86.7 % and NPV : 97.4 %**

# Real-time PCR, very promising , but ...

- **Still an expensive technology**
- **Logistic**
  - 24/24 hours and 7/7 days
  - In the lab?
  - In the obstetrical department ?
- **In combination with prenatal screening strategy ?**
- **No antimicrobial result**
  - In the future detection of R genes, but mixed flora !

# Antimicrobial resistance



# Clinically relevant Antimicrobial resistance ?

AB agent	IAP	Therapy	Resistance
Penicillin	X	X	“No”, but ↗ MIC
Erythromycine	X	X	10 - 30 %
Clindamycin	X	X	Up to 20 %
Vancomycin	(X)	(X)	No
Fluoroquinolone		(X)	Few cases
Aminoglycoside		(X)	No HLR



# Susceptibility to penicillin

- **Very few « not S » isolates recently characterized in Japan**
  - Mutation in *pbp* genes, especially in *pbp2x*
  - MIC= 0.25 -1 mg/L

*Noriyuki Nagano et al, AAC 2008*

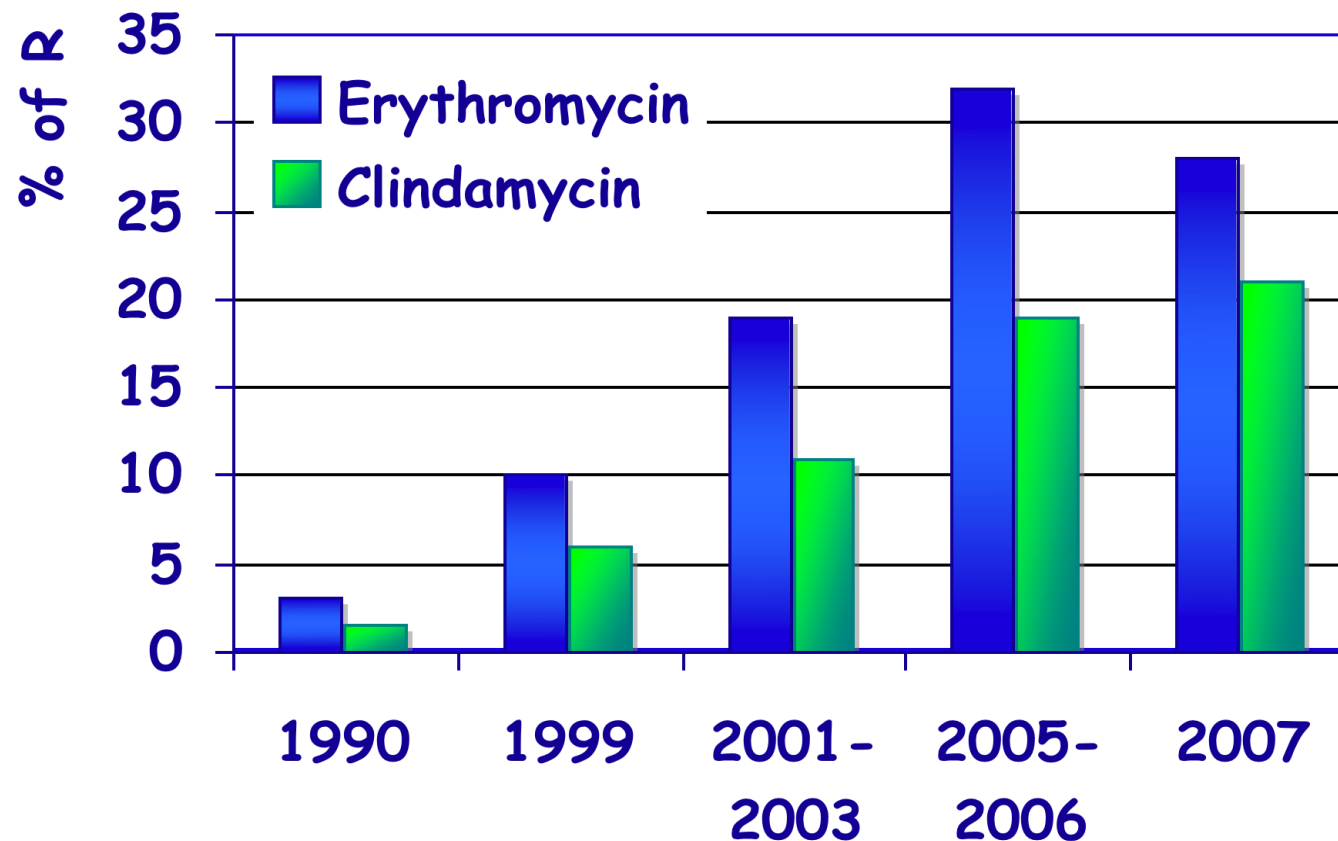
- **To recommend to all laboratories**
  - **To send « non-S » isolate to reference lab.**

# AST interpretation criteria

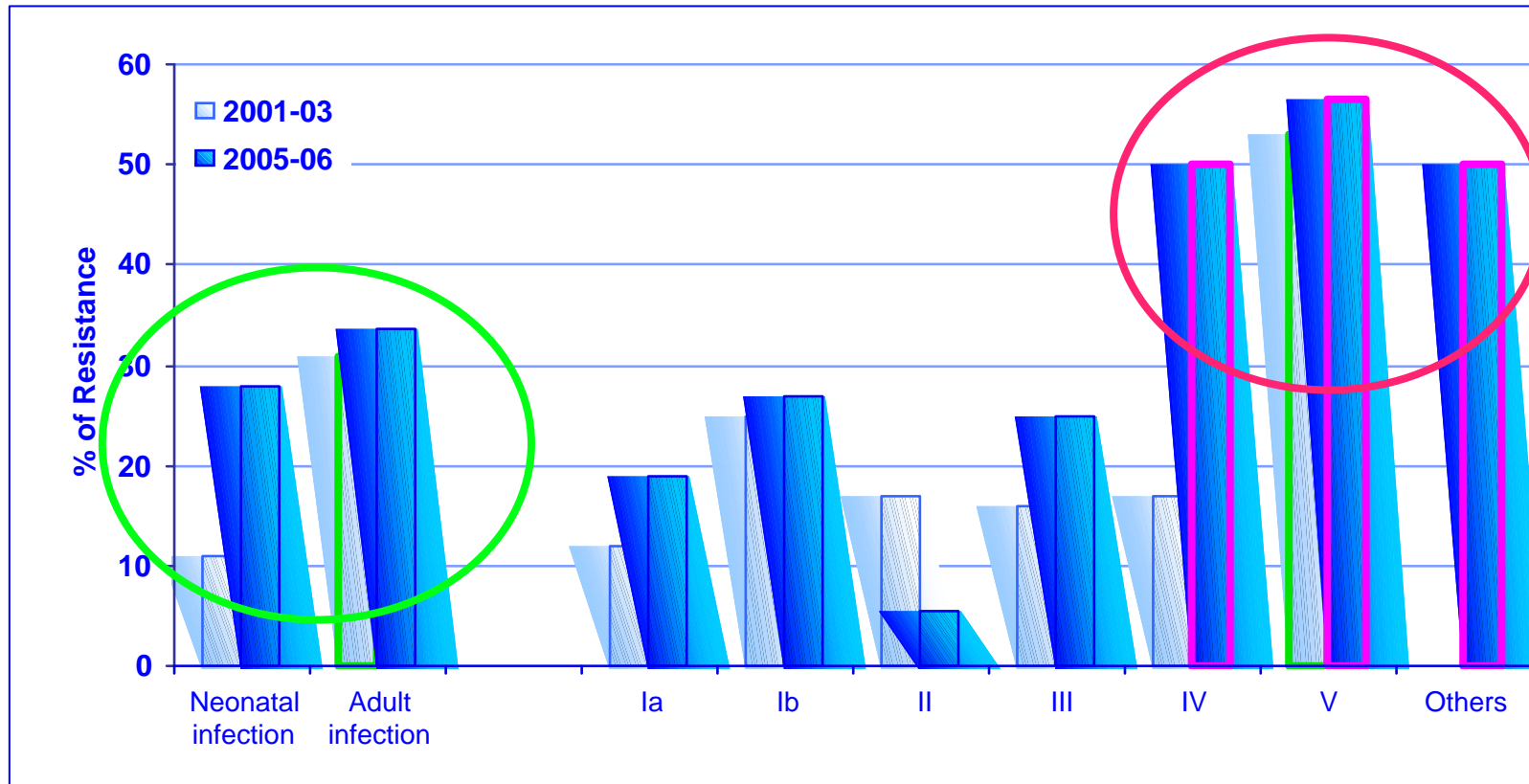
*CLSI 2009 (Diffusion MH +blood) & EUCAST 2009*

	CLSI			CLSI			EUCAST		
	Zone Diameter (mm)			MIC (mg/L)			MIC (mg/L)		
	S	I	R	S	I	R	S	I	R
<b>Penicillin</b>	≥ 24	-	-	≤ 0.12	-	-	≤ 0.25	-	-
<b>Erythromycin</b>	≥ 21	16-20	≤ 15	≤ 0.25	0.5	≥ 1	≤ 0.25	0.5	≥ 1
<b>Clindamycin</b>	≥ 19	16-18	≤ 15	≤ 0.25	0.5	≥ 1	≤ 0.5	-	≥ 1

# Erythromycin and clindamycin resistance Evolution among Belgian GBS isolates



# Erythromycin Resistance of Belgian clinical GBS isolates



**2001-2003 187 invasive isolates, Melin et al, ICAAC 2003, #C2-81**

**2005-2006 178 invasive isolates, Melin et al, ICAAC 2007 #C2-168**

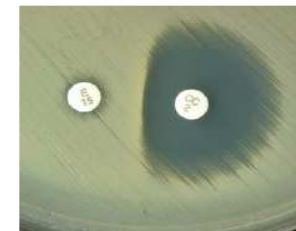
# MLS Resistance phenotypes

## *D-test recommended*

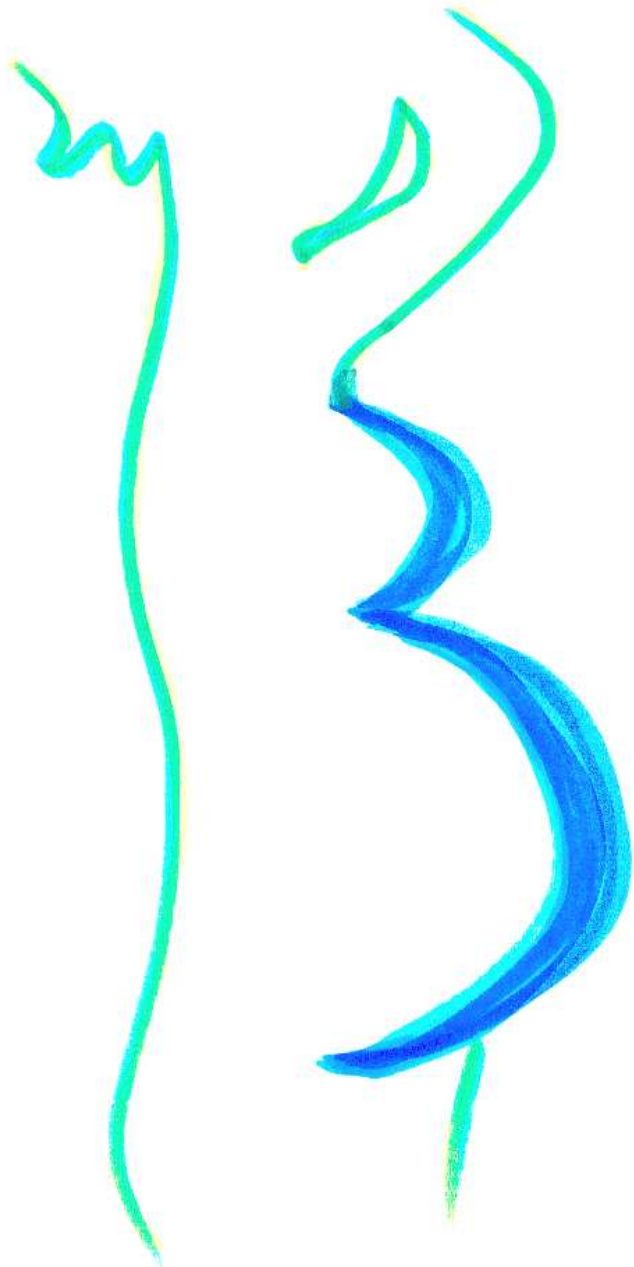
Phenotype	%	Ery MIC <sub>50</sub> / MIC <sub>90</sub> (mg/L)
MLS <i>Constitutive</i>	45	>256 / >256
<i>Inducible</i>	34	4 / >256
M	21	4 / 12

*P.Melin, LISSSD 2008 P215*

- **Dtest**
  - cMLS Erythro R & Clinda R
  - iMLS Erythro R & Clinda S//R with Dtest +
  - M Erythro R & Clinda S with Dtest -
- **Vitek2:** not always reliable, to be improved



Neither macrolides no lincosamides should no longer be used  
without susceptibility testing



## SUMMARY

- **Culture-based GBS prenatal screening**
  - To optimize critical factors
  - Improved by selective differential agars
  - False +/False - !
- **Rapid intrapartum screening**
  - Real time PCR
    - Yes but costs, logistic, ...
- **Antimicrobial R**
  - Surveillance of Penicillin by NRC
  - To perform AST for macrolides/lincosamides