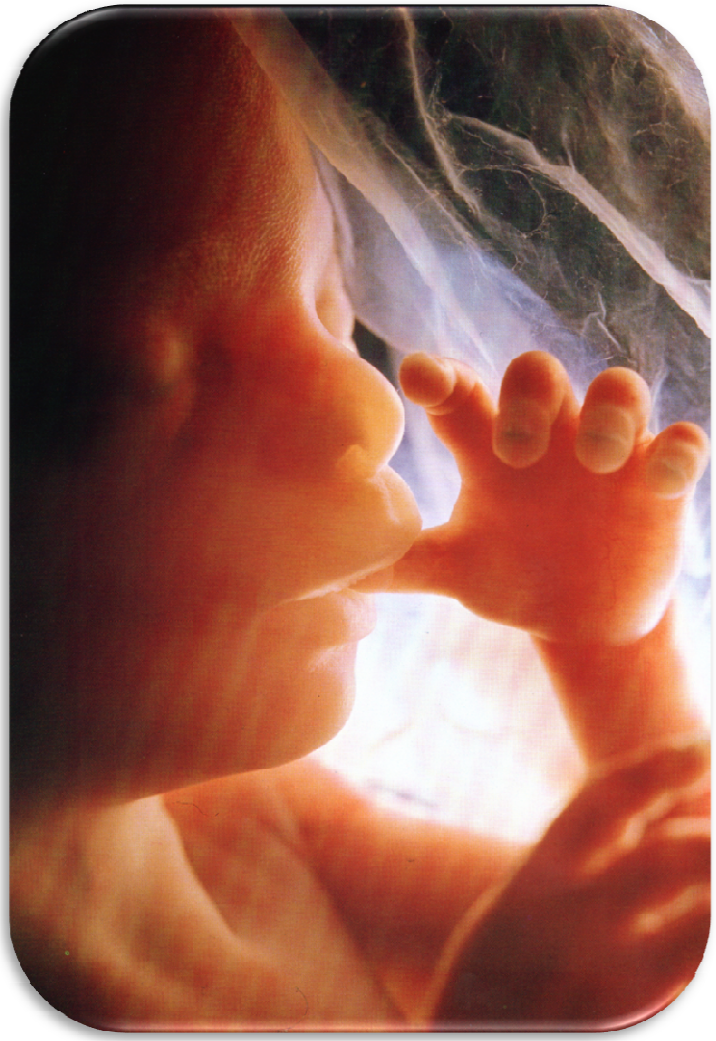


Prevention for GBS perinatal disease: Which improvements for GBS screening?

Pierrette Melin

National Reference Centre for GBS
Microbiology, University of Liege
Medical Microbiology, University Hospital of Liege

INTRODUCTION & BURDEN

Streptococcus agalactiae or GBS

Gram positive cocci
- **Catalase -**
- **β-hemolytic**
- **CAMP test +**
- **Hippurate +**
- **Esculine-**
- **Orange pigment**

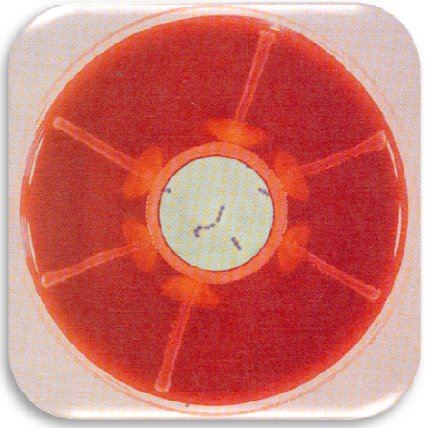
10 capsular serotypes (Ia, Ib, II-IX)

1887, Noccard-Mollereau, bovine mastitis

1933, Group B Antigen

1964, severe neonatal sepsis

➢**1970, N° 1 in neonatal infections**



Group B streptococcal diseases in neonates

- Since the 1970s, leading cause of life-threatening infections in newborns
  - Neonatal illness/death
  - Long-term disabilities

GLOBAL public health major concern !

Also in developing countries

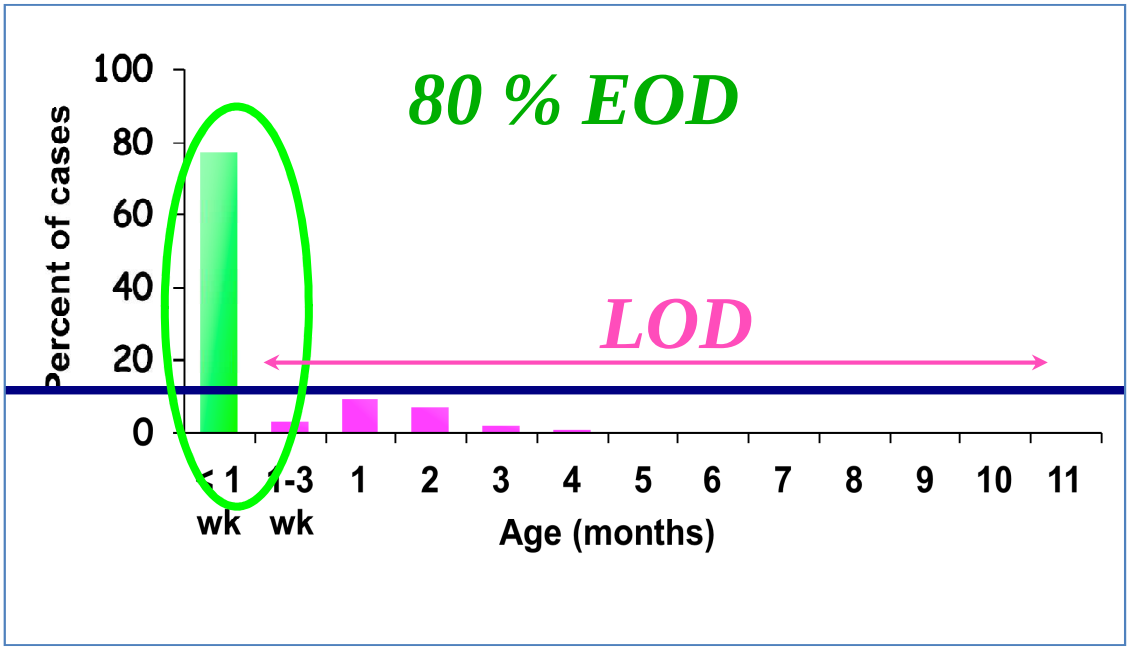
- Maternal morbidity
  - Along pregnancy
  - Peripartum
- Serious diseases among elderly and adults with underlying diseases
  - Significant mortality

GBS Neonatal Infections

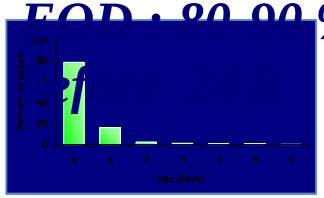
A. Schuchat, Clin Microb Rev 1998;11:497-513

GBS Neonatal Infections

A. Schuchat, Clin Microb Rev 1998;11:497-513



EOD : 80-90 % occur



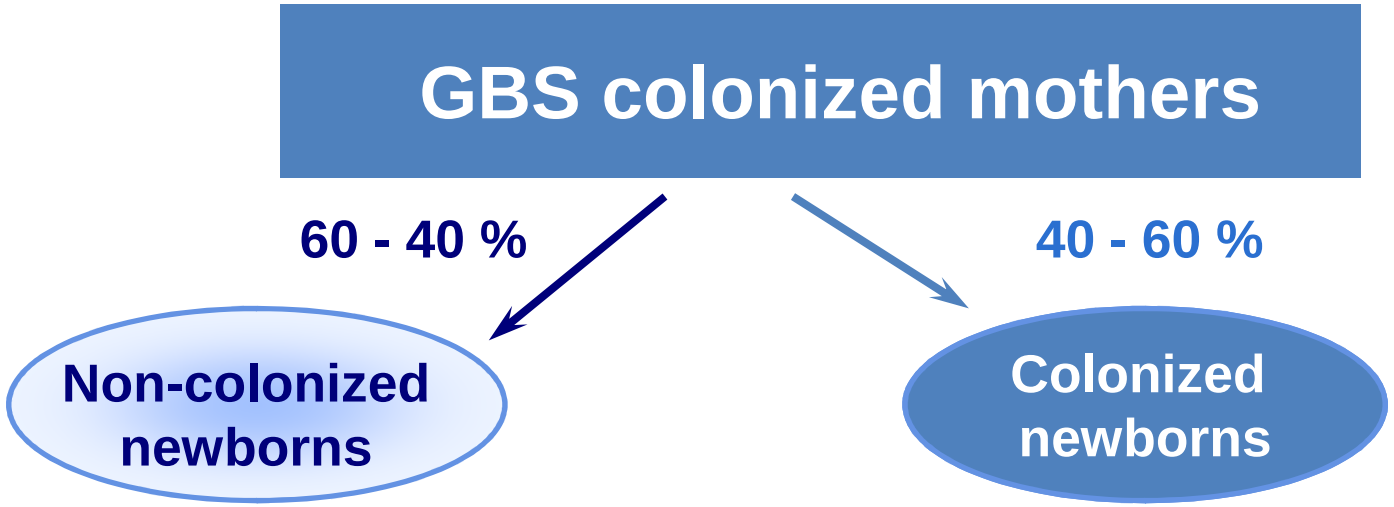
Burden of neonatal GBS early onset diseases in European countries

Location	Incidence per 1,000 live-births	*Reference*
Spain	2 (1996) to 0.45 (2008)	*Lopez Sastre et al. Acta Pediatr 2005*
Belgium	2	*Melin, Indian J Med Res 2004*
Eastern Europe	0.2 - 4	*Trijbels-Smeulders, Pediatr Infect Dis J 2004*
Western Europe	0.3 - 2	
The Netherlands	1.9	
Scandinavia	0.76 - 2	
Southern Europe	0.57 - 2	

- Carriage rate ?
- Ethnicity ?
- Sub-reporting?
- Systematic diagnostic approach?
- Virulence?

Data assessing more accurately the true burden are needed

GBS EOD vertical transmission



GBS EOD vertical transmission

GBS colonized mothers

60 - 40 % → **Non-colonized newborns**

40 - 60 % → **Colonized newborns**

96 - 98 %

Asymptomatic

GBS EOD vertical transmission

GBS colonized mothers

60 - 40 %

40 - 60 %

Non-colonized newborns

Colonized newborns

Risk factors

2 - 4 % GBS EOD

96 - 98 %

Asymptomatic

sepsis
pneumonia
meningitis
long term sequelae

GBS maternal colonization

Risk factor for early-onset disease (EOD) :
vaginal GBS colonization at delivery

- **GBS carriers**
  - **10 - 35 % of women**
  - **Clinical signs not predictive**
  - **Dynamic condition**
  - **Intestinal reservoir**
  - **Prenatal cultures late in pregnancy can predict delivery status**

Additional Risk Factors for Early-Onset GBS Disease

- **Obstetric factors:**
  - **Prolonged rupture of membranes,**
  - **Preterm delivery,**
  - **Intrapartum fever**
- **GBS bacteriuria**
- **Previous infant with GBS disease**
- **Immunologic:**
  - **Low specific IgG to GBS capsular polysaccharide**

Risk factors

No difference in occurrence either in GBS Positive or Negative women, except intrapartum fever

Lorquet S., Melin P. & al.

J Gynecol Obstet Biol Reprod 2005

GBS EOD - Belgian data

- **Incidence**
  - **1985 -1990: 3/1000 live births**
  - **1999, estimation : 2/1000 live births**
  - **2010, estimation : < 1/1000 live births**
- **Meningitis : 10 %**
- **Mortality : 5 -10 %**
- **60 % EOD (130 cases) : WITHOUT any maternal/obstetric risk factor except colonization**
- **Prenatal screening**
  - **Recto-vaginal cultures : 13-35 % GBS Positive**

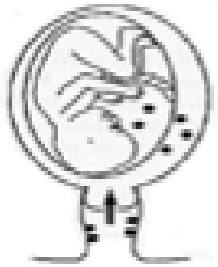
P. Melin - 2001, 2007 - Reference laboratory for GBS.

Stages in the pathogenesis of GBS neonatal EOD : *Bacterial & individual factors*

Meningitis

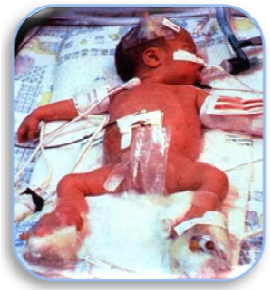
Colonization : adhesion to epithelial cells
different virulence factors (pili, scpB, …)

Brain barrier
Pili,
β-hemolysin, …



Ascendant **transmission** (amnionitis)

GBS pathogenesis

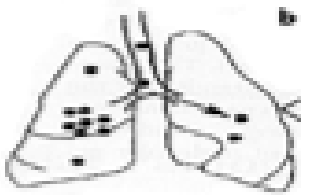


Sepsis

IL1, IL6, TNF α,
PGE2, TxA_2 ,

Bacteria
Peptidoglycan
β-hemolysin, …

Resistance to phagocytose
- Capsule
- C5a peptidase
- …..

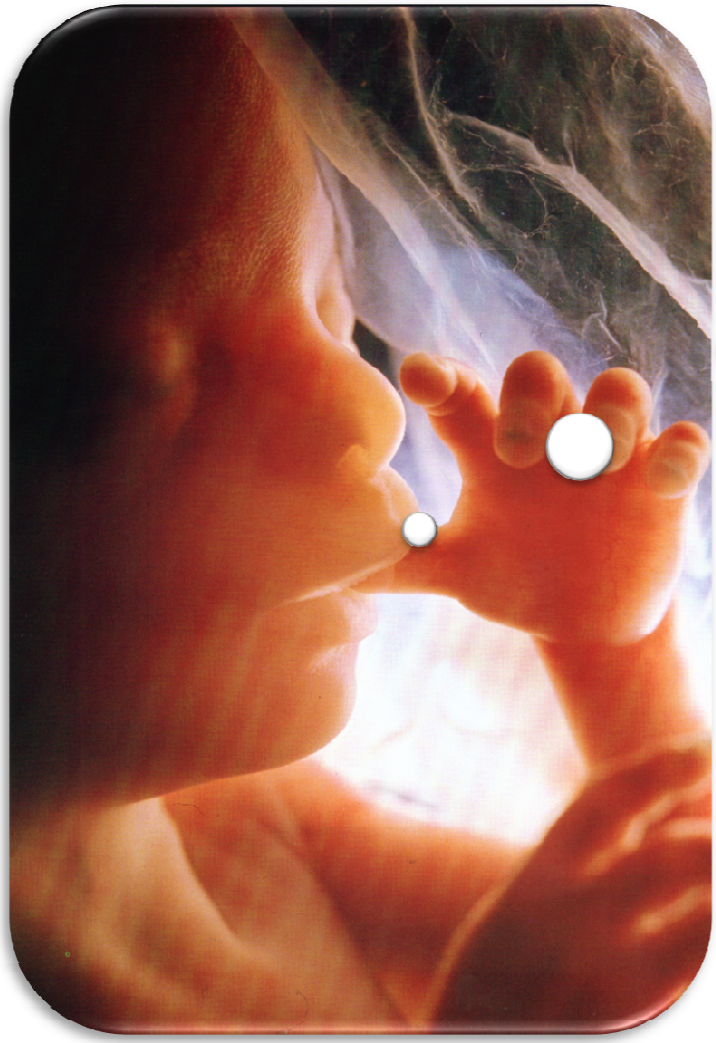


β-hemolysin, invasins (**pneumonia**)

Phagocytes cells,
Antibodies, Complement

- **Universal prenatal screening-based strategy**
- **Risk-based strategy**
- **No guideline**

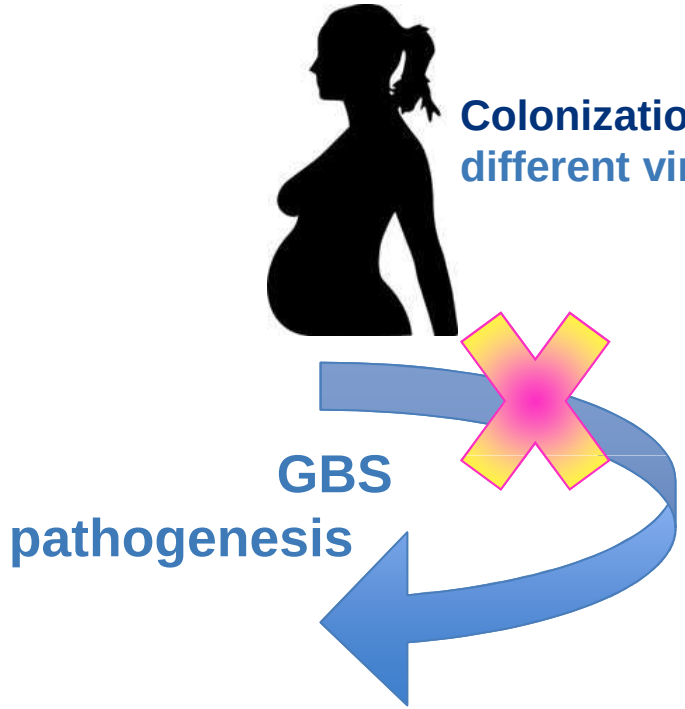
GUIDELINES FOR PREVENTION OF GBS PERINATAL DISEASE



Which prevention strategy for GBS perinatal diseases ?

- **Intrapartum antibioprophylaxis**
- **Immunoprophylaxis**

Stages in the pathogenesis of GBS neonatal EOD : *Bacterial & individual factors*



Colonization : adhesion to epithelial cells
different virulence factors (pili, scpB, …)

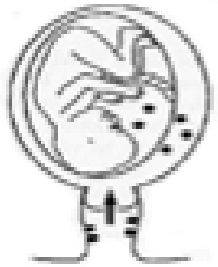
Intrapartum antibioprophylaxis
> 4 (2) hours before delivery

Stages in the pathogenesis of GBS neonatal EOD : *Bacterial & individual factors*

GBS vaccine

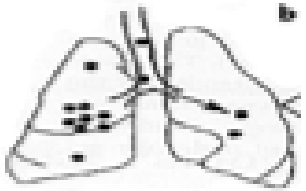
« still expected »

Colonization : adhesion to epithelial cells
different virulence factors (pili, scpB, …)



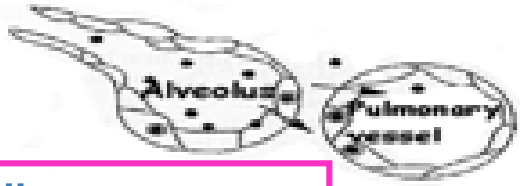
Ascendant transmission (amnionitis)

GBS pathogenesis



β-hemolysin, invasins (pneumonia)

Resi… phag…
- Ca…
- C… pe… ase
- …..



Phagocytes cells,
Antibodies, Complement

Prevention of perinatal GBS EOD

- **Intrapartum antibiotics**
  - **Highly effective at preventing EOD in women at risk of transmitting GBS to their newborns (≥ 4 h)**

    (clinical trials in late 80s)

Risk-based strategy
or
Screening-based strategy

Prevention of perinatal GBS EOD

- Screening-based strategy

INTRAPARTUM ANTIMICROBIAL PROPHYLAXIS

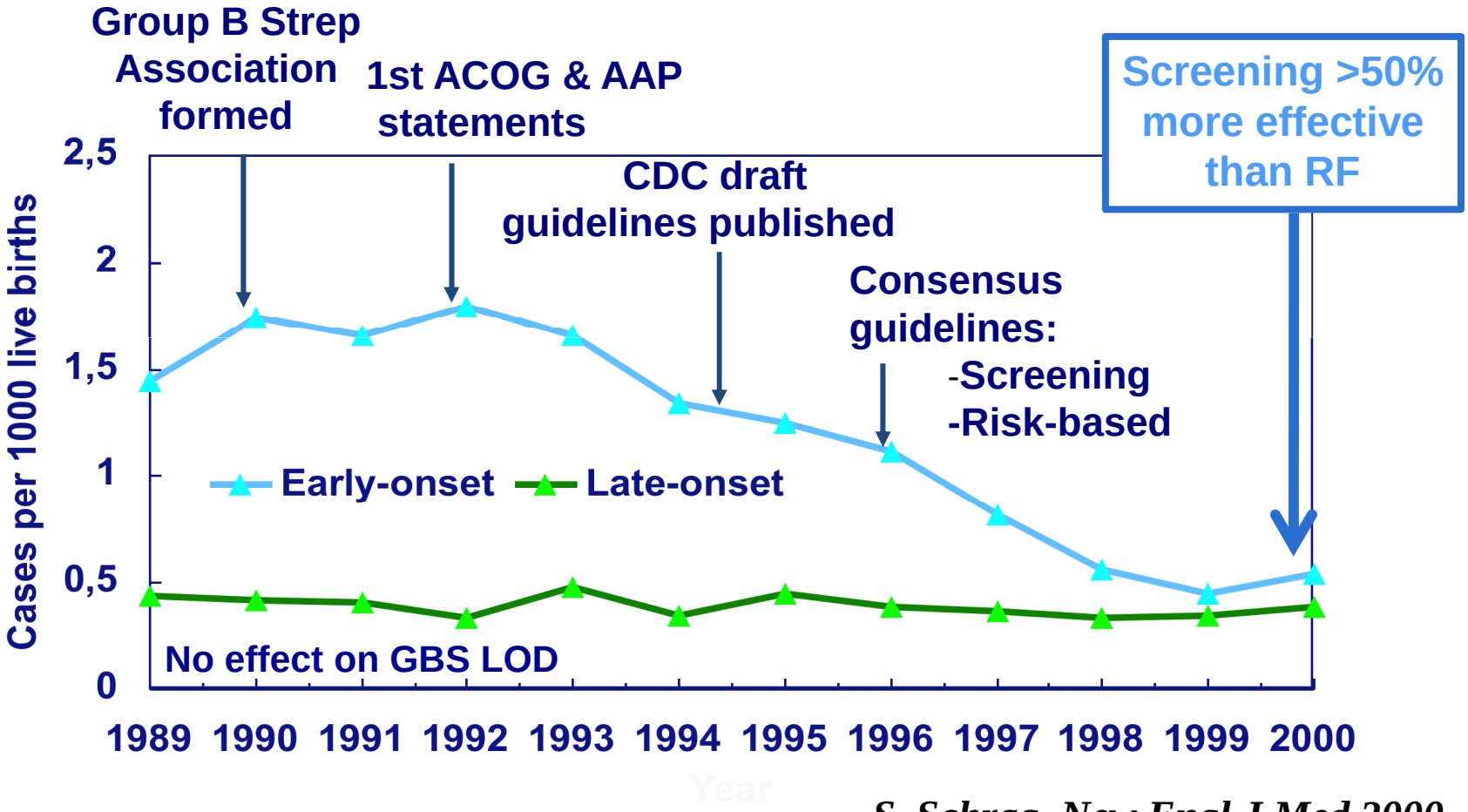
Main goal :

- To prevent 70 to 80 % of GBS EO cases

Secondary :

- To reduce peripartum maternal morbidity

Impact of prevention practices Early- and Late-onset GBS Diseases in the 1990s, U.S.



S. Schrag, New Engl J Med 2000
Schrag S. et al. N Engl J Med 2002; 347:233-9

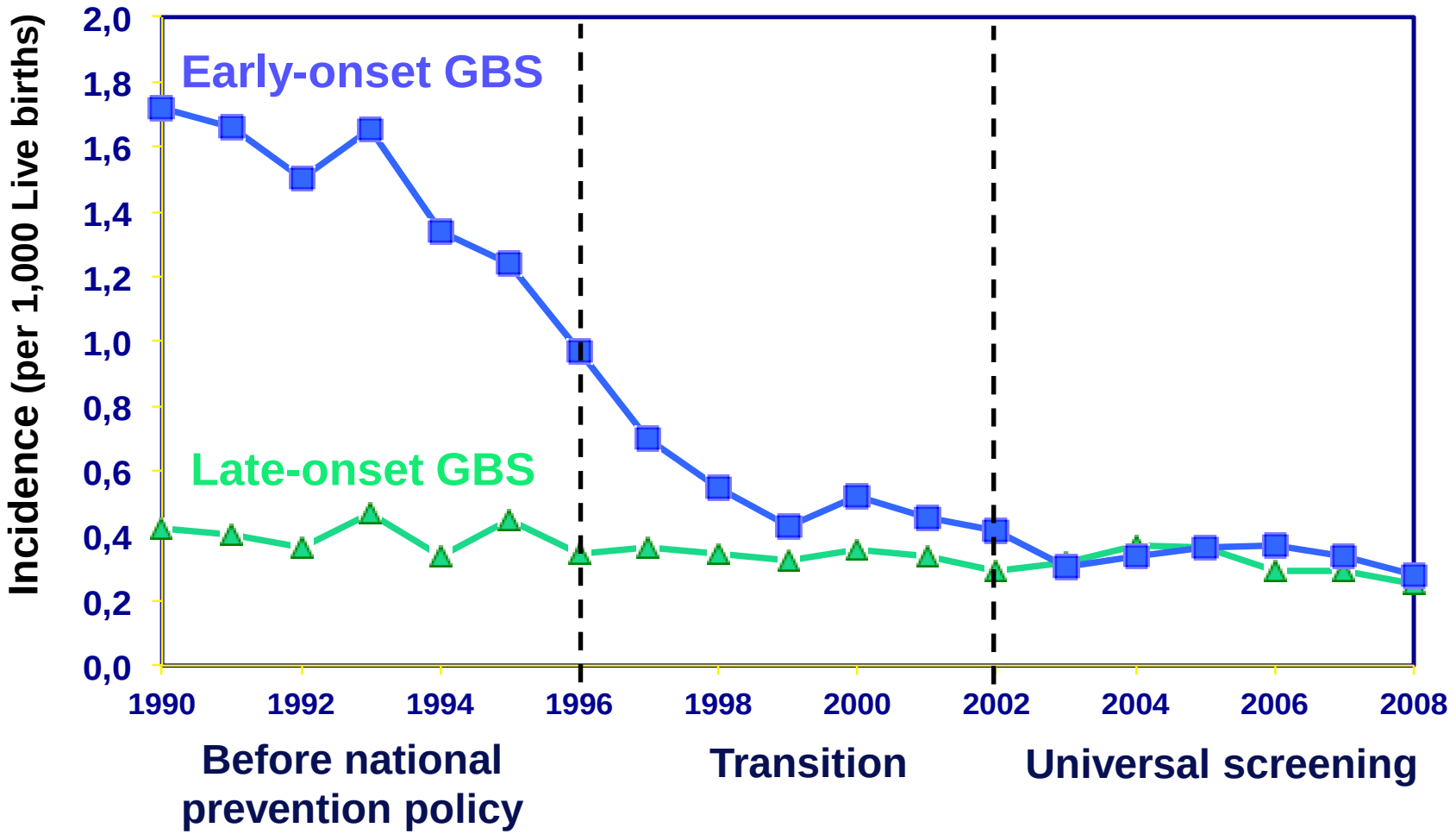
Why is Screening more protective than the risk-based approach ?

Schrag S. et al. N Engl J Med 2002; 347:233-9

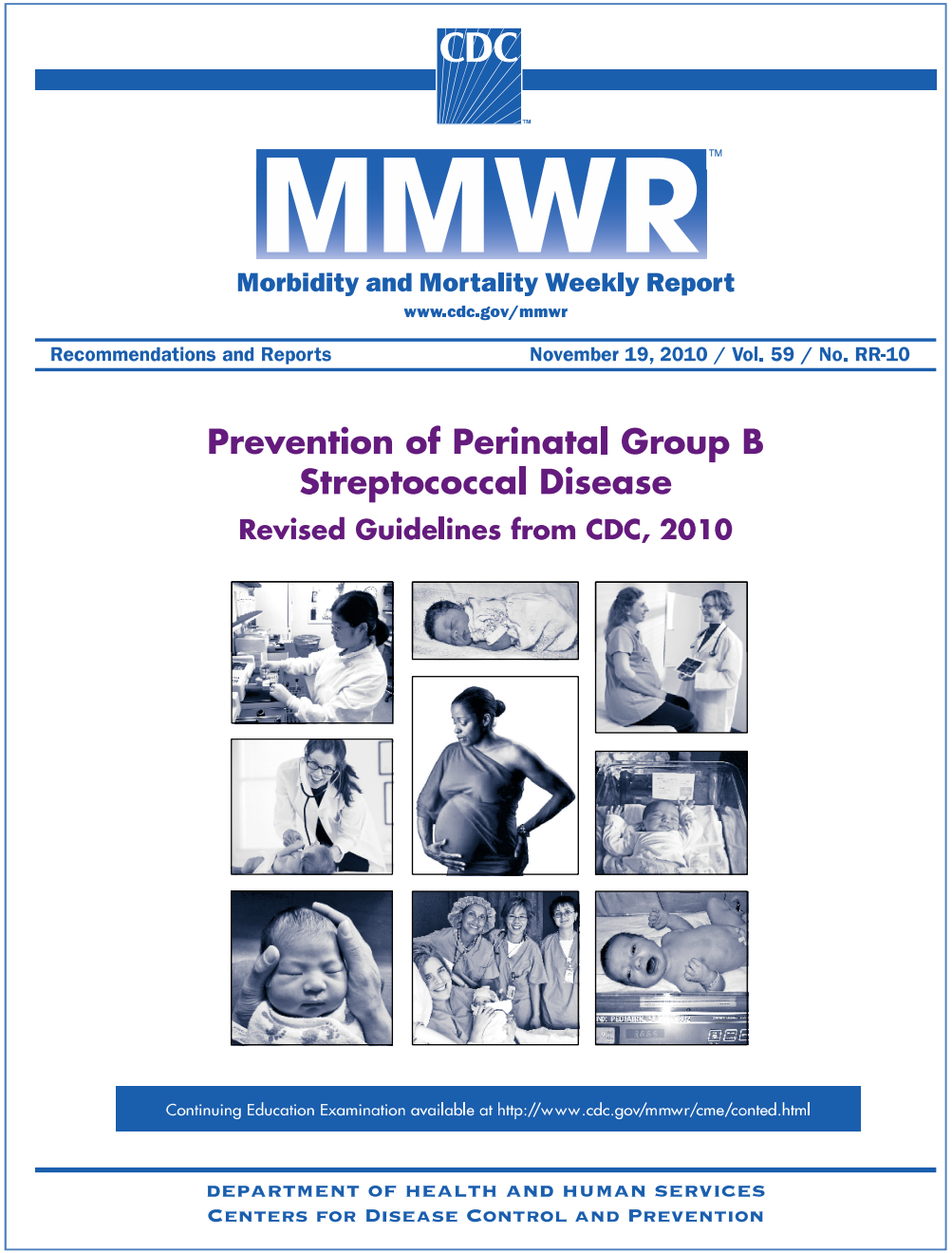
Broader coverage of « at-risk » population

- **Captures colonized women without obstetric RF**
- **High level of compliance with recommendations**
- **Enhanced compliance with risk-based approach cannot prevent as many cases as universal screening**

Impact of prevention practices
Early- and Late-onset GBS Diseases, U.S.



Incidence of early- and late-onset invasive group B streptococcal disease in selective Active Bacterial Core surveillance areas, 1989-2008 (CDC 2010)



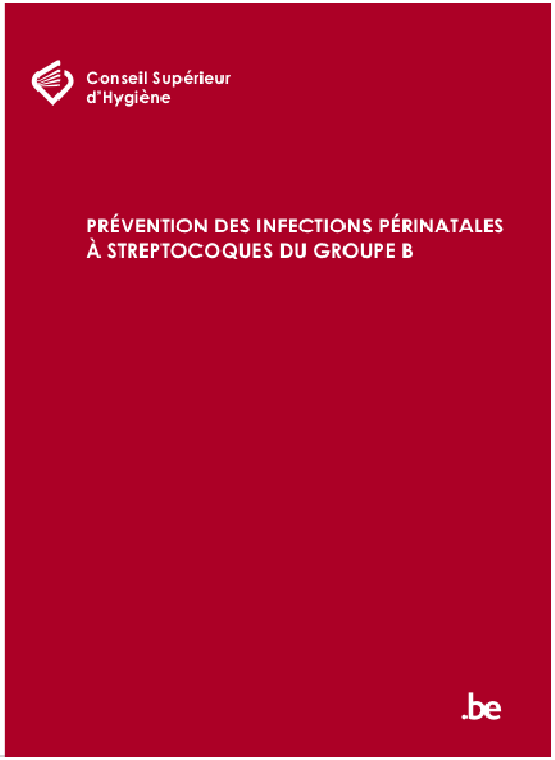
CDC, USA, MMWR, Vol 59 (RR-10) August 2010

Endorsed by

- ***AAP***
- ***ACOG***

SHC, Belgium July 2003

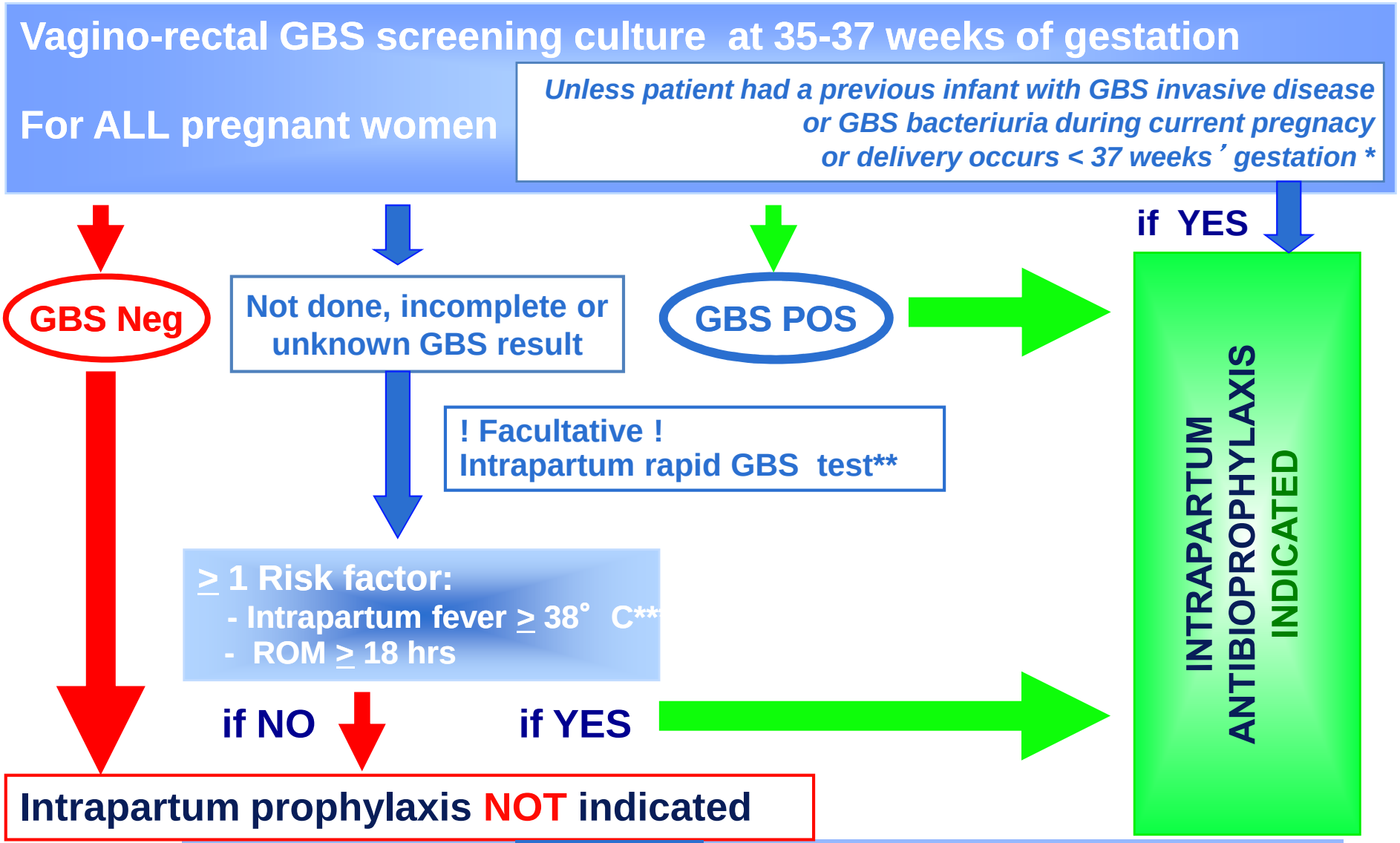
Revision ongoing

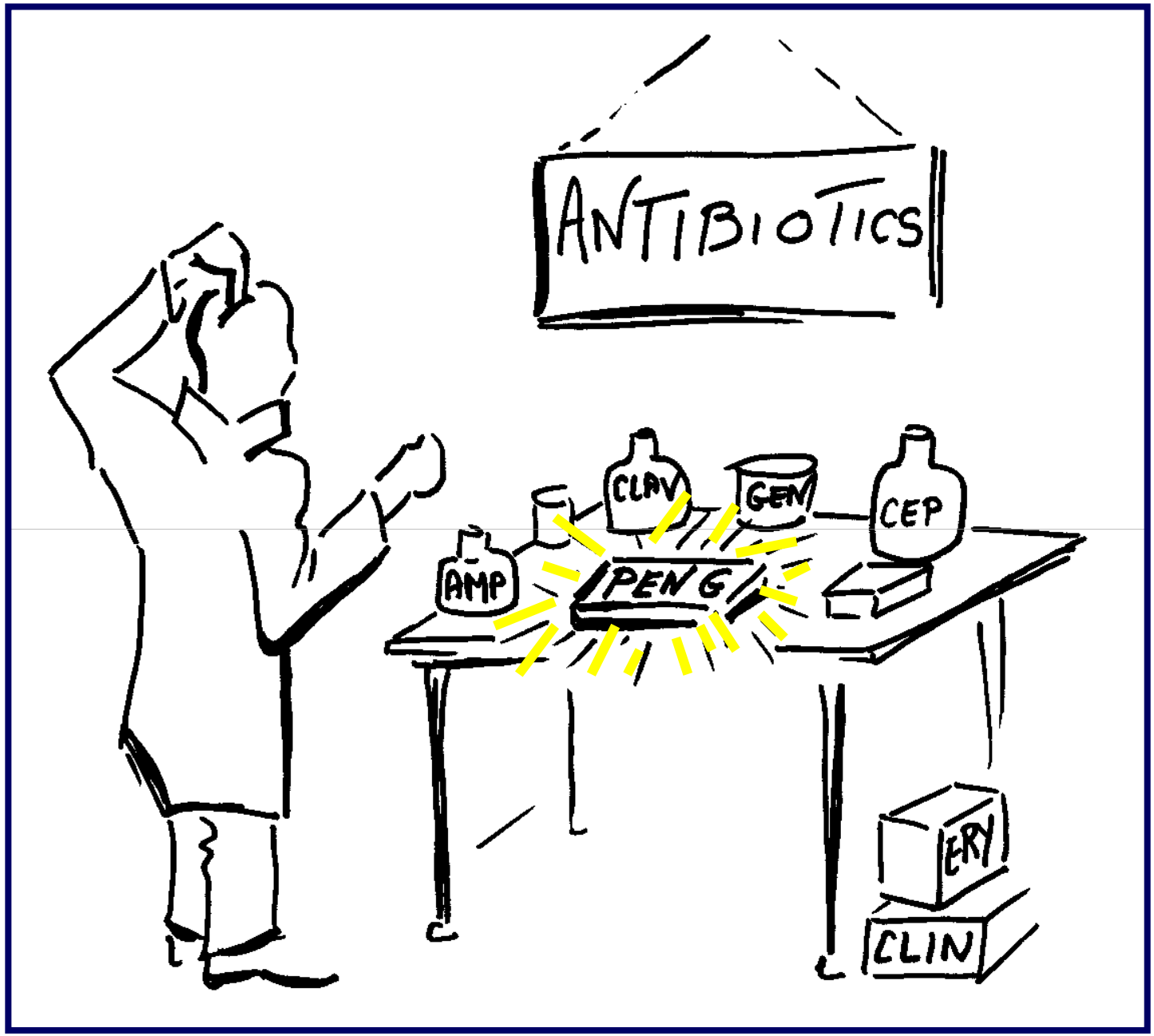


European strategies for prevention of GBS EOD

- Intrapartum antibioprophylaxis recommended
  - Screening-based strategy
    - Spain, 1998, 2003, revised 2012
    - France, 2001
    - Belgium, 2003, revision ongoing 2012
    - Germany, 1996, revised 2008
    - Switzerland, 2007
  - Risk-based strategy
    - UK, the Netherlands, Denmark
- No guidelines
    - Bulgaria, …

Universal screening-based strategy for prevention of GBS perinatal disease




ANTIBIOTICS
AMP
CLAV
GEN
PEN G
CEP
ERY
CLIN

Remaining burden of GBS EOD
Missed opportunities

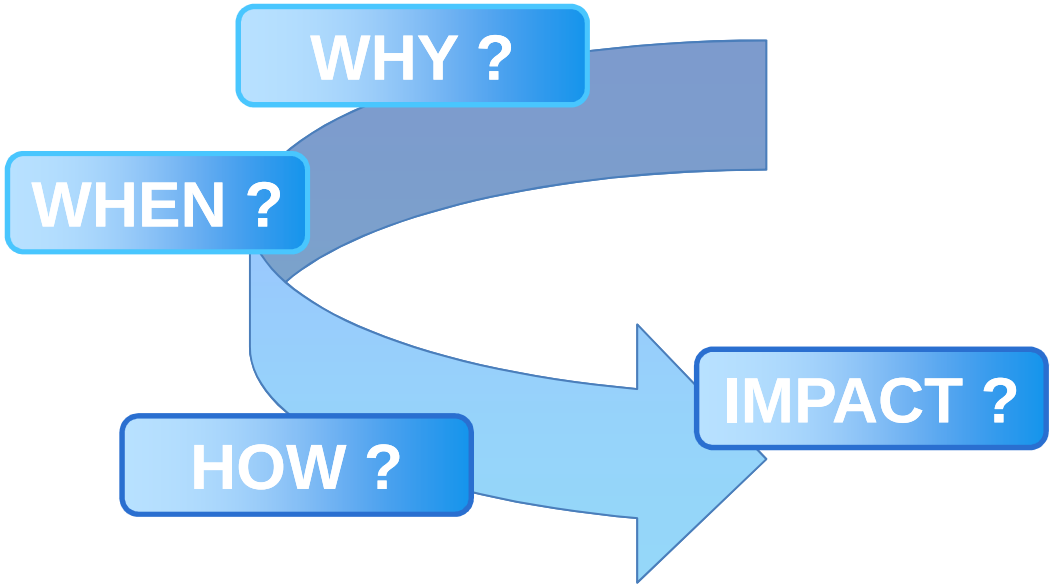
In spite of universal screening prevention strategy

In spite the great progress

Cases still occur

- **Among remaining cases of EOD**
  - **Some may be preventable cases**
    - **Missed opportunities for (appropriate) IAP**
    - **False negative screening**

Van Dyke MK, Phares CR, Lynfield R et al. N Engl J Med 2009
CDC revised guidelines 2010
Poyart C, Reglier-Poupet H, Tazi et al. Emerg Infect Dis 2008
DEVANI project, unpublished data 2011



SCREENING
FOR GBS COLONIZATION

Antenatal GBS culture-based screening

Goal of GBS screening

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

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

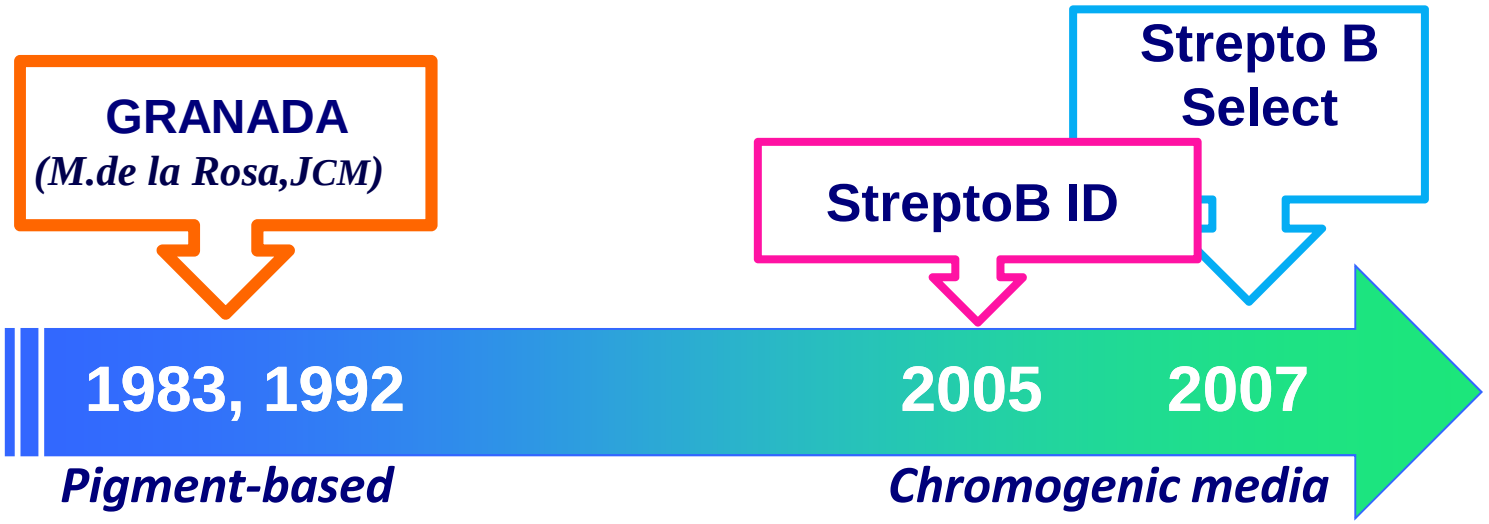
From direct plating on blood agar
Evolution of culture methods

Use of selective enrichment broth

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

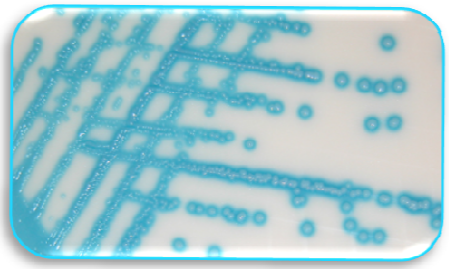
Use of differential agar media

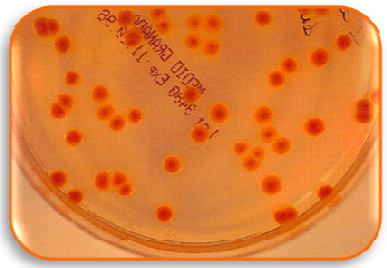
Recommended by some European guidelines (+ CDC 2010)

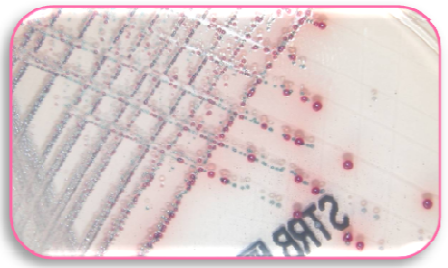


Which agar or which combination?

+/- Blood agar







Workload - costs - extra-testing - non β-hemolytic GBS detection to be considered

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/condition**

  (non nutritive medium: Amies/Stuart or Granada like tube) (type of swab)(Length and T°)
- **Request form** — **To specify prenatal « GBS » screening**
- **Laboratory procedure**

(CDC 2010 - Belgian SCH 2003)

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/condition**

  (non nutritive medium: Amies/Stuart or Granada like tube) (type of swab)(Length and T°)
- Request form — To specify prenatal « GBS » screening
- Laboratory procedure

(CDC 2010 - Belgian SCH 2003)

Crucial conditions to optimize SCREENING

Transport-collection system & transport-storage condition

- **Specimen storage in transport medium and detection of group B streptococci by culture.**

Rosa-Fraile M. et al. J Clin Microbiol 2005, 43: 928-930

Recovery of group B streptococci (GBS) was assessed in 1,204 vaginorectal swabs stored in Amies transport medium at 4 or 21°C for 1 to 4 days either by direct inoculation onto Granada agar (GA) or by culture in blood These data indicate that viability of GBS is not fully preserved by storage of vaginorectal swabs in Amies transport medium, mainly if they are not stored under refrigeration.

➢ Belgian Guidelines (2003, SHC)

"Specimens should be placed in a non-nutritive transport medium (e.g., Amies or Stuart's without charcoal). In these conditions, viability of GBS is warranted for at least 48 h at room temperature or in a fridge (2 - 8° C).

Specimen labels should clearly identify that specimens are for group B streptococcal culture. Swabs should reach the lab within 48 h of collection."

Crucial conditions to optimize SCREENING

Transport-collection system & transport-storage condition

Preliminary results (2012, NRC GBS)

- **Use of a selective enrichment Lim broth** ***(BD, Copan, bioMérieux)***
  - **At RT° up to 35° C**
  - **Between 4-8° C**

- **Use of a selective enrichment Granada medium** ***(bioMérieux)***
  - **At RT° up to 35° C**
  - **Between 4-8° C**

Crucial conditions to optimize SCREENING

Transport-collection system & transport-storage condition

Preliminary results (2012, NRC GBS)

- **Use of a selective enrichment Lim broth** ***(BD, Copan, bioMérieux)***
  - **At RT° up to 35° C**
    - **Rapid important amplification of GBS initial inoculum**
    - **Sustained viability > 4 days**
  - **Between 4-8° C**
    - **≥ 24 hours, continuous decrease of life GBS**

- **Use of a selective enrichment Granada medium** ***(bioMérieux)***
  - **At RT° up to 35° C**
    - **Rapid important amplification of GBS initial inoculum**
    - **Sustained viability at RT°**
    - **Abrupt lost of viability at 35° C ≥ 48-72h**
  - **Between 4-8° C**
    - **≥ 24 hours, continuous decrease of life GBS**

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 positive women at time of delivery**
    - **Continuing occurrence of EO GBS cases**
  - **False-positive**
    - **Positive prenatal screening /negative at time of delivery**
    - **Unnecessary IAP**

Need for more accurate predictor of intrapartum GBS vaginal colonization

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**
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    - **Positive prenatal screening /negative at time of delivery**
    - **Unnecessary IAP**

Need for more accurate predictor of intrapartum GBS vaginal colonization

Prenatal culture-based screening combined with *illumigene*® Group B Streptococcus assay

A loop mediated isothermal amplification (LAMP) assay by Meridian Bioscience, Inc

- **Broth enrichment followed by *illumigene*® GBS**
  - **Speed and accuracy**

Evaluation of the *illumigene*® GBS

242 vagino-rectal swabs

CHU Lg
& AZ St-Lucas Gent

And

Granada agar

18 – 48h *at 35˚ C/ana*

LIM broth

Overnight subculture at 35˚ C and

Granada agar

Strep B select

illumigene GBS

18 – 48h ana

at 35˚ C air

Primary culture GBS Pos (1-4+)/Neg

Enrichment GBS Pos (1-4+)/Neg

illumigene GBS Pos/Neg/Invalid

Evaluation of the *illumigene*® GBS

		GBS culture		
		Positive	Negative	
illumigene GBS	Positive	45	2	47
	Negative	5	188	193
		50	190	240

GBS Positive cultures: 20.7%

illumigene GBS *vs* GBS reference culture (all discrepancies were retested)

Sensitivity	90.0 %
Specificity	98.9 %
PPV	95.7 %
NPV	97.4 %
Efficiency	97.1 %

Evaluation of the *illumigene*® GBS

		GBS culture		
		Positive	Negative	
illumigene GBS	Positive	45	2 : PCR pos	47
	Negative	5	188	193
		50	190	240

GBS Positive cultures: 20.7%

illumigene GBS *vs* GBS reference culture /GBS DNA

Sensitivity	90.0 %	
Specificity	98.9 %	→ 100%
PPV	95.7 %	→ 100 %
NPV	97.4 %	
Efficiency	97.1 %	

Evaluation of the *illumigene*® GBS

		GBS culture		
		Positive	Negative	
illumigene GBS	Positive	45	2 : PCR pos	47
	Negative	2 positive 3 very rare GBS	188	193
		50	190	240

GBS Positive cultures: 20.7%

illumigene GBS *vs* GBS reference culture /GBS DNA

Sensitivity	90.0 %	→ 95.7%
Specificity	98.9 %	→ 100%
PPV	95.7 %	→ 100 %
NPV	97.4 %	→ 99 %
Efficiency	97.1 %	

Evaluation of the *illumigene*® GBS

- **Easy to perform**
  - **But training very important**
    - **Molecular amplification method**
      - **Need for different skill**
      - **Workflow**
- **Invalid results**
  - **CHU Liege**
    - **1 negative retested “invalid”**
  - **AZ Sint Lucas, Gent, decreased rate with experience**
    - **5 resolved as negative**
    - **3 resolved as positive**
- **Short hands-on-time**
- **Short turn-around-time**

Evaluation of the *illumigene*® GBS

- Speed and accuracy
- Good comparison to reference culture method
  - 100% specificity and positive predictive value
  - High sensitivity and negative predictive value
  - Identification of an 0.8% additional GBS positive specimen
  - Overall cost and logistic to be considered

Prenatal culture-based screening: Limiting factors

- **Unknown GBS status at presentation for delivery**
  - **Screening performed but result not available**
  - **Women with no prenatal care**

→ **Risk based strategy**

- **60% at GBS risk not identified**
- **> 10% of unnecessary IAP**

Need for rapid accurate predictor of intrapartum GBS vaginal colonization

Alternative to GBS prenatal screening: intrapartum screening
Theranostic approach

Turnaround time

collect specimen at admission

Specimen Analysis "POCT" ?

Results

Optimal management of patient

30-45 minutes, 24 hrs/7 d, robust

Benitz et al. 1999, Pediatrics, Vol 183 (6)

Intrapartum screening theranostic approach: expected advantages

- **Inclusion of women without prenatal screening/care**
- **Identification of women with change of GBS status after 35-37 wks gestation**
- **Increased accuracy of vaginal GBS colonization status at time of labor & delivery**

IAP addressed to right target

- **Reduction of inappropriate/unnecessary IAP**
- **Broader coverage of « at GBS risk women »**

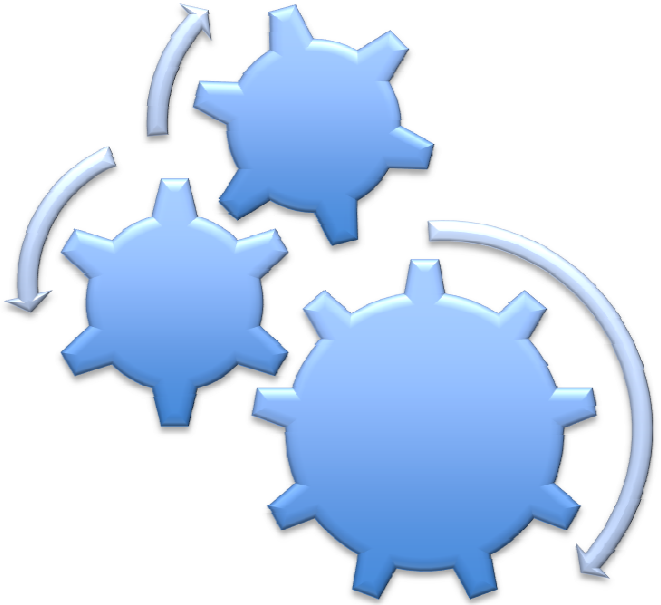
Improvement of prevention

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 (35-45 min)** (can be performed as a POCT)

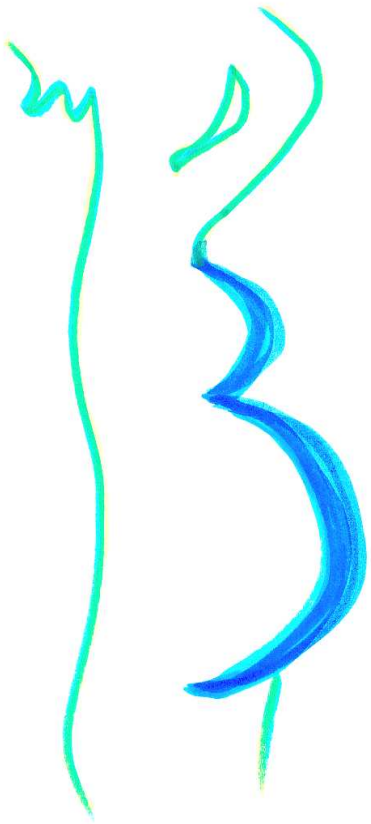
Real-time PCR, very promising, BUT …

- **Rapid, robust & accurate technology**
- **Still an expensive technology (specific equipment)**
  - **Cost effective ?**
    - ***Need for more cost-effective clinical study***
- **Logistic**
  - **24 hours 7 days**
  - **In the lab?**
  - **In the obstetrical department as a POCT ?**
- **In combination with prenatal screening strategy ?**
  - **CDC 2010 : for women with premature delivery or no prenatal care**
- **No antimicrobial result**
  - **In the future detection of R genes, but mixed microbiota !**



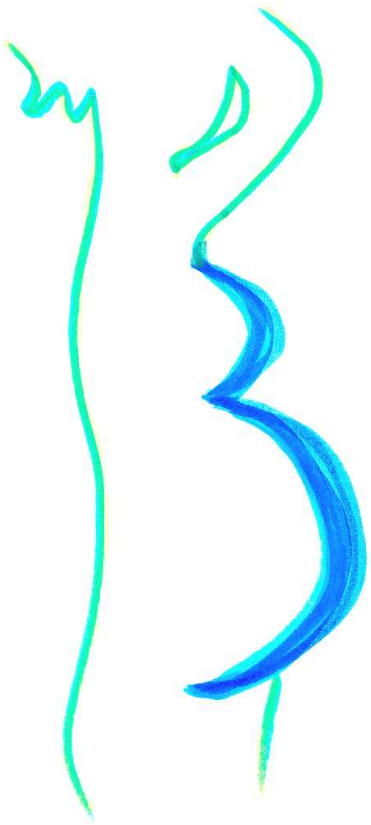
CONCLUSION
Take home messages

In Europe, as globally



- **Neonatal GBS diseases**
  - **EOD and LOD, a public health concern**
  - **IAP efficient for prevention of EOD**
    - **Best strategy still a matter of debate**
    - **Not 100% efficient**
    - **No effect on LOD**
  - **IAP not widely recommended**
  - **Need better data assessing more accurately the true burden**
- **GBS vaccine eagerly expected**

Summary



“Screening” Prevention strategies

- **Culture-based GBS prenatal screening**
  - To optimize critical factors
  - Improved by selective differential agars
  - False +/False - !
- **Culture-LAMP combined GBS prenatal screening**
  - High sensitivity and negative predictive value
  - 100% specificity and positive predictive value
  - Identification of an 0.8% additional GBS positive specimen
- **Rapid intrapartum screening**
  - Real time PCR
    - Yes but costs, logistic, …
    - Need for more clinical and cost effectiveness trial

Evaluation of *illumigen*® GBS

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Meridian for supplying the test kits