



Towards a European Consensus for prevention of Perinatal Group B Streptococcal Disease

THE LABORATORY OLD & NEW TOOLS TO DETECT GBS


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CONTENT

- **Introduction & burden**
 - History and historical context of perinatal GBS disease
 - Early and contemporary epidemiology
 - Pathogenesis and risk factors
- **Prevention**
 - Strategies through
 - Maternal intrapartum chemoprophylaxis - Guidelines
 - Maternal immunization
- **Screening**
 - Old and new tools to detect GBS
- **Take home messages**


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INTRODUCTION & BURDEN

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Streptococcus agalactiae or GBS




Gram positive cocci
β-hemolytic
Encapsulated

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

Rebecca Lancefield 1895-1981

1887, Nocard-Mollereau, bovine mastitis
1933, Group B Antigen
1964, severe neonatal sepsis, Eickhoff et al N Eng J med
>1970, N° 1 in neonatal infections



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Group B streptococcal diseases in neonates

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

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Group B streptococcal diseases in neonates

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



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

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Group B streptococcal diseases in neonates

- Since the 1970s, leading cause of life-threatening infections in newborns

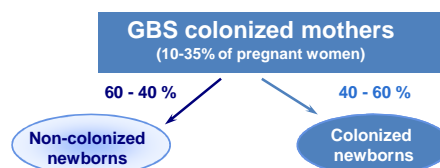
- Neonatal illness/death
- Long-term disabilities

GLOBAL health major challenge !
Also in developing countries

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

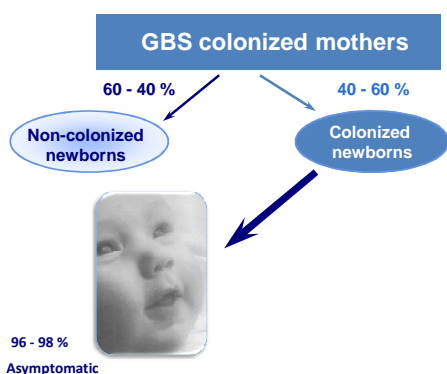
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GBS EOD vertical transmission



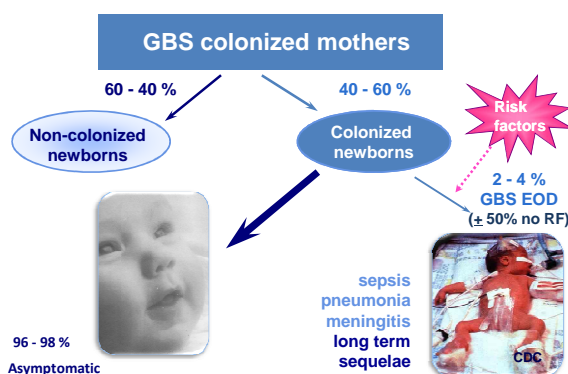
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GBS EOD vertical transmission



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GBS EOD vertical transmission



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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:

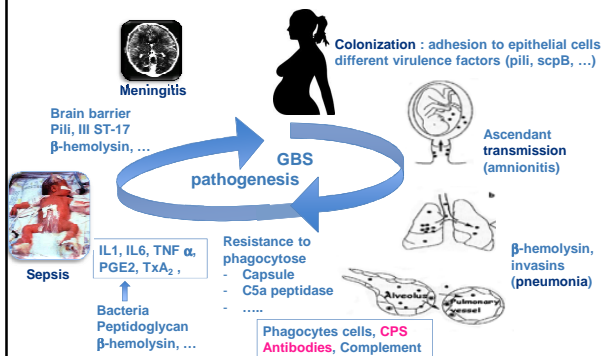
- Low specific IgG to GBS capsular polysaccharide

*: 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

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

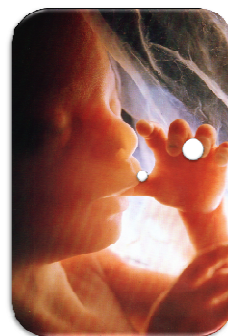


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- Universal antenatal screening-based strategy
- Risk-based strategy
- No guideline

GUIDELINES FOR PREVENTION OF GBS PERINATAL DISEASE

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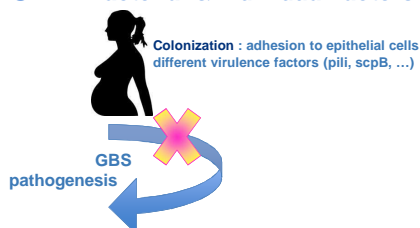


Which prevention strategy for GBS perinatal diseases ?

- Intrapartum antibioprophylaxis
 - Immunoprophylaxis
- Key strategy
« nearly within reach »

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

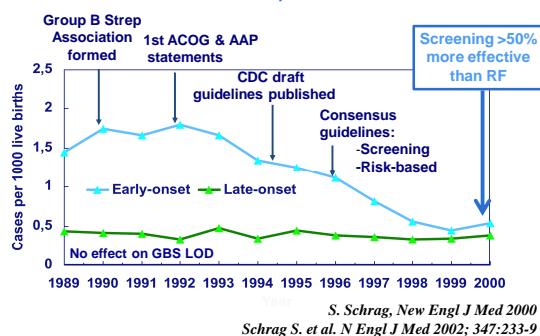


Intrapartum antibioprophylaxis
> 4 (2) hours before delivery

Highly effective in preventing GBS EOD (1st clinical trials in late 80s)

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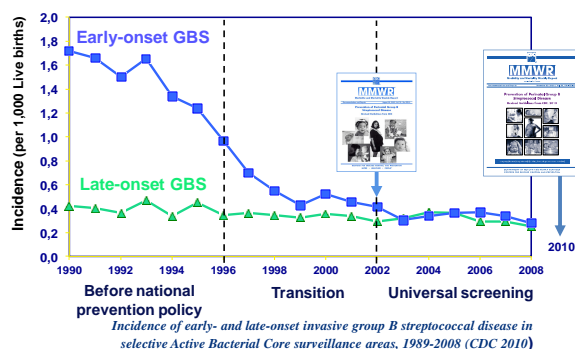
Impact of prevention practices Early- and Late-onset GBS Diseases in the 1990s, U.S.



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

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

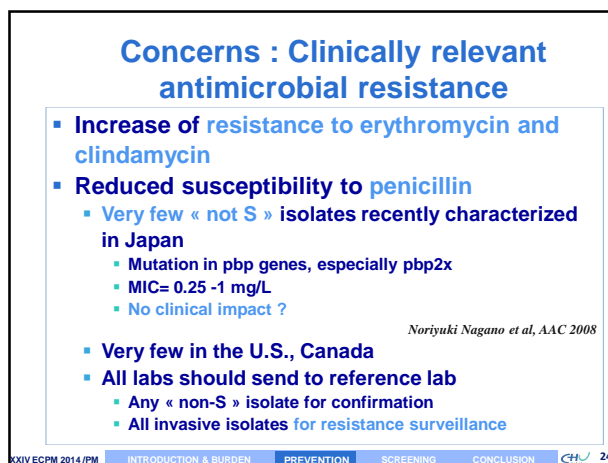
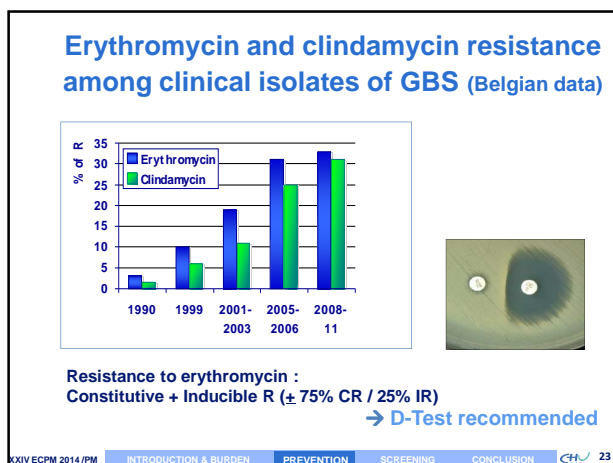
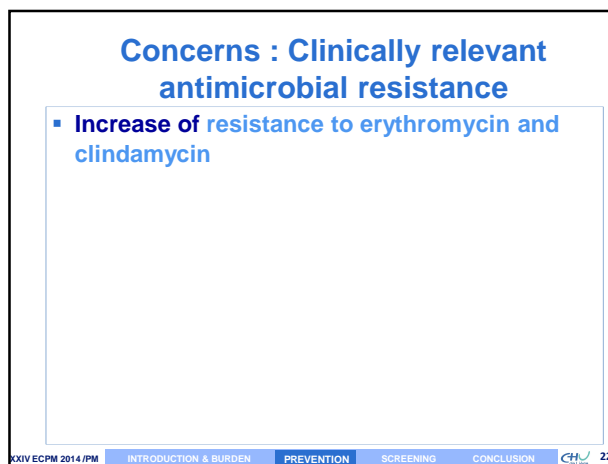
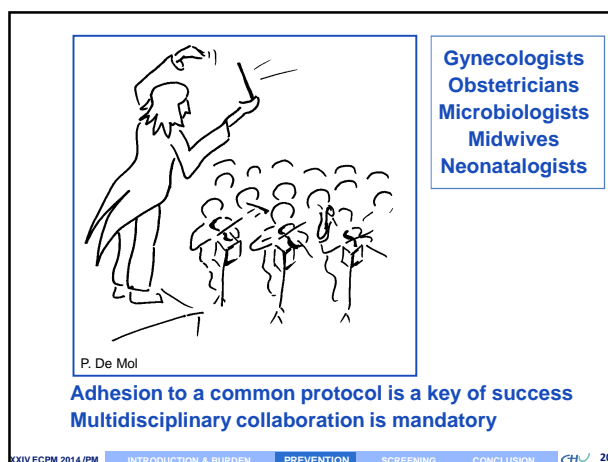
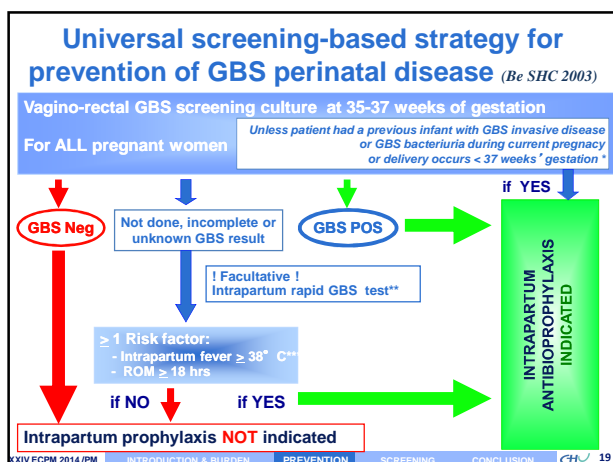


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European strategies for prevention of GBS EOD

- Intrapartum antibioprophylaxis recommended
 - Screening-based strategy
 - Spain, 1998, 2003, revised 2012
 - France, 2001
 - Belgium, 2003, revision ongoing 2013
 - Germany, 1996, revised 2008
 - Switzerland, 2007
 - Risk-based strategy
 - UK, the Netherlands, Denmark
- No guidelines
 - Bulgaria, ...

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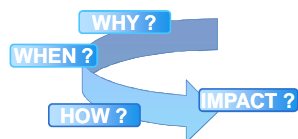
Remaining burden of GBS EOD Missed opportunities

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

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Specimen collection
Processing
Culture or non culture approach?

SCREENING FOR GBS COLONIZATION

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Antenatal GBS culture-based screening

Goal of GBS screening

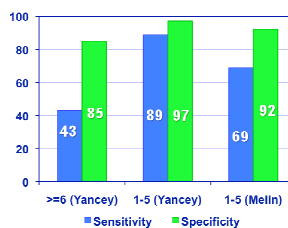
To predict GBS vaginal (rectal) colonization at the time of delivery

- Critical factors influencing accuracy
 - Swabbed anatomic sites (distal vagina + rectum)
 - Timing of sampling
 - Screening methods
 - Culture
 - Procedure
 - Media
 - Non-culture

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Optimal time for screening 35-37 weeks gestation

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



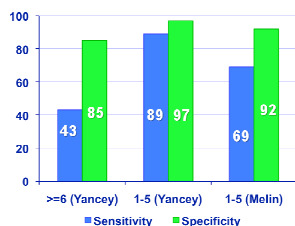
Not 100 % as
colonization is dynamic

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

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Optimal time for culture-based screening 35-37 weeks gestation

Culture-based screening done 1 to 5 or ≥ 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
antenatal screening !

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

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Antenatal culture-based screening: Limiting factors

- Positive and negative predictive values

False-negative results

- Failure of GBS culture (reduced viability during transport, oral ATB, feminine hygiene) or new acquisition
- Up to 1/3 of GBS positive women at time of delivery

Need for more accurate predictor of
intrapartum GBS vaginal colonization

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From direct plating on blood agar Evolution of culture methods

Use of selective enrichment broth (Lim broth, e.g.)

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

Use of differential agar media

Recommended by some European guidelines (+ CDC 2010)

GRANADA
(M.de la Rosa, JCM)

StreptoB ID

Strepto B
Select

Brilliance
StrepB

1983, 1992

2005

2007

2012

Pigment-based

Chromogenic media

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Standard procedure



Vagino-rectal swab or
Vaginal & rectal swabs

Inoculate swab(s) in 1 Lim broth

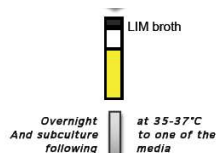


Overnight
And subculture
following



at 35-37°C
to one of the
media

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Granada
agar

StrepB
Select

ID
StrepB

Anaero

Ambient air

Ambient air

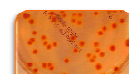
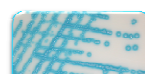
48 h at 35-37°C



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Which agar or which combination?

+/- Blood agar



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

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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)

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Crucial conditions to optimize SCREENING

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- WHO** ALL the pregnant women
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- Request form** To specify prenatal « GBS »
screening
- Laboratory procedure**

(CDC 2010 - Belgian SCH 2003)

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Crucial conditions to optimize SCREENING

Transport-collection system & transport-storage condition

- Type of swab: Nylon flocked >> regular fiber swab

Nylon Flocked Swab

Superior sample collection and release

Collected sample

>80% of the sample analyte released*

Regular Fiber Swab

Sample stays trapped in fiber matrix

Trapped sample

Sample dispersion, dilution and entrapment in the fiber matrix

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Crucial conditions to optimize SCREENING

Transport-collection system & storage condition

- Recommendations CDC, USA (2010)
 - Non nutritive media: Amies or Stuart without charcoal
 - Storage at 4° C or RT 1-4 days
 - Or Granada like tubes ??
- Recommendations CSS, Belgium (2003)
 - Non nutritive media: Amies or Stuart without charcoal
 - Storage maximum 48h at 4° C

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Crucial conditions to optimize SCREENING

Transport-collection system & 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.

Viability of GBS NOT fully preserved by storage of vaginorectal swabs in Amies transport medium, mainly if not stored under refrigeration.

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ICAAC 2013
53rd ICAAC | SEPT 10-13 | Denver, CO

IMPROVEMENT OF TRANSPORT CONDITION OF SWABS FOR GROUP B STREPTOCOCCAL (GBS) SCREENING

P. Melin, M. Dodémont, G. Sarlet, R. Sacheli, J. Descy, C. Meex, P. Huynen, MP. Hayette
National Reference Centre for GBS, University Hospital of Liège, Liège, Belgium

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Crucial conditions to optimize SCREENING

Transport-collection system & storage condition (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

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Results: Recovery of GBS in Lim BD at 4° C, RT and 35° C

Time (h)	4°C (CFU)	RT (CFU)	35°C (CFU)
0	10	10	10
24	5	100	10000
48	3	10000	10000
72	3	10000	10000
96	1	10000	10000

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Transport conditions to be recommended for optimizing GBS antenatal screening

Belgian Health Superior Council, 2013

- **Transport system**
 - Use of a **selective enrichment Lim broth with a flocced swab** (BD, Copan, bioMérieux, i.e.)
- **Transport and storage condition**
 - At RT* (up to 35° C)
 - As soon as possible
 - Viability sustained at least 4 days
- **Remark**
 - If use of Amies or Stuart medium (non nutritive medium)
 - To be processed as soon as possible within 24 hours (max 48 h)

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Antenatal 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



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Evaluation of the *illumigene*® GBS assay for antenatal screening from Lim broth

- **Speed and “accuracy”**
- **Good comparison to reference culture method**
- **“Easy” to perform BUT not as easy as claimed and training very important**
 - 95% sensitivity and 100% specificity
 - Identification of an 0.8% additional GBS positive specimen
 - Overall cost and logistic to be considered

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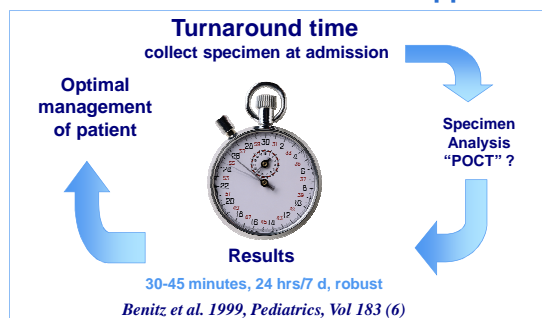
Antenatal culture-based screening: Limiting factors

- **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

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Alternative to GBS antenatal screening: Intrapartum screening Theranostic approach



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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)



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The Xpert GBS™ Advantage: Simplicity

- Fully automated process reduces handling time to just minutes
- Random access for flexibility and workflow optimization
- Rapid results to improve patient management
- Fully integrated reagent and instrument system for accuracy and reproducibility

1. Insert swab into cartridge and break at mark

2. Dispense Reagent 1 into port 1

3. Dispense Reagent 2 into port 2

4. Insert cartridge and start assay

Total hands-on time = 2 minutes

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Xpert GBS for intrapartum screening
(selected paper amongst many others)

Diagnostic Accuracy of a Rapid Real-Time Polymerase Chain Reaction Assay for Universal Intrapartum Group B Streptococcus Screening

Najoua El Helali, Jean-Claude Nguyen, Aïcha Ly, Yves Giovangrandi and Ludovic Trinquart

Clinical Infectious Diseases 2009;49:417–23

■ **968 Pregnant women**

■ **Intrapartum Xpert GBS, Cepheid** (performed in lab)

■ vs intrapartum culture

antepartum culture (French recom.)
(vaginal swab/CNA-BA)

Sensitivity	98.5%	PPV	58.3%
Specificity	99.6%	NPV	92.1%
PPV	97.8%		
NPV	99.7%		

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Xpert GBS for intrapartum screening
(selected paper amongst many others)

Cost and effectiveness of intrapartum group B streptococcus polymerase chain reaction screening for term deliveries.

El Helali N, Giovangrandi Y, Guyot K, Chevet K, Gutmann L, Durand-Zaleski I

Obstet Gynecol 2012 Apr;119 (4):822-9

2009	2010
Antenatal screening	Xpert GBS intrapartum screening
11.7% GBS POS	Performed by midwives as a POCT !!
	16.7% GBS POS
	Less GBS EOD & less severe
	Cost neutral per delivery

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Xpert GBS for intrapartum screening
(selected paper amongst many others)

Real-Time PCR Assay Provides Reliable Assessment of Intrapartum Carriage of Group B *Streptococcus*

Michelle J. Alfa, Shadi Sepehri, Pat De Gagne, Michael Helawa, Gunwat Sandhu, and Godfrey K. M. Harding

JCM, Sept. 2010, p. 3095–3099

■ **205 Pregnant women**

■ **Intrapartum Xpert GBS, Cepheid**

■ vs intrapartum culture (with Lim enrichment step)

24.5% GBS pos

Sensitivity	91.7%
Specificity	99.3%
PPV	97.7%
NPV	97.3%

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Real-time PCR, very promising, BUT ...

- **Rapid, robust & accurate technology**
- **Still an expensive technology (specific equipment)**
 - Cost effective ?
 - Need for more cost-effectiveness clinical study
 - 2014 NRC GBS - CHULg & UIA
- **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
- **Drawback: no antimicrobial result**
 - In the future detection of R genes, but mixed microbiota !

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CONCLUSION

Take home messages

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In Europe, as globally

Neonatal GBS diseases

- EOD and LOD, a global 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
- New tools to improve GBS detection

GBS vaccine eagerly expected

- Appears to be within reach

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Summary

"Screening" Prevention strategies

- **Culture-based GBS antenatal screening**
 - False +/False -
 - To optimize critical factors
 - Improved by selective differential agars
 - Expected improvement from transport system
- **Rapid intrapartum screening**
 - Real time PCR
 - Yes but costs, logistic, ...
 - Need for more clinical and cost effectiveness trials
 - No result for clindamycin susceptibility

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Prevention strategy for GBS EOD: TOWARDS A EUROPEAN CONSENSUS ?

Answers from the next
speakers ?



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