



DEVANI

European Training Course on
Microbiological Procedures for Group B
Streptococcal
Diagnosis, Screening & Typing

Archbishop Makarios III Hospital & Cyprus Oncology
Centre,
Nicosia, Cyprus
Friday 29th May 2009



Training Course Organisers

Health Protection Agency Centre for Infections, London, UK

&

ALTA, Siena, Italy

&

Novartis Vaccines & Diagnostics, Siena, Italy

&

Istituto Superiore di Sanità, Rome, Italy

&

**Paediatric Department, Archbishop Makarios III Hospital, Nicosia,
Cyprus**

&

Cyprus Institute of Biomedical Sciences (CIBS)

Training Course Teachers and Presenters

Dr Baharak Afshar

Health Protection Agency Centre for Infections, London, UK

Dr Roberta Creti

Istituto Superiore di Sanità, Roma, Italy

Dr Manuel de la Rosa

Servicio Andaluz de Salud, Hospital Universitario Virgen de las Nieves, Granada,
Spain

Dr Androulla Efstratiou

Health Protection Agency Centre for Infections, London, UK

Dr Knud Poulsen

Aarhus Universitet, Aarhus, Denmark

Dr Pavla Krizova

National Institute of Public Health, Prague, Czech Republic

Dr Pierrette Melin

Centre Hospitalier Universitaire de Liège, Liège, Belgium

Dr Martin Musílek

National Institute of Public Health, Prague, Czech Republic

Dr Graziella Orefici

Istituto Superiore di Sanità, Rome, Italy and ALTA, Siena, Italy

Trainees

Belgium

Dr Grisel Rodriguez Cuns

Centre Hospitalier Universitaire de Liège, Liege,
Belgium

Mrs Cecile Meex

Belgian Reference Centre for Group B Streptococci,
Liege, Belgium

Bulgaria

Dr Antoneta Decheva

National Center of Infectious and Parasitic Diseases,
Sofia, Bulgaria

Czech Republic

Dr Jana Kozakova

University of Public Health, Prague, Czech Republic

Dr Jitka Motlova

National Institute of Public Health, Prague, Czech Republic

Denmark

Mr Lasse Berg Hansen

Aarhus University, Denmark

Mr Anders Jensen

Aarhus University, Aarhus, Denmark

Germany

Dr Kerstin Blessing

Centre of Paediatrics and Adolescent Medicine,
Freiburg, Germany

Mr Fabian Lander

University Medical Centre, Freiburg, Germany

Italy

Dr Roberta Bernardini

University of Rome, Rome, Italy

Ms Roberta Cozzi

Novartis Vaccines & Diagnostics, Siena, Italy

Dr Filippo Ferrari

Azienda Ospedaliero–Universitaria Policlinico, Modena,

Italy

Dr Sceida Khacheh

Novartis Vaccines & Diagnostics, Siena, Italy

Dr Annibale Raglio

USC Microbiology and Virology AO Ospedali Riuniti,
Bergamo, Italy

Spain

Dr Jorge Cabrera Alvargonzalez

C/ Juan de la Cierva n°2 3°B Granada, Spain

Miss Eva Gonzalez Cid

Hospital Universitario Virgen de las Nieves, Granada,
Spain

UK

Mrs Karen Broughton

Health Protection Agency Centre for Infections,
London, UK

Dr Anna Vickers

Health Protection Agency Centre for Infections,
London, UK

Programme

TRAINING COURSE PROGRAMME

Friday 29th May 2009

**Archbishop Makarios III Hospital, Nicosia, Cyprus
Conference Room, First Floor**

Group 1 (9 trainees):

9.00–11.00

Laboratory diagnosis and screening for GBS

(M. de la Rosa, P. Melin, R. Creti, G. Orefici)

Laboratory procedures for processing clinical specimens for GBS antenatal screening cultures:

	Time Presentation+ Discussion	Teacher
Specimen collection		
<ul style="list-style-type: none"> • Optimal time of specimen collection • Correct specimen type and method of collection • Adequate quantity and appropriate number of processing 	15 minutes + 5 minutes	Pierrette Melin
Specimen transport and storage		
<ul style="list-style-type: none"> • Time between specimen collection and processing • Special consideration to minimise deterioration 	15 minutes + 5 minutes	Manuel de la Rosa
Specimen processing and identification		

<ul style="list-style-type: none"> Primary isolation media and GBS differential agar media (Granada and chromogenic agar): presentation, principle, pros and cons 	20 minutes + 5 minutes	Manuel de la Rosa
<ul style="list-style-type: none"> Prenatal GBS culture-based screening protocols: Comparison of their performances DEVANI recommended protocol for GBS prenatal culture-screening 	20 minutes + 5 minutes	Pierrette Melin
<ul style="list-style-type: none"> Preventing perinatal group B streptococcal disease: bacterial identification, typing and susceptibility testing 	20 minutes + 10 minutes	Roberta Creti

11.00–11.30

Coffee

**Archbishop Makarios III Hospital, Nicosia
Conference Room, Second Floor**

Group 2 (9 trainees):

9.00–10.00

Characterisation of GBS from clinical samples and non-culture diagnosis (B. Afshar, A. Efstratiou)

Non-culture diagnostic assays: real-time PCR assay, other PCR-based methods, *commercial and 'in-house'*.

10.00–11.00 **Serotyping methods for GBS (practical session)**

(B. Afshar, A. Efstratiou)

- GBS confirmation using the Prolex latex kit (Pro-Lab Diagnostics)
- GBS serotyping using the Strep-B latex kit (Statens Serum Institut, Denmark)

11.00–11.30

Coffee

At 11.30, the two groups will exchange sessions.

13.30–14.30

Lunch

**Cyprus Oncology Centre, Nicosia
Lecture Theatre**

Both groups will attend this session together.

14.30–15.00

Molecular characterisation of GBS

Chairs: Dr. Androulla Efstratiou (Health Protection Agency Centre for Infections, London – UK) and Dr. Graziella Orefici (Istituto Superiore di Sanità, Rome – Italy)

14.30–14.40

Multiplex PCR assay for capsular typing of GBS
(Knud Poulsen, Denmark)

14.40–14.50

Multilocus Sequence Typing (MLST) for GBS
(Pavla Krizova & Martin Musílek, Czech Republic)

14.50–15.00
repeat (VNTR)

**Identification of variable–number tandem–
sequences in GBS**
(Baharak Afshar, UK)

15.00 –15.20

Tea

15.20–16.00

General Discussion

16.00

End of training course

Antenatal Screening Culture for GBS Colonization

ANTENATAL SCREENING CULTURE FOR GBS COLONIZATION

PREANALYTICAL CONSIDERATIONS

I. RATIONALE

Group B streptococcal (GBS) disease is the leading bacterial infection associated with illness and death among newborns in industrialized countries. GBS (*Streptococcus agalactiae*) is transmitted vertically from colonized women to their infants prior to, or during birth. Early-onset GBS infection (occurring less than 7 days of life) can result in severe, potentially fatal infection in newborns (sepsis, pneumonia, meningitis).

A substantial number of GBS infections can be prevented with preterm screening of pregnant women and subsequent intrapartum antimicrobial prophylaxis to GBS colonized women. In general, penicillin G is the preferred agent for prophylaxis specific for GBS colonization because of its empiric efficacy against GBS, and its narrow spectrum of activity.

II. PRINCIPLE OF SELECTIVE CULTURE FOR GBS

In the setting of strategy for prevention of GBS perinatal disease, the optimal time for performing antenatal cultures is between 35 and 37 weeks' gestation. As GBS carriage is highly variable, GBS antenatal cultures are not always good predictors of maternal GBS status at presentation for delivery. To improve sensitivity and predictive values of the GBS antenatal screening, culture techniques that maximize the

likelihood of GBS recovery are required for prenatal screening. Important factors that influence the accuracy of detection of GBS maternal colonization are the choice of culture media, the body sites sampled and the timing of the sample. The yield of GBS-positive culture is increased by sampling the anorectum in addition to the lower vaginal area, because the gastrointestinal tract is a major reservoir of GBS. This can be performed using a single swab or two different swabs. After collection, swabs must be placed in a non-nutrient transport medium as Amies or Stuart. The use of a selective broth medium that inhibits the growth of competing organisms, Gram-negative enteric bacilli and other normal flora significantly increases the yield of GBS culture and is recommended since it has been found to be the most sensitive method to detect female colonization. The most widely used selective medium is Todd-Hewitt broth with nalidixic acid and colistin (e.g. Lim broth) or nalidixic acid and gentamicin (according to C.Baker's formula, e.g. Trans-Vag broth) further sub-cultured on blood agar plate. However, this enrichment broth is not totally selective for GBS, and other Gram-positive cocci may as well be enriched by this method, possibly hiding GBS and leading to false-negative results.

For this reason, use of selective and differential media for subcultures can improve screening sensitivity as well as shortening the turnaround time, and is already recommended in some European countries for antenatal GBS-screening cultures. Several options are now available. Granada agar was the first and most widely used medium in Spain and Belgium. On Granada agar, β -hemolytic strains of GBS produce orange colonies that are clearly differentiated from the background flora. For recto-vaginal cultures, the sensitivity of Granada agar for the detection of GBS is superior to that of blood agar. Easy to read and with 100% specificity, workload and turnaround time are reduced. Recently, two selective and differential chromogenic media have been launched: ChromID Strepto B (bioMérieux, France) in 2006 and StrepBSelect (Biorad, France) in 2007. Compared with culture on blood agar, these new chromogenic media significantly increase the sensitivity of GBS

screening and can also replace, with true benefit, the blood agar. But these chromogenic media are not fully specific, so all presumptive colonies of GBS must be confirmed by a specific antigenic detection test.

To conclude, in the setting of a maternal GBS screening program, efforts to improve screening for GBS status remain important. Correct laboratory processing of culture specimens plays a critical role in successful implementation of the screening policy. The use of selective differential media can improve antenatal culture sensitivity and has already been recommended in different European countries, such as Spain and Belgium.

III. SPECIMEN COLLECTION, TRANSPORT AND HANDLING

Rayon or Dacron, fiber or flocked, swabs, with non-nutritive transport media (e.g., Amies or Stuart's \bar{r} preserve the viability of the organism by providing moisture, and buffering to maintain the pH.

Collection (cf. appendix 1)

- At 35–37 weeks of gestation, collection of specimen(s) for culture may be done by physician or other qualified caregivers (or either self-collected by the patient, with appropriate instruction). This involves swabbing the distal vagina (vaginal introitus), followed by the rectum (i.e., through the anal sphincter). A unique swab for both sites of collection is rational but two different swabs can be used. Because lower vaginal as opposed to cervical cultures are recommended, cultures should not be collected by speculum examination.
- Immediately after specimen collection, swabs are inserted into the transport media.

Specimen transport

- One or both swabs placed in their transport medium are submitted as soon as possible to the laboratory (within the day); if any delay

is expected, refrigerate specimens (2–8°C) for a maximum of 48 hours. In these conditions, GBS can remain viable.

- All specimens should be labeled and patient name, hospital medical record number, other patient demographics, date and time of collection should be specified on the accompanying requisition form.
- Order should clearly identify that specimens are specifically submitted for group B streptococcal culture.

IV. MATERIALS & CULTURE MEDIA

Culture media

Selective streptococcal broth:

- Lim broth = Todd Hewitt broth supplemented with colistin (10mg/L) and nalidixic acid (15 mg/L), available from various vendors.
- Todd Hewitt broth supplemented with gentamicin (8mg/L) and nalidixic acid (15 mg/L) can be used but this formula requires the addition of 5 % defibrinated sheep blood for increased recovery of GBS, available from various vendors.

Agar media:

- Granada plates (BioMedics, bioMerieux, Becton Dickinson)
- ChromID Strepto B (bioMerieux)
- StrepBSelect (BioRad)
- (Blood agar with/without colistin and nalidixic acid)

Identification methods

- Gram stain procedure
- Catalase test
- Other tests for identification of catalase–negative Gram–positive cocci

- Various latex or co-agglutination serologic tests for streptococcal grouping

Other supplies

- Incubator at 35°C, ambient air
- Self-contained anaerobe-generating system (if use of Granada agar).
- Incubator at 35°C + 5% CO₂ or self-contained CO₂-generating system (if use of blood agar).

ANALYTICAL CONSIDERATIONS

V. QUALITY CONTROL

- Verify that media meet expiration date and QC parameters.
- Test each lot of selective broth with QC strains. The suggested QC organisms and expected results include: *Streptococcus agalactiae* ATCC 12386, moderate to heavy growth and *Escherichia coli* ATCC 25922, partial to complete inhibition. Separate broths are inoculated with the above QC organisms: 1 ml suspension of 18–24 h broth cultures containing either 1,000 or less CFU/ml for GBS and 100,000 CFU/ml for *E.coli*. QC tests are incubated at 35°C ± 2°C in an aerobic atmosphere and examined for up to 3 days for growth.
- Test each lot of agar with a QC GBS strain to verify the ability of the media to produce colonies with the expected characteristics and color. Always refer to manufacturers' product insert.
- Do not use media found to be deficient or that appears compromised.
- Refer to individual test procedures for further QC requirements.

VI. PROCEDURE

Inoculation

- Upon reception, swabs for GBS prenatal screening cultures are inoculated into selective enrichment broth medium. If both a vaginal and rectal swab is submitted from one patient, both swabs may be placed into one enrichment broth. If using broth with gentamicin/nalidixic acid, add 5 % of sterile defibrinated sheep blood to each tube. Sheep blood is not added to LIM broth.
- Optional, before inoculation of the selective broth: in addition to, and not instead of the broth inoculation, some laboratories may choose to inoculate the swab to primary solid media such as CNA sheep blood agar or to a selective streptococcal medium as Granada agar or a specific chromogenic agar, immediately upon receipt. Plate(s) should be streaked for isolation.

Incubation

- Incubate selective direct plate(s) at 35 to 37°C in the appropriate atmosphere: blood agar with 5% CO₂, Granada agar anaerobically* and chromogenic agars in ambient air.
** : If anaerobic incubation is not possible, alternatively Granada plates can be incubated in air if a coverslip is placed over the inoculum. This method is somehow inferior to the anaerobic incubation.*
- Incubate broth at 35 to 37°C in ambient air, or in CO₂.
- Subculture the broth after 18 to 24 h of incubation to a Granada agar plate or to a GBS selective chromogenic agar as StrepBSelect (BioRad) or ID Strepto B agar (bioMerieux), if GBS have not been isolated on direct plate(s).
- Incubate the subculture plate(s) at 35 to 37°C in the appropriate atmosphere for 48 h.

Culture examination

- After overnight incubation, observe plates for suggestive GBS colonies and identify them. If negative after overnight incubation, reincubate an additional 24 hours before reporting a negative result.

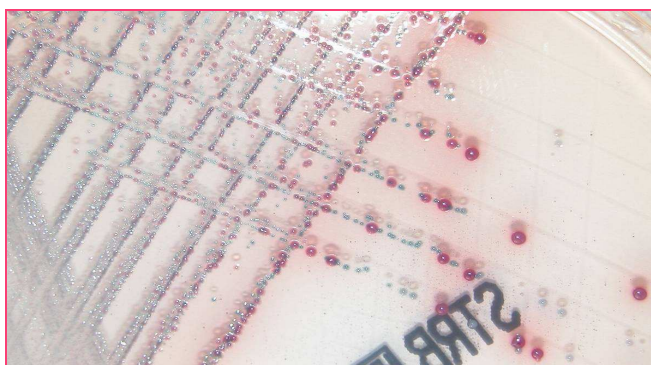
- Upon Granada medium, the development of orange or red colonies is specific (100%) of GBS. (cf. figure 1).
- On StrepBSelect, suggestive colonies of GBS are pale to dark blue turquoise and on ID Strepto B, suggestive colonies of GBS are pale pink to red. (cf. figures 2 & 3). On these chromogenic media, colonies suggestive of GBS should be specifically identified with a test for GBS antigen detection.
- On blood agar, suggestive colonies of GBS are gray, translucent, with a surrounding zone of beta-hemolysis (or no hemolysis: very rare).

Figures 1, 2 & 3: *GBS typical suggestive colonies on different selective and differential agars after 48 hours incubation in appropriate atmospheres:*

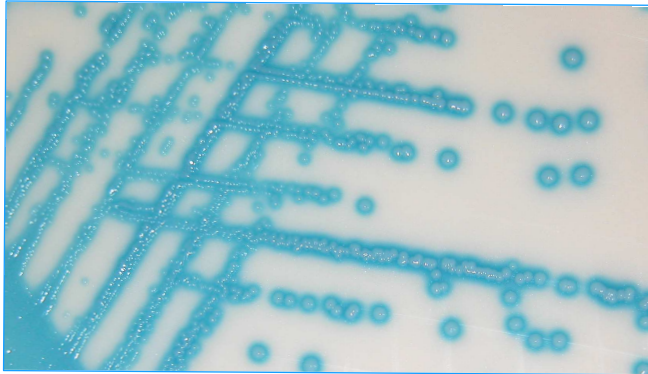
1- Granada agar: orange colonies;



2- ID Strepto B agar: pink to red colonies;



3- StrepB Select agar: blue-turquoise colonies.



Identification of group B streptococcus: detection of group B antigen

Suspicious colonies (catalase negative, Gram positive cocci) should be identified with an accurate and reliable standardized method for streptococcal serologic grouping, such as slide latex- or co-agglutination. If growth is insufficient or heavily mixed, suspicious colonies should be sub-cultured to a fresh plate. Genetic probe is a useful option and may be used to identify suspicious GBS colonies.

Long term storage of GBS isolates

- Use of a CryoBank Beads system for freezing GBS cultures is the more convenient system, but other types of cryo-storage can be used.
- From a fresh 18 hours plate culture of GBS, make a heavy cloudy suspension in the storage broth in the tube.
- Immediately place the tube in a -70°C freezer.

POSTANALYTICAL CONSIDERATIONS

VII. SUGGESTED REPORTING

- When no GBS is recovered, report as: **No group B streptococcus isolated.**
- When GBS is recovered, report as: **Presence of group B streptococcus, or Group B streptococcus isolated.**
Report the presence of group B streptococci but do not report any enumeration.

VIII. INTERPRETATION

- A positive culture indicates colonization with GBS, which may or may not indicate infection.

IX. LIMITATION

- False-negative cultures can result from inability to recognize non-hemolytic colonies on Granada or blood agar.
- Due to the large load of various bacteria in vaginal and rectal flora, false-negative results can be observed even with selective differential media as chromogenic media when they are used for direct inoculation of rectal or vagino-rectal swabs. As recommended in this procedure, subcultures from selective enrichment is mandatory.
- In addition, even with ideal sampling and culture procedures, maternal factors such as use of oral antibiotics or a variety of feminine hygiene products before specimen collection can lead to failure to culture GBS.

- False-positive results can be caused by misinterpretation of the confirmatory tests.

X. REFERENCES

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- Boyer KM, Gotoff SP. Prevention of early-onset neonatal disease with selective intrapartum chemoprophylaxis. *N Engl J Med*, 1986; 314: 1665-9
- Schuchat A, Group B streptococcus. *Lancet*, 1999; 353:51-6
- Dillon HC, Gray E, Pass MA, *et al.* Anorectal and vaginal carriage of group B streptococci during pregnancy. *J Infect Dis*, 1982; 145: 794-9
- Yancey MK, Schuchat A *et al.* The accuracy of late antenatal screening cultures in predicting genital group B streptococcal colonization at delivery. *Obstet Gynecol*, 1996; 88: 811-5
- Goodman JR *et al.* Longitudinal study of group B streptococcus carriage in Pregnancy. *Infect Dis Obstet Gynecol*, 1997;5: 237-43
- Melin P *et al.* Evaluation of the StrepB Select Agar for the Detection of Group B Streptococci from Vaginal and Recto-vaginal Specimens In: *Program and*

abstracts of the 18th European Congress of Clinical Microbiology and Infectious Diseases, Barcelona, Spain 2008 American Society for Microbiology, 2000.

XI. APPENDIX

Appendix 1: Gynecologist's memo for collecting clinical specimens for antenatal GBS screening cultures.

Appendix 2: Laboratory procedure for processing clinical specimens for antenatal GBS screening cultures:

- **Standard procedure**
- **Optional procedure**

Appendix 1

Gynecologist's memo for collecting clinical specimens for antenatal GBS screening cultures

ANTENATAL GBS SCREENING

WHEN

Collect specimen at **35–37 weeks** of gestation.

WHO

ALL pregnant women at that time of pregnancy.

WHICH SPECIMEN

Lower vagina (introitus)
& **rectum** (through the anal sphincter)

MATERIAL

One (or two) swab(s) for both sites of collection
placed in non-nutritive transport medium
(e.g. Amies or Stuart's without charcoal).

STORAGE & TRANSPORT

Transfer specimens to the laboratory within the day.
If any delay, refrigerate specimens (2 to 8°C), maximum 48 hours.

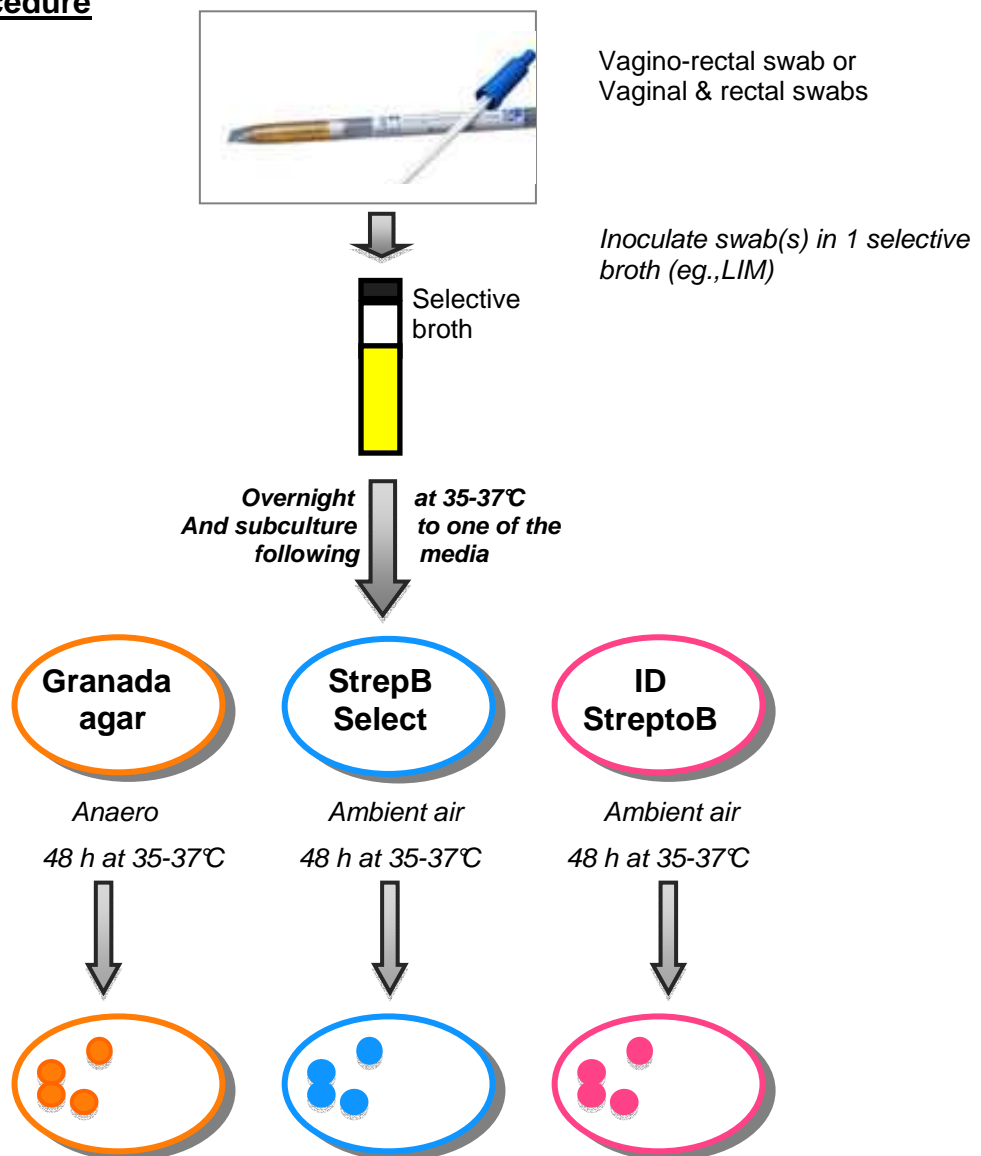
REQUESTING FORM

Clearly order culture specifically for "GBS Screening".

Appendix 2

Laboratory procedure for processing clinical specimens for GBS antenatal screening cultures

Standard procedure



POSITIVE GBS Screening if	Orange colonies = GBS	Blue-turquoise colonies = suggestive GBS Id. to confirm	Pink colonies = suggestive GBS Id. to confirm
Negative GBS Screening if	No orange colonies	No blue-turquoise colonies	No pink colonies

Optional procedure



Vagino-rectal swab or
Vaginal & rectal swabs



AND



Inoculate swab and streak to at least one of the following media



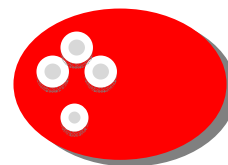
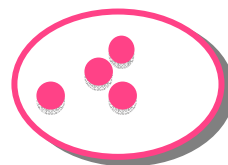
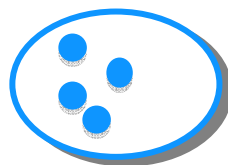
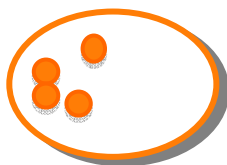
Selective broth
(eg., LIM)

Overnight at 35-37°C



**If no GBS identified on direct plate(s):
proceed as in standard procedure.**

48 h at 35-37°C in appropriate atmosphere



POSITIVE GBS Screening if	Orange colonies = GBS	Blue-turquoise colonies = suggestive GBS Id. to confirm	Pink colonies = suggestive GBS Id. to confirm	Beta-hemolytic colonies = suggestive GBS Id. to confirm
Negative GBS Screening if	No orange colonies	No blue-turquoise colonies	No pink colonies	No beta-hemolytic colonies

Serotyping Methods for GBS

GBS confirmation using the Prolex latex kit (Pro-Lab Diagnostics)



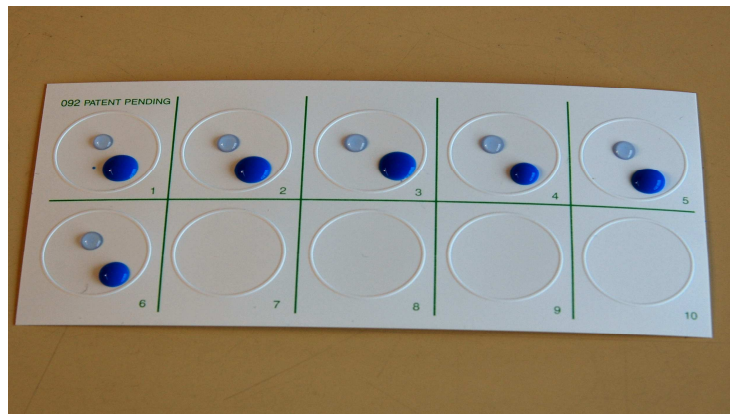
MATERIALS

- Prolex kit, Prolab Diagnostics (Cat number: PL030)
- Disposable Bacteriological loops 1 μ l
- Gilson Pipetman P20 or P100, Anachem
- Plastic capped test tubes or similar
- Disposable plastic Pasteur pipettes
- Rubber or plastic test tube rack

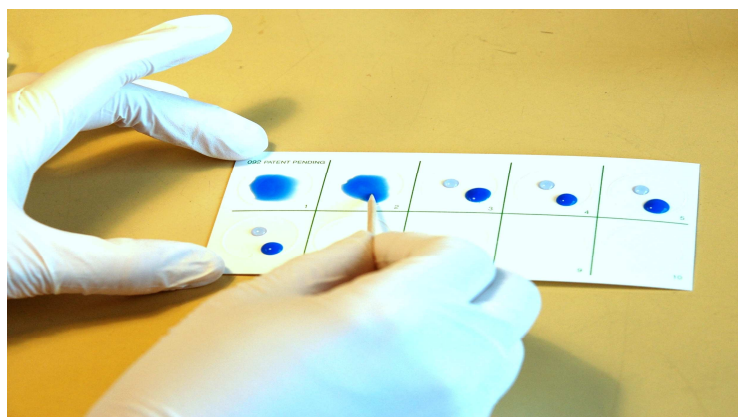
METHOD

Gloves and safety glasses should be worn for this procedure.

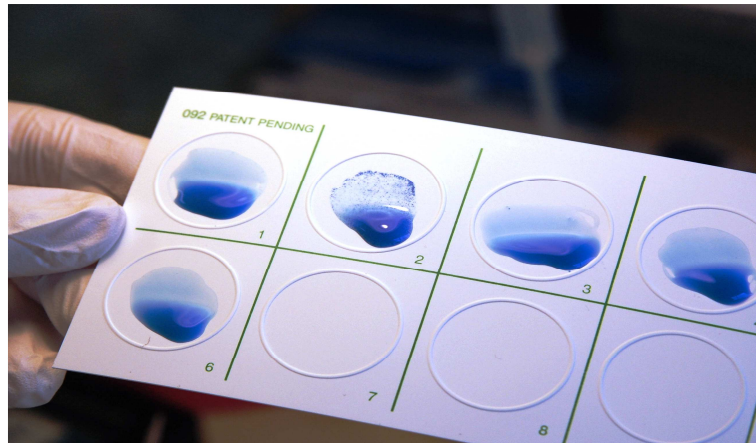
1. Allow all reagents and samples to reach room temperature before use.
2. Dispense 1 drop of Extraction Reagent 1 (Yellow cap) into an appropriately labelled plastic capped test tube or similar for each culture to be grouped.
3. Use a 1µl loop to make a light suspension of the culture, 5 colonies should be sufficient.
4. Add 1 drop of Extraction Reagent 2 (Red cap) to each tube.
5. Mix the reaction by tapping the tube with a finger for 5 – 10 seconds.
6. Add 4 – 5 drops of Extraction reagent 3 (Blue cap) to each tube, tap as above to mix.
7. Resuspend each of the latex suspensions by shaking vigorously for a few seconds.
8. Holding the dropper bottle vertically, dispense one drop (20ul) of each blue latex suspension onto a separate circle on the test card.
9. It is important to hold the dropper bottle vertically, and to ensure that the drop forms at the **tip** of the nozzle. If the nozzle becomes wet an incorrect volume will form **around** the end and not at the tip: if this occurs dry the nozzle before progressing
10. Using a plastic disposable pipette or a Gilson (P20 or P100), place one drop (20ul) of extract beside each drop of latex suspension.



11. Mix the blue latex and the extract with the sticks provided in the kit, using the complete area of the reaction circle. Use a fresh separate stick for each circle, and discard into a "Dispojar" for autoclaving.



12. Gently rock the card allowing the mixture to flow slowly over the entire test area for a maximum of 1 minute.
13. At one minute, observe for agglutination. The card should be held at normal reading distance (25 to 35cm) from the eyes. Do not use a magnifying lens. The patterns obtained are clear-cut and can be recognised easily under all normal lighting conditions.



14. Record agglutination and grouping result on relevant worksheet and enter result onto LIMS.
15. Discard the used test cards into plastic discard autoclave bin for safe disposal.
16. Ensure that the reagents are returned to the refrigerator after use.

RESULTS

1. **POSITIVE RESULT:** A significantly rapid strong clumping of the blue latex particles to form an agglutination pattern in only one of the latex reagents indicates specific identification of the streptococcal isolate. A weak reaction with a single blue latex reagent should be repeated using a heavier inoculum. The repeated test is considered positive if a visible agglutination occurs with only one of the latex blue reagents. Delayed weak reactions in the other latex suspensions should be ignored
2. **NEGATIVE RESULT:** No visible agglutination of the blue latex particles.
3. False negative or false positive reactions can occur if inadequate amounts of culture or extraction reagents are used.

QUALITY CONTROL

Routine quality control procedures for each Prolex™ lot are carried out by Prolab Diagnostics. This involves testing each of the kit components with extracts of each of the streptococcal groups A, B, C, D, F and G using ATCC strains. Additionally each blue latex suspension is tested for the absence of cross reactions against extracts of the following ATCC organisms *E.coli* (ATCC 25922), *K.pneumoniae* (ATCC 13883), *S.aureus* (ATCC 25923) and *H.influenza* type b (ATCC 10211).

a) **Test of the reactivity of the latex suspensions (Positive Control Procedure)**

The positive control, provided with the kit, is used to check the performance of the individual blue latex reagents. The blue latex reagents should show obvious agglutination with the positive control. The positive control procedure does not demonstrate the specificity of the blue latex reagents nor ensure that the extraction step was performed correctly and is functioning.

b) **Test for specificity of agglutination (Negative Control Procedure)**

Use normal saline solution to test for the absence of autoagglutination with the blue latex reagents.

c) **Test of enzyme extraction procedure**

Carry out the complete test procedure using a stock culture of known group. (NCTC strains are stored frozen in glycerol blood broths at -80°C). Occasional tests with a variety of known groups

should be employed to evaluate the accuracy and efficiency of the complete test system, including the operator.

INTERPRETATION AND LIMITATIONS OF THE TEST

1. **STRONG RAPID AGGLUTINATION IN MORE THAN ONE LATEX SUSPENSION (but not all)** indicates that the extract may contain a mixture of streptococci, or other bacteria containing cross-reacting antigens. Replate for purity check and/or further identification procedures.
2. **POSITIVE REACTIONS WITH BOTH GROUP D AND G LATEX REAGENTS**
Some strains of group D streptococci have been found which appear also to possess group G antigen. These will react with both D and G latex and may be confirmed as group D by bile-aesculin or PYRA test as set out in **SOP R6408**, "Identification of Streptococci and Related Genera – Additional Tests".
3. **WHEN AGGLUTINATION IS WEAK**
Proceed as in 5.1 (a) to examine the effectiveness of the latex reagents. Some isolates of streptococci, notably *S.oralis*, *S.mitis*, *S.sanguis* may produce weak cross-reactions with streptococcal latex reagents.
4. **AGGLUTINATION OF ALL THE LATEX SUSPENSIONS**
A stringy or thread-like appearance, indicates contamination with another organisms, which should be eliminated by further sub-culture. Occasional false positive results may occur with organisms from unrelated genera, for example, Klebsiella, Escherichia or Pseudomonas.
5. **IF NONE OF THE LATEX SUSPENSIONS SHOW AGGLUTINATION**
It is likely that the culture does not belong to any of the groups covered in the test. However, some strains of group D and group F may yield false negative results, see **6.6**, below.

6. FALSE NEGATIVE RESULTS

May be due to the use of few organisms for extraction, particularly with group D strains – some of which yield less antigen than other groups, (especially *Streptococcus bovis* and *Enterococcus faecium*), and group F strains (*S.milleri*, group) which have minute colonies – some of which adhere strongly to the agar surface.

7. The existence of antigens common to organisms from heterologous species or genera has been demonstrated in some streptococci, so cross-reactions may occur eg; the group D antigen may be common to organisms of streptococcal groups Q,R,S and other genera such as *Lactobacillus sp.* *Listeria monocytogenes* may cross react with the Group B and/or G streptococcal latex reagents, since *L.monocytogenes* exhibits similar antigenicity to Group B and G streptococci.

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GBS serotyping using the standard Strep-B Latex kit (Statens Serum Institut, Denmark)



MATERIALS

- Columbia blood agar (COL) plate
- Saline buffer
- 1 ml plastic pipettes
- Glass slides
- 1 µl plastic disposable loops
- Strep B latex kit, SSI, Denmark (Cat No 54991)
- Sterile 3x1/2 inch plastic capped tube, Marathon Supplies, FALCON 2054
- Bench lamp
- Black tile



METHODS

Gloves and safety glasses should be worn for this procedure.

1. Pipette 250 μ l PBS into a sterile plastic capped tube.
2. Using a 1 μ l plastic loop, make a heavy suspension of the test organism from the cultured Columbia blood agar plate.



3. Pipette 20 μl of bacterial suspension onto latex reaction card (or a glass microscope slide).
4. Use a 1 μl plastic loop to transfer a loopful of latex reagent to each drop of suspension and mix briefly.
5. Rotate latex reaction card (or glass slide) for 10–15 seconds and look for agglutination.
6. Test bacterial suspension with all 10 antisera from the Strep-B-Latex kit (SSI, Denmark).

This method does not follow the manufacture's instructions but has been validated against all group B type strains and numerous wild strains.

Multiplex PCR assay for capsular typing of GBS

(Dr Knud Poulsen, Aarhus University, Denmark)

Protocol for quick purification of DNA from Gram-positive bacteria for PCR:

(Woo *et al.*, 2001, J. Clin. Microbiol. 39: 3147–3155)

1. Pick up a loop-full (1 μ l loop) of bacteria from the agar plate.
2. Suspend in 100 μ l distilled H₂O.
3. Mix (Vortex).
4. Transfer 20 μ l to a new tube (store the remaining 80 μ l at -20 °C as a reserve for later use).
5. Add 80 μ l of 0.05 M NaOH. Mix (Vortex)
6. Incubate at 60 °C for 45 min.
7. Add 9.2 μ l of 1M Tris-HCl pH 7.0. Mix and spin down briefly. Store at -20 °C.

For PCR: dilute this stock solution 1:100 in distilled water. Use 5 μ l for PCR in a 25 μ l reaction.

Multiplex PCR for capsular typing of GBS:

(Poyart *et al.*, 2007, J. Clin. Microbiol. 45: 1985–1988)

The method is based on two multiplex PCR's, one with primer mix I and one with primer mix II. Mix I contains the primer pairs specific for serotypes Ia, Ib, II, III, and IV. Mix II contains primer pairs specific for serotypes V, VI, VII, and VIII. Besides, the method includes a GBS specific PCR targeting the *dltS* gene. The newly described serotype IX is detected in a separate PCR described by Kong *et al.*, 2008, J. Clin. Microbiol. **46**: 2745–2750.

CPS type-specific primers for multiplex PCR and primers for GBS specific *dltS* PCR.

Primer name	Sequence (5' to 3')	Gene target(s)	Amplicon size(s) (bp)	GenBank accession no. of targeted operon
Ia-F	GGTCAGACTGGATTAATGGTATGC	<i>cps1aH</i>		AB028896
Ia-R	GTAGAAATAGCCTATATACGTTGAATGC	<i>cps1aH</i>	521 and 1,826	
Ib-F	TAAACGAGAATGGAATATCACAAACC	<i>cps1bJ</i>		AB050723
Ib-R	GAATTAACCTCAATCCCTAAACAATATCG	<i>cps1bK</i>	770	
II-F	GCTTCAGTAAGTATTGTAAGACGATAG	<i>cps2K</i>		AY375362
II-R	TTCTCTAGGAAATCAAATAATTCTATAGGG	<i>cps2K</i>	397	
III-F ^a	TCCGTACTACAACAGACTCATCC	<i>cps1a/2/3I</i>		AF163833
III-R ^a	AGTAACCGTCCATACATTCTATAAGC	<i>cps1a/2/3J</i>	1,826	
IV-F	GGTGGTAATCCTAAGAGTGAAGTGT	<i>cps4N</i>		AF355776
IV-R	CCTCCCAATTTTCGTCCATAATGGT	<i>cps4N</i>	578	
V-F	GAGGCCAATCAGTTGCACGTAA	<i>cps5O</i>		AF349539
V-R	AACCTTCTCCTTCACACTAATCCT	<i>cps5O</i>	701	
VI-F	GGACTIONGAGATGGCAGAAGGTGAA	<i>cps6I</i>		AF337958

VI-R	CTGTCCGACTATCCTGATGAATCTC	<i>cps6I</i>	487	
VII-F	CCTGGAGAGAACAATGTCCAGAT	<i>cps7M</i>		AY376403
VII-R	GCTGGTCGTGATTTCTACACA	<i>cps7M</i>	371	
VIII-F	AGGTCAACCACTATATAGCGA	<i>cps8J</i>		AY375363
VIII-R	TCTTCAAATTCCGCTGACTT	<i>cps8J</i>	282	
dltS-F	AGGAATACCAGGCGATGAACCGAT	<i>dltS</i>		AL766853
dltS-R	TGCTCTAATTCTCCCCTTATGGC	<i>dltS</i>	952	

1. For the multiplex PCR (with primer mix I, II, or the dltS specific primers) use the AmpliTaq Gold PCR system with GeneAmp 10x PCR buffer II (Applied Biosystems / Roche).

Set up the reaction on ice:

12 µl H₂O

2.5 µl 10 x PCR buffer II

2.5 µl MgCl₂ (25 mM)

0.5 µl dNTP's (10 mM of each, Roche)

2 µl primer mix (5 pmol / µl of each)

0.5 µl AmpliTaq Gold polymerase (5U / µl)

5 µl DNA

Total: 25 µl

2. Thermo cycling program:

Initial denaturation at 94 °C for 2 min.

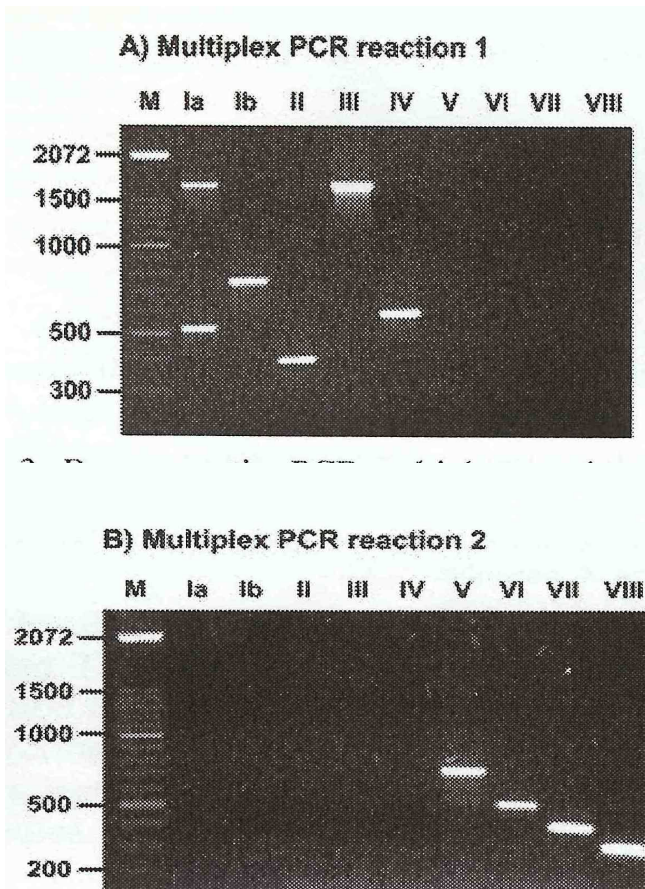
35 cycles of: 94 °C for 60 sec, 55 °C for 45 sec, 72 °C for 90 sec.

Extension at 72 °C for 7 min.

3. Analyse the products by 1.5 % agarose gel electrophoresis and stain with EtBr.

4. To verify the sizes run the products along with products of known serotypes.

(note: the 1,826 bp band in serotype Ia may be weak)



(Poyart *et al.*, 2007, J. Clin. Microbiol. **45**: 1985–1988)

PCR for detection of CPS type IX.

5. Use the primers described by Kong *et al.*, 2008, J. Clin. Microbiol. **46**: 2745–2750.

IXS1: 5'-GCTCATTTACAACCTTGTAGACGGC-3'

IXA: 5'-GCCATATCAGAGCAAATATGTCATATATC-3'

These primers specifically amplify a 346 bp product from serotype IX strains.

6. For this PCR we use the thermo cycling program:

Initial denaturation at 94 °C for 2 min.

30 cycles of: 94 °C for 60 sec, 65 °C for 60 sec, 72 °C for 120 sec.

Ekstension at 72 °C for 7 min.

(note: serotype IX strains may also amplify a product in the multiplex PCR with either mix I or mix II).

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Other Useful References

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- Wen et al., Use of a serotype-specific DNA microarray for identification of group B Streptococcus (*Streptococcus agalactiae*). J. Clin. Microbiol. 2006; 44: 1447–52.

Useful websites:

- MLST website: www.mlst.net
- DEVANI website: www.devaniproject.org
- GBS Support website: www.gbss.org.uk