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Collection of GBS samples at 35-37 weeks of gestation and isolation V.2

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Pregnant women's recto-vaginal samples were collected to identify *Streptococcus agalactiae* (Group B Streptococci, GBS).

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protocol

Palacios-Saucedo GdC, Rivera-Morales LG, Vázquez-Guillén JM, Caballero-Trejo A, Mellado-García MC, Flores-Flores AS, González-Navarro JA, Herrera-Rivera CG, Osuna-Rosales LE, Hernández-González JA, Vázquez-Juárez R, Barrón-Enríquez C, Valladares-Trujillo R, Treviño-Baez JD, Alonso-Téllez CA, Ramírez-Calvillo LD, Cerda-Flores RM, Ortiz-López R, Rivera-Alvarado M

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Remove each swab from the packaging and insert the swab 2 cm into the vagina, followed by

- 1 the rectum, inserting the swab 1 cm through the anal sphincter. Three swabs were collected for each patient.
- 2 The First swab was inoculated on Todd-Hewitt broth supplemented with gentamicin 8 µg/ml and nalidixic acid 15 µg/ml (THB, Becton-Dickinson, NJ, USA). The second swab was inoculated into LIM enrichment broth (Todd-Hewitt broth with 1% yeast extract, 15 µg/ml nalidixic acid, and 10 µg/ml colistin, Becton-Dickinson, New Jersey, USA). Both swabs were incubated for 18-24 hours at 37° C in 5% CO₂. The third swab was submerged into an appropriate transport medium (ESwab, Copan Diagnostics, Brescia, Italy). Finally, the three swabs were subcultured onto Petri dishes with Columbia agar (OXOID LTD, Basingstoke, Hampshire, England) with 5% sheep blood and incubated for 18-24 hours at 37° C to confirm GBS identification afterward.
- 3 Criteria for GBS identification
 - a) Gram-positive coccus.
 - b) Negative Catalase test.
 - c) CAMP test with wedge-shaped hemolysis in the presence of *Staphylococcus aureus* ATCC 25923.
 - d) Positive hippurate hydrolysis.
 - e) Observation of growth with color change from white to orange pigment after 18-24 h incubation in the Strep B Carrot Broth® chromogenic medium (Hardy Diagnostics®, Santa María, CA, USA).
 - f) Growth mauve colonies into CHROMagar™ StrepB base.