

GBS PNA FISH®

Streptococcus agalactiae Culture Identification Kit



REF KT010



Intended Use

GBS PNA FISH is a qualitative nucleic acid hybridization assay intended for identification of *Streptococcus agalactiae* from turbid Lim broth cultures obtained from vaginal and rectal swabs of pregnant women between 35 and 37 weeks gestation.

The GBS PNA FISH does not provide susceptibility results. Culture isolates are needed for performing susceptibility testing as recommended for penicillin-allergic women. Subculture to a solid media for additional testing.

The GBS PNA FISH Assay is used as an aid in the detection of *Streptococcus agalactiae* from turbid Lim broth cultures.

IVD For *in vitro* diagnostic use.

Summary and Explanation

Streptococcus agalactiae (Group B *Streptococcus* (GBS)) a leading cause of preventable infectious disease in neonates.

GBS PNA FISH is a fluorescence *in situ* hybridization (FISH) method using PNA probes hybridizing to *S. agalactiae*-specific ribosomal RNA sequences to identify *S. agalactiae*.

The test provides rapid identification of *S. agalactiae* on smears made from Lim broth cultures obtained from vaginal and rectal swabs incubated overnight at 35-37 °C.

Principle of the Procedure

A mixture of fluorescein-labeled *S. agalactiae*-specific PNA probes is added to a smear prepared from turbid growth in Lim broth after overnight incubation. Hybridization is performed at 55°C for 30 min. The hybridization is followed by a post-hybridization wash at 55°C for 30 min. with Wash Solution. Finally, the smear is mounted with Mounting Medium and examined by fluorescence microscopy.

Reagents

GBS PNA FISH is comprised of the following kit components:

Fixation Solution

Fixation Solution

3 mL phosphate-buffered saline with detergent.

GBS PNA

GBS PNA

1.5 mL PNA probes in hybridization solution. Contains 30% formamide.

60x Wash Solution

60x Wash Solution

50 mL Tris-buffered saline with detergent.

Mounting Medium

Mounting Medium

3 mL photobleaching inhibitor in glycerol.

Precautions

IVD For *in vitro* diagnostic use.

For professional use, only, by personnel trained in laboratory techniques and experienced in fluorescent microscopy.

Safety Precautions

The GBS PNA contains 30% formamide. May cause harm to the unborn child. Keep out of reach of children. Avoid exposure - obtain special instructions before use. Material Safety Data Sheet is available on request. Formamide is non-hazardous once diluted into Wash Solution during the wash step.

Establish precautions against microbiological hazards.

Do not eat, drink, smoke, apply cosmetics, store or prepare foods within the designated work area.

Dispose of reagents in accordance with federal, state and local regulations.

Technical Precautions

Reagents must not be used after the expiration dates printed on the labels.

Reagents are provided at fixed concentrations. Assay performance may be affected if the reagents are modified in any way or are not stored under the recommended conditions as detailed in "Storage of Kit Components".

Avoid microbial contamination of reagents.

Avoid any cross-contamination of samples and reagents, as this may give rise to erroneous results.

Do not allow dropper bottle tip to touch the smear as this may cause cross contamination of material between slides, or cause contamination of the reagent.

Do not use other filters than the Dual Band Filter (AC003).

Do not use other microscope slides than the Microscope slides (AC001).

It is important that the microscope is functioning properly. Make sure that the microscope bulb is correctly adjusted and has not aged beyond its specified lifetime.

Storage and Preparation of Kit Components

To ensure optimal kit performance, it is important that kit components are stored and prepared according to the following instructions.

Storage

Store kit components at 2-8°C. Place kit components at room temperature prior to use and return the kit components to 2-8°C after use.

Preparation of Wash Solution

Prepare working strength Wash Solution by adding 4 mL of 60x Wash Solution followed by 240 mL of fresh deionized or distilled water directly to the Staining Dish. Store remaining concentrate at 2-8°C.

Preparation of Mounting Medium

The Mounting Medium should be left at room temperature for at least 5 min. before use.

Specimen Collection and Preparation

Preparation of Smears

- Follow laboratory's current SOP on Lim broth workup.
- Place one drop of Fixation Solution on a well on the microscope slide.
- Transfer 10-50 µL of turbid Lim broth growth to the Fixation Solution and mix gently to emulsify.
- Fix the smears by either heating them for 20 min. at 55-80°C or allow the smears to air-dry and fix them by methanol-fixation or by flame-fixation.
- Immerse the slide in 80% or 96% ethanol for 5-10 min and leave to air-dry until the smear is dry (approximately 10 min.).

Test Procedure

Material Provided

GBS PNA FISH® KT010

Each kit contains sufficient material for testing 50 cultures. Reagents are supplied ready for use except where indicated. The expiration date of the kit is as indicated on the outer box label.

Material Required and Available From AdvanDx.

Microscope Slides	1-well microscope slides	AC001
Coverslips	Coverslips, 22 x 22 mm, Thickness: 0.15 mm	AC002
Dual Band Filter	Dual band filter	AC003
Staining Dish	Staining dish with cover and slide holder	AC004
PNA FISH Workstation	Slide warmer (55 ± 1°C)	AC005
Waterbath	Waterbath (55 ± 1°C)	AC006
GBS Control Slide	GBS Control Slide	CS010

Contains a positive control prepared from liquid culture containing *S. agalactiae*, ATCC# 13813, and negative control prepared from liquid culture of *S. pyogenes*, ATCC# 12384.

Material Required but not Provided

- Water, deionized or distilled.
- Fluorescence microscope equipped with a 60x or 100x oil objective.
- Immersion oil. Must comply with the microscope objective and be non-fluorescent.

Assay Procedure

All steps are performed at room temperature unless otherwise stated. Before starting the assay procedure, prepare working strength Wash Solution in the Staining Dish, add cover and start preheating in the Water Bath (55 ± 1°C). Do not reuse Wash Solution, but prepare fresh working strength Wash Solution for each run.

Hybridization

- Add one drop of GBS PNA to the well on the microscope slide with the smear.
- Add coverslip. Avoid air bubbles.
- Incubate for 30 ± 5 min. at 55 ± 1°C.

Stringent Wash

- Immerse slide in preheated Wash Solution at 55°C and carefully remove the coverslip. Often, the coverslip slides off by gently agitating the slide in the Wash Solution. Occasionally, the coverslip must be pushed off with forceps.
- Incubate for 30 ± 5 min. at 55 ± 1°C.
- Allow the slide to air dry.

Mounting

- Add one drop of Mounting Medium to the smear.
- Add coverslip. Avoid air bubbles.
- Examine slide as described below within 2 hours.
- Do not expose the slides to direct sunlight or other strong light sources as this may lead to fluorescence bleaching.

Quality Control

Control material should be tested in accordance with guidelines or requirements of local, state, and/or federal regulations or accrediting organizations, including controls grown in liquid media.

Quality control for fluorescent testing should be done each time testing is performed. The QC results should be able to monitor for appropriate testing conditions, particularly those affecting hybridization stringency and cell wall penetration, since PNA methodology is designed to optimize cell wall penetration.

Use AdvanDx GBS Control Slides (CS010) or prepare smears from cultures of laboratory or reference strains of *S. agalactiae*, e.g. ATCC 13813 as a Positive Control and *S. pyogenes*, e.g. ATCC 12384 as a Negative Control as described above under Specimen Collection and Preparation. *S. agalactiae* must test green-positive and *S. pyogenes* must test negative in accordance with the Interpretation of Results. When using an AdvanDx GBS Control Slide (CS010), simply remove slide from pouch and follow the PNA FISH procedure starting with the hybridization step.

Procedural Notes

Temperature Control:

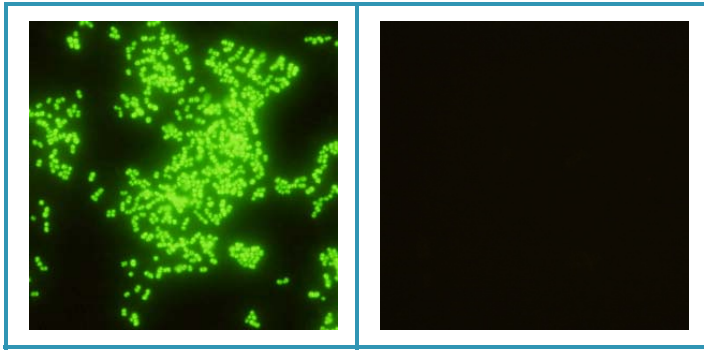
It is important that the temperature of the Slide warmer/Incubator has reached 55°C prior to starting the hybridization and that the Wash Solution has reached 55°C prior to immersion of the slides. The temperature should be checked using a thermometer as outside temperature readings may not always be accurate.

Interpretation of Results

Examine slides using a fluorescence microscope. The smear appears in general pale yellow.

Positive - multiple bright green fluorescent cocci in multiple fields of view identifies *S. agalactiae*.

Negative - non-fluorescent.



Representative example of green-positive *S. agalactiae* (left), and negative (right) test results.

Troubleshooting

False positive Control and Sample test results may occur if the Dual Band Filter (AC003) is not used, or by contamination of the specimens.

False negative Control or Sample test results may occur if AdvanDx Microscope Slides (AC001) are not used or if the temperature is not accurately controlled during hybridization and washing.

Please refer to the Precautions and Limitations sections in this product insert or contact AdvanDx.

Limitations

False Negative result may occur when specimens are below the LoD of 10^5 CFU/mL or due to mixed growth. Subculturing to a solid media may be needed to demonstrate growth below the LoD or the presence of mixed growth.

False negative results may also be due to error in assay technique.

False positive green autofluorescence may occur if a standard FITC filter is used instead of the Dual Band Filter (AC003).

The type and condition of the instrumentation used will influence the visual appearance of the image obtained. The fluorescence may vary due to the type of microscope employed, the light source and the level of rRNA in the cells. Each laboratory should establish its own criteria for reading the results using appropriate controls.

S. agalactiae ATCC 1487, a non human strain, may give a borderline weak signal/result.

The product has only been validated with Lim broth cultures.

Expected Results

The expected *S. agalactiae* positive result rate from Lim broth cultures of vaginal swabs is 10-30% (3).

Performance Characteristics

Clinical Studies

The performance of GBS PNA FISH was evaluated on 636 Lim broth cultures obtained from vaginal and rectal swabs at three clinical trial sites in the United States (FL, PA and OH) and is summarized below where the results are compared to results obtained by conventional methods.

Study	Positive Agreement	Negative Agreement
A-1	98.0% (48/49) 95% CI (89.2-100)	86.8% (164/189) 95% CI (81.1-91.3)
A-2	98.4% (61/62) 95% CI (91.3-100)	93.2% (164/176) 95% CI (88.4-96.4)
B	89.2% (33/37) 95% CI (74.6-97.0)	98.1% (157/160) 95% CI (94.6-99.6)
C-1	98.4% (63/64) 95% CI (91.6-100)	100% (137/137) 95% CI (97.8-100)
C-2	100% (62/62) 95% CI (95.3-100)	99.3% (138/139) 95% CI (96.1-100)

Study Site A1 - SBA and Streptocard™ Enzyme Latex Test

	SBA + Streptocard™ Enzyme Latex Test	
	+	-
GBS PNA FISH	+	48
	-	1
Total	Agreement	Agreement
	98.0% (48/49) 95% CI (89.2-100)	86.8% (164/189) 95% CI (81.1-91.3)

Study Site A2 – Selective Streptococcus agar with 5% Sheep Blood to SBA and BBL™ Streptocard™ Acid Latex Test

	Selective Streptococcus agar with 5% Sheep Blood and SBA + BBL™ Streptocard™ Acid Latex Test	
	+	-
GBS PNA FISH	+	61
	-	1
Total	Agreement	Agreement
	98.4% (61/62) 95% CI (91.3-100)	93.2% (164/176) 95% CI (88.4-96.4)

Study Site B - SBA and PathoDx Strep Grouping Test

	SBA + PathoDx	
	+	-
GBS PNA FISH	+	33
	-	4
Total	Sensitivity	Specificity
	89.2% (33/37) 95% CI (74.6-97.0)	98.1% (157/160) 95% CI (94.6-99.6)

Study Site C1 - SmartCycler® Smart GBS Test

SmartCycler Smart GBS			
		+	-
GBS PNA FISH	+	63	0
	-	1	137
Total		Agreement 98.4% (63/64) 95% CI (91.6-100)	Agreement 100% (137/137) 95% CI (97.8-100)

Study Site C2- SBA and Remel® Streptex Test

SBA + Remel® Streptex			
		+	-
GBS PNA FISH	+	62	1
	-	0	138
Total		Sensitivity 100% (62/62) 95% CI (95.3-100)	Specificity 99.3% (138/139) 95% CI (96.1-100)

Analytical Sensitivity

The detection limit for *S. agalactiae* was determined to be approximately 10⁵ colony-forming units per mL by serial dilutions of a *S. agalactiae*-positive culture. This is consistent with the analytical sensitivity of slide-based staining techniques.

Analytical Specificity

GBS PNA FISH has been tested on 36 laboratory and reference strains comprising *Streptococcus agalactiae* and other species appearing as Gram-positive cocci in pairs or chains. Most (7/8) *S. agalactiae* tested green-positive. *S. agalactiae* ATCC 51487 gave borderline signal which may have been due to its unusual growth requirements. All (36) other species representing 18 fungal species and 18 other bacterial species, including some common species of vaginal flora, all (36/36) tested negative by GBS PNA FISH.

Reproducibility Studies

Panels of ten strains were analyzed by GBS PNA FISH at three separate sites over three separate days by one operator.










Expected Result	Site 1	Site 2	Site 3	Between Site Agreement
Positive	100% (6/6)	100% (6/6)	100% (6/6)	100% (18/18)
Negative	100% (24/24)	100% (24/24)	100% (24/24)	100% (72/72)
Total	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90)

Bibliography

1. **Baron, E. J.** 1998. Processing and interpretation of blood cultures, chap. 2.3. In: H.D. Isenberg (ed.), Essential procedures for clinical microbiology, ASM Press, Washington DC.
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3. **Morven, S., M. S. Edwards, and C. J. Baker.** 2001. Group B streptococcal infections, p. 1099-1101. In J.S. Remington, and J.O. Klein (ed.), Infectious Diseases of the Fetus and Newborn. 5th ed. WB Saunders Company, Philadelphia, PA.
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Definitions

	Product code/catalog number
	Consult the instructions for use
	Contains sufficient for <n> tests
	Manufacturer
	Authorized representative
	<i>In vitro</i> diagnostic medical device
	Use by
	Batch code
	Storage temperature limitations

Technical Advice and Customer Service

For all inquiries, please contact AdvanDx or your local distributor.



AdvanDx, Inc.
400 TradeCenter
Woburn, MA 01801
USA

Tel: +1 781 376 0009
Fax: +1 781 376 0111

techsupport@advandx.com
www.PNA-FISH.com



AdvanDx A/S
Bygstubben 11
2950 Vedbæk
Denmark

Tel: +45 45 16 07 99
Fax: +45 45 16 07 98

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The product must not be used for Slide-Based human Cytochemistry, ISH-based Cancer Cytogenetics and Flow Cytometry.

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Purchase of this kit licenses its use under Patent numbers: US 5,985,563; US 5,888,733; US 6,395,474; US 6,357,163; US 5,539,082; US 7,223,833; EP 862,650; EP 804,456

