


STANTON TERRITORIAL HEALTH AUTHORITY

Yellowknife, Northwest Territories

TITLE: Hippurate	Revision Date: 20-April-2018	Issue Date: 20-April-2016
Document Number: MIC53100	Status: Approved	
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Approved by: S. Asmussen, Manager of Diagnostic Services	Signed by: 	

PURPOSE:

This test determines the ability of bacterial to hydrolyze sodium hippurate for the identification of *Streptococcus agalactiae*, *Campylobacter jejuni*, *Gardnerella vaginalis*, *Listeria species* and other aerobic bacteria. The presence of the enzyme hippurate hydrolase is visualized by the **purple** color when ninhydrin reagent oxidizes the amino acid, glycine, produced during the hippurate hydrolysis.

REAGENTS and/or MEDIA:

- glass test tubes
- Hippurate discs BD REF#231723
- Ninhydrin reagent BD REF#261201
- Sterile distilled water
- Wooden stick

SPECIAL SAFETY PRECAUTIONS:

Containment Level 2 facilities, equipment, and operational practices for work involving infectious or potentially infectious materials or cultures.

- Lab gown must be worn when performing activities with potential pathogens.
- Gloves must be worn when direct skin contact with infected materials is unavoidable.
- Eye protection must be used where there is a known or potential risk of exposure to splashes.

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- All procedures that may produce aerosols, or involve high concentrations or large volumes should be conducted in a biological safety cabinet (BSC).
- The use of needles, syringes, and other sharp objects should be strictly limited.

QUALITY CONTROL:

Test with a known positive and negative control each time test is performed:

Positive: *Streptococcus agalactiae* ATCC12386

Negative: *Streptococcus pyogenes* ATCC19615



PROCEDURE INSTRUCTIONS:

Step	Action
Performing Hippurate Test	
1	Label a test tube for each sample being tested, including Positive and Negative controls.
2	Add a Hippurate disk and 0.4mL of sterile water to each test tube.
3	Heavily inoculate the tube with a loopful of the Test and QC organisms. Mix until a smooth suspension is obtained.
4	Incubate at 35°C for 2 hours.
5	Remove from incubator and add 5 drops of Ninhydrin reagent and shake gently.
6	Reincubate tubes for 10 mins and read reactions.

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INTERPRETATION OF RESULTS:

Action		
IF	THEN	
Deep purple-blue color development	Positive reaction	
No color change or light purple	Negative reaction	

EXPECTED RESULTS:

Organism	Expected Results	BBL Reagent	Commercial Reagent
<i>Streptococcus agalactiae</i> ATCC 12386	+	+	+
<i>Streptococcus pyogenes</i> ATCC 19615	-	-	-
<i>Campylobacter coli</i> 802	-	-	-
<i>Campylobacter coli</i> 834	-	-	-
<i>Campylobacter jejuni</i> 1118	+	+	+
<i>Campylobacter jejuni</i> ATCC 29428	+	+	+
<i>Gardnerella vaginalis</i> ATCC 14028	+	+	+
<i>Gardnerella vaginalis</i> JH3	-	-	-
<i>Gardnerella vaginalis</i> BD12	+	+	+
<i>Gardnerella vaginalis</i> BD16	-	-	-
<i>Gardnerella vaginalis</i> BD11	+	W+	W+
<i>Gardnerella vaginalis</i> BD10	+	+	+
<i>Gardnerella vaginalis</i> BD8	-	-	-
<i>Gardnerella vaginalis</i> BD7	-	-	-
<i>Gardnerella vaginalis</i> BD6	+	W+	W+
<i>Gardnerella vaginalis</i> BD3	+	W+	W+
<i>Gardnerella vaginalis</i> BD2	+	+	+
<i>Gardnerella vaginalis</i> BD1	+	W+	+
<i>Gardnerella vaginalis</i> BD5	+	+	+
<i>Gardnerella vaginalis</i> JH4	+	+	+
<i>Gardnerella vaginalis</i> JH7	+	+	+
<i>Gardnerella vaginalis</i> BD9	-	-	-
<i>Gardnerella vaginalis</i> BD14	-	-	-
<i>Gardnerella vaginalis</i> BD15	+	+	+
<i>Gardnerella vaginalis</i> AMMS17	+	+	+
Control (no organism)	-	-	-

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REFERENCES:

- BD . (2010, 06). Ninhydrin Reagent Droppers.
- BD. (2010). BBL Taxo Differentiation Disks Hippurate.

REVISION HISTORY:

REVISION	DATE	Description of Change	REQUESTED BY
1.0	31Jul13	Initial Release	Darrach (A)
2.0	31Mar16	Update of "Special Safety Precautions" to reflect risk assessment recommendations.	C. Russell

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