HIPPURATE DISKS

- For in vitro use only - Catalogue No. DH45

Our Hippurate Disks are a rapid test designed to detect an organism's ability to enzymatically hydrolyze hippurate.

This characteristic and methodology is useful for the identification and differentiation of:

1. *Campylobacter jejuni* (+) from other campylobacter species (–), and more specifically *Campylobacter coli* (–) and *Campylobacter laridis* (–)
2. *Streptococcus agalactiae* (+) from other human β-hemolytic streptococci (usually –)
3. *Legionella pneumophila* (+) and *L. feeleii* (V) from other *Legionella* and *Legionella*-like species (–)
4. *Actinobacillus lignieresii* (+) from *Actinobacillus equuli* (–)
5. *Listeria grayi* (–) from *L. innocua* (+), *L. ivanovii* (+) and *L. monocytogenes* (+)
6. *Brevibacterium iodinum* (–) from *B. casei* (+), *B. epidermidis* (+) and *B. linens* (+)
7. *Mobiluncus mulieris* (–) from *M. curtisii* subsp. *curtisii* (+) and *M. curtisii* subsp. *holmesii* (+)
8. Aid in the identification of *Gardnerella vaginalis* (+)

Organisms possessing the enzyme hippurate hydrolase (hippuricase) can hydrolyze the peptide bond in hippurate releasing glycine and benzoic acid as end products. Glycine can then be detected by the addition of Ninhydrin Reagent, which through a complex reaction produces a purplish-blue end product that is readily visible. Since ninhydrin reagent can react with other free amino acids or proteins present in culture media to produce a false positive reaction; it is essential that that test inoculum be derived from a solid agar medium and not a broth medium. Great care must also be taken during the inoculation process to ensure that gouging of the agar surface does not occur when scraping off colonies for testing.

Recommended Procedure

1. Into a small, sterile test tube (13 x 100 mm) add 0.4-mL of sterile distilled water and one Hippurate Disk.
2. Heavily inoculate the tube with a few colonies from a pure, overnight (24-hour) culture of the test organism grown on a solid medium. Shake or vortex to ensure that the suspension is homogenous.
3. Incubate for 2 hours at 37°C (a 24 hour incubation period is required for *Legionella*).
4. Slowly add 0.2-mL (five drops) of Ninhydrin Reagent (Cat No. RN70) down the side of the tube to form an overlay. Do not shake.
5. Reincubate the tube at 37°C and check for color change after 10 and 20 minutes. Do not incubate tubes for longer than 30 minutes.

Interpretation of Results

Positive: Presence of a blue or purple color (Hippurate hydrolyzed)

Negative: Colorless or gray color (Hippurate not hydrolyzed)

- *A positive result is the development of a blue or purple color regardless of intensity*
- *The inoculum must be derived from a solid agar medium such as sheep blood agar*
- *Do not incubate tubes longer than 30 minutes or false positives may occur*

Quality Control

Organism	Expected Result
Campylobacter jejuni ATCC 33291	+ve (Purple color change)
Streptococcus agalactiae ATCC 13813	+ve (Purple color change)
Campylobacter coli ATCC 33559	–ve (No color change)
Streptococcus pyogenes ATCC 19615	–ve (No color change)

Storage and Shelf Life

Our Hippurate Disks should be stored at -20°C. At this temperature they have a shelf life of 26 weeks from the date of manufacture.

Ordering Information

Cat#	Description	Format
DH45-25	Hippurate Disks	25/vial

References

1. Hwang M, Ederer GM. Rapid hippurate method for presumptive identification of group B streptococci. J Clin Micro 1975; 1:114-5.
2. Greenwood JR, Pickett MJ. Salient features of *Haemophilus vaginalis*. J Clin Micro 1979; 9:200-4.
3. Skirrow MB, Benjamin J. Differentiation of enteropathogenic *Campylobacter*. J Clin Path 1980; 33:11.
4. Harvey SM. Hippurate hydrolysis by *Campylobacter fetus*. J Clin Micro 1980; 11:435-7.
5. Hebert BA. Hippurate hydrolysis by *Legionella pneumophilia*. J Clin Micro 1981; 13:240-2.
6. Cacho JB, Aguirre PM, Hernanz A, Velasco AC. Evaluation of a disk method for detection of hippurate hydrolysis by *Campylobacter* spp. J Clin Micro 1989; 27:359-60.
7. Murray, P.R., E. Baron, M. Pfaller, F. Tenover, R. Yolken. Manual of clinical microbiology. 7th ed. Washington: ASM, 1999.
8. MacFaddin JF. Biochemical tests for the identification of medical bacteria, 3rd ed. Philadelphia: Lippincott Williams & Wilkins, 2000.

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