



## O129 DISKS

- For in vitro use only -

Catalogue No. DD10 & 11

Our O129 Disks are used for the differentiation of vibrios from other gram-negative bacteria.

Shewan and Hodgkiss recognized the sensitivity of vibrios to the vibrio-static agent O129 (2,4-diamino-6,7-di-isopropyl-pteridine phosphate) in 1954. O129 was found to be useful in the differentiation of vibrios from other gram-negative bacteria especially aeromonads, which are characteristically resistant to O129. Among the genera, different species of vibrios show different sensitivities to O129; this can be used as a diagnostic feature as antimicrobial disks of different concentrations can be used to determine their degree of sensitivity. O129 disks are offered at two concentrations: 10- $\mu$ g and 150- $\mu$ g. Methods for standardized disk antimicrobial susceptibility testing are employed, with any zone of inhibition around O129 disks being regarded as sensitive. Salt containing media (0.5%) must be used for the testing procedure, as sodium ions stimulate the growth of all *Vibrio* species and are required by most.

### Recommended Procedure

1. Obtain a pure, fresh culture of the test organism.
2. Using a sterile swab, streak a sample of the organism onto a non-selective Blood Agar Plate (containing 0.5% NaCl) in three directions to obtain a heavy, confluent growth.
3. Aseptically place one 10 $\mu$ g and 150 $\mu$ g O129 Disk onto the agar surface. Ensure that the disks are situated suitably apart from each other to avoid meeting of zones.
4. Incubate aerobically at 35°C for 24 hours.
5. Observe for zones of inhibition.

### Interpretation of Results

**Sensitive:** Zone of inhibition around both O129 disks

**Partially Sensitive:** Zone of inhibition around the 10- $\mu$ g disk; no zone around 150- $\mu$ g disk

**Resistant:** No zone of inhibition around both O129 disks

| Organism                   | MIC (mg/mL) | Disk Test |
|----------------------------|-------------|-----------|
| <i>Aeromonas</i> species   | 400         | R         |
| <i>Vibrio</i> species      |             |           |
| <i>V. natriegens</i>       | 40-60       | PS        |
| <i>V. alginolyticus</i>    | 1-50        | PS        |
| <i>V. parahaemolyticus</i> | 15-40       | PS        |
| <i>V. harveyi</i>          | 10-20       | PS        |
| <i>V. campbellii</i>       | 3-20        | PS        |
| <i>V. cholerae</i>         | 2-7.5       | S         |
| Non-O1 cholera vibrios     | 2-7.5       | S         |
| <i>V. metschnikovii</i>    | 2-7.5       | S         |
| <i>V. anguillarum</i>      | 1-5         | S         |
| <i>V. pelagia</i>          | 1-5         | S         |
| <i>Plesiomonas</i> species | 2-40        | Variable  |

R = resistant    PS = partially sensitive    S = sensitive

Results from the O129 disk test should be interpreted with conjunction with other tests including the results of the salt requirement test for reliable identification of pathogenic *Vibrio* species.

- *Vibrio cholerae* O129-resistant strains have been reported especially from recent epidemics. Non-O1 *Vibrio cholerae* O129 resistance has also been reported

## Quality Control

| <u>Organism</u>                              | <u>Expected Results</u> |
|--|-------------------------|
| <i>Vibrio metschnikovii</i><br>ATCC 7708     | Sensitive               |
| <i>Vibrio parahaemolyticus</i><br>ATCC 17802 | Partially sensitive     |
| <i>Aeromonas hydrophilia</i><br>ATCC 49140   | Resistant               |

## Storage and Shelf Life

Our O129 Disks should be stored at -20°C. At this temperature they have a shelf life of 26 weeks from the date of manufacture.

## References

1. Shewan JM, Hodgkiss W. Nature 1954; 63:208-9.
2. Ramamurthy T, Pal A, Pal SC, Nair GB. Taxonomic implications of the emergence of high frequency of occurrence of 2,4-diamino-6,7-diisopropylpteridine-resistant strains of *Vibrio cholerae* from clinical cases of cholera in Calcutta, India. J Clin Microbiol 1992; 30:742-3.
3. Isenberg HD, Ed. Clinical microbiology procedures handbook, Vol 1. Washington, DC: ASM, 1992.
4. Murray PR, Baron EJ, Pfaller MA, Tenover FC, Tenover RH, Eds. Manual of clinical microbiology. 7<sup>th</sup> ed. Washington, DC: ASM, 1999.
5. MacFaddin JF. Biochemical tests for the identification of medical bacteria, 3rd ed. Philadelphia: Lippincott Williams & Wilkins, 2000.

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