CAMP IDENTIFICATION SYSTEM

A 3 test biochemical system for the presumptive identification of thermophilic Campylobacter spp.

Description

The MAST ID™ CAMP Identification System, developed at the Preston Public Health Laboratory by Bolton and colleagues, consists of three biochemical tests which permit speciation of the most commonly isolated thermophilic enteropathogenic campylobacters. The tests are for hippurate hydrolysis, indoxyl acetate hydrolysis and urease activity and require pure cultures of organisms taken from selective or non-selective media.

The indoxyl acetate test has been evaluated by several workers for campylobacters. The test involves the hydrolysis of indoxyl acetate giving a dark blue colour due to release of the indoxyl group. Tests have shown that C.coli, C.jejuni (all subsp.), H.fennelliae, A.cyaerophila and C.upsaliensis can hydrolyse indoxyl acetate while other species of campylobacter cannot.

The urease test is based on Christensen's urea medium. In this test the urease positive variant of C.lari (UPTC), H.nitrofigilis and H.pylori give a positive result.

The hippurate test is based on the method described by Bolton et al. In this test C.jejuni (all subsp.) gives a positive result while all other campylobacters are negative.

Packing and Ordering Information

The test system provides the three biochemical tests in tubes and a developing reagent, with sufficient reagents for ten tests. Order code: CAMP-ID.

Stability and Storage

MAST ID™ CAMP IDENTIFICATION SYSTEM should be stored at 2-8°C in the containers provided and may be used until the expiry date given on the label. Do not expose reagents to bright light.

Allow to equilibrate to room temperature before opening.

Shelf life - 2 years from date of manufacture.

Instructions For Use

1. Make a dense suspension of organisms equivalent to a McFarland standard no. 5 in 0.1% sterile peptone water. Organisms should be taken from fresh pure cultures grown on selective media, e.g. MAST Preston Blood Free Agar (DM251) or from MAST Columbia Agar (DM115) (with 5-7% whole blood) incubated under microaerobic conditions at 37°C for 24 or 48 hours.

Note:- MAST Peptone Water (DM185) is recommended as some other manufacturers peptone waters do not perform well with some of the tests. Alternatively a water suspension can be made if the suspensions are to be used immediately for these tests.

2. Add 0.5ml of the bacterial suspension to the urease test (grey capped tube) and the hippurate test (black capped tube). Seal the tubes and incubate at 37°C for 4 hours.

Note:- gently tap the tube before adding the bacterial suspension to ensure that the dried reagents are at the bottom of the tube for reconstitution. After adding the bacterial suspension and capping, shake the tube well to ensure that the reagents are resuspended.

3. Remove the swab from the Indoxyl Acetate tube (white capped tube), briefly dip in sterile deionised water then scrape several colonies from
a suitable culture plate. Replace the swab in the tube, seal it and incubate the tubes at 37ºC for 30 minutes.

4. After 4 hrs incubation layer ninhydrin reagent onto the surface of the Hippurate tube by adding 2 drops of Ninhydrin Developing Reagent. Seal the tube and leave at room temperature for up to 10-15 minutes.

Results and Interpretation

A. Reading the Results

1. Read the indoxyl acetate test after 30 minutes. A positive result is indicated by a dark blue colouration of the swab around the organism as a result of hydrolysis of the indoxyl acetate and release of the indoxyl group. A negative result is indicated by an absence of blue colouration with colouration due only to the presence of bacteria.

Note: Many organisms apart from campylobacters will give a rapid, positive indoxyl acetate result. Beware of contaminant colonies.

2. Read the urease test after 4 hours at 37ºC. A positive result is indicated by a pink colouration and a negative result is indicated by a lack of colour change i.e. an orange-yellow colouration.

3. Read the hippurate test 10 - 15 minutes after adding the Ninhydrin Developing Reagent. A positive result is indicated by a bright purple colouration and a negative result is indicated by a lack of colouration i.e. clear. It is suggested that known positive and negative control organisms should be used with the hippurate test to aid the interpretation of results.

B. Interpretation of Results

For clinical isolates the following table should be used to identify the campylobacter species. The table lists the thermophilic enteropathogenic strains of campylobacter which represent about 99% of clinical isolates.

Limitations of Use

Pure cultures of freshly grown organisms must be used. These cultures must have been initially characterised as campylobacters by simple tests e.g. morphology, oxidase and motility.

Table 1. Interpretation of Results

<table>
<thead>
<tr>
<th>Organism</th>
<th>Hippurate Test</th>
<th>Indoxyl Acetate Test</th>
<th>Urease Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. jejuni (all subsp)</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>C. coli</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>C. lari</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C. lari (UPTC)</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>C. upsaliensis*</td>
<td>-</td>
<td>+/w+</td>
<td>-</td>
</tr>
</tbody>
</table>

* Check catalase reaction. C.upsaliensis are catalase negative or give weak results.

References

4. Christensen WB. Urea decomposition as a means of differentiating Proteus and paracolon cultures from each other and from Salmonella and Shigella. J Bact. 1946; 52: 461.