

Evaluation of the hippurate test as a method for the differentiation between *Campylobacter jejuni* and *Campylobacter coli*



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Introduction

Identification of *Campylobacter* spp. is well known to be problematic because of their complex taxonomy, biochemical inertness, and fastidious growth requirements. *Campylobacter coli* and *C. jejuni* are the more frequently isolated and identified from human and animal sources.

From the epidemiological point of view species differentiation is important and necessary to trace transmission routes of campylobacters, and their incidence and behaviour in the environment and in animals.

Discrimination between the two closely related species *C. jejuni* and *C. coli* is often based on hippurate hydrolysis, where *C. jejuni* gives a positive reaction and *C. coli* does not. In fact, in ISO 10272-1:2006, hippurate is the only test mentioned to distinguish *C. jejuni* from *C. coli*.

The presence of the enzyme hippurate hydrolase is visualized by the purple colour formed when ninhydrin reagent oxidizes the amino acid (glycine) produced during hippurate hydrolysis.

The aim of the present study was to evaluate the ability of the hippurate test to differentiate *C. jejuni* and *C. coli* and to evaluate discrepancies on results readings between technicians due to method and colour subjectivity.

Materials and Methods

Hippurate hydrolysis test was performed on 100 strains of *Campylobacter* (45 *C. coli* and 55 *C. jejuni*) following ISO 10272-1 standard. *C. coli* ATCC 43478 and *C. jejuni* ATCC 33560 were used as negative and as positive controls, respectively.

A tube containing 0,4 ml of a sodium hippurate solution were inoculated with a full loop of inoculum and incubated for 4h at 37°C ± 1°C. After incubation 0,2 ml of a ninhydrin solution was added followed by 10 more min. of incubation at 37°C ± 1°C. A positive reaction is indicated by appearance of a dark violet colour, a pale violet colour or no colour change indicates a negative reaction (Fig.1).

The results were read by more than one technician. Duplicate assay were performed on 10% of the strains.

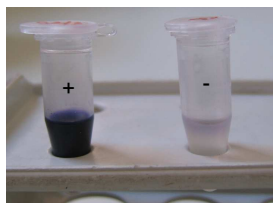


Figure 1: Hippurate hydrolysis positive (+) and negative (-) reactions

Results

47 strains were identified as *C. jejuni* and 43 as *C. coli* by all technicians. Different interpretations occurred with 10% of the strains. Figure 2 shows some examples of the test: while tubes N° 2, 8, 10, 15 and 17 were classified as positive and tubes N° 1, 3, 4, 6, 9, 11, 16 and 19 were classified as negative, tubes N° 5, 7, 12, 13, 14, 18 and 20 were classified as positive or negative depending on the technician who read the tubes.

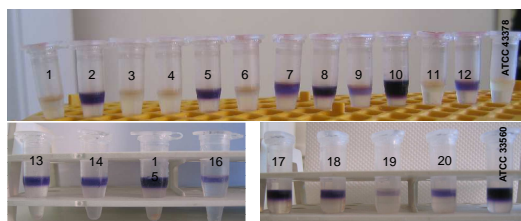


Figure 2: Results of hippurate hydrolysis test for 20 isolated strains, and *C. coli* ATCC 43478 and *C. jejuni* ATCC 33560

Two of the 10 duplicates performed were not concordant. Differences in colours can be seen in Figure 3.

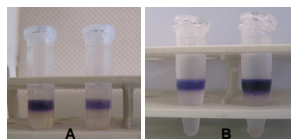


Figure 3: Hippurate hydrolysis test (duplicated assays) for two strains.

Conclusion

The present study clearly shows the subjectivity of the hippurate hydrolysis test. 10% of the strains had different result interpretations. Moreover, 20% of duplicates had discrepant results.

The colour of tubes 13 and 14 are very similar (Fig. 2), although these 2 strains were previously identified by genetic methods as *C. jejuni* (13) and (14) as *C. coli*.

It could be very difficult to decide when the pale violet ends (negative result) and the dark violet (purple) starts.

It should be noted that some strains of *C. jejuni* can be weakly positive and give false-negative results, other amino acids or peptides present in culture media or produced during the incubation can react with ninhydrin and give false-positive results and the colour developed could be badly interpreted.

This study demonstrates that, when there is a need to distinguish between *C. jejuni* and *C. coli*, other methodologies should be used instead of the hippurate hydrolysis test.

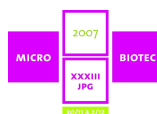
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