IDEXX Summary #2O:

Topic: Production of indole is not an effective indicator of *E. coli*

Title: "Identification of *E. coli* from drinking water: a comparison of phenotypic and genotypic methods"

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Report Highlights:

- Results of the study show that production of indole is a poor indicator of *E. coli*
- An examination of 6,000 isolates of "*E. coli*" isolated from water showed that the traditional confirmatory test, based on production of indole from tryptophan at 44°C was inaccurate.
- Using the International Standards Organization procedure for the detection of *E. coli* in drinking water resulted in a false positive rate of approximately 30%
- The overall conclusion shows that production of β-G-glucuronidase is a far superior marker to indole production for accurately identifying *E. coli*

Identification of *E.coli* from drinking water: a comparison of phenotypic and genotypic methods

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E.coli remains the indicator of choice for the detection of fecal contamination in water and many methodologies are employed. Until relatively recently, methods were based upon membrane filtration or most probable number techniques and isolates required "confirmation" using phenotypic tests. Application of PCR to the identification of isolates growing on membranes has also been used.

We have conducted an examination of over 6000 isolates of *"E.coli"* isolated from water using a variety of phenotypic and genotypic tests. The results of these studies showed that the traditional confirmatory test, based on production of indole from tryptophan at 44°C was inaccurate. In fact using the International Standards Organization procedure for the detection of *E.coli* in drinking water resulted in a false positive rate of approximately 30%. These organisms were phenotypically identified as *Klebsiella oxytoca*. Subsequent molecular analysis using PCR or *in situ* hybridization with an *E.coli*-specific probe clearly showed that these organisms were not *E.coli*. Testing the ability of these organisms to produce the enzyme β-D-glucuronidase also clearly distinguished them from *E.coli*.

The relationship between the identification obtained using molecular methods and that obtained using phenotypic methods depends on the method used for primary isolation. Isolates were grouped according to whether they were isolated at 35°C by membrane filtration, 44°C or by enzymatic methods such as defined substrate technology. For the membrane filtration methods *E.coli* was identified based upon lactose fermentation and production of indole from tryptophan. For glucuronidase-based methods, organisms which produced both β-D-galactosidase and β-D-glucuronidase were considered to be *E.coli*.

The numbers of strains of *E.coli* examined were: isolated at 35°C on membrane filters (1638), isolated at 44°C on membrane filters (2105) and by enzymatic media (2793). When these strains were examined using tests for the production of indole from tryptophan and β-D-glucuronidase and by molecular methods, significant differences were found in the proportion that confirmed as *E.coli* (Table 1).

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Table 1. Number of strains determined to be *E.coli* using PCR, production of indole and production of β-G-glucuronidase

Method used for primary isolation	Strains Examined	+ molecular	+ indole	+ glucuronidase
MF (35°C)	1638	1202	1638	1197
MF (44°C)	2105	2024	2105	2007
Enzymatic	2793	2790	2780	2793

It is clear from the results shown above that production of indole is a poor indicator for *E.coli* when the original isolation occurs at 35°C, and can result in false positive *E. coli* identification. In these situations, *Klebsiella oxytoca* can grow on selective media and produces indole. However, *K. oxytoca* is seldom recovered on selective media at 44°C and consequently the differences between the various methods for identifying *E.coli* are much smaller when isolates were originally isolated at 44°C. When enzyme methods were used to isolate the organism, the difference between the three identification methods was negligible.

The overall conclusion from these experiments was that in the absence of definitive molecular methods for the confirmation of *E.coli*, production of β-G-glucuronidase is a far superior marker to indole production for accurately identifying *E.coli*.

The ability of a small number of commercially available kits for the identification of *Enterobacteriaceae* to discriminate between *Klebsiella oxytoca* and *E.coli* was also investigated. The results of this study showed that those kits which did not include a test for the production of β-D-glucuronidase frequently misidentified *Klebsiella oxytoca* as *E.coli* and in up to 15% of tests incorrectly identified *E.coli* as *Klebsiella* or other members of the *Enterobacteriaceae*.

Examination of the organisms which were identified as *E.coli* using biochemical test kits but which were not found to be *E.coli* using molecular tests showed that the use of a simple test for the production of β-D-glucuronidase correctly identified the organism in over 98% of cases.

We conclude that methods for the identification of *E.coli* vary significantly and that the addition of a simple test for the ability to express the enzyme β-D-glucuronidase leads to an accurate identification in almost all cases whereas tests for production of indole from tryptophan and test kits which rely heavily upon this characteristic can often give misleading results. Thus we recommend the inclusion of tests for the production of β-D-glucuronidase as a primary screen in the identification of *E.coli*.

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