

Uncovering the Myths of Rapid Bacterial Testing and Identification

In today's modern microbiology laboratory, many scientists recognize the benefits of rapid bacterial testing and identification methods. With the rapid evolution of technology, many rapid methods have been developed that significantly reduce time to detection and final identification of bacteria whilst reducing the cost of the testing process and allowing the release of time sensitive products more quickly.

However there are still many microbiologists who are still using traditional, costly and much slower methods. Why is this? Mostly due to the fact that many myths surround quicker methods which can scare away microbiologists from taking advantage of them. In this article, we will attempt to dispel some of these myths and uncover the truth and immense benefits of choosing rapid methods for presumptive to final identification of key pathogens.

Consider a food laboratory testing time sensitive product. However, due to their traditional Listeria detection method, they can not release product to the supermarket for 5 or more days after production. This reduces the effective shelf life time in which it could be purchased and used. The costs involved with this time sensitive process are very high. If a product could be released after only 2 days of testing, it would be fresher and have a longer shelf life, not to mention the significantly reduced labour costs. This quicker product release time can save companies millions of dollars and provide a definitive competitive edge.

However, all of this being said, there are still many microbiologists who haven't embraced rapid methods. This is mainly due to the lack of understanding of the products available, and the myths surrounding their use in the first place.

- *The performance of short screens of biochemical tests is adequate for the identification of key groups of organisms to the genus level.*

This approach must be taken cautiously. Currently there are some 139 different species recognized as belonging to the Family *Enterobacteriaceae*. When many of these simple schemes were first developed, as few as 35 species belonging to this family were recognized. Even with the application of 24 biochemical tests such as provided with the Microgen® GN A + B biochemical identification kits, there are still some species which are very close related biochemically and may need the inclusion of further information (colonial morphology, site or source of isolation) or the performance of additional tests to differentiate.

- *True rapid systems which provide identifications within 4-6 hours of inoculation are as reliable as those systems which require overnight incubation.*

Incorrect... The achievement of identification after such a short incubation period relies on the identification system being inoculated with a very large number of bacteria

(MacFarland 9 - 10). To achieve this density of organisms, multiple colonies must be selected and in doing so the potential always exists to select different organisms and inoculate the system with mixed cultures. Working from a single colony with a system which requires overnight incubation eliminates this problem, ensuring no mixed culture, accurate results and confidence in reporting.

- ***It is not possible to detect Listeria and/or Listeria monocytogenes from initial sample to biochemical ID in less than 5 days?***

Using the FDA BAM recommended ALOA Chromogenic Media in conjunction with the Microgen Listeria ID strips (in AOAC approval process) you can successfully achieve presumptive to final identification confirmation in 2 days! If you have a liquid sample, you can directly streak the ALOA plate with that sample. Incubate the plate at 37°C for 24 hours. If the sample is not liquid, combine 25g of sample with 225ml of Demi Half Fraser broth. Incubate the mixture for 24 hours at 30°C. After this enrichment,

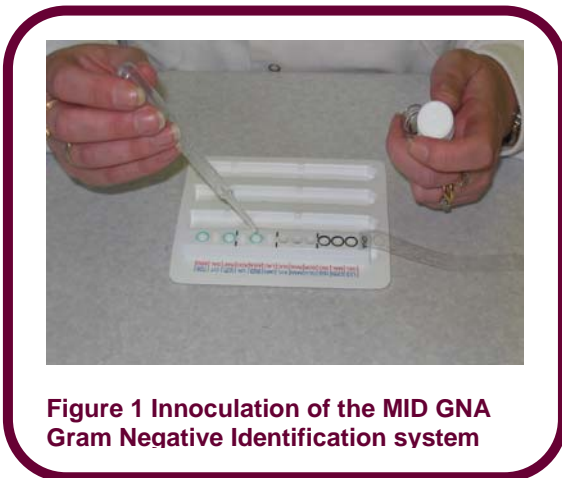


Figure 1 Inoculation of the MID GNA Gram Negative Identification system

subculture 0.1ml of the enrichment broth onto ALOA and incubate for 24 hours at 37°C to yield results. A solid sample may be combined with the broth and masticated using a Pulsifier™. If you have a presumptive positive colony on the ALOA plate, you can then

biochemically confirm with the Microgen® Listeria ID kit. Simply inoculate the strip and within 24 hours you will have a confirmed identification of the colony being investigated.

- ***It is not possible to detect Salmonella and/or Salmonella spp. From an initial sample to biochemical ID in less than 5 days?***

The use of ASAP chromogenic selective media in conjunction with the Microgen® GN A identification strip will allow the rapid isolation and confirmation of *Salmonella spp.*. The ASAP will isolate all *Salmonella* serotypes including *S. typhi*, *S. paratyphi*, *S. arizonae*, H2S-ve, non-motile, lactose positive, sucrose positive in 24 hours. Clinically, the ASAP medium may be used for stool examination. Inoculate the plates directly from the samples or from an enrichment broth. In the Food Industry, simply inoculate the plates from an appropriate selective enrichment culture broth. If you have a presumptive positive for *Salmonella* on the ASAP media, *Salmonella spp.* will appear as pink to purple colonies. Confirmation can then be made using the Microgen® GN A Gram negative identification panel. Simple inoculation of the strips will prove in 24 hours the presence and identification of the following *Salmonella spp.*: *Salmonella Group I*, *S.typhi*, *S.cholerae-suis*, *S.paratyphi A*, *Salmonella Group II*, *Salmonella Group IIIa*, *Salmonella Group IIIb*, *Salmonella Group IV*, *Salmonella Group V*, *Salmonella Group VI*. Using the Microgen GNA + B panel biochemistry identification strips will also identify *S.gallinarum* and *S.pullorum*.

- ***I have to test and confirm by traditional microbiology that conforms to standard methods.***

Untrue... The Microgen® GN A+B and Listeria ID biochemical Identification kits are based on international standards organization (ISO) standard methods. These standard methods substrates are

provided in a convenient 12 microwell strip, ensuring optimal convenience and ease of use. Each and every standard method substrate included in the microwells can be directly correlated as a standard method substrate.

- ***Biochemistry ID kits are difficult to assemble, inoculate, handle, and read.***

The Microgen[®] biochemical ID kits are extremely easy to use. Unlike other methods on the market, there is absolutely no product assembly and no water addition. Inoculation is as simple as pipetting 100ul of inoculum solution into a microwell. The lid then simply pops back on the microwell unit. The convenient and compact size and microwell format (only 5 inches by ½ inch total size) ensures that the strips can be stacked in an incubator without worry of tipping over

- ***I do not need a Haemolysis test for Listeria Identification.***

The two virulent species of *Listeria* (*L. monocytogenes* and *L. ivanovii*) both produce a Phospholipase C (phosphatidylinositol specific) enzyme. Mutant strains of *L. monocytogenes* that

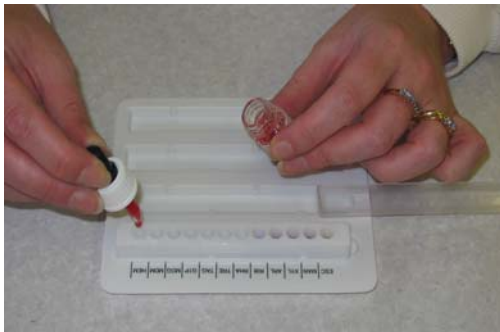


Figure 2. Addition of Haemolysin Reagent to the MID Listeria ID System

lack the Phospholipase C enzyme are avirulent or their virulence is significantly reduced. The detection of haemolysin provides a more practical method of discriminating between virulent and avirulent *Listeria* species. As the detection of the haemolytic

activity is fundamental to the identification of *Listeria* species (in particular *L. monocytogenes* and *L. ivanovii*) and the interpretation of this reaction is sometimes difficult, we have developed a highly sensitive, easily interpreted and stable microwell haemolysin test (Figure 1). This test is used in combination with 11 additional carbohydrate utilization tests to form the Microgen[®] Listeria ID system for *Listeria* species identification. The haemolysin test is simply performed by adding 1 drop of the specially stabilized sheep red blood cells to the last well (well 12) of the Microgen[®] Listeria ID test panel. If the organism being identified produces haemolysin, the red blood cells will rapidly be lysed and the cellular contents released into the suspending medium. The contents of the well will appear as a homogeneous red/brown solution. Alternatively, if the organism being identified does not produce haemolysin, the stabilized red blood cells will remain intact. These cells will settle to the bottom of the microwell forming a distinct red layer with a clear supernatant.

- ***Interpreting the results of a biochemistry ID test is extremely difficult and confusing.***

The Microgen[®] Identification System Software for Windows is a simple to use; yet extremely comprehensive data analysis system for the interpretation of results generated using any of the Microgen ID systems. Classically, organisms are identified using a range of biochemical or growth characteristic tests with the results being compared to the accumulated results of known cultures of similar organisms. This is straightforward when only a few organisms make up the database being considered and only a small number of tests are required to differentiate them. This task becomes significantly more complicated as the numbers increase. To simplify this process a computer aided probability based approach such as that employed in the Microgen Identification System Software may be used. The Microgen

Identification System Software also recommends a range of supplementary tests that may be used to further differentiate the species selected as the most likely identification choices. Software use is extremely simple. Once you have read the identification strips, you can obtain a simple octal code which is input into the software which in turn will generate a result complete with the probability of identification and if appropriate, additional test recommendations.

- *I can not perform biochemical identification directly from chromogenic selective media.*

With the Microgen Listeria ID and the GNA+B identification kits, the organisms can be identified from selective or non-selective agar. Some other kits require incubation of the organism on a non-selective agar which would increase your test and confirmation time another 24 hours or more.

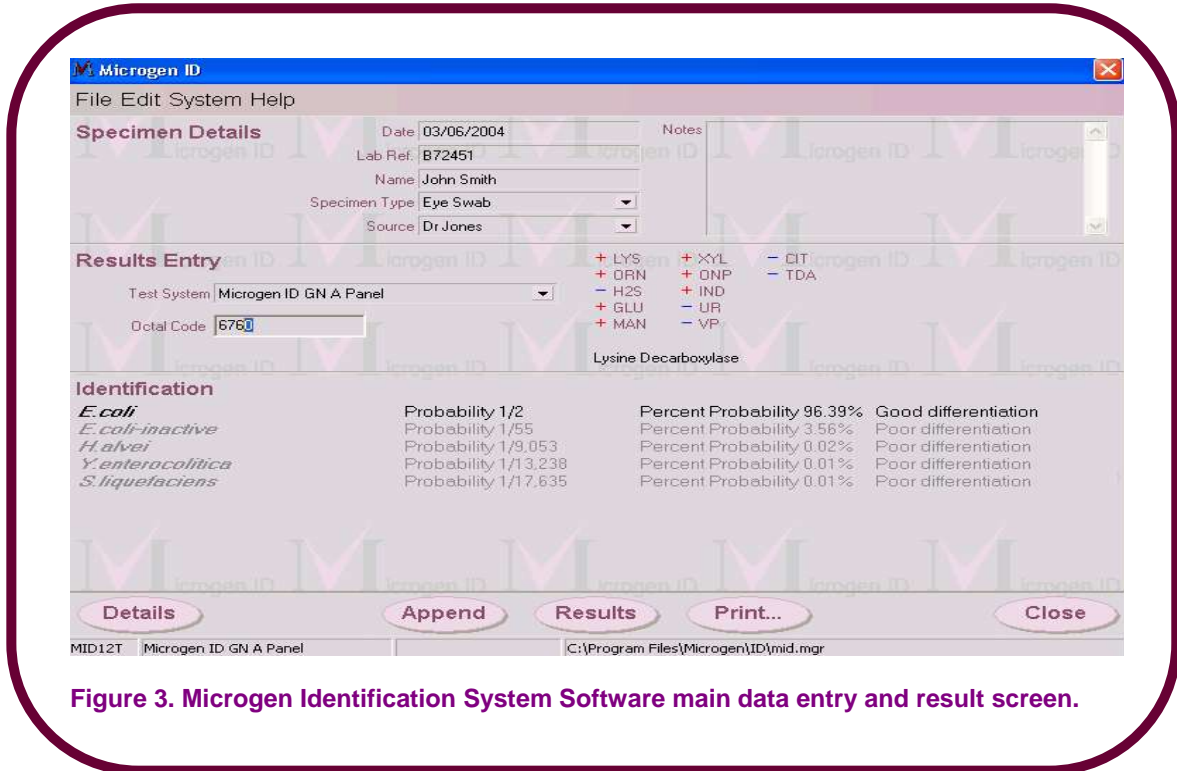


Figure 3. Microgen Identification System Software main data entry and result screen.

FOR MORE INFORMATION ON THE MICROGEN IDENTIFICATION PRODUCTS AND THE MICROGEN IDENTIFICATION SYSTEM SOFTWARE OR ANY OF THE OTHER MICROGEN PRODUCTS FEATURED, PLEASE CONTACT YOUR LOCAL MICROGEN DISTRIBUTOR OR COMPLETE THE INFORMATION REQUEST FORM ENCLOSED WITH THE RELEVANT RESPONSE CODE AND RETURN IT TO MICROGEN BIOPRODUCTS

MICROGEN BIOPRODUCTS LTD
 1 Admiralty Way
 Camberley
 Surrey
 United Kingdom GU15 3DT
 Phone: +44 (0)1276 600081
 Fax: +44 (0)1276 600151
 E-mail: sales@microgenbioproducts.com
 Web: www.microgenbioproducts.com

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