

# PERFORMANCE TESTED METHODS<sup>SM</sup>

## AOAC RESEARCH INSTITUTE NEWS

# Microgen *Listeria* ID Test Kit Granted PTM Status



### Microgen Bioproducts Ltd.

Microgen *Listeria* ID (MID-67) for Confirmation of Individual *Listeria* species from Isolated Colonies on Selective (or Nonselective) Agar Plates

PTM Status: September 21, 2004

Certificate No.: 060402

**L***isteria* species are a common source of contamination in a wide variety of foods and food ingredients. The genus comprises six different species that have distinct characteristics in terms of metabolism and potential to act as a human pathogen. The most commonly found species are *Listeria monocytogenes*, a pathogenic species, and *Listeria innocua*, considered as nonpathogenic.

Microgen Bioproducts Ltd. has developed a biochemical identification system that enables laboratories to rapidly confirm which *Listeria* species have been isolated directly from the selective isolation plate. The product needs only one colony and delivers results in 18–24 hours. The unique feature of the product is an in-well hemolysis test which is based on the ability of a *Listeria* species isolate to lyse red blood cells, one of the key pathogenicity markers that provides a clear discrimination between *Listeria monocytogenes* (hemolytic) and *Listeria innocua* (nonhemolytic).

### Summary of Validated Claims

The Microgen *Listeria* ID kit has been validated under the AOAC Research Institute's Performance Tested Methods<sup>SM</sup> Program to confirm that the product identifies all six members of

the genus correctly and that the serotype of the isolate does not affect the result. Furthermore, studies were undertaken to confirm that non-*Listeria* (closely related bacteria) would not be mistaken for *Listeria* species by the product. The method was also directly compared to the FDA/BAM online method.

The product's claim to be able to confirm *Listeria* species colonies from a range of selective and nonselective media was also confirmed during the extensive ruggedness studies.

### Description of the Method

Microgen *Listeria* ID has been designed to enable users to generate rapid confirmation of the identity of any *Listeria* species that are isolated from

food or food ingredient samples. The product is normally used on "*Listeria*-like colonies" that have been isolated on a selective agar plate. One colony is then taken and suspended in the *Listeria* suspending broth (supplied in the kit). The bacterial suspension is then added to all 12 wells of the micro-well strips provided. Finally the hemolysin reagent (containing stabilized red blood cells) is added to well 12. The micro-well strip is then incubated for 18–24 hours in a nonfan-assisted incubator at 35°–37°C.

The substrate reactions are easily read the next day: well 1 is esculin which should have turned black for all *Listeria* species isolates tested; the next 10 wells are sugars and will have either remained purple (negative) or

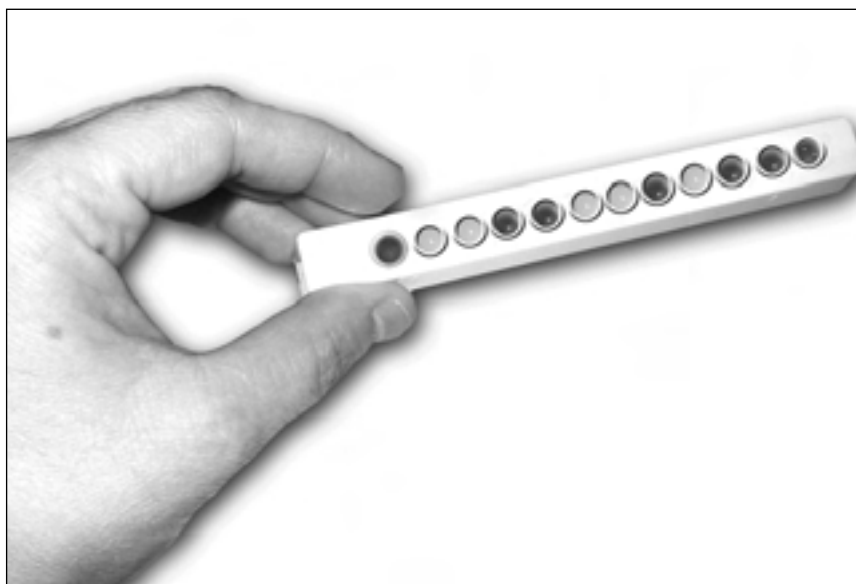


Figure 1. Microgen *Listeria* ID Test micro-well strip.

turned yellow (positive; see Figure 1). The final hemolysis well will either show a button of red blood cells with clear liquid (negative) or a cloudy liquid with no red cell button visible (positive/hemolytic; see Figure 2). The results are recorded and used to produce a four-digit code that is input into the dedicated software program. The program analyzes the four-digit code and suggests the most probable *Listeria* species to generate the code that has been input.

### Test Kit Features

The Microgen *Listeria* ID kit offers several advantages over the conventional method and other miniaturized biochemical test systems for *Listeria* species confirmation. First, the product can be used on bacterial colonies taken directly from selective agar plates. Most other systems require the test to be performed on colonies from nonselective plates. Furthermore, only one colony is required per test so there is no problem with multiple species contamination as all distinct colonies can be tested separately. Second, the multi-well strip is a self-contained test system that delivers the complete result without recourse to additional confirmatory tests, such as CAMP or a separate blood plate for hemolysis. Third, the manufacturer has produced a

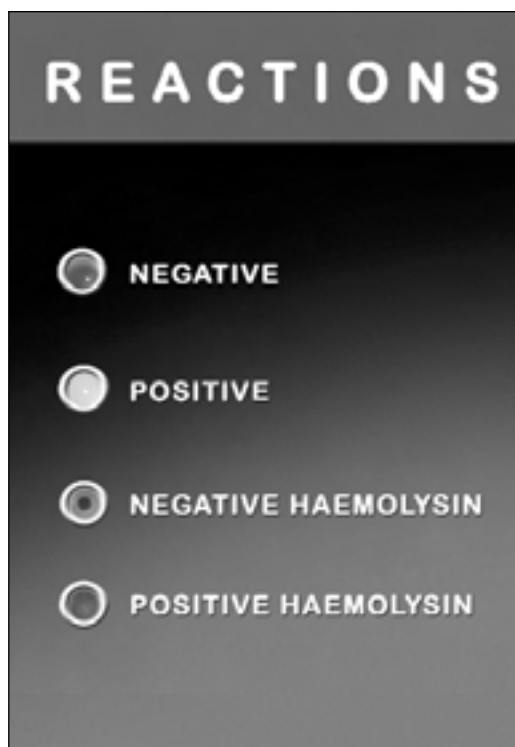


Figure 2. Microgen *Listeria* ID substrate reactions.

dedicated software program that interprets the results achieved in the test strip and delivers a most probable species result to confirm the identity of the isolate under test. Finally, the product now incorporates a feature that ensures the introduction of a non-*Listeria* species isolate into the test system will be identified by the software program and prompt the operator to go back to reconfirm that the isolate under test is indeed a member of the genus *Listeria*, using standard pretests of

gram stain (positive), oxidase (negative), catalase (positive), and motility at 25°C but nonmotile at 37°C.

### Independent Validation

Campden & Chorleywood Food Research Association performed an external validation study on behalf of the AOAC RI. Comparing the Microgen *Listeria* ID with the online FDA/BAM method, they found that the two methods delivered equivalent results. “The Microgen *Listeria* ID Test was easy and quick to carry out compared to performing the identification procedures in the FDA/BAM method. In addition, the Microgen Test gave an identification result within 24 hours of inoculating the micro-well strip, whereas the FDA/BAM method took several days to obtain an identification result. Also, the Microgen Identification System Software was simple to use.”

### Internal Validation

**Inclusivity:** A total of 91 confirmed *Listeria* species isolates were tested using the Microgen *Listeria* ID product and all were successfully confirmed by the kit.

**Exclusivity:** A total of 32 non-*Listeria*s (closely related bacteria) were tested in the Microgen *Listeria* ID product and all were successfully rejected by the system as non-*Listeria*s.

**Method comparison:** Ten *Listeria* species [five different *Listeria monocytogenes* serotypes strains and one strain each of the other five species

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Table 1. Summary of method comparison results <sup>a</sup>							
<i>Listeria</i> species	ATCC No.	NCTC No.	Serotype	ID code	Microgen ID result	Probability	FDA/BAM result
<i>L. monocytogenes</i>	35152	7973	1/2A	4547	<i>L. monocytogenes</i>	1/1	<i>L. monocytogenes</i>
<i>L. monocytogenes</i>	NA	10887	1/2B	4547	<i>L. monocytogenes</i>	1/1	<i>L. monocytogenes</i>
<i>L. monocytogenes</i>	NA	NA	1/2C	4547	<i>L. monocytogenes</i>	1/1	<i>L. monocytogenes</i>
<i>L. monocytogenes</i>	NA	11994	4B	4547	<i>L. monocytogenes</i>	1/1	<i>L. monocytogenes</i>
<i>L. monocytogenes</i>	NA	4883	4C	4547	<i>L. monocytogenes</i>	1/1	<i>L. monocytogenes</i>
<i>L. innocua</i>	33090	11288	6A	4546	<i>L. innocua</i>	1/1	<i>L. innocua</i>
<i>L. seeligeri</i>	35967	11856	1/2B	5445	<i>L. seeligeri</i>	1/1	<i>L. seeligeri</i>
<i>L. welshimeri</i>	35897	11857	6B	5566	<i>L. welshimeri</i>	1/1	<i>L. welshimeri</i>
<i>L. ivanovii</i>	19119	11846	NA	5455	<i>L. ivanovii</i>	1/3	<i>L. ivanovii</i>
<i>L. grayi</i>	25400	NA	NA	6642	<i>L. grayi</i>	1/2	<i>L. grayi</i>

<sup>a</sup> NA = Not applicable; ATCC = American Type Culture Collection, USA; NCTC = National Collection of Type Cultures, UK.

(TEFs), which scale the potency of each chemical relative to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), the most potent known form of dioxin. To date, only three studies have been completed, all involving PCBs (which are members of the dioxin-like class).

Gilliom suggests that something similar might be possible with pesticides. Instead of referring to TEFs, however, pesticide studies use the term "pesticide toxicity index" (PTI).

It's too much to ask such a simple formula to reveal whether any given stream sample is toxic to wildlife. Gilliom says, but at least it provides a quick way to compare the toxicity of samples from various sites. When this is done with the USGS's 3000 samples, he says, agricultural and urban streams wind up the most toxic to fish and crustaceans. Mixed-use streams, even though they contain more complex mixtures, carry lower toxicity indexes, presumably because chemicals are present in lower concentrations.

But other researchers warn that PTIs may provide false rankings, particularly for substances from different chemical families.

One problem, says Michael Lydy, an environmental toxicologist at Southern Illinois University, is that one chemical might activate an enzyme pathway that alters the toxicity of another chemical. If the second chemical's metabolite is more toxic than the parent pollutant, the mixture might be much more toxic than either of its constituents.

Alternatively, if one chemical activates a metabolic pathway that rapidly detoxifies the other, the mixture might be of lower toxicity than its now-degraded constituent.

In another paper presented at the SETAC meeting, Lydy's colleague, Jason Belden, also of Southern Illinois University, examined the synergism between two pesticides from different chemical classes: esfenvalerate (a pyrethroid insecticide) and chlorpyrifos (an organophosphate insecticide) in fathead minnows. He found that the mixture was roughly twice as toxic as would be predicted from either a simple additivity or PTI-style approach.

But Belden observed no such synergism in a second species: aquatic midges. That indicates yet another complexity in the study of chemical

mixtures: the effects may be species-dependent.

Complicating matters even further, a study by Lydy's colleague Jonathan Maul and ecotoxicologists at Arkansas State University found that malathion was 76% more toxic to aquatic invertebrates if the researchers used chemical cues to trick the invertebrates into believing that predators were in the vicinity. No predators were actually present, but the "predator stress" represented yet another form of synergism, making the invertebrates more susceptible to the pesticides. This effect, however, was not observed for dicotophos, another compound in the organophosphate class of pesticides.

What this means, Maul said, is that by examining toxicities in highly controlled laboratory settings, ecotoxicologists may be underestimating the actual risk to aquatic organisms. Rather, it is necessary to consider their impact in the context of the ecological processes of specific ecosystems. ■

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(*innocua*, *seeligeri*, *welshimeri*, *ivanovii*, and *grayi*) were tested in direct comparison with the online FDA/BAM method with complete agreement in the results achieved. See Table 1.

**Ruggedness:** A range of ruggedness studies were performed that confirmed (1) the kit was capable of withstanding the extremes of temperature during international shipping, (2) the shelf-life of 1 year from date of manufacturing, and (3) the ability to use the product on colonies isolated on a range of selective agar plates (including chromogenic agars). The strip is a complete testing system—no other tests are required, e.g., no CAMP test.

### Conclusions

The food industry must be vigilant in controlling microbiological contami-

nation throughout its production processes. One of the key bacterial pathogens that can find its way into a wide range of food products is *Listeria monocytogenes*. While a number of the *Listeria* species are not considered as potential human pathogens, *Listeria monocytogenes* certainly is. It is important that food-testing laborato-

ries isolating *Listeria* species from food manufacturing environments can rapidly confirm with which *Listeria* species they are dealing. The Microgen *Listeria* ID Kit delivers a complete, rapid, reliable, and easy to use system for discriminating *Listeria monocytogenes* from other *Listeria* species isolates. ■

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### Contact and Ordering Information

Microgen *Listeria* ID (MID 67) is currently available in the United States from:

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Mention of trade names or commercial products is for identification only and does not constitute preference over similar ones not mentioned. If you are interested in submitting an article regarding a test kit that has been granted *Performance Tested Methods*<sup>SM</sup> status, contact Deborah McKenzie at dmckenzie@aoac.org.