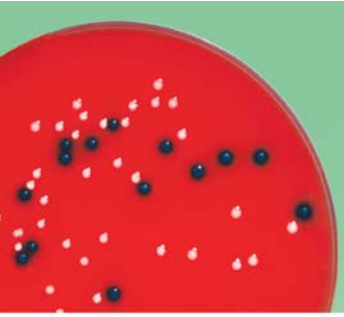


FOOD TESTING

RAPID'*L.Mono*™ Chromogenic Media

PERFORMANCE TESTED* AOAC RESEARCH INSTITUTE LICENSE NUMBER 030406

- **Fast results**
- **Easy identification by colony color**
- **Cost-effective detection**
- **Choice of plates or bottled media**
- **Simple protocol**
- **Independent test validation**
- **Complete solutions for all of your *Listeria* testing needs**

Detect *Listeria* Species in 24 Hours

Listeria monocytogenes is an extremely dangerous bacterial pathogen that can cause:

- Serious health problems and death, particularly in immunosuppressed individuals, newborns, and the elderly
- Stillbirths and miscarriages in pregnant women

Symptoms of listeriosis include:

- Fever
- Fatigue
- Nausea and vomiting
- Diarrhea

Of all reported cases of listeriosis:

- 90% of patients require hospitalization
- There is a mortality rate of 30% (higher in vulnerable individuals)

Infection is caused by eating food that is contaminated with *L. monocytogenes*. The bacterium is dangerous because it can grow slowly at refrigerated temperatures (Mead et al. 1999).

Serious outbreaks of listeriosis continue to plague the food industry. In 2002, a multistate outbreak of listeriosis led to a recall of 27.4 million pounds of ready-to-eat turkey and chicken products (Centers for Disease Control and Prevention 2002). This, along with other large outbreaks, has sparked new government regulations for testing for the presence of *Listeria* spp. in food and in the environment.

A rapid culture method for detection of *Listeria* spp. is necessary to:

- Cut down on time to results
- Ensure accurate results

Now FDA/BAM Recommended

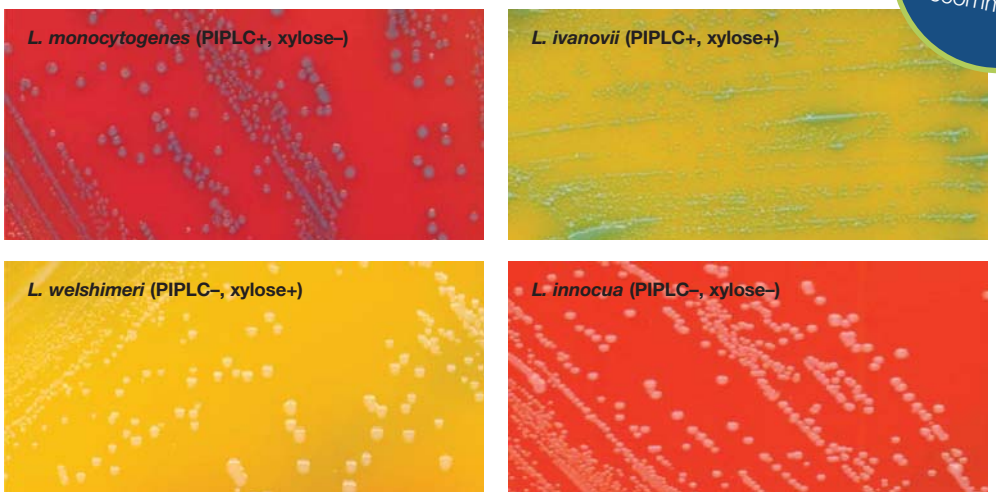


Fig. 1. *Listeria* species on RAPID'*L.Mono* chromogenic medium.

BIO-RAD

Detection of *Listeria* Species

The principle of RAPID'*L.Mono* chromogenic medium relies on the specific detection of the phosphatidyl-inositol phospholipase C (PIPLC) activity of *L. monocytogenes* and the inability of this species to metabolize xylose.

Only two *Listeria* species demonstrate PIPLC activity:

- *L. monocytogenes*
- *L. ivanovii*

The addition of xylose to the medium allows for differentiation of these two species:

- *L. ivanovii* produces bluish-green colonies with a distinct yellow halo, based on its ability to metabolize xylose
- *L. monocytogenes* produces blue colonies lacking a halo

Other nonpathogenic species of *Listeria* do not exhibit PIPLC activity and produce white colonies on RAPID'*L.Mono* chromogenic medium. These species are:

- *L. welshimeri*
- *L. innocua*
- *L. seeligeri*
- *L. grayi*

L. welshimeri will metabolize xylose, producing white colonies with a yellow halo.

The selective supplement contained in the medium inhibits the majority of interfering flora, including:

- Gram-positive and gram-negative bacteria
- Yeasts and molds

References

Centers for Disease Control and Prevention, Public Health Dispatch: Outbreak of Listeriosis — Northeastern United States, 2002, Morb Mortal Wkly Rep 51, 950–951 (2002)

Mead PS et al., Food-related illness and death in the United States, Emerg Infect Dis 5, 607–625 (1999)

* Samples of RAPID'*L.Mono* were independently evaluated by the AOAC Research Institute and were found to perform to the producer's specifications as stated in the test kit's descriptive insert. The producer certifies this kit conforms in all respects to the specifications originally evaluated by the AOAC Research Institute as detailed in the Performance Tested Certificate number 030406.

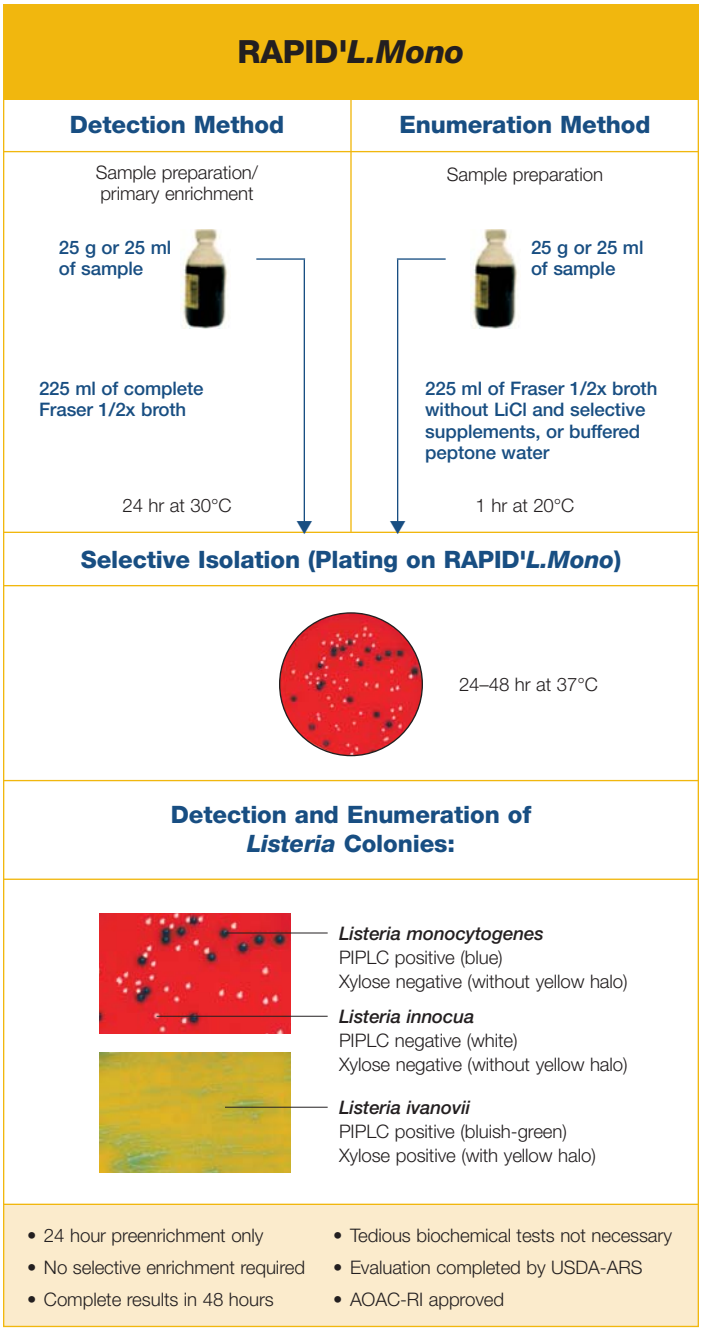
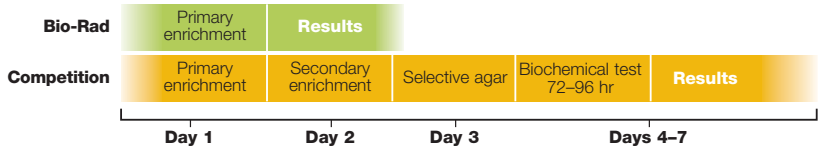


Fig. 2. Protocols for the detection and quantitation of *Listeria* spp. using RAPID'*L.Mono* chromogenic medium.



The New Blue Plate Special: Advances in Chromogenic Media

When Lissa Gilliam, Quality Assurance Supervisor with Dynamic Foods, orders up a blue plate special, she doesn't necessarily mean what's on the menu at the cafeteria chain the company supplies. She may be referring to an advanced chromogenic culture media plate.

The Lubbock, TX-based food manufacturer uses Bio-Rad Laboratories' RAPID'*L.mono* in its in-house laboratory as a secondary confirmation method for the identification of *Listeria monocytogenes*. If *L. monocytogenes* is present in a sample, the colony will appear blue in color on the chromogenic media plate. "If we receive a presumptive positive for *Listeria monocytogenes*, we conduct secondary confirmation tests," says Gilliam. "We set up an AOAC-approved identification method and, at the same time, we also streak the RAPID'*L.mono* plate. What this does is give us peace of mind because within 24 hours we can be certain to a high degree that we either are or aren't dealing with *Listeria monocytogenes*, whereas confirmatory results from the AOAC-approved identification method might take five to six days for confirmation after a presumptive."

For Dynamic Foods, which operates a bakery, a red meat processing department and a cooked foods plant that produces assorted sauces, gravies, non-meat casseroles, puddings, pie fillings and assorted ready-to-cook and ready-to-heat items all in one facility, the difference between 24 hours and five days is extremely important. In addition to the cafeteria chain, Dynamic Foods also boasts vigorous outside sales of custom products for foodservice and retail outlets, making approximately 250 food products in total, which translates into 200 to 250 independent product samples each month for testing in the company's in-house laboratory, says Gilliam.

"We try to fill custom orders as quickly as we can while taking care of our cafeteria chain orders and that means we try to get the fastest turnaround time possible for our customers while maintaining high standards of quality and safety. With the high volume of production at the plant and our company's commitment to testing product for *Listeria* to get the most information on our products before shipping them, turnaround time becomes an even bigger issue. Being able to use a chromogenic media that so rapidly and clearly differentiates *L. monocytogenes* from non-target organisms helps us to achieve both of these goals," she adds.

SEEING THE DIFFERENCE MAKES THE DIFFERENCE

The development of selective chromogenic media is arguably one of the first rapid microbiological methods developed for use in the food industry, notes Brad Crutchfield, Vice President, Life Sciences with Bio-Rad Laboratories, a leader in life science and clinical diagnostic products. In essence, a chromogenic medium is based on classical culture microbiology, only without the wait.

"The demand is for a faster way to accurately identify organisms. In food processing environments, the requirement is for rapid, positive identification of a pathogen. This adds value to a negative test result, creating a high level of assurance in releasing a safe and wholesome product. At the same time, many microbiologists still like to see colonies on the plate, but recognize that the standard culture method, although highly accurate, does not fit into a modern food processing environment where time is more highly valued than ever. A selective chromogenic medium not only offers the food microbiologist a way to reduce the time between test result and product release, but ensures that the target organisms are not positively identified."

The principle of chromogenics is very simple: The agar contains a substrate, or chemical that is specific to certain organisms. Bacteria streaked onto this medium will either use or not use this chemical based on the organism's biochemical pathways, causing an enzymatic reaction signified by a color change of the bacteria or colony. For example, the RAPID'*L.Mono* medium relies on the specific detection of phospholipase of *L. monocytogenes* and on the inability of this species to metabolize xylose. After a 24- to 48-hour incubation, *Listeria monocytogenes* form characteristic blue colonies without a yellow halo. Colonies formed by other species of *Listeria* are white, with or without a yellow halo.

The ability to differentiate the target organism so clearly is where chromogenic media really shines. "The usefulness of chromogenic media is that it reduces the the chance that the target pathogen will be missed because all of the other colonies on the plate look the same and you pick the wrong ones," says Crutchfield.

Gilliam adds that her company is eagerly anticipating the AOAC approval of the method, noting that the Dynamic Foods laboratory continues to use the RAPID'*L.mono* as a secondary method alongside an AOAC International-approved method with "completely consistent results."

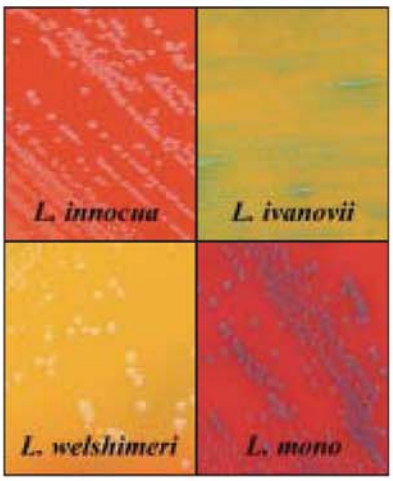
"When this receives approval, we will probably use it alone," she says. "It is a much improved method for us to use, primarily because of the reduced time it takes for set up and completion. The fact is, if you have a presumptive, the conventional identification test requires several additional enrichment steps to confirm the presence of the organism, which takes several days. Whereas with the Rapid'*L.mono* plate, once you get the presumptive, you can streak from the enrichment broth, and by the next morning know what you have."

Reprinted from Food Safety Magazine, June/July 2003, with permission of the publishers.

Color-Coded Colonies Make Test Reading a Breeze

Listeria contamination is an issue that can never totally be conquered. It lingers in drains and in soil, dormant and waiting to attack when you least expect it, and the results can be catastrophic. Fever, vomiting, stillbirths and death can all occur when the pathogen strain of this bacterium is ingested.

Often, companies will test their food production lines for indicator organisms. These are organisms that, if present on plant environment surfaces, can indicate poor sanitization conditions. *Listeria* spp. are used as indicators of the pathogenic *Listeria monocytogenes*.



The best defense against *Listeria* contamination is vigilant testing. And the faster or more easily you can get test results, the sooner you can get products out the door.

Traditional testing methods for *Listeria* take days for test confirmation and the results are less than crystal clear. PALCAM media, for example, requires 48 hours for preenrichment, and microorganisms other than *Listeria,* such as staphylococci or enterococci, may grow. The time spent waiting for test results and deciphering what they mean delays product ship dates, diminishing product shelf-life and adding to overall manufacturing costs.

The industry needs a clearer and more rapid indicator of *Listeria,* to test finished products and to evaluate the overall environment. Bio-Rad Laboratories, a leading rapid microbiology diagnostic test kit company based in Hercules, CA, has responded with its RAPID'L.Mono test, a selective chromogenic medium for detection and enumeration of *Listeria* spp. in food products for animal and human consumption and in the environment. It returns results in half the time than traditional cultures but still gives microbiologists the hands-on experience of seeing the colonies on the plate. With RAPID'L.Mono, enumeration of colonies by species is possible, allowing the user to gather data on the efficiency of their surface cleaning procedures.

APPLE ORCHARD PROJECT PROVES VERACITY OF THE RAPID'L.MONO TEST

But is it as reliable? The answer is yes, according to Carol Ziel, university research associate for Iowa State University, Ames, IA. Last year, Ziel set out to find *Listeria* in an apple orchard and decided to evaluate the accuracy of the Bio-Rad tool as an environmental test for *Listeria.* She sampled raw cider, processed cider, apples, processing equipment and soil to be sure she was covering all of the obvious environmental hot spots. All of the samples came back free of *Listeria monocytogenes,* says Ziel.

Ziel knew the RAPID'L.Mono test was faster and easier to read than traditional *Listeria* tests using PALCAM agar, but she wanted to prove that the results were just as reliable. When she didn't find any *L. monocytogenes* in her orchard samples, Ziel spiked a new set from the same environmental sources, testing some with PALCAM and some with the RAPID'L.Mono test. The differences, she says, were quickly evident.

The PALCAM culture took about a day longer to develop, and in the test results the colonies were dark in color with no clear visual indicator of species. This lack of clarity required Ziel to randomly pick colonies off the sample to test their species.

The rapid test from Bio-Rad, on the other hand, color codes its colonies. *Listeria monocytogenes* produces blue colonies (PIPLC positive) without yellow halo (xylose negative); whereas *Listeria ivanovii* produces greenish-blue colonies (PIPLC positive) with yellow halo (xylose positive) and other *Listeria* spp. colonies are white (PIPLC negative). This distinctive color-coding process means that even as the culture is developing the user can see exactly what is there. And because there is only a 24-hour preenrichment, results come faster.

"The spiked cider sample was beautiful," Ziel says. "It was crisp and clean with white and colored colonies. It was a picture-perfect plate."

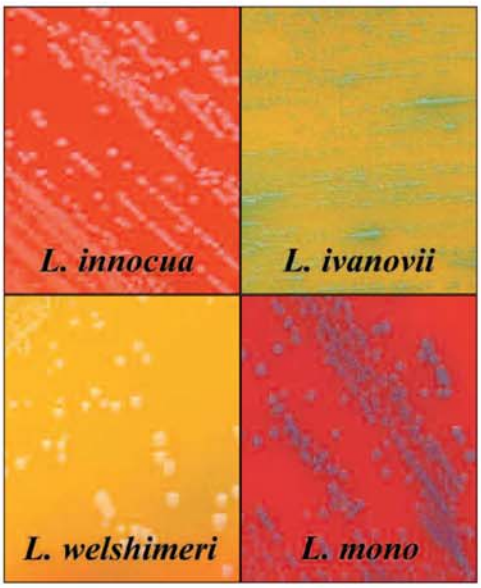
Similarly the spiked soil sample tested with the RAPID'L.Mono was far easier to read than the PALCAM test. "Even though the number of colonies on the plate were too numerous to count, you could see the blue colonies pop out from the others," Ziel says. She was able to easily pick those out and go back to identify them. "Even if you have 200 colonies, the color on the RAPID'L.Mono culture makes them very easy to see."

Ziel sees the Bio-Rad test as a great benefit to labs, not just for end products, but for hygiene testing. "For Hazard Analysis and Critical Control Points (HACCP), it's a great plate that gives you quantifiable proof of what you've got," she says. "It's much easier to use."

"This test has real potential for environmental testing," Ziel adds, noting that the ability to get rapid test results is key to successful sanitation efforts in the food processing facility. "The old method was like looking for a needle in a haystack."

Combination Meat Processing Plant Doesn't Duck *Listeria* Testing

When ready-to-eat meat (RTE) products are processed in the same facility as raw meats, frequent, reliable *Listeria* testing is a vital part of a food safety program. *Listeria* is a problem that never goes away because the organism can lay dormant and survive in drains and in soil for months, waiting for the right conditions to thrive.



"In a plant where ready-to-eat and raw foods are handled, *Listeria* is always a concern," says the biotechnology center supervisor at a busy duck and chicken processing plant based in the Midwest. "You have to be more vigilant."

Even though the plant fully separates its ready-to-eat duck and other poultry product processing from its raw poultry processing areas, and it redesigned the drains throughout the facility to avoid backflow issues from raw to RTE areas, which can lead to *Listeria* growth, she says, frequent *Listeria* testing is still a top priority for the company.

The quality assurance team at the plant conducts *Listeria* testing on four to five duck samples each day of processing, six days a week, as well as taking 12 to 15 environmental sponge samples each day for *Listeria* testing to ensure that no *Listeria* is present in the food or in the plant environment.

Bio-Rad Test is Faster and More Effective

Unlike Rapid'L.Mono, traditional testing methods for *Listeria* take four days for test confirmation and the results can be less than crystal clear. PALCAM media, for example, requires 48 hours for pre-enrichment, and microorganisms other than *Listeria*, such as staphylococci or enterococci, may grow. The time spent waiting for test results and deciphering what they mean delays product ship dates, diminishing product shelf life, adding to overall manufacturing costs.

Rapid'L.Mono returns results in half the time it takes traditional cultures but still gives the microbiologist the opportunity and confidence of seeing the colonies on the plate. The results are also easier to read and interpret because the Bio-Rad test color codes its colonies—*Listeria monocytogenes* produces blue colonies without yellow halo whereas *Listeria ivanovii* produces greenish-blue colonies with yellow halo and other *Listeria* spp. colonies are white. This distinctive color coding process means that even as the culture is developing on the plate, the food company can see exactly what species is present, eliminating the risk of misinterpreting test results.

The ability to differentiate the target organism so clearly is where chromogenic media really shines, notes Brad Crutchfield, Vice President, Life Sciences with Bio-Rad Laboratories. "A potentially contaminated food sample typically does not contain a large number of cells leading to the possibility that the few *Listeria monocytogenes* colonies in a sea of non-pathogenic *Listeria innocua* may be missed with standard culture methods. With Rapid'L.Mono, these colonies stand out, providing a presumptive result for *Listeria monocytogenes.*"

"We love Rapid'L.Mono because it's fast, inexpensive and unbelievably sensitive," agrees the biotechnology center supervisor at the duck processing facility. Her team implemented the Bio-Rad test 18 months ago, replacing its other *Listeria* monitoring tools after a thorough test comparison process. Previously, the facility had used another vendor's test in combination with the *Listeria* test from the United States Department of Agriculture (USDA).

When the plant first considered using the Bio-Rad test, it ran all three tests simultaneously for several weeks to verify the accuracy of Rapid'L.Mono. Eventually, the quality assurance team dropped the other two tests, notes the supervisor. "They were unnecessarily redundant," she says. In fact, the comparison testing process showed that Rapid'L.Mono picked up *Listeria* that the other tests missed. "If we have any *Listeria* it will grow with this test. You couldn't possibly miss it."

The processing facility is happy with the test's ultra-sensitivity and the fact that if any *Listeria monocytogenes* is found, it is instantly identifiable when those colonies turn bright blue. In other tests, colony growth may appear dark or cloudy with no clear visual indicator of which ones are *Listeria monocytogenes.* "Now, we can eliminate the possibility of *Listeria monocytogenes* right away, which we love," she says. "We've never been able to do that before."

Once the 24-hour pre-enrichment is complete, the full results for Rapid'L.Mono are available in 48 hours—half the time it took to get results from the USDA test—which means the plant can get product out of the door faster. "We hold everything until we get our *Listeria* test results back," she says. "This test bought us two more days of shelf life. Our sales guys love that."

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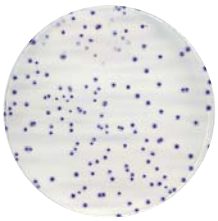
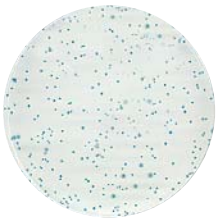
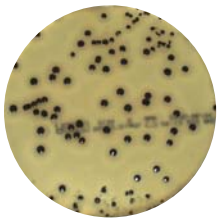
Other Chromogenic Media



E. coli



Other coliforms



Staphylococcus

RAPID'*E.coli*™ 2 Medium

- Detects and enumerates *E. coli* and other coliforms in 24 hours
- Detection based on easy-to-read color-change reaction
- Counting of colonies is effortless

RAPID'*Staph*™ Medium

- Complete results in 24 hours
- Allows enumeration of coagulase-positive *Staphylococcus aureus*
- Highly selective

Ordering Information

Catalog #	Description
RAPID'*L.Mono* Chromogenic Medium	
355-5294	RAPID'*L.Mono* Agar, bottled medium, 190 ml
356-3694	RAPID'*L.Mono* Agar, 90 mm petri dishes, 20
Buffered Peptone Water	
355-4179	Buffered Peptone Water, ready-to-use, 225 ml bottle, 6
356-4684	Buffered Peptone Water, dehydrated, 500 g
Fraser Broth	
355-4658	Fraser 1x Complete Broth, ready-to-use, 50 x 10 ml tubes
355-5797	Fraser 1/2x Complete Broth, ready-to-use, 255 ml bottle, 6
356-4604	Fraser Broth Base, 500 g
356-4615	Fraser 1x Broth Supplement, freeze-dried, box of 10 vials
356-4616	Fraser 1/2x Broth Supplement, freeze-dried, box of 10 vials
Baird-Parker + RPF Medium	
357-8618	Baird-Parker With Rabbit Plasma Fibrinogen (RPF) Agar Kit, 6 bottles x 90 ml + 6 supplement vials
RAPID'*E.coli* 2 Medium	
355-5299	RAPID'*E.coli 2* Medium, ready-to-use, 6 bottles x 100 ml
356-4024	RAPID'*E.coli 2* Base, dehydrated, 500 g
RAPID'*Staph* Medium	
356-3960	RAPID'*Staph* Medium, ready-to-use, 20 dishes x 90 mm
356-4704	RAPID'*Staph* Base, dehydrated, 500 g
355-4201	Egg Yolk With Potassium Tellurite, 5 ml vial
355-4205	Egg Yolk With Potassium Tellurite, 25 ml bottle
356-2682	0.2% Sulfamethazine Solution, 2.5 ml vial

BIO-RAD

Bio-Rad Laboratories, Inc.

Life Science Group

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