IFU

Instructions for Use

COMPACT DRY™ LS

Cat. no. LS100	Compact Dry™ LS, 60x75mm Tray with 10x60mm Well	100 trays/box

INTENDED USE

Hardy Diagnostics Compact Dry™ LS is a ready-to-use test method recommended for the isolation and enumeration of *Listeria* spp. in raw materials, finished products, or on environmental surfaces pertaining to food and related industries.

This product is not intended to be used for the diagnosis of human disease.

SUMMARY

The importance of food cleanliness cannot be understated. The Centers for Disease Control and Prevention (CDC) estimates that 48 million Americans get sick and 3,000 die each year from foodbourne disease.(1) *Listeria monocytogenes*, the causative agent of listeriosis, is the third leading cause of food related deaths. Listeriosis affects primarily pregnant, elderly, or immunocompromised individuals, and has a mortality rate of 20-30%.(2)*L. monocytogenes* and other *Listeria* spp. are gram-positive, catalase-positive bacilli that grow well in cold temperatures and haline environments, both of which are commonly used to limit microbial propagation.(3) While *L. monocytogenes* is the only common human pathogen in this genus, the presence of other *Listeria* contaminants indicates that suitable conditions exist for *L. monocytogenes* to grow, and that action should be taken to eliminate these conditions and potential sources of contamination.

Compact Dry™ LS is a ready-to-use chromogenic medium for performing *Listeria* spp. counts that contains dehydrated culture media and a cold water-soluble gelling agent in a non-woven cloth matrix. The medium is instantly hydrated when inoculated with a sample, and capillary action diffuses the sample evenly over the matrix to form a gel within seconds. Compact Dry™ LS contains a chromogenic substrate that yields a colored reaction when utilized and permits the differentiation of *Listeria* spp. from other types of bacteria.

Compact Dry™ LS performs comparably to the FDA MPN method for enumeration of *Listeria* spp.(6) Compact Dry™ LS utilizes ready-to-use trays that save space and greatly reduce the time needed to perform microbiological testing. Compared to other commonly used culture systems, Compact Dry™ has a longer shelf life, can be stored at room temperature, does not require manual sample spreading, is rigid, stackable and easy to label, and allows for direct colony picking for further subculture.

FORMULA

Compact Dry™ LS contains dehydrated culture media, a gelling agent, and a chromogenic substrate to assist in differentiating *Listeria* spp. from other bacteria that may be present.

Final pH 7.2 +/- 0.2 at 25°C.

STORAGE AND SHELF LIFE

Storage: Upon receipt, store at 1-30ºC. away from direct light. Media should not be used if there are any signs of deterioration, contamination, or if the expiration date has passed. Product is light and temperature sensitive; protect from light, excessive heat, moisture, and freezing. If foil pouch is opened and not all plates are used, return remaining plates to pouch and reseal until next use. Opened packages should be used as soon as possible.

The expiration date on the product label applies to the product in its intact packaging when stored as directed. The product may be used and tested up to the expiration date on the product label and incubated for the recommended quality control incubation times as stated below.

Refer to the document "Storage" for more information.

PRECAUTIONS

This product may contain components of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not guarantee the absence of transmissible pathogenic agents. Therefore, it is recommended that these products be treated as potentially infectious, and handle observing the usual universal blood precautions. Do not ingest, inhale, or allow to come into contact with skin.

This product is for laboratory use only. It is to be used only by adequately trained and qualified laboratory personnel. Observe approved biohazard precautions and aseptic techniques. All laboratory specimens should be considered infectious and handled according to "standard precautions." Refer to the document "Guidelines for Isolation Precautions" from the Centers for Disease Control and Prevention.

For additional information regarding specific precautions for the prevention of the transmission of all infectious agents from laboratory instruments and materials, and for recommendations for the management of exposure to infectious disease, refer to CLSI document M29: *Protection of Laboratory Workers from Occupationally Acquired Infections.*

Sterilize all biohazard waste before disposal.

Refer to the document "Precautions When Using Media" for more information.

PROCEDURE

Prior to Use: Refer to listed references for appropriate methods of collection, preparation, and dilution of samples under investigation. [1-5]

For environmental samples or to test uneven surfaces of equipment, swab surface of test area with EnviroTrans™ (e.g. Cat. no. SRK05 or SRK35).

For raw material and food testing, prepare and dilute samples using an appropriate diluent such as Dilu-Lok™ II (e.g. Cat. no. D590 or D599).

General Dilution Guidelines:

For Making 1:10 Serial Dilutions

1. Using a sterile pipet or scoop, aliquot 10ml or 10gm of test sample to a 90ml pre-filled Dilu-Lok II™ dilution vial to yield a 1:10 dilution. Mix thoroughly.

2. From the 1:10 dilution vial in step 1, use a fresh sterile pipet and aliquot 10ml from this dilution vial into a second 90ml pre-filled Dilu-Lok II™ vial to yield a 1:100 dilution. Mix thoroughly.

3. Continue aliquoting 10ml dilutions into 90ml pre-filled Dilu-Lok II™ vials until the desired concentration of test sample is achieved. Each subsequent dilution increases by a factor of 10. A separate sterile pipet should be used with each dilution. Each subsequent dilution increases by a factor of 10. A separate sterile pipet should be used with each dilution.

For Making 1:100 Serial Dilutions

1. Using a sterile pipet or scoop, aliquot 1ml or 1gm of test sample to a 99ml pre-filled Dilu-Lok II™ dilution vial to yield a 1:100 dilution. Mix thoroughly.

2. From the 1:100 dilution vial in step 1, use a fresh sterile pipet and aliquot 1ml from this dilution vial into a second 99ml pre-filled Dilu-Lok II™ vial to yield a 1:10,000 dilution. Mix thoroughly.

3. Continue aliquoting 1ml dilutions into 99ml pre-filled Dilu-Lok II™ vials until the desired concentration is achieved. Each subsequent dilution increases by a factor of 100. A separate sterile pipet should be used with each dilution.

Method of Use Direct Inoculation:

1. It is recommended that samples are incubated at 20°C for 1 hour in buffered peptone water before inoculating to Compact Dry LS.

2. Remove the set of four trays from the foil pouch and separate each individual tray by gently bending along the connecting edge until each tray snaps free. Alternatively, if setting up a dilution series of the same sample, trays can be left connected to facilitate reading similar samples. Trays that are not used immediately should be resealed in the foil pouch. Refer to the "Storage and Shelf Life" section for proper storage of unused trays.

3. Remove the lid of the tray using two fingers to hold down one end of the lid and the thumb to lift the opposite end. Lids are easier to remove using a "peel back" method as opposed to a "pull off" method.

4. Inoculate by pipetting 1ml of sample directly to the center of a dry tray well, being careful not to touch the surface of the matrix with the pipet tip. Once dispensed, the sample will automatically diffuse across the surface by capillary action to form a gel; manual spreading of the inoculum is discouraged. Remember to account for the sample inoculum when calculating the dilution series.

5. Replace the lid and label the tray with appropriate information, including the sample dilution factor.

6. Invert the tray and incubate, upside down with the medium on top, at 35-37°C for 24-48 hours.

7. Count colonies using the Hardy Diagnostics Wizard™ Compact Dry™ plate reader (Cat. no. CDR1) designed exclusively for use with Compact Dry™. See the Wizard™ Compact Dry™ instruction manual. Alternatively, colonies can be counted when illuminated from the backside of the tray to calculate CFU/ml using the Scan® 100 colony counter (Cat. no. 435000) or comparable back lighting. If the colony count is high, use the 1cm x 1cm molded grid on the back of the tray to assist in colony counting. Use a sheet of white paper with gridded lines to diffuse the light if the molded grids in the tray are difficult to visualize with a light box.

Method of Use Membrane Filtration:

1. Remove the lid on a Compact Dry™ plate and pipette 1.0ml of sterile purified water into the middle of the plate to activate the matrix just before use.

2. Using a membrane filtration set-up, filter 100-250ml of a water sample under reduced pressure through a sterile 0.45µm membrane filter.

3. Keep the set-up running and rinse the inside of the filter funnel using 20-30ml of sterile purified water. Repeat this step two to three times to ensure all of the sample has been filtered through the membrane.

4. Remove the membrane from the funnel using sterile forceps (e.g. Cat. no. 800000) and apply it filter side up (trap side away from the matrix) to the surface of the pre-moistened Compact Dry™ plate. Gently press the membrane onto the surface, making sure to remove bubbles so the membrane is completely flush and centered on the matrix.

5. Replace the lid and incubate the tray right-side-up using the information outlined above.

6. Count colonies and record results as outlined above.

INTERPRETATION OF RESULTS

After incubation, read trays using the Wizard™ Compact Dry™ plate reader (Cat. no. CDR1) or read colonies against a white or illuminated background such as with the Scan® 100 colony counter (Cat. no. 435000) or comparable back lighting.

Blue-colored colonies are indicative of *Listeria* spp. Count the total number of blue colonies to obtain a *Listeria* spp. count. The growth area is 20cm^2. If the colony count is high, the total count can be obtained by multiplying the average number of colonies observed in one 1cm x 1cm square grid by 20.

Bacteria other than *Listeria* spp. may grow, such as *Proteus mirabilis*, but will produce colorless or brown colonies and should not be included in the total *Listeria* count. Some other bacteria, such as some *Enterococcus* species, will not grow but may turn the media a light purple color. These should not be included in the total *Listeria* count.

LIMITATIONS

It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on colonies from pure culture for complete identification of bacteria and/or fungi.

During inoculation, do not touch the surface of medium and be careful to avoid any contamination by airborne microorganisms.

During incubation, keep cap tight on plates to avoid any possible dehydration.

A dilution may be needed when the sample has a dark color.

When the sample is viscous (thick), pipetting the sample on several points on a plate or an additional dilution may be needed for an even suspension.

When the sample contains an enzyme, it may react with the enzyme substrate in the dry sheet and affect the color.

If the nature of sample does affect the reaction of the medium, inoculate only after the factor is eliminated by means of dilution and other techniques. (e.g. samples with high viscosity, colored, reactive with chromogenic substrate, and with a high or low pH).

It is recommended to use a stomacher and filter homogenized sample afterwards to eliminate carry over of tiny particles of foodstuff onto the surface of the medium.

Counting colonies may be difficult against a dark background. For best results, count colonies using the Hardy Diagnostics Wizard™ Compact Dry™ plate reader (Cat. no. CDR1) or with the tray held against a white or illuminated background such as with the Scan® 100 colony counter (Cat. no. 435000).

If using a light box, the molded grid lines or colonies may be difficult to view due to excessive brightness. Diffuse the light using a sheet of white, gridded (1cm x 1cm) paper underneath the tray to facilitate colony counting.

Colonies are not distinguishable on trays if concentrations are above 100 CFU/ml, as high colony counts will result in the whole surface becoming colored or no color development. The sample should be diluted to a concentration of less than 100 CFU/ml for best use.

Refer to the document "Limitations of Procedures and Warranty" for more information.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, swabs such as EnviroTrans™, applicator sticks, scoops, dilution buffers such as Dilu-Lok™ II, other culture media, Wizard™ Compact Dry™ plate reader (Cat. no.

CDR1), Scan® 100 colony counter (Cat. no. 435000), incinerators, and incubators, etc., as well as serological and biochemical reagents, are not provided.

QUALITY CONTROL

End users can anticipate the following typical performance characteristics when testing with CompactDry™.

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
Listeria monocytogenes ATCC® 7644	J	24-48hr	35-37°C	Aerobic	Growth;blue colonies
Listeria ivanovii ATCC® 33090	J	24-48hr	35-37°C	Aerobic	Growth; light blue-blue colonies
Escherichia coli ATCC® 25922	J	24-48hr	35-37°C	Aerobic	Inhibited

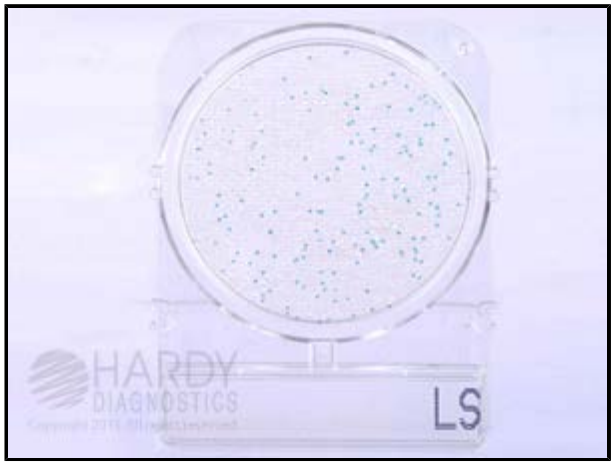
* Refer to the document "Inoculation Procedures for Media QC" for more information.

USER QUALITY CONTROL

Check for signs of contamination and deterioration. Users of commercially prepared culture media may be required to perform quality control testing to demonstrate growth or a positive reaction and to demonstrate inhibition or a negative reaction (where applicable). See the following reference for more specific information. (1-5)

PHYSICAL APPEARANCE

Compact Dry™ LS should appear dry, free of particles, and light yellow in color.



Listeria monocytogenes (ATCC® 19115) grown aerobically on Compact Dry™ LS for 24 hours at 35 degrees C.

Listeria monocytogenes (ATCC® 19115) grown aerobically on Compact Dry LS for 24 hours at 35 degrees C. The organisms were plated at a concentration of 10^6 CFU/ml, demonstrating confluent growth.

REFERENCES

1. *Estimating Foodbourne Illness: An Overview*. 2014. Centers for Disease Control and Prevention.

2. Samaswamy, Vidhya, et al. 2007. Listeria - Review of Epidemiology Pathogenesis. *Jour. of Microbiolog, Immunology & Infection*. 40: 4-13.

3. Ryser, Elliot T., and Elmer H. Marth, eds. 2007. *Listeria, listeriosis, and food safety*. CRC Press.

4. Association of Official Analytical Communities. *Official Methods of Analysis*. AOAC, Washington, D.C.

5. American Public Health Association. *Standard Methods for the Examination of Dairy Products.* APHA, Washington, D.C.

6. APHA Technical Committee on Microbiological Methods for Foods. *Compendium of Methods for the Microbiological Examination of Foods.* APHA, Washington, D.C.

7. U.S. Food and Drug Administration. *Bacteriological Analytical Manual.* Arlington, VA http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm2006949.htm

8. Teramura, Hajime, et al. 2011. Evaluation of the dry sheet medium (Compact Dry LS) for screening of total Listeria count in food samples. *African Journal of Microbiology Research* 5.30: 5419-5426.

IFU-10348[A]

Manufactured for and distributed by:

HARDY DIAGNOSTICS

1430 West McCoy Lane, Santa Maria, CA 93455, USA
Phone: (805) 346-2766 ext. 5658
Fax: (805) 346-2760
Website: HardyDiagnostics.com
Email: TechnicalServices@HardyDiagnostics.com
Ordering Information

Distribution Centers:
California · Washington · Utah · Arizona · Texas · Ohio · New York · Florida · North Carolina

The Hardy Diagnostics manufacturing facility and quality management system is certified to ISO 13485.

HDQA 2207I [F]