

Instructions for Use

ORANGE SERUM AGAR

Cat. no. G91	Orange Serum Agar, 15x60mm Plate, 11ml	10 plates/bag
Cat. no. G181	Orange Serum Agar, 15x100mm Plate, 18ml	10 plates/bag

INTENDED USE

Hardy Diagnostics Orange Serum Agar is used for the cultivation and enumeration of aciduric, putrefactive microorganisms, such as *Lactobacillus* spp., *Bacillus* spp., *Leuconostoc* spp., *Clostridium* spp., yeasts and molds in citrus fruit juices and juice concentrates.⁽⁵⁾

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

SUMMARY

The microbial population of citrus foods and juices varies greatly depending on how foods are processed and preserved. A small percentage of contaminated fruit can "seed" the operating equipment with spoilage organisms. Equipment that is used for fruit juice preparations is often found to be a significant source of contamination. There are many specific operations, or areas, where microbial buildup can occur such as presses, extractors, finishers, mills, pipelines and conveyors. *Geotrichum candidum* has been labeled the "machinery mold" because of its tendency to accumulate on fruit processing equipment. Aerobic plate counts can provide an index for assessing the sanitation of citrus fruit and juice processing equipment.⁽⁸⁾

In the production of citrus concentrates, juice may be held in stainless steel tanks for 30 to 120 minutes before high-temperature evaporation. It is during this holding period that the product is most susceptible to microbial spoilage. The spoilage of unpasteurized fruit juices is most often due to aciduric organisms such as lactic acid bacteria and yeast with *Saccharomyces*, *Candida* and *Torulopsis* being the most commonly isolated spoilage organisms.⁽⁸⁾

Commercially prepared and packaged citrus fruit juices and products have a pH range of 2.9 to 4.0. For example, the pH of orange juice is usually 3.0 to 4.0 and other acidic foods like tomato juice have pH that ranges from 3.9 to 4.4.⁽⁷⁾ Because of the low pH of fruits and fruit juices, aciduric molds and yeast are the microbes that are most often encountered in contaminated citrus products. Of the aciduric bacteria, the lactic acid group, primarily *Lactobacillus* and *Leuconostoc* spp., is most often observed.⁽⁸⁾ On occasion, *C. pasteurianum* is responsible for the spoilage of products with low pH such as tomatoes, pears, figs and pineapples.⁽⁷⁾ While potential contaminants of food products, pathogenic bacteria are not usually encountered in citrus fruit products due to the low pH and the pasteurization process during manufacturing. However, non-pasteurized apple cider has been reported to be responsible to over 200 cases of salmonellosis. Studies have shown that *Salmonella enterica* is able to survive up to 30 days in apple juice with a pH of 3.6.⁽⁸⁾

Foodborne yeasts and molds include several hundred species. These organisms, due to their wide range of pH and temperature tolerances, as well as their assortment of hydrolytic enzymes, have the ability to grow in most low pH food. Contamination of foods by yeast and molds result in substantial losses to the producer, processor and the consumer. Several of the foodborne molds, and possibly yeast, may be hazardous to human and animal health due to

their ability to produce mycotoxins. Yeast and molds may also elicit allergic reactions or infections in those individuals who are aged or those receiving chemotherapy or antibiotics.⁽⁷⁾

Orange Serum Agar is specially formulated for the isolation, cultivation and enumeration of the aciduric bacteria, mold and yeasts seen in citrus foods, juices and other foods with low pH values. Orange Serum Agar contains: casein peptone as a nitrogen source, yeast extract to provide B-complex vitamins to stimulate growth, and dextrose as a carbohydrate source. In order to create an acidic media that favors the recovery of aciduric organisms, orange powder is added to the media, while potassium phosphate serves as a buffer.

FORMULA*

Ingredients per liter of deionized water:*

Casein Peptone	15.5gm
Dextrose	4.0gm
Orange Powder	3.5gm
Yeast Extract	3.0gm
Dipotassium Phosphate	2.5gm
Agar	17.0gm

Final pH 5.5 +/- 0.3 at 25°C.

* Adjusted and/or supplemented as required to meet performance criteria.

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-8°C. away from direct light. Media should not be used if there are any signs of deterioration (shrinking, cracking, or discoloration), contamination, or if the expiration date has passed. Product is light and temperature sensitive; protect from light, excessive heat, moisture, and freezing.

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 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

1. Filter juice sample through a sterile bacteriological membrane filter, pore size 0.45µm.⁽⁸⁾
2. Place the membrane filter on the surface of prepared Orange Serum Agar plates. Avoid trapping bubbles between the agar and filter.
3. Invert plates and incubate for 1-5 days at 35°C.

Consult listed reference for additional information regarding the Orange Serum Agar membrane filtration.⁽⁸⁾

INTERPRETATION OF RESULTS

Determine the microbial colony count and note the morphology of each specific colony type. However further tests must be performed on all growth in order to differentiate and identify organisms.⁽⁵⁾ Microscopic examination must be performed on all molds for complete identification.

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.

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, other culture media, swabs, applicator sticks, incinerators, and incubators, etc., as well as serological and biochemical reagents, are not provided.

QUALITY CONTROL

Hardy Diagnostics tests each lot of commercially manufactured media using appropriate quality control microorganisms and quality specifications as outlined on the Certificate of Analysis (CofA) and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media*. The following microorganisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
<i>Lactobacillus acidophilus</i> ATCC® 4356	A	1-5 days	35°C	Aerobic	Growth
<i>Weissella paramesenteroides</i> ATCC® 33313	A	1-5 days	35°C	Aerobic	Growth
<i>Aspergillus brasiliensis</i> ATCC® 16404	G	1-5 days	35°C	Aerobic	Growth

* Refer to the document "[Inoculation Procedures for Media QC](#)" for more information.

USER QUALITY CONTROL

End users of commercially prepared culture media should perform QC testing in accordance with applicable

government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory quality control procedures or regulation, perform quality control testing to demonstrate growth or a positive reaction and to demonstrate inhibition or a negative reaction, if applicable. Hardy Diagnostics quality control testing is documented on the certificate of analysis (CofA) available from Hardy Diagnostics [Certificate of Analysis](#) website. Also refer to the document "[Finished Product Quality Control Procedures](#)," and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media* for more information on the appropriate QC procedures. See the references below.

PHYSICAL APPEARANCE

Orange Serum Agar should appear slightly opalescent, and light to medium amber in color.

REFERENCES

1. Anderson, N.L, et al. Cumitech 3B; *Quality Control and Quality Assurance Practices in Clinical Microbiology*, Coordinating ed., A.S. Weissfeld. American Society for Microbiology, Washington, D.C.
2. Jorgensen., et al. *Manual of Clinical Microbiology*, American Society for Microbiology, Washington, D.C.
3. Tille, P., et al. *Bailey and Scott's Diagnostic Microbiology*, C.V. Mosby Company, St. Louis, MO.
4. Isenberg, H.D. *Clinical Microbiology Procedures Handbook*, Vol. I & II. American Society for Microbiology, Washington, D.C.
5. MacFaddin, J.F. *Media for Isolation, Cultivation, Identification, Maintenance of Bacteria*, Vol. I. Williams & Wilkins, Baltimore, MD.
6. *Quality Assurance for Commercially Prepared Microbiological Culture Media*, M22-A2. Clinical and Laboratory Standards Institute (CLSI - formerly NCCLS), Wayne, PA.
7. Landry, W.L., Schwab, M.J., Lancette, G.A. 1995. *Bacteriology Analytical Manual*, 8th ed. AOAC International, Gaithersburg, MD.
8. Vanderzant, C. and Splittstoesser, D.F. 1992. *Compendium of Methods for the Microbiological Examination of Foods*. American Public Health Association, Washington, D.C.

ATCC is a registered trademark of the American Type Culture Collection.

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

HDQA 2207D [D]