IFU

Instructions for Use

HARDY DIAGNOSTICS
A Culture of Service™

STANDARD METHODS AGAR

Cat. no. G13	Standard Methods Agar, 15x100mm Plate, with lid label, 18ml	10 plates/bag
Cat. no. G43	Standard Methods Agar, 15x100mm Plate, 18ml	10 plates/bag
Cat. no. G44	Standard Methods Agar, 15x100mm Plate, without label, 18ml	10 plates/bag
Cat. no. H53	Standard Methods Agar, 15x150mm Plate, 68ml	10 plates/bag
Cat. no. Q21	Standard Methods Agar, 20x125mm Tube, 18ml Deep	20 tubes/box
Cat. no. U95	Standard Methods Agar, 4oz. Glass Bottle, 100ml	20 bottles/box
Cat. no. U295	Standard Methods Agar, 8oz. Glass Bottle, 200ml	12 bottles/box
Cat. no. U297	Standard Methods Agar, 500ml Polycarbonate Bottle, 500ml	12 bottles/box
Cat. no. U395	Standard Methods Agar, 16oz. Glass Bottle, 400ml	12 bottles/box

INTENDED USE

Hardy Diagnostics Standard Methods Agar is recommended for use in determining the microbial content in dairy products, food, water samples, and other material of sanitary importance.

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

SUMMARY

Standard Methods Agar is a modified formulation of Tryptone Glucose Skim Milk Agar that was developed by Bowers and Hucker. [5] Yale showed that this modified version is more effective in plate count procedures on milk and dairy products.

Standard Methods Agar is equivalent to the formulation of Plate Count Agar (Tryptone Glucose Yeast Agar) as listed in *Standard Methods for the Examination of Water and Wastewater* , 19th ed., AOAC, and USP. [1-4] The American Public Health Association (APHA) recommends use of the medium for performing the "standard plate count" on dairy products. [6]

Bacterial growth nutrients are provided by peptone, yeast extract, and glucose. B-complex vitamins are primarily supplied by yeast extracts. Glucose serves as an energy source. These nutrients, together with the nutrient factors present in the dairy products to be evaluated will support the growth of the majority of organisms found in the dairy samples.

FORMULA

Ingredients per liter of deionized water:*

Pancreatic Digest of Casein	5.0gm
Yeast Extract	2.5gm
Glucose	1.0gm
Agar	15.0gm

Final pH 7.0 ± 0.2 at 25ºC.

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

STORAGE AND SHELF LIFE

Storage: Upon receipt store plated media at 2-8ºC. away from direct light. Tubed and/or bottled media should be stored at 2-30ºC. 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 dating 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.

Refer to the document "Storage" on the Hardy Diagnostics Technical Document website 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." The "Guidelines for Isolation Precautions" is available from the Centers for Disease Control and Prevention at www.cdc.gov/ncidod/dhqp/gl_isolation.html.

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 M-29: *Protection of Laboratory Workers from Occupationally Acquired Infections: Approved Guideline.*

Sterilize all biohazard waste before disposal.

Refer to the document "Precautions When Using Media" on the Hardy Diagnostics Technical Document website for more information.

Refer to the document SDS Search instructions on the Hardy Diagnostics website for more information.

PROCEDURE

Specimen Collection: Consult listed references for information on specimen collection and processing of food, dairy, water samples, and other materials of sanitary significance. [1-8]

Prior to inoculation, warm prepared media to room temperature.

For melting bottled media: Autoclave at 121ºC. for one to three minutes or until melted. Alternatively, a covered, boiling waterbath (100ºC.) can be used. There should be enough water in the waterbath to reach the media line. A covered waterbath will help to reach and maintain the temperature. Heat in waterbath until melted.

Spread Plate Method:

1. Prepare decimal dilutions in sterile diluent to obtain 30-300 CFU per plate.

2. Aseptically inoculate agar surface with 0.1ml of well mixed diluted sample.

3. Using a sterile spreader device, spread the dilution evenly over the surface of the agar.

4. Incubate plates aerobically for 48 +/- 2 hours at 35ºC.

Pour Plate Method:

1. Melt agar by placing in a boiling waterbath until liquified.

2. Cool media to 45-50ºC. Maintain in a 45-50º waterbath until ready to pour.

3. Prepare decimal dilutions in sterile diluent to obtain 30-300 CFU per plate.

4. Place a 1ml inoculation into a sterile petri plate.

5. Aseptically pour approximately 18ml of the cooled media (45-50ºC.) over the inoculum. Carefully swirl the plate to mix the inoculum evenly.

Note: After autoclaving, do not heat media longer than three hours at 45-50ºC. Sterile solidified medium can only be remelted once.

6. Allow media to solidify.

7. Incubate plates aerobically for 48 +/- 2 hours at 35ºC.

INTERPRETATION OF RESULTS

Following incubation, examine the plates for growth. Count the number of colonies and express in number of colony forming units (CFU) per gram or milliliter of sample; take into account the dilution factor. If duplicate plates were set-up, express the average for the two plates in terms of the number of microorganisms per gram or milliliter of sample. Consult listed references for additional information on interpretation and enumeration of microbial growth on this medium. [1-8]

Precipitated zones of para-casein are indicated by white to off-white zones surrounding colonies. Transparent inner zones surrounding white zones indicate digestion of para-caseinate. The presence of caseolytic microorganisms are indicated by either of these reactions.

LIMITATIONS

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

Refer to the document "Limitations of Procedures and Warranty" on the Hardy Diagnostics Technical Document website 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 Certificates of Analysis (CofA). The following organisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
Staphylococcus epidermidis ATCC ® 12228	A	24hr	35°C	Aerobic	Growth
Escherichia coli ATCC ® 25922	A	24hr	35°C	Aerobic	Growth
Enterococcus faecalis ATCC ® 29212	A	24hr	35°C	Aerobic	Growth

* Refer to the document "Inoculation Procedures for Media QC" on the Hardy Diagnostics Technical Document website 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 certificates of analysis (CofA) available from Hardy Diagnostics Certificates of Analysis website. In addition, refer to the following documents on the Hardy Diagnostics Technical Document website for more information on QC: "Introduction to Quality Control" and "Finished Product Quality Control Procedures," or see reference(s) for more specific information.

PHYSICAL APPEARANCE

Standard Methods Agar should appear slightly opalescent, and light amber in color.

Staphylococcus epidermidis (ATCC ® 12228) colonies growing on Standard Methods Agar (Cat. no. G43). Incubated aerobically for 24 hours at 35ºC.

Escherichia coli (ATCC ® 25922) colonies growing on Standard Methods Agar (Cat. no. G43). Incubated aerobically for 24 hours at 35ºC.

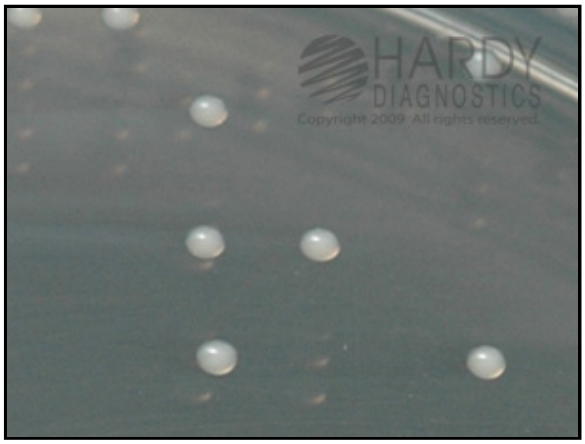
REFERENCES

1. American Public Health Association. *Standard Methods for the Examination of Water and Wastewater,* APHA, Washington, D.C.

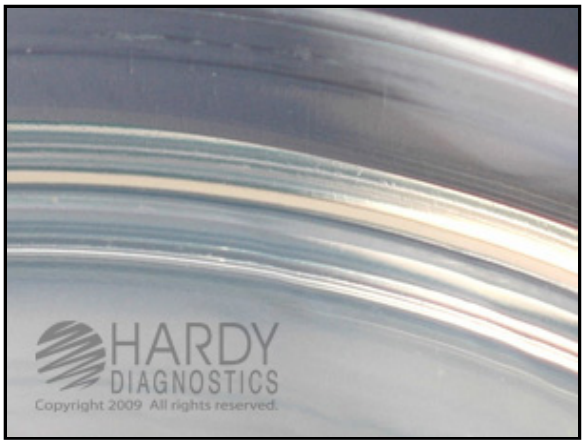
2. Association of Official Agricultural Chemists, 10th ed. p. 737; 1965.

3. Association of Official Analytical Chemists. *Official Methods of Analysis* ^{sm}, AOAC, Washington, D.C.

4. *United States Pharmacopoeia and National Formulary* (USP-NF). Rockville, MD: United States Pharmacopeial



Enterococcus faecalis (ATCC ® 29212) colonies growing on Standard Methods Agar (Cat. no. G43). Incubated aerobically for 24 hours at 35ºC.



Uninoculated plate of Standard Methods Agar (Cat. no. G43).

Convention.

5. Bowers and Hucker. 1944. *Tech. Bull* ., p. 228. N.Y. State Exp. Station.

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

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

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

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.