

# Instructions for Use

## SODIUM CHLORIDE (NACL) 6.5% MEDIA

<a href="#">Cat. no. R27</a>	NaCl 6.5% Broth without Indicator, 13x100mm Tube, 3ml	20 tubes/box
<a href="#">Cat. no. L22</a>	NaCl 6.5% Agar without Indicator, 16x100mm Tube, 5.5ml Slant	20 tubes/box
<a href="#">Cat. no. K49</a>	NaCl 6.5% Broth with Indicator, 13x100mm Tube, 2ml	20 tubes/box
<a href="#">Cat. no. R26</a>	NaCl 6.5% Broth with Indicator, 13x100mm Tube, 1ml	20 tubes/box

### INTENDED USE

Hardy Diagnostics NaCl 6.5% Media are recommended for use in salt tolerance testing to differentiate *Enterococcus* from non-enterococcal group D streptococci.

### SUMMARY

Salt Tolerance Broth was derived from a medium modification designed by Hajna.<sup>(3)</sup> Quadri made further modifications by adding salt, dextrose and bromcresol purple to an infusion broth.<sup>(7)</sup> The medium was designed to aid in the differentiation of *Enterococcus* spp. from streptococci by determining the ability of enterococci to grow in the presence of 6.5% NaCl.

Salt acts as a selective agent and interferes with membrane permeability and osmotic equilibrium. Salt tolerant organisms will produce heavy growth in the broth and on solid agar within 48 hours. Organisms that are capable of growing in the presence of a high salt concentration will also ferment dextrose. Dextrose fermentation produces an acid reaction which results in the bromcresol purple indicator turning yellow. Appearance of a yellow color change in broth with indicator is indicative of a positive salt tolerance test. Growth of organisms on high salt agar is also indicative of salt tolerance, but the omission of bromcresol purple means no color reaction will occur. Organisms that do not grow on NaCl 6.5% agar are not salt tolerant. *E. faecalis*, *E. zymogenes*, *E. liquifaciens*, and *E. durans* are among the *Enterococcus* species that are salt tolerant.

### FORMULA

Ingredients per liter of deionized water:\*

Sodium Chloride	65.0gm
Brain Heart Infusion Broth	25.0gm

In addition, Cat. no. K49 and R26 contains:

Dextrose	2.0gm
Bromcresol Purple	30.0mg

Cat. no. L22 contains the above in addition to:

Agar	25.0gm
------	--------

Final pH 6.9 +/- 0.3 at 25°C.

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

## STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-30°C. away from direct light. NaCl 6.5% Broth with Indicator (Cat. no. R26) and NaCl 6.5% Broth without indicator (Cat. no. R27) should be stored 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 Precautions for blood. Do not ingest, inhale, or allow to come into contact with skin.

This product is for *in vitro* diagnostic 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

Specimen Collection: This product is not intended for primary isolation of patient specimens. It should be used only with cultures of isolated organism. This product is used in conjunction with other biochemical tests to identify cultures of isolated organism. Information on specimen collection may be found in standard reference texts.<sup>(2,4-6)</sup>

Method of Use: Prior to inoculation, the medium should be warmed to room temperature. Using a light inoculum from an overnight culture, inoculate the medium and incubate aerobically at 35°C. for 24-48 hours. Examine media for turbidity/growth or the presence of colonies on media without indicator. Media with indicator should be examined for a color change from purple to yellow.

## INTERPRETATION OF RESULTS

A positive salt tolerance test is indicated by growth and/or turbidity in media without indicator. Media containing indicator will produce a color change from purple to yellow. A negative test is denoted by no growth and/or no color

change. A negative test indicates the organism is not capable of growing in a high salt concentration.

## 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.

Infusion broth with 6.5% NaCl may produce slow reactions thereby making test interpretation difficult.

*Streptococcus agalactiae* (group B) will grow in the media, however it does not produce an acid reaction. Turbidity, but no color change will be present.

A light inoculum must be used when inoculating broth. Too heavy an inoculum may produce turbidity, thus resulting in a false-positive result.

## 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	
<b>NaCl 6.5% Media without Indicator:</b>					
<i>Enterococcus faecalis</i> ATCC® 29212	E	24-48hr	35°C	Aerobic	Growth
<i>Streptococcus pyogenes</i> ATCC® 19615	E	24-48hr	35°C	Aerobic	Inhibited
<i>Streptococcus bovis</i> ATCC® 9809	E	24-48hr	35°C	Aerobic	Inhibited
<b>NaCl 6.5% Media with Indicator:</b>					
<i>Enterococcus faecalis</i> ATCC® 29212	E	24-48hr	35°C	Aerobic	Growth; media turns yellow at 48 hours
<i>Streptococcus pyogenes</i> ATCC® 19615	E	24-48hr	35°C	Aerobic	Inhibited; media remains purple
<i>Streptococcus bovis</i> ATCC® 9809	E	24-48hr	35°C	Aerobic	Inhibited; media remains purple

\* 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

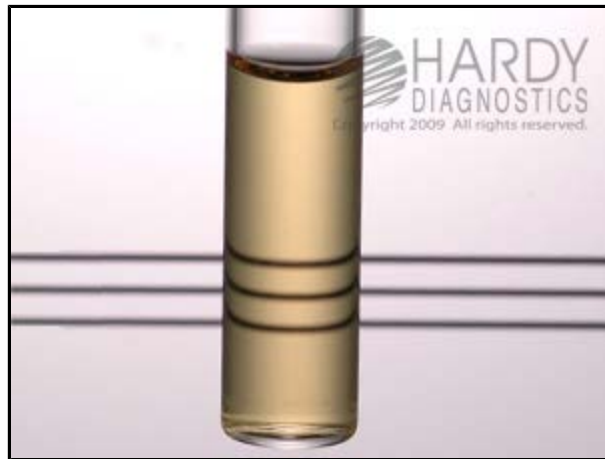
end users check for signs of contamination and deterioration and, if dictated by laboratory quality control procedures or regulation, perform quality control testing to demonstrate growth or a positive reaction and to demonstrate inhibition or a negative reaction, if applicable. Hardy Diagnostics quality 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

- NaCl 6.5% Media without Indicator should appear clear, and light amber in color.
- NaCl 6.5% Media with Indicator should appear clear, and purple in color.



*Enterococcus faecalis* (ATCC® 29212) growing in NaCl 6.5% Medium without Indicator (Cat. no. R27). Incubated aerobically for 24 hours at 35°C.



*Streptococcus pyogenes* (ATCC® 19615) inhibited in NaCl 6.5% Medium without Indicator (Cat. no. R27). Incubated aerobically for 24 hours at 35°C.



*Enterococcus faecalis* (ATCC® 29212) growing in NaCl 6.5% Medium with Indicator (Cat. no. R26). Incubated aerobically for 24 hours at 35°C.



*Streptococcus pyogenes* (ATCC® 19615) inhibited in NaCl 6.5% Medium with Indicator (Cat. no. R26). Incubated aerobically for 24 hours at 35°C.

## REFERENCES

1. Anderson, N.L., et al. *Cumitech 3B; Quality Systems in the Clinical Microbiology Laboratory*, Coordinating ed., A.S. Weissfeld. 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.

3. Hajna, A.A. and C.A. Perry. 1943. *American Journal of Public Health*; 33:550-58.
4. Isenberg, H.D. *Clinical Microbiology Procedures Handbook*, Vol. I, II & III. American Society for Microbiology, Washington, D.C.
5. MacFaddin, J.F. 1985. *Media for Isolation, Cultivation, Identification, Maintenance of Bacteria*, Vol. I. Williams & Wilkins, Baltimore, MD.
2. Jorgensen., et al. *Manual of Clinical Microbiology*, American Society for Microbiology, Washington, D.C.
7. Quadri, S.M., et al. 1978. *Journal of Clinical Microbiology*; 7:238.
8. *Quality Assurance for Commercially Prepared Microbiological Culture Media*, M22. Clinical and Laboratory Standards Institute (CLSI - formerly NCCLS), Wayne, PA.

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

IFU-10602[C]



1430 West McCoy Lane, Santa Maria, CA 93455, USA

Phone: (805) 346-2766 ext. 5658

Fax: (805) 346-2760

Website: [HardyDiagnostics.com](http://HardyDiagnostics.com)

Email: [TechnicalServices@HardyDiagnostics.com](mailto: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.

Copyright© 2020 by Hardy Diagnostics. All rights reserved.

HDQA 2207F [D]