Instructions for Catalog # 083A

WasteWatR™ Coliform MicrobE™ - SM9221

Revision 090119

Description:

- This standard consists of two glass vials each containing one gelatin tablet and a desiccant pouch (Sample A and B). The bacteria are contained in the gelatin tablets. Two sterile, 100 mL hydrating fluids are also provided. Sample A contains a certified number for both Colony Forming Units (CFU) and Most Probable Number (MPN) index and Sample B contains no microorganisms.
- This standard is not preserved.
- The glass vials containing the bacteria should be stored at 4±2°C.
- The hydrating fluid can be stored at room temperature.
- This product is intended to be used as a quality control check of the entire analytical process for the analytes/matrix included in the standard for SM 9221 or equivalent multiple tube fermentation methods.
- ERA suggests that when subsampling this product prior to analysis you use a minimum sample size of at least 50 mL. Using a smaller sample size may invalidate the assigned value and/or uncertainty shown on the certificate of analysis.
- The certified values apply to the diluted sample after following the stated dilution instructions.

Helpful Hints:

- The bacteria are in a lyophilized form in the gelatin tablets and each sample must be hydrated per the following instructions prior to analysis.
- ERA recommends that the quality control guidelines in *Standard Methods for the Examination of Water and Wastewater*, Section 9020B be followed to determine media acceptability prior to analysis.
- "EPA strongly recommends that laboratories evaluate the false-positive and negative rates for method(s) they use for monitoring total coliforms. . . . with the intent that if the method they choose has an unacceptable false-positive or negative rate, another method can be used." – 40 CFR 141.21 f.3.12
- To avoid reporting any false positive results, follow the coliform verification steps as indicated in the method you are using.
- This standard contains viable microorganisms and should be analyzed **immediately** after being hydrated.
- When performing Multiple-Tube Fermentation, run a series of tubes that would yield a result consistent with the manufacturing range of 20 to 2400 MPN index/100 mL.

Instructions:

1. Remove the vials from refrigeration and allow to warm to room temperature.
2. Carefully open the hydrating fluid that has been stored at room temperature.
3. One at a time, open the bacteria sample vials and aseptically transfer the gelatin tablets into the hydrating fluid.
4. Properly dispose of the empty glass vials and desiccant pouches.
5. Reseal the bottles that now contain the bacteria samples.
6. With the bottle tightly closed, shake the sample for a few seconds. Observe the sample to confirm that the gelatin tablet has dissolved. If the tablet has not completely dissolved, shake for a few more seconds.
7. Analyze the inoculated samples using your normal procedure.

Safety:

- ERA products may be hazardous and are intended for use by professional laboratory personnel trained in the competent handling of such materials. Responsibility for the safe use of these products rests entirely with the buyer and/or user. Safety Data Sheets (SDS) for all ERA products are available through our website. www.eraqc.com
- **ERA microbiology standards contain live microorganisms** and should be used only by individuals with bacteriological training.
- Properly disinfect any spills and sterilize used containers by autoclaving before disposal.

LIMITED USE LABEL LICENSE

PLEASE READ THIS AGREEMENT.

THIS LIMITED USE LABEL LICENSE ("LULL") IS BETWEEN ENVIRONMENTAL RESOURCE ASSOCIATES, INC. ("ERA"), AND YOU (THE "END USER"). THIS LULL GOVERNS THE TERMS UNDER WHICH THE END USER MAY PURCHASE OR OTHERWISE OBTAIN PRODUCTS ("PRODUCTS") FROM ERA PURSUANT TO THIS LULL. THE PRODUCTS INCLUDE CERTAIN MATERIALS OWNED BY A THIRD PARTY ("LICENSOR") WHICH HAS GRANTED A LICENSE TO ERA FOR THE PURPOSE OF THIS LULL. ERA IS WILLING TO GRANT END USERS THE RIGHT TO PURCHASE AND USE THE PRODUCTS ONLY IF THE END USER ACCEPTS ALL OF THE TERMS OF THIS LULL.

IF YOU ARE ACTING ON BEHALF OF A CORPORATION OR OTHER ENTITY, THEN YOU REPRESENT THAT YOU HAVE THE AUTHORITY TO ENTER INTO THIS LULL ON BEHALF OF THAT CORPORATION OR ENTITY. IN SUCH EVENT, "YOU" REFERS TO YOUR CORPORATION OR ENTITY. BY ACCESSING AND USING THE PRODUCTS, THE END USER ACKNOWLEDGES THAT THE END USER HAS READ THIS LULL, UNDERSTANDS IT, AND AGREES TO BE BOUND BY IT. IF THE END USER DOES NOT AGREE TO ANY OF THE TERMS IN THIS LULL, THE END USER SHOULD NOT UTILIZE THE PRODUCTS.

1. **SCOPE OF USE**. *You may only use the Products provided to you for the purpose of carrying out a proficiency testing program in your laboratory.* The Products are not intended for use in humans. End User agrees that Products designated as Biosafety Level 2 or 3 constitute known pathogens and that other Products not so designated and may be pathogenic under certain conditions. End User assumes all risk and responsibility in connection with the receipt, handling, storage, disposal, transfer and use of the Products including without limitation taking all appropriate safety and handling precautions to minimize health or environmental risk. End User agrees that any activity undertaken with the Products will be conducted in compliance with all applicable guidelines, laws and regulations.
2. **RESTRICTIONS.** End User may not use, copy, modify or transfer the Products, to others in whole or in part except as expressly provided in this LULL. The Products contain trade secrets and intellectual property of ERA and the Licensor, and the End User may not reverse engineer, replicate, alter, or tamper with the Products, or authorize any third party to do any of the foregoing. The rights granted hereunder to the End User are personal to the End User, and any attempt by the End User to transfer any of the rights, duties, or obligations hereunder is void and shall terminate this LULL. An End User may not rent, lease, loan, resell for profit, or distribute the Products, or any part thereof in any way.
3. **OWNERSHIP.** The Products are the property of ERA. The Products are provided to the End User for use only under the terms of this LULL, and ERA and the Licensor reserve all rights not expressly granted to the End User.
4. **TERMINATION.** This LULL will terminate immediately without notice to the End User if the End User breaches a term of this LULL, or if the End User does not pay ERA, any amounts owed by End User. Further, in the event of a termination or expiration of any agreement between ERA and the Licensor on all or a part of the Products, the End User's right to access and use the Products may also terminate or expire.
5. **WARRANTY DISCLAIMER.** THE PRODUCTS SHALL BE COVERED BY THE APPLICABLE ERA STANDARD WARRANTY, A COPY OF WHICH IS SUPPLIED WITH THE PRODUCTS OR UPON REQUEST. NO OTHER WARRANTIES ARE PROVIDED BY ERA OR THE LICENSOR OF ANY KIND, EXPRESS OR IMPLIED, INCLUDING BUT NOT LIMITED TO ANY IMPLIED WARRANTIES OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, TYPICALITY, SAFETY, ACCURACY, AND NON-INFRINGEMENT. END USER'S REMEDIES UNDER ERA WARRANTY ARE LIMITED TO REPAIR OR REPLACEMENT OF THE PRODUCT THAT FAILED TO CONFORM TO ERA'S APPLICABLE STANDARD WARRANTY PROVIDED THAT SUCH FAILURE IS REPORTED TO ERA WITHIN FIVE WORKING DAYS OF END USER'S RECEIPT OF THE PRODUCT.
6. **COMPLIANCE WITH LAW.** END USER IS SOLELY RESPONSIBLE FOR COMPLIANCE WITH ALL APPLICABLE FOREIGN AND DOMESTIC, FEDERAL, STATE, AND LOCAL STATUTES, ORDINANCES, AND REGULATIONS.
7. **EXPORT LAW.** The Products are subject to U.S. export control laws and may be subject to export or import regulations in other countries. End User shall not to export the Products under any circumstances whatsoever. In any case, the End User will indemnify and hold ERA, its affiliated companies, and the Licensor harmless from any and all claims, losses, liabilities, damages, fines, penalties, costs and expenses (including attorney fees) arising from, or relating to, any breach by the End User of the End User's obligations under this section.
8. **LABORATORY QUALIFICATION ASSURANCE.** The microorganisms and subsequent growth on culture media deriving from the Products, are considered to be bio-hazardous. Government agencies do regulate the disposal of these materials. By entering into this LULL, End User confirms that its laboratory procedures comply with the handling and disposal of these bio-hazardous materials, and that End User's laboratory staff is qualified and properly trained to receive, process and store lyophilized microorganisms. End User acknowledges that the lyophilized microorganisms are for in-vitro use only and are to be used according to their intended use.
9. **INDEMNIFICATION.** End User hereby agrees to indemnify, defend and hold ERA, its affiliated companies, and the Licensor harmless against any third party claims, losses, expenses, and damages (including reasonable attorney's fees) arising out of or relating to the use, receipt, handling, storage, transfer, disposal and other activities related to the Products. Any resolution of a claim subject to this indemnification agreement will be subject to written consent by ERA.
10. **LIMITATION OF LIABILITY.** IN NO EVENT WILL ERA, OR THE LICENSOR BE LIABLE FOR ANY INDIRECT, SPECIAL, INCIDENTAL PUNITIVE, EXEMPLARY, OR CONSEQUENTIAL DAMAGES OF ANY KIND IN CONNECTION WITH OR ARISING OUT OF THIS LULL OR PRODUCTS (WHETHER IN CONTRACT, TORT, NEGLIGENCE, STRICT LIABILITY, STATUTE OR OTHERWISE) EVEN IF ERA OR THE LICENSOR HAVE BEEN ADVISED OF THE POSSIBILITY OF SUCH DAMAGES. IN NO EVENT SHALL ERA'S AND THE LICENSOR'S CUMULATIVE LIABILITY EXCEED THE FEES PAID BY END USER UNDER THIS LULL FOR THE TWELVE (12) MONTH PERIOD PRECEDING THE DATE OF THE EVENT GIVING RISE TO THE CLAIM. End User agrees that the limitations of liability set forth in this LULL shall apply even if a limited remedy provided hereunder fails of its essential purpose.
11. **INTELLECTUAL PROPERTY.** ERA and the Licensor shall retain ownership of their respective right, title and interest in the Products. The Products are subject to the restrictions noted in the "Scope of Use" section above. End User expressly acknowledges that ERA retains all right, title and interest in any trademarks registered or owned by ERA, the ERA trade name, and the ERA catalog marks. End User expressly agrees not to use any of ERA's trademarks, ERA's trade names, ERA's catalog marks, in any way without ERA's prior written agreement.
12. **GOVERNING LAW.** This LULL shall for all purposes be governed by and interpreted in accordance with the laws of the State of Colorado.
13. **WAIVER.** No failure to enforce any term of this LULL shall constitute a waiver of such term in the future.

14.ASSIGNMENT. Neither this LULL nor any of the End User's rights or obligations hereunder may be assigned by the End User in whole or in part without the prior written approval of ERA. Any other attempted assignment shall be null and void.

15.SEVERABILITY. If any part of this LULL is for any reason found to be invalid, illegal or unenforceable, the validity, legality and enforceability of the remaining provisions of this LULL shall not be affected.

16.COMPLETE AGREEMENT. This LULL is the complete and exclusive statement of the agreement between ERA and the End User, and supersedes any proposal or prior agreement, oral or written, and any other communications between the parties in relation to the subject matter of this LULL. No waiver, alteration or modification of this LULL shall be valid unless made in writing and signed by ERA.

Microbiology Technical Guide to Getting it Right the First Time

Introduction:

Before you start the analysis of your quantitative WasteWatR™ Coliforms Quality Control sample (Cat. # 083A) please read through this guide. This document is intended to walk you through all the important steps and ask all the right questions to help ensure that your technique and procedures are correct. This guide is not intended to supersede any specific Quality Assurance Plan or method-specific quality control that you are currently doing. It is only a supplement intended to get you thinking about how you currently perform the method and point out aspects of the process that we at ERA have observed to be common causes of poor method performance. And if you need any help at any step of the way, don't hesitate to call ERA for technical assistance at 1-800-372-0122; our Microbiology Team is here to help!

General Quality Control:

Sterility Checks - Laboratories should perform sterility checks for the procedure in use. For membrane filter procedures check sterility of media, membrane filters, buffered dilution water, pipets, flasks and dishes, and all equipment at the beginning and end of each series of samples, using buffered dilution water as the sample. For multiple-tube procedures, check sterility of media, buffered dilution water, and glassware. Each new batch of the above specific materials should be checked prior to use.

Positive Controls - For each new batch of media and method apparatus, check analytical procedure by testing a positive control to demonstrate that the media produces the expected reaction to the organism under test. In order to ensure identity and traceability, reference cultures used for positive controls should come from a recognized national collection, organization, or manufacturer recognized by an accrediting authority. These cultures may be a single-use preparation or cultures maintained by procedures that ensure the continued purity and viability of the organism.

Negative Controls - For each new batch of media and method apparatus, check analytical procedure by testing a negative control to demonstrate that the media does not demonstrate the typical positive reaction of the target organism. In order to ensure identity and traceability, reference cultures used for negative controls should come from a recognized national collection, organization, or manufacturer recognized by an accrediting authority. These cultures may be a single-use preparation or cultures maintained by procedures that ensure the continued purity and viability of the organism.

Method Evaluation - All methods in use in the laboratory should be evaluated for their ability to produce acceptable results prior to first use. For quantitative microbiology methods this can be accomplished by participation in an approved Proficiency Testing program or the analysis of a Quality Control sample.

Hydration of Pellet - The initial hydration of the gelatin tablet supplied with ERA samples should be performed using the instructions provided. Please do not deviate from the instructions as the certified values and acceptance intervals are based on customer data and deviations could result in an incorrect outcome.

Once hydration of the pellet is complete, perform your analysis immediately. Waiting more than 30 minutes to perform your analysis could have an affect on results. Gently shake the sample prior to taking aliquots for analysis.

Culture Media - Quality of culture media is critical. Never prepare media from raw ingredients if a source of dehydrated media is available. If preparing dehydrated media, follow instructions closely. Always check pH and make any adjustments that are necessary. Always evaluate media prior to first use. Never use media outside of its expiration date and never use media that has not been stored according to the manufacturer's specifications. Confirm that prepared media and dehydrated media's ingredients and proportions match specifications for method in use.

Dilution and Rinse Water - Only hydrate the gelatin tablet using the hydrating fluid supplied by ERA. To perform dilutions and apparatus rinsing, do not use DI or distilled water. ERA recommends Phosphate Buffer or Peptone water as it is not uncommon for DI or distilled water to cause inhibitory effects. Reference SM 9050C 20th Edition for the preparation of Buffered water or Peptone water.

Thermometers - Use thermometers that are graduated in increments appropriate to the analysis being performed. Check the accuracy of thermometers semiannually against a certified NIST thermometer. Use the correction factors, if any, listed on your traceable thermometer's Certificate of Accuracy.

Water Bath Incubators - Verify that your water bath maintains the desired test temperature, such as 44.5°C ± 0.2°C. Keep an appropriate thermometer immersed in the water bath, monitor and record the temperature twice daily at least 4 hours apart. The stability and uniformity of temperature distribution is important. For best operation, equip your water bath with a gable cover, use a water circulator and place incubator in an area where room temperature is maintained between 16-27°C (60-80°F).

Air Incubators - Verify that your incubator maintains the appropriate test temperature, such as 35°C ± 0.5°C. Also, verify that cold samples are incubated at the test temperature for the required time. Check and record the temperature twice daily for the shelves in use. The thermometer should be submerged in water or glycerine. Place incubator in an area where room temperature is maintained between 16-27°C (60-80°F). When you open the door of an air incubator the internal temperature can radically change and once the door is closed the temperature does not immediately return to the desired temperature. This time to return to appropriate incubation temperature may be significant, so opening and closing the incubator should be minimized.

Method Specific Issues – Multiple-Tube Fermentation:

Multiple-Tube Fermentation - Results reported as "> value" will be scored as that value. It is therefore critical that appropriate dilutions are run. We would recommend that QC samples are run at dilutions to simulate the PT scenario.

Dilution Scheme - If performing Multiple-Tube Fermentation, run a series of tubes that would yield a result consistent with the manufacturing range of 20-2400 MPN index/100 mL. For example, a series of 15 tubes at 10 mL, 1 mL, and 0.1 mL aliquots will cover an MPN range of <2 to >1600 MPN/100 mL. If desired to cover a wider range of values alter the dilution series.

Degassing Broth - To avoid a false positive result due to dissolved gas in the media broth, pre-warm the room-temperature broth tubes for 30 minutes in a 35°C incubator and remove any gas that develops in the fermentation tubes prior to inoculation. A small gas bubble during sample interpretation may indicate insufficient degassing prior to inoculation.

Broth Concentration - For an MPN method, confirm that you are using the appropriate broth strength as described in the specific method in use.

References:

Standard Methods for the Examination of Water and Wastewater, 20th edition.

2003 NELAC Standard, Quality Systems, Appendix D, June 2003.