Instructions for Catalog # 083
WasteWatR™ Coliform MicrobE™

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.
- 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. Both typical and atypical colonies need to be verified.
- This standard contains viable microorganisms and should be analyzed **immediately** after being hydrated.
- When choosing sample sizes for analysis, note that the manufacturing range for this product is 20 to 2400 CFU/100 mL (MPN index/100 mL). Given this range, select sample portions that will most likely yield a membrane filter in the ideal counting range. We recommend that the sum volume of the portions selected be equal to the total sample volume provided.

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.

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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. # 083) 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 – Membrane Filtration:

Membrane Filtration PT Results - PT results reported as "too numerous to count" will be evaluated as "No Evaluation". It is therefore critical that appropriate dilutions are run. We would recommend that QC samples are run at dilutions to simulate the PT scenario.

Proper Dilution Scheme - When performing Membrane Filtration, selection of sample size will be governed by expected bacterial density. The assigned value for all ERA QC and PT sample lots will fall in the range of 20-2400 CFU/100 mL. Given this range, select sample portions that will most likely yield a membrane filter in the ideal counting range (20-80 CFU/100 mL for Total Coliform and 20-60 CFU/100 mL for Fecal Coliform). We recommend that the sum volume of the portions selected be equal to the total sample volume provided. If selected portions do not yield an ideal count, report results as dictated by Standard Method 9222B section 6.

Example: For the initial sample provided, dilutions of 50 mL, 30 mL, 12 mL, 5 mL, 2 mL and 1 mL are aliquoted from the sample being tested and diluted with buffered dilution water to 100 mL final volume and each sample is filtered and plated. If the sample result is in the middle to higher portion of the manufacturing range, an ideal count should result on one of your plates. If it is in the lower portion of the range, you may need to use multiple plates to determine the reported result.

Filter Paper - Use 47mm diameter, 0.45 µm pore diameter filter paper. Defective filter paper exhibits one or more of the following observations: Inhibition of colony development at the grid lines, abnormal spreading of colonies, poor or absent colony sheen on the surface of the filter on mEndo agar, brittleness, decreased recovery, severe wrinkling, and areas with no absorption. When the filters are floated on reagent water, the water should diffuse uniformly and completely in 15 seconds.

Media Broth for Broth and Pad - The pad must retain 1.8-2.2 mL of broth. Confirm this using a calibrated syringe by recording the volume of broth in the single-use container minus the excess volume not absorbed by the pad.

Application of Filter - Poor contact of the filter paper to the pad reduces media uptake for the organisms. Achieve maximum contact of the filter paper to the pad by placing an edge of the filter paper to the pad and vigorously rolling the filter onto the pad. A final tap of the non-inverted Petri Dish on the lab bench will also provide additional contact.

Method Specific Issues – Quanti-Tray:

Result Interpretation - Most ONPG-MUG detection systems (Colilert/Colisure etc.) have interpretation time windows when results are considered definitive. Some examples would be 18-22 hours using Colilert-18 and 24-28 hours for Colilert. That is to say that if a sample has not changed to a positive result at the end of the normal incubation the result could still be positive if it changes prior to an additional specified period of time. Follow the manufacturer's procedure closely.

Quanti-Tray (51-well tray) - This method exhibits a maximum counting range of >200 MPN per 100 mL without dilution. A dilution scheme of ERA's 100 mL microbiological product must be performed to cover ERA's manufacturing range.

Quanti-Tray/2000 (97-well tray) - This method exhibits a maximum counting range of >2419 MPN per 100 mL without dilution. No dilution of ERA's 100 mL microbiological product has to be performed.

Comparators - Comparators are for distinguishing threshold positive results from negative results. These are available for some ONPG-MUG systems and should be used if available.

References:

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

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