

ALKEN-MURRAY CORPORATION	TITLE: COLIFORM TEST	NO. QC-4	
MICROBIOLOGICAL DIVISION	QUALITY CONTROL	PAGE 1 OF 2	REV: 6

Revised by Valerie Anne Edwards on 11/28/2005

# ALKEN-MURRAY CORPORATION

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## QUALITY CONTROL METHOD - 4

Detection of Total Coliform and E. Coli Organisms

### Description:

This quality control procedure is designed to verify that no *E. coli*, and especially no *E. coli* 0157:H7 are present in Alken-Murray's microbial formulas, using Defined Substrate Technology. This procedure replaces the IDEXX method that required a special device (SimPlate) to allow a count to be made, which was too time consuming. We have also replaced using IDEXX Colilert because the color in some of our products (Alken Nu-Bind and Alken Bio-Nutrient 9) invalidated that test procedure. This new procedure uses the BBL ChromAgar G137 for easier and faster results. There are two options for using this media, to test our products for contamination, which will fail the lot, and for distributors to test ponds for the presence of sewage contamination, thereby assisting with correct product selection. A trained laboratory technician should be employed to utilize this procedure.

### Equipment & Supplies:

*CHROMagar 0157 from BD* (various vendors)  
 Culture Media & Supplies Tryptic Soy Agar (QC-22) with or /wo 2.25% glucose  
 Weber Sterile Phosphate Buffer (with magnesium) in 99 ml bottle  
 Kovac's Reagent for indole test (QC-29) <http://www.alken-murray.com/QC29.pdf>  
 Sulfide-Indole-Motility - SIM (QC-85) <http://www.alken-murray.com/QC85.pdf>  
*bioMerieux* API20E test kit <http://www.biomerieux.com>  
*bioMerieux* ampoule tube stand  
*bioMerieux* sterile 0.85% saline ampoules, both 1 ml and 5 ml  
 LabSafety or Weber Scientific sterile Pasteur pipets (*individually wrapped, dispsable*)  
 sterile cotton swabs (*individually wrapped, disposable*)  
*bioMerieux* McFarland Standard set  
 Eppendorf air displacement pipettor with sterile filter tips (1 - 100 µl or 100 - 1000 µl)  
 Microbial turntable (optional)  
 Distilled water  
 Incubator set to 35°±0.5°C or 44°±0.5°C  
 Sterile gloves  
 3 - 4 mm sterile platinum loop or pre-sterilized disposable blue 1 µl loop  
 Bunsen burner or Oxford Bacticerator III  
 Disinfectant and isopropyl alcohol to prevent contamination of sample

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### Test Procedure:

1. Prepare Alken-Murray bacterial formula for testing by awakening microbes from dormancy as detailed in QC-1, <http://www.alken-murray.com/QC1.pdf>  
Alternatively, if testing pond water, skip this step..
2. Aseptically inoculate plates using the streak for isolation (4 locations) using a sterile 3 - 4 mm platinum loop
3. During same session, stab-inoculate QC 85 - SIM tube
4. Incubate plates for 24 ± 2 hours at 35° - 37°C

### Interpreting Results

1. Examine the plates for growth at 24 hours.
2. Small BLUE colored colonies 1 to 1.5 mm in diameter is a presumptive positive test for the presence of non-pathogenic *E. coli*. Confirm this test with the indole spot test (Kovac's Reagent) and *bioMerieux* API 20E protocol.
3. Small MAUVE colored colonies are presumptive for pathogenic *E.coli* 0157-H7.
4. Alken-Murray's *Enterobacter cloacae* strains, will appear as colorless colonies. Alken-Murray's *Pseudomonas* will be inhibited on this medium.
5. Add Kovac's Reagent to SIM tube.
6. Confirm any positives in item 3 or 4 with *BioMerieux* API 20E test. Follow procedures in literature for that test.
7. Record results in log book and computer.
8. Mark any positive lot numbers for disposal, check with bookkeeping for list of any clients who already received this lot number and give list to Ken, so that clients can be notified and replacements sent as soon as possible.
9. Verify which workers handled blending of lot numbers testing positive and schedule them for retraining in factory hygiene (Purell wash of hands after bathroom use or eating, before donning gloves and at end of work day) and personal protection clothing, including lab coat, N-95 dust mask for powders or surgical mask for liquids, latex or nitrile examination gloves, safety glasses or goggles and/or face shield. Gloves should be changed whenever leaving blending area or lab for any reason.

### Limitations

1. Color-blind individuals may encounter difficulty reading this test. Get verification.
2. Confirm any positives with *bioMerieux* API 20E test kit