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### MEMORANDUM

**To: All NPDES Permittees Who Must Perform *E.coli* Testing**  
**From: Bruno Pigott, Assistant Commissioner**  
**Office of Water Quality**  
**Subject: Policy for Reporting “Too Numerous to Count” (TNTC) Data for *E. coli* Testing**  
**Date: July 1, 2005**

Utilization of this TNTC policy should not become necessary in any but the rarest of situations. Any questions regarding this policy should be directed to Barbara McDowell at (317) 233-6464 or bmcowell at idem.IN.gov.

#### For Testing Methods Utilizing a Membrane Filter:

NPDES permits require that the monthly average of *E. coli* be less than 125 colonies per 100 milliliters (mL) of filtered sample. *Standard Methods for the Examination of Water and Wastewater* 20<sup>th</sup> Edition – pages 9-59, indicate that the allowable maximum number of colonies per plate (filter) is 200. The optimum count is in the range of 20 to 80 colonies, with an ideal sample yielding about 50 colonies. (If no filter has a count falling in the optimum range, meaning in the range of 20 to 80 colonies, total the colonies on all filters and report as number per 100 mL) (See 20<sup>th</sup> Edition – pages 9-61 for detailed examples.)

Even though filtration of 100 milliliters (or lesser volumes) normally produces an acceptable colony count for disinfected effluent, occasionally the count for these normally acceptable dilutions may exceed 200 colonies per plate.

**To prevent such an occurrence we are strongly recommending that laboratory personnel routinely run a 1.0 milliliter dilution along with the normally acceptable dilutions for each test.**

**If all dilutions for that test, including the 1.0 mL dilution, result in plates (filters) that are deemed TNTC, the number reported for the 1.0 mL test should be 63,200. This number should be reported on the Monthly Report of Operations (MRO) as the *E.coli* result for that day and should be included in the monthly average calculation.**

Justification: If the 1.0 mL plate is deemed TNTC, then the actual count is likely to be somewhere between 20,000 (maximum count for a 1.0 mL dilution) and 200,000 (maximum count for a 0.1 mL dilution). **The TNTC number of 63,200 is the geometric mean of those two numbers.**

**For the Colilert Using Quanti-tray Procedure:**

Even though filtration of 100 milliliters (or a lesser volume that you have determined) normally produces an acceptable Quanti-tray, very occasionally these normally acceptable dilutions may result in all wells on the Quanti-tray fluorescing.

**To prevent such an occurrence we are strongly recommending that laboratory personnel routinely run a 10.0 milliliter dilution along with the normally acceptable dilution for each test.**

**If all dilutions for that test, including the 10.0 mL dilution, result in trays that are fully fluoresced, the number reported for the 10.0 mL test should be 76,000. This number should be reported on the MRO as the *E.coli* result for that day and included in the monthly average calculation.** (However, if your facility is using an Excel spreadsheet MRO supplied by IDEM, you do not have to alter the default value assigned to TNTC results by the spreadsheet.)

Justification: If the 10.0 mL tray is fully fluoresced, then the actual count is likely to be somewhere between 24,192 (maximum count for a 10.0 mL dilution) and 241,920 (maximum count for a 1.0 mL dilution). **The reportable number of 76,000 is the approximate geometric mean of those two numbers.**