

viruses when monitoring is required, according to this publication, is the American Society of Testing and Materials (ASTM) Method D4994–89. Although Method D4994–89 was validated in a multi-laboratory study, the Method achieves only partial recovery of virus from sewage sludge and laboratories are sometimes allowed to use their own standard virus plaque assays. This results in wide variations in virus levels and types recovered from various sewage sludge samples, calling into question the utility of the method. Furthermore, Method D4994–89 is labor intensive, making it difficult for many laboratories to undertake.

Several groups have proposed simpler methods which may yield higher virus recoveries than Method D4994–89. However, limited data are available to evaluate these methods. EPA supports the concept of performance-based methods, and the PEC would accept data from simpler methods, if shown to be at least as effective as Method D4994–89. Therefore, the Agency has developed a research plan to improve analytical methods for viruses and anticipates this work to be completed in 2005. The goal is to have improved methods with higher sensitivity, specificity, and accuracy for detecting viruses in sewage sludge. One objective in this plan is to demonstrate whether other methods are comparable to Method D4994–89. Methods will be compared for their ability to recover viruses that are naturally present in sewage sludge in addition to their ability to recover seeded viruses.

The plaque assay was used for virus detection in the initial round-robin testing of Method D4994–89. This quantitative assay relies upon the development of virus-induced plaques within cell culture monolayers. A most probable number (MPN)-based method for measuring cytopathic effect (CPE) in cell cultures may prove a more useful assay as this is reported capable of detecting viruses at 2-to 100-fold lower concentrations than plaque assays, with the higher sensitivities observed for environmental water samples.

The plaque assay and the MPN-based CPE assays are limited because it fails to detect many of the most important human enteric viral pathogens. Thus, they may provide limited data on whether viral pathogens are inactivated by sewage sludge treatment processes. A new assay has been developed that combines the advantages of cell culture (e.g., detection of infectious particles only) and polymerase chain reaction (PCR) techniques for rapid detection of important human viral pathogens. The Agency will evaluate this integrated cell

culture—PCR (ICC–PCR) assay to determine whether previously undetectable human enteric viral pathogens are present in sewage sludge.

Method validation will be accomplished by comparing Method D4994–89 using plaque, MPN, and ICC–PCR assays for seeded and unseeded sewage sludge types. EPA will develop standard operating procedures (SOP) to be further tested on a wide variety of sewage sludge types.

The final objective will be to determine the appropriate virus type to use in seeding viruses in sewage sludge. Utilizing the method described in the SOP, virus recoveries will be compared using a range of virus types, including poliovirus, coxsackievirus, echovirus, and others to be determined. If possible, the Agency will determine recoveries before and after a sewage sludge treatment process. It is estimated that this project will take two years. Products include publication of scientific papers describing the method comparisons and a SOP detailing the optimal method for validation studies.

Project 3c: Development and Validation of Analytical Methods for Fecal Coliform in Sewage Sludge

Fecal coliform bacteria are used as indicators of treatment process effectiveness in the production of Class A and Class B sewage sludge. This ongoing project identifies available methods for enumerating fecal coliforms in sewage sludge, selects the most appropriate methods, determines minimum performance characteristics that must be met, and evaluates these methods in quantifying such organisms using multiple laboratories.

EPA will use multiple laboratories to update and evaluate protocols for assaying fecal coliforms in sewage sludge using multiple tube fermentation techniques and test the method on treated sewage sludge samples using independent laboratories. Samples of Class A and B sewage sludge from full-scale wastewater treatment facilities will be assayed with and without known amounts of *Escherichia coli*, a species of fecal coliform. The Agency will compare the relative performance of individual laboratories performing such tests and develop acceptable standards. The final product, anticipated to be completed in 2005, will be a draft EPA Method 1680 entitled “Fecal Coliforms in Treated Sewage Sludge by Multiple-Tube Fermentation Procedures.”

Project 3d: Development and Validation of Analytical Methods for *Salmonella* in Sewage Sludge

Many serovars of *Salmonellae* can cause gastroenteritis and typhoid fever. *S. enterica* serovar Typhi is the causative agent for typhoid fever. These bacteria may be used to demonstrate treatment effectiveness of Class A sewage sludge. This project will identify available methods for enumerating *Salmonella* in treated sewage sludge, select the most appropriate methods, evaluate minimal performance characteristics that must be met, and evaluate these methods in quantifying such organisms using multiple laboratories. EPA will develop and test the method on treated sewage sludge samples.

The Agency will update and evaluate protocols for assaying *Salmonella* in sewage sludge using multiple tube fermentation techniques among multiple laboratories. Samples of Class A sewage sludge from full-scale wastewater treatment facilities will be assayed with and without known amounts of *Salmonella*. EPA will compare the relative performance of individual laboratories performing such tests and develop acceptable standards. The final product, to be completed in late FY 2004, will be a draft EPA Method 1682 titled “*Salmonella* in Sewage Sludge by Modified Semisolid Rappaport-Vassiliadis (MSRV) Medium.”

Project 4: Field Studies of Application of Treated Sewage Sludge

EPA will initiate field studies to evaluate management techniques for treated sewage sludge in order to determine whether the pathogen and chemical requirements of part 503 are being met. These studies, that relate to certain categories discussed in the Agency’s preliminary strategy of April 9, 2003, notice (68 FR 17385–17386, 17388–17390), will measure selected indicators of microbial, chemical, and particulate emissions from sewage sludge land application sites and will study the fate of contaminants in the soil to which biosolids are applied. Data resulting from these studies may also be appropriate for inclusion in future risk assessments of biosolids application scenarios.

EPA plans to work with State, Regional, USDA, and other partners to conduct field studies of land application practices at up to five sewage sludge land application sites. Field sampling at actual application sites will involve a variety of media and methods to characterize airborne and soil-bound