

holding time of 96 hours is clearly indicated in the Methods and can be found on page 10, section 8.2.1 of the April 2001 versions of Method 1622 and Method 1623.

Although footnote 17 of the proposal inaccurately stated the technique for calculating holding time, the underlying methods themselves described this technique correctly. The footnote has been corrected to indicate that holding time is properly calculated from the time of sample collection to elution for samples shipped to the laboratory in bulk and calculated from the time of sample filtration to elution for samples filtered in the field.

V. Response to Major Comments

EPA encouraged public participation in this rulemaking and requested comments on the methods proposed for *E. coli*, enterococci, *Cryptosporidium*, and *Giardia*. EPA also requested any data that would support comments on specific test methods. Fourteen stakeholders provided comments addressing over 25 issues. These stakeholders included four laboratories, seven regulatory authorities, and three industries/industry groups.

The following sections summarize major comments received on the proposed rule and EPA's response. The complete Response to Comments document can be found in the Docket for today's final rule.

A. *E. coli* and Enterococci Methods for Wastewater Analysis

Several commenters requested that the methods for *E. coli* and enterococci be approved for the analysis of wastewater samples. Since these methods were not validated in wastewater, they are not approved for use in that matrix. EPA is in the process of validating methods for the analysis of *E. coli* and enterococci in wastewater and plans to propose test methods for these bacterial indicators by the end of 2004.

B. *Cryptosporidium* and *Giardia* Methods for Wastewater and Biosolids Analysis

Several comments advocated the use of EPA Method 1622 and 1623 for the analysis of wastewater and biosolids samples; other comments requested that EPA modify and approve the methods for use in those matrices. EPA has not validated these methods for those uses. Thus this final rule applies only to ambient water. If EPA develops water quality criteria for *Cryptosporidium* and *Giardia* at a future time, EPA may validate EPA Methods 1622 and/or 1623 for use in the NPDES Program.

C. Limitations of Determinative Technique of Proposed *Cryptosporidium* and *Giardia* Methods and Potential for False Positives

Several comments expressed concern regarding the subjectivity and limitations of the immunofluorescence assay (IFA)-based determination procedure in EPA Methods 1622 and 1623 and the related potential for false positives. EPA acknowledges that IFA relies on analyst training and experience for reliable results. However, EPA Methods 1622 and 1623 provide the analyst with three microscopy tools to aid in the identification of potential target particulates during microscopic examination. The methods provide detailed, progressive criteria for determining whether a particulate is a *Cryptosporidium* oocyst or a *Giardia* cyst based on the use of these tools and include the use of immunomagnetic separation (IMS) as the sample cleanup procedure to minimize the transfer of non-target particulates to the slide. Nonetheless, the inherent technical judgement involved in the determinative step in EPA Methods 1622 and 1623, combined in some cases with interfering materials and/or cross-reactivity of the antibody stain, may still lead to false positives or false negatives. Although other determinative techniques that are currently under development have the promise of providing less-subjective assessments of the presence of *Cryptosporidium* oocysts and *Giardia* cysts in a sample, these techniques are not yet validated and are therefore not yet appropriate for EPA approval for ambient water monitoring. Extensive details on the performance of EPA Methods 1622 and 1623, including inter- and intra-laboratory precision and recovery of the methods at multiple laboratories and on a variety of ambient water types (*i.e.*, validation), are provided in the Results of the Interlaboratory Validation Study of EPA Method 1622 (EPA-821-R-01-027), the Results of the Interlaboratory Validation Study of EPA Method 1623 (EPA-821-R-01-028) and the Implementation and Results of the Information Collection Rule Supplemental Surveys (EPA-815-R-01-003), which were included in the docket for the proposal. Given the robustness of the validation procedure, the Agency is confident that although the IFA technique requires specialized training, overall, the methods will provide for valid *Cryptosporidium* and *Giardia* precision and recovery for use in ambient waters.

D. Application of Performance-Based Measurement System (PBMS) Concept to EPA Methods 1622 and 1623

Several commenters recommended that the performance of alternate antibody reagents be evaluated for EPA Methods 1622 and 1623 using a quantitative PBMS approach. EPA agrees with the comments, and considers the PBMS Tier 2 validation approach described in Methods 1622 and 1623, Section 9, to be appropriate for antibody stains and IMS. However, EPA does not believe that the PBMS Tier 2 validation approach is adequate to assess the comparability of methods with different determinative techniques, such as comparing a polymerase chain reaction (PCR)-based method to an IFA-based method. Use of a different determinative technique is generally considered to be a different method, rather than a modified version of a method because it is usually very difficult to compare methods that use different determinative techniques. For example, the filtration/IMS/IFA technique employed in Methods 1622 and 1623 differs considerably from genetic tests because the former measures the infective form of *Cryptosporidium* and *Giardia*, while the latter measures genetic material (DNA or RNA). Similarly, the membrane filtration method for bacteria differs from an MPN method for bacteria because the former is a direct quantitative method, whereas the latter employs a qualitative statistical index rather than an actual enumeration of the number of organisms present in the sample. An appropriate approach for these comparisons would be to perform side-by-side tests. This approach is outlined in the draft guidance document, EPA Microbiological Alternate Test Procedure (ATP) Protocol for Drinking Water, Ambient Water, and Wastewater Monitoring Methods, Guidance (EPA-821-B-03-004).

VI. Statutory and Executive Order Reviews

A. Executive Order 12866: Regulatory Planning and Review

Under Executive Order 12866 (58 FR 51735 (October 4, 1993)), the Agency must determine whether the regulatory action is "significant" and therefore subject to Office of Management and Budget (OMB) review and the requirements of the Executive Order. The Executive Order defines "significant regulatory action" as one that is likely to result in a rule that may:

(1) Have an annual effect on the economy of \$100 million or more, or adversely affect in a material way the