

other than bacteriology, ASM suggested that it was their experience that these reagents and stains perform as well as the reagents surveyed for bacteriology.

- Molecular amplification control procedures should adhere to standards outlined in the NCCLS document "Molecular Diagnostic Methods for Infectious Diseases, MM3-A, 1995." At a minimum, control procedures for these tests should validate cell lysis, absence of inhibitors, absence of contamination, and adequate amplification. The following controls should be included with each run:

- Positive control (low range of assay sensitivity).
- One to five negative controls.
- Internal control.
- Quantitative assays should include two to three standards of known copy number. For microbial genotyping, control procedures should include at least two isolates of the same species being tested. One isolate should have the same phenotype as the unknown, and one should be a different phenotype.

*Response:* Our responses to the above recommendations are set forth below.

We agree that the mycology requirement for control testing of nitrate assimilation on auxanographic media is not relevant for the large majority of laboratories performing fungal identification, and have deleted that requirement. If laboratories use the procedure, they will be required, as stated formerly at § 493.1218(f) (now at § 493.1256(e)(1)) to test the medium and reagents with each batch (prepared in-house), lot number (commercially prepared), and shipment when prepared or opened. This will be the same control testing as required for other reagents and media used for fungal identification procedures.

The language formerly at § 493.1233(c) (now at § 493.1264(c)) requires laboratories to check permanent stains each month of use by using a fecal sample control. This terminology does not preclude the use of a fecal sample as a control or a commercially prepared control slide. The requirement remains as written in existing CLIA regulations; however, we will note this clarification in Appendix C of the State Operations Manual (CMS Pub. 7).

We recognize ASM's concern that the mycobacteriology decontamination process be monitored and adequately controlled to ensure that the decontaminating agent is of the proper strength to kill contaminating organisms without destroying mycobacteria (especially *Mycobacterium tuberculosis*). However, the method they

suggested for doing this is only one way in which it may be accomplished. There are a number of other ways in which this process may be controlled (for example, monitoring the contamination rate over time to ensure the appropriate organisms are being killed). In an effort to maintain flexibility in CLIA regulations, in this final rule, we are not adding this ASM proposed control requirement to those for mycobacteriology. As noted formerly at § 493.1103(a) (now at § 493.1232), the laboratory must establish and follow written policies and procedures that assure optimum integrity of patient specimens from the time they are collected until testing has been completed and results reported. In addition, former § 493.1103(a) (now at § 493.1242(a)(6)) requires laboratories to have and follow written policies and procedures for specimen processing, and former § 493.1703 (now at §§ 493.1249(a) and (b)) requires the monitoring and assessment of these policies and procedures, and the implementation of corrective actions to resolve problems that are identified. These requirements ensure that the processing of mycobacterial specimens is monitored, assessed, and controlled, while allowing the laboratory to use any of several acceptable methods to do so.

We agree with ASM that, whenever possible, the frequency for control testing should be standardized for all microbiology subspecialties. Frequencies for individual reagents and stains are not specified in CLIA regulation for mycology and virology. For parasitology, a frequency requirement (to test once a month) is only given for permanent stains. The frequency requirement for all other reagents and stains in these subspecialties is the default contained in the general control procedure requirements that are now at § 493.1256(e)(1) and (2).

We agree appropriate requirements for molecular amplification procedures are needed, and that the NCCLS standards are an excellent reference for laboratories to use. Requirements addressing most of the recommendations made by ASM for amplification procedures are included in CLIA regulations, although not as specifically as suggested by this organization. CLIA regulations require the laboratory director to have control procedures to monitor the complete analytic process. For amplification procedures this includes, in general, validating cell lysis and ensuring absence of inhibitors, absence of contamination, and adequate amplification. The CLIA requirements

for control procedures for all tests are now at § 493.1256(d). This provision requires all laboratories to follow manufacturer's instructions for control testing, and to, at minimum, conduct a test that includes two control materials of different concentrations (a positive and negative control are required for qualitative tests) on each day patient specimens are tested. CLIA regulations require that if the laboratory determines additional numbers or types of controls, or a greater frequency of running controls is needed to detect immediate error and monitor test performance over time, the numbers, types, and or frequency of controls must be increased accordingly.

While we agree with the recommendation made by ASM describing the positive and negative controls that should be used for molecular amplification procedures, the CLIA control requirements are minimum requirements and do not specify that a positive control must be at the low range of assay sensitivity, or that more than one negative control be tested daily. Likewise, these minimum requirements do not specify the types of controls that must be included with microbial genotyping, but only that two controls must be tested each day patient specimens are tested.

However, if test system instructions specify such control testing, or if the laboratory determines (during its initial evaluation of the test system at § 493.1253) that more controls are needed, the additional control testing must be performed.

For molecular amplification procedures, ASM also recommended the inclusion of an internal control in each run, primarily to detect inhibition of the amplification process. We agree that for some amplification procedures the presence of inhibitors or interfering substances in certain specimens may cause false negative test results, and that for these procedures, a control system is necessary to detect inhibition. However, as noted by NCCLS, inhibitors are not a significant source of false negative results for every test, and if inhibitors or interfering substances are encountered only rarely, NCCLS does not recommend running controls for inhibition. Therefore, we have added a requirement at § 493.1256(d)(3)(v) that states, if reaction inhibition is a significant source of false negative results, the laboratory must include a control system to detect such inhibition.

In response to the ASM recommendation that quantitative assays include two to three standards of known copy number, as stated above, under CLIA regulations, quantitative