**Section 450.1150 Quality Control System Methodologies**

a) Hematology

1) Manual Procedures

A) Each procedure shall be checked or recalibrated each day of use with standards (calibrators) or reference materials covering the range of expected values. See Section 450.520 for checking dilutors and samplers.

B) Hemoglobin – methodology shall be calibrated monthly with standards that cover at least three concentrations and a zero point.

C) Hematocrit – Optimum packing time of microhematocrit centrifuges shall be determined before being placed into use and after major adjustments or repairs. The speed of the microhematocrit centrifuge shall be checked monthly. Tolerance limits shall be established. Timer checks shall be performed monthly. Tolerance limits shall be established.

D) Red and White cell counts – The hemocytometer counting chamber and coverslip shall be maintained in a condition that does not interfere with cell recognition or the volume of the chamber. Coverslips certified by the Bureau of Biological Standards shall be used. Counts shall be performed with certified pipettes or pipettors whose accuracy has been determined by the manufacturer.

E) Platelet counts – Manual platelet counts shall be performed by counting both sides of the chamber. Tolerance limits shall be established. A procedure to compare platelet results with the differential blood film shall be established.

F) Differential Leukocyte count – Blood smears shall be prepared and stained by a method which produces smears in which morphologic cell features can be evaluated. Cellular morphology shall be examined and platelets estimated routinely with the differential count.

2) Automated Procedures

A) Particle Counting and Hemoglobin

i) Calibration techniques shall follow the manufacturer's specifications.

ii) The director shall establish criteria for high and low counts and determine the policy for verification. Tolerance limits shall be established for duplicate testing.

iii) Background counts shall be performed daily on diluent and lysing agents.

iv) Reference materials shall be used each, or after each run to assess precision.

v) Each procedure shall be checked or recalibrated each 8 hours, if the instrument is used during the 8 hour period, with standards (calibrators) or reference materials covering the range of expected values.

B) Differential counts

i) The manufacturer's specifications shall be followed with respect to operation, calibration, and the use of reference materials.

ii) The director shall establish a policy for the review of all abnormal differentials that indicate an abnormal cellular, morphology or abnormal platelet enumeration.

3) Coagulation studies

A) Two levels of reference materials for prothrombin and or partial thromboplastin times shall be used during each 8 hours when the instrument is used, Action limits shall be established.

B) If available commercially, two levels of reference materials shall be included in each run for all other coagulation procedures. Patient specimens shall be performed in duplicate and tolerance limits established.

b) Chemistry

See Section 450.1120 for general quality control requirements. See Section 450.520 for checking dilutors and samplers.

1) Manual-Automated procedures which use a Spectrophotometer or Photometer

A) Calibration of the optical component of each instrument shall be done in accordance with the instrument manufacturer's instructions.

B) Each procedure shall be recalibrated at least every three months or more frequently in accordance with the following:

i) Procedures which are linear shall include at least 3 standard concentrations (calibrator) (unless the instrument manufacturer specifies that 3 calibrators are not necessary to determine procedure in linearity and calibration over the reportable range) including one at the highest level of the reportable range and one near the threshold (cutoff).

ii) Procedures which are non-linear over the reportable range shall include (unless the instrument manufacturer specifies that procedure calibration over the reportable range can be accomplished in another manner) a minimum of 5 standard concentrations (calibrator).

iii) The procedure is recalibrated when major instrument maintenance has been performed.

iv) The procedure is recalibrated in accordance with the manufacturer's recommendations and when a reagent lot number is changed.

v) The procedure is recalibrated when the quality control program reflects an unusual trend or the controls fall outside acceptable limits.

C) At a minimum, one reference material and one calibrator or two reference materials with different concentrations shall be used for each analyte in each run of unknown specimens, except, when prepackaged reagent analyzers are used, one reference material and one calibrator or two reference materials with different concentrations shall be used once in each 24 hour period in which the analyzer is used for that analyte.

2) Atomic Absorption Flame Photometers

A) The atomization rate shall be checked each day of use.

B) Each run of unknown specimens shall include two levels of reference materials.

C) Calibration and operation techniques shall follow the manufacturer's specifications.

D) Each procedure shall be recalibrated each day of use.

3) Chromatography

A) A standard (calibrator) shall be included with each batch of unknown specimens.

B) Calibration and operation techniques shall follow the manufacturer's specifications.

C) Reference materials (spiked samples) shall be included in each batch of unknown specimens and are treated the same as unknowns.

4) Electrophoresis

A) The linearity of a densitometer shall be checked each day of use.

B) Reference materials for comparison of migration patterns and stain intensity shall be included with each run.

5) Ion Selective Electrode

A) The manufacturer's recommendations shall be followed with respect to calibration and control procedures.

B) Reference materials shall be included with each run.

6) Radioimmunoassay

A) The stability of radioisotope counting equipment shall be checked each day of use with an appropriate radioactive reference source. Tolerance limits shall be established.

B) Background counts shall be performed each day of use and tolerance limits established.

C) Each procedure shall include calibrators (standards) as recommended by the reagent manufacturer.

D) Reference materials shall be included with each run.

E) The duration of the counting times shall follow the recommendations of the instrument manufacturer.

7) Mass Spectrometry

A) Mass spectrometers shall be tuned daily.

B) Procedures for checking air leaks and determining ion ratios shall be available and followed.

C) Ion ratios shall be determined for each instrument and each assay if appropriate for the instrument.

D) If ion ranges are used, criteria shall be available for designating a positive.

c) Urinalysis

1) Specific gravity equipment shall be calibrated with distilled water and one other solution of known refractive index each day of use.

2) Screening or qualitative chemical urinalysis shall be checked daily by use of suitable reference materials.

3) Calibration and the use of reference materials for equipment which utilizes automatic readers shall follow the recommendations of the manufacturer.

d) Bacteriology-mycology

1) Each unit of media shall be properly labeled to indicate identity, date of preparation-receipt and expiration date.

2) Each batch of media shall be tested before use, or concurrently with selected organisms, for selectivity, sterility, enrichment, and biochemical response, as currently required under 42 CFR 493.1256(e)(4).

3) Appropriate ATCC strains shall be available and maintained.

4) All reagents, strips, discs, and antisera shall be properly labeled as to lot number and expiration date and checked each day of testing with organisms that produce positive and negative reactions.

5) An adequate incubation system shall be used and shall be appropriate for the kinds of organisms isolated and volume of work. CO2 incubators shall be checked daily to insure that CO2 concentration is maintained within established tolerance limits.

6) Flow charts may be used to indicate all steps to be employed to isolate and identify all organisms.

7) The daily log or worksheet shall reflect all tests and test results which lead to the isolation and identification of all microorganisms.

8) Staining materials shall be checked each day of use against organisms with the expected staining characteristics.

9) A wire loop used for quantitative tests shall be calibrated prior to placing into use and quarterly thereafter.

10) Agar Disc Diffusion methods:

A) The agar disc diffusion test shall be checked with each new batch of media and at least once each seven days with stock cultures of Escherichia coli ATCC 25922, Staphylococcus aureus ATCC 25923, and Pseudomonas aeruginosa ATCC 27853. Zone sizes shall be recorded for each antimicrobial agent. Limits shall be established.

B) Petri dishes used shall have a diameter not less than 150 mm and contain no more than 12 discs.

C) Susceptibility tests shall be performed on pure cultures only.

D) A barium sulfate turbidity standard shall be used for the Kirby-Bauer method.

11) Minimum Inhibitory Concentration (MIC) Methods:

A) The MIC test shall be checked with each new batch of media and at least once each seven days with stock cultures of Escherichia coli ATCC 25922, Staphylococcus aureus ATCC 29213, and Pseudomonas aeruginosa ATCC 27853. The MIC values shall be recorded for each antimicrobial agent. Tolerance limits shall be established.

B) When trimethoprim-sulfamethoxazole is included in the battery of antibiotics, Streptococcus faecalis ATCC 29212 shall also be included as a control.

12) Automated susceptibility testing systems shall follow the quality control requirements specified by the manufacturer or at a minimum those specified under item 11 above.

e) Parasitology

1) A calibrated ocular micrometer shall be available for determining the size of ova and parasites when size is a critical factor.

2) The laboratory shall have an atlas and reference collection of prepared slides, transparencies or gross specimens. The collection shall include organisms which the laboratory encounters and reports from patient specimens.

3) Permanent stains shall be used for the examination of intestinal protozoa and other parasites where internal structure is critical for proper identification.

4) Concentration methods shall be routinely employed on all stool specimens negative for ova and parasites by direct examination methods. Concentration techniques shall be capable of detecting all cases of clinically significant parasites likely to be encountered in the community.

f) Immunology-Serology-Immunochemistry

Kits purchased for serological testing shall be used in accordance with the manufacturer's instructions.

1) VDRL/RPR

A) Non-reactive, minimally reactive, and reactive reference materials shall be included with each run.

B) The needle delivery shall be verified within plus or minus two drops per ml each time a new needle is used, when control patterns cannot be reproduced, and when the antigen does not drop clearly from the needle.

C) The revolutions per minute of the rotator shall be checked each week of use and be within the recommended tolerance limits.

D) Each new lot of antigen and reference materials shall be checked with non-reactive, weakly reactive and reactive reference materials before being placed into use.

E) Ambient temperature in the test area shall be maintained between 23 degrees Centigrade and 29 degrees Centigrade.

F) The antigen for VDRL testing shall be prepared fresh each day of use.

2) Qualitative tests

Positive and negative controls shall be included in each run. Each new lot of reagents and reference materials shall be parallel checked with one of known reactivity before being placed into use.

3) Quantitative tests

Each quantitative test shall include with each run a negative control, where applicable, a positive control of known titer or controls of graded reactivity. Each new lot of reagents and reference materials shall be parallel checked with one of unknown reactivity before being placed into use.

g) Immunohematology

1) ABO grouping reagents and Rh typing sera shall conform to the requirements of licensure under 21 CFR 600-680. Any facility which produces their own products shall adhere to these same requirements.

2) All antisera, ABO reagent red cells, anti-human globulin (Coombs) shall be tested each day of use with a positive control.

3) Antibody screening reagent red cells shall be tested each day of use with at least one known antibody.

4) All antisera except ABO antisera shall be tested each day of use with a negative control.

5) The reagent manufacturer's protocol for testing shall be followed.

6) An autologous cell control is required when samples are being tested for Rh type. An autologous cell control is not required to accompany the Rh type when testing donor samples.

h) Histopathology

1) All special stains shall be controlled by use of positive tissues.

2) All tissue specimens shall be kept in a preservative until microscopic examination and diagnosis have been completed by the pathologist.

3) All stains shall be filtered prior to each day of use.

4) All tissue processing solutions shall be changed or rotated on a regularly scheduled basis.

5) The quality of stains shall be evaluated daily by the director and suboptimal stains corrected immediately.

6) All gross tissue specimens received shall be properly labeled and securely packaged so as to maintain absolute certainty of identification throughout processing, recording and storage.

7) Slides shall be identified with permanent labels and stored so they are readily accessible. Paraffin blocks shall be adequately identified, indexed, stored in a cool place and protected against damage by heat for at least 2 years. Wet tissue specimens shall be retained until a diagnosis has been made. The slide and a copy of the report shall be filed for at least 10 years.

8) The laboratory shall request that the tissue request shall contain the name, birthdate, name of the surgeon, clinical information and the date of surgery.

i) Cytogenetics

1) Special Equipment

A) Incubators shall be on special emergency lines.

B) Laminar Flow Hoods shall be used.

C) Karyotyping facilities shall be available with the production of hard copies.

2) Culture Initiation of Specimens

A) At least two (2) containers for each patient

B) Maximum of 1% patient failure (i.e. failure to provide a report as defined in Section 450.1150(j)(3)), for blood, amniotic fluid and chorionic villus samples in a period not to exceed 30 calendar days. If in excess of 1%, the laboratory director shall contact the Department and stop performing the tests until the laboratory can demonstrate a patient failure rate of less than one percent.

C) For other tissues higher patient failure rates are acceptable.

i) Skin and products of conceptions: maximum of 20% failure in a period not to exceed 30 calendar days. If in excess of 20%, the laboratory director shall contact the Department and stop performing the tests until corrective action is demonstrated.

ii) Bone Marrow: maximum of 5-10% failure in a period not to exceed 30 calendar days. If in excess of 5-10%, the laboratory director shall contact the Department and stop performing the tests until corrective action is demonstrated.

3) Analysis and Interpretation

A) Counting Chromosomes

i) At least 11-20 metaphases from the two containers shall be counted for routine blood, amniotic fluid, skin, products of conception, and chorionic villus specimens.

ii) For the Fragile-X chromosome, a minimum of 100 metaphases is required before reporting a negative result. Control values for Fragile-X shall be maintained.

iii) If a clinically significant hypermodal metaphase or a structurally abnormal chromosome is detected, 20 additional cells (or 10 additional centers) in each of the two cultures shall be analyzed.

iv) If 2 clinically significant hypomodal metaphases are detected, repeat steps in subsection (3)(A)(iii).

B) Karyotypes

i) A 400 band resolution is minimum.

ii) At least two banded karyotypes (hard copies) shall be prepared for routine bloods, amniotic fluids, chorionic villus specimens, skins, and products of conception.

iii) For bone marrows, at least 25 metaphases shall be photographed and analyzed. A minimum of 20 cells shall be analyzed for the presence of the Philadelphia chromosome and other markers for chronic myelogenous leukemia.

C) Reporting and Interpretation

i) All reports shall adhere to the current International System of Cytogenetic Nomenclature.

ii) All abnormal findings should be accompanied by a recommendation to consult a Geneticist.

D) Documentation

In addition to other documentation required for any laboratory, documentation of power failure, failure rate, contamination, labeling discrepancy, poor or no growth, poor slide quality, interpretive dilemmas, and diagnostic errors shall be maintained.

4) Archives

Retention of adequate slides, films, hard copies and reports in order to re-analyze any cases challenged, shall be in accordance with the State statute of limitations.

j) Toxicology – Controlled Substances (Drugs of Abuse)

Laboratories which perform tests for controlled substances shall meet all pertinent requirements of the Act and regulations. In addition, the following items shall apply to toxicology laboratories.

1) The laboratory shall demonstrate proficiency as required under Section 450.720, except, the laboratory shall discontinue providing confirmatory testing if for two consecutive testing periods the laboratory either fails to report results for confirmatory testing or for two consecutive testing periods the laboratory fails to confirm the presence of any substance in any proficiency testing specimen or on one occasion falsely confirms and reports the presence of substances not in the test specimen. Reinstatement to offer confirmatory testing shall require errorless performance in two subsequent proficiency testing surveys.

2) The director shall provide confirmatory testing of specimens whenever initial screening shows the presence of controlled substances. The confirmatory testing shall use different principles of chemistry and be at least as sensitive as the testing used for screening purposes. Drug screening may be performed on-site with confirmatory testing at a licensed laboratory or licensed toxicology laboratory.

3) Reports from the laboratory shall include limits of detection (LOD) for methods utilized and identify the method used to confirm positive screening results. Only specimens confirmed positive shall be reported positive for a specific drug or metabolites.

4) Each analytical run of specimens shall have at least three reference specimens including: a specimen containing no drug or metabolites; a specimen with a known amount of standard at or near the threshold (cutoff), and one additional reference specimen. Documentation that currently used methodology does not allow carryover to contaminate the testing of a subject's specimen, shall be maintained. A minimum of 10 percent of all test samples analyzed per batch shall be a mixture of reference specimens indicated in this subsection (j)(4).

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