D5531
(Rev. 140, Issued: 05-29-15, Effective: 05-29-15, Implementation: 05-29-15)

§493.1265 Standard: Virology

(a) When using cell culture to isolate or identify viruses, the laboratory must simultaneously incubate a cell substrate control or uninoculated cells as a negative control material.

Interpretive Guidelines §493.1265(a)

When condition-level deficiencies in Virology are identified in any or all phases of testing, use D5010.

Any laboratory testing patient specimens for the Human Papillomavirus (HPV) must enroll and successfully participate in a CMS-approved proficiency testing program for HPV beginning in 2008. Laboratories should refer to Subpart H for further information. The laboratory's CLIA certificate must include the subspecialty of Virology. The

laboratory must also be in compliance with all of the CLIA regulations governing the preanalytic, analytic, and post analytic phases of testing including proficiency testing and personnel requirement.

Cell Culture

For commercially purchased cell culture media, the requirement for media quality control checks is satisfied by visually examining the media for sterility and ensuring the ability of the media to sustain cell life. If the media is prepared or produced in the laboratory, use D5477:

- Each component of cell culture media should be checked for sterility using bacterial culture techniques. In addition, fetal bovine serum must be checked for toxicity using cell culture systems;

- The combined product (e.g., Hanks, Eagles and Earles) should be checked for sterility using bacterial culture techniques and the ability to propagate growth with cell cultures; and

- Cell culture systems should be checked for mycoplasma contamination at regular intervals established by the laboratory.

Non-Culture Methods

1. For other non-culture identification (e.g., antigen identification) systems that are used for viral identification, the laboratory is not required to maintain live viral cultures for quality control purposes. However, positive and negative controls are required to evaluate the detection phase, if such controls are available commercially or in the laboratory. Use D5449 and/or D5453 as appropriate.

2. If organism controls are not available, a previously extracted viral antigen as the positive control plus a previously confirmed negative control of the same matrix as the patient sample may be used. Use D5485. A positive organism control must be subjected to the extraction process if such a control is available in the laboratory. Use D5453.

3. For fluorescent stains, the control requirements are met by using virus-infected cells for a positive control among uninfected cells for a negative control. Use D5475.

The intent of the regulations is for the laboratory to have methodologies available to isolate and identify the viruses that are etiologically related to the clinical disease for which services are offered. For example, if a laboratory offers services only for Herpes testing, it must have available host systems for the isolation and/or test methods for the identification of the Herpes virus. If the laboratory is not using the appropriate host system, use D3007.

“Host system” is defined as the animal, egg or cell culture model, which supports the propagation of viruses.

Clinical information important for the determination and selection of the proper host system should include (Use D5305):

- Clinical symptoms of the patient;
- Age of the patient;
- Source of the specimen;
- Date of onset of clinical symptoms;
- Recent travel information of patient;
- Test request; and
- Date of specimen collection.

Cell culture is the host system used most frequently. The specific cell line (type) is usually selected based upon its known sensitivity and susceptibility to different viruses. For example, the cell lines to be used as host systems for the following clinical specimens could be:

- Upper respiratory infection specimens: Primary Monkey Kidney (PMK), Human Fetal Diploid Lung (HFDL), or equivalent;
- Enteric specimens: PMK, Human Fetal Diploid Kidney (HFDK), or equivalent;
- Urine specimens: HFDL, PMK, or equivalent;
- Genital specimens: Human Foreskin (HFD), Vero (Continuous Monkey Kidney), or equivalent;
- Vesicular lesions: HFDL, PMK, BSC-1 (Monkey Cell Line), or equivalent; and
- Tissues or Spinal fluids: PMK, Vero, BSC-1, HFDK or HFDL, or equivalent.

Prior to the inoculation of the cell cultures, the laboratory should check the cell culture systems for the following:

- The age of the cell culture monolayer (no more than 7-10 days post “seeding”) (Use D5417);
- Maintenance media that is free from inhibitory substances (Use D5477); and

- Sterility (visual observation for turbidity) (Use D5477).

Uninoculated cell substrate controls are used to determine whether the specificity of a test system has been ensured. Generally, an uninoculated cell control for each cell line that is inoculated is used per inoculation day to determine whether the consequent cytopathic effect (CPE) in the cells inoculated with patient specimen was caused by specific etiologic agent(s), or caused by the nonspecific deterioration of the cells themselves. Often, as monolayer host cells age, the cells deteriorate, exhibiting "rounding" and "pulling-apart." This cell change may be confused with CPE if uninoculated cells are not available to compare with the inoculated cells.

Probes §493.1265(a)

How does the laboratory determine the specific cell line to be used as the host system? Use D3007 or D5411 as applicable.

When reviewing the laboratory's identification procedures for the clinical diseases for which services are offered, how does the laboratory rule out the presence of Clostridium difficile toxin in those cell cultures in which the patient specimen exhibits non-specific effects unrelated to viral cytopathic effect (CPE)? Use D3007 or D5411 as applicable.

If presumptive reports are issued based on CPE, how does the laboratory confirm the identification reported? Use D3007 or D5411 as applicable.

For tests such as hemagglutination inhibition and viral neutralization in which antisera must be standardized, how has the laboratory determined the optimum dilution of the antisera to ensure maximum sensitivity and specificity? Use D5437.

Neutralization Tests

How does the laboratory standardize its dilution of the viral isolate and control virus to the appropriate Tissue Culture Dose 50 or equivalent, each time the test is performed? Use D5437.

How many varieties of uninoculated cell cultures does the laboratory use to check each new lot of anti-serum or serum pool for toxicity? Use D5477 or D5479 as applicable.

Hemagglutination Inhibition Tests

After having determined the hemagglutination titer, how does the laboratory determine the working dilution of the viral isolate (i.e., usually 4 Hemagglutination units)? How does the laboratory ensure that this working dilution is correct for isolates and controls? Use D5421 or D5423 as applicable.

How often and for which hemagglutination inhibition tests does the laboratory include a

serum/cell/buffer control and a cell/buffer control? Use D5425.

Does the laboratory include one known virus or viral antigen specific to each antisera used in the test procedure? Use D5449.

Direct Immunofluorescence Tests

How does the laboratory determine which immune serum conjugate(s) to use when identifying viruses using antisera that react with viruses that are etiologically similar (e.g., an antigen test for specimens from patients with flu-like symptoms that identifies Respiratory Syncytial Virus, Influenza, and Parainfluenza)? How does the laboratory ensure the specificity of this conjugate for the specific virus being identified? Use D5421 or D5423 as applicable.

How does the laboratory rule out non-specific reactivity for each conjugate used? Use D5421 or D5423 as applicable.

Indirect Immunofluorescence Tests

Has the laboratory determined the optimum dilution of its anti-species, e.g., antibody to host system or cell culture (such as anti-PMK, conjugated immune serum)? Use D5421 or D5423 as applicable.

Has the laboratory determined the optimum dilution of the virus specific immune serum? Use D5421 or D5423 as applicable.

Determine whether the laboratory is checking positive and negative reactivity using (Use D5475):

- Uninoculated cells plus immune serum plus anti-species conjugate (negative control); and

- Viral antigen or known virus infected cells plus immune serum plus anti-species conjugate (positive control).

Determine whether the laboratory checks each new batch or shipment of conjugate using known virus infected cells plus PBS plus anti-species conjugate. Use D5471.

§493.1265 Standard: Virology

(b) The laboratory must document all control procedures performed, as specified in this section.

Interpretive Guidelines §493.1265(b)

QC records must identify the host cell cultures employed, the number of tubes or plates

inoculated or uninoculated, maintenance medium used, the number of times the patient specimen was sub-cultured, the specific sub-culture or passage in which the virus was identified, the CPE observed, and post inoculation date of observations. If the deficiency is due to absence of dates of testing and observations, use D5787.

§493.1267 Standard: Routine chemistry

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For blood gas analyses, the laboratory must perform the following: Interpretive Guidelines §493.1267(a)-(d)

When condition-level deficiencies in Routine Chemistry are identified in one or more phases of testing, use D5016.

Control materials generally are not available to verify the reportable range at the very high range of patient results. When necessary, the laboratory may verify the results by splitting patient samples and assaying them on two different blood gas analyzers.

Quality control records should include lot numbers, date prepared/opened, expiration dates, the actual measurements, reaction and/or observations and demonstrate that controls were tested as required.

Do not dictate the acceptable format for documentation.

Probes §493.1267(a)-(d)

For blood gas testing, do the records include barometric pressure and room temperature, as necessary?

Do the records of a laboratory that moves from testing site to testing site demonstrate the performance of control samples following transport of equipment when such activity affects test performance specifications and/or instrument calibration?