

**Supplemental Assay Method for Examination of Porcine Cells for Porcine Parvovirus Contamination**

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**United States Department of Agriculture  
Center for Veterinary Biologics  
Testing Protocol**

**SAM 801**

**Supplemental Assay Method for Examination of Porcine Cells for Porcine  
Parvovirus Contamination**

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**Supplemental Assay Method for Examination of Porcine Cells for Porcine Parvovirus Contamination**

**1. Introduction**

This Supplemental Assay Method (SAM) describes an *in vitro* test procedure for examining porcine origin vaccine production cells for the presence of porcine parvovirus. The procedure uses several methods, e.g., immunofluorescence, hemagglutination, and aniline dye staining.

**2. Materials**

**2.1 Equipment/instrumentation**

Equivalent equipment or instrumentation may be substituted for any brand name listed below.

**2.1.1** Four or more sterile plastic or glass tissue culture containers having at least 75-cm<sup>2</sup> surface area

**2.1.2** At least 1 rack of sterile Leighton tubes with coverslips or 2 flats of Tech slides

**2.2 Reagents/supplies**

Equivalent reagents or supplies may be substituted for any brand name listed below.

**2.2.1** Enough production cells and media to seed 2 containers from each production lot of cells to be tested

**2.2.2** A supply of pretested serum that is normally used by the manufacturer. In the case that porcine serum is used, it shall be negative by either serum neutralization (SN) or hemagglutination inhibition (HI) for porcine parvovirus antibodies.

**2.2.3** A supply of medium that is normally used to propagate production of swine cells and will grow a monolayer in 6 to 7 days

**2.2.4** An anti-porcine parvovirus conjugate. An initial supply will be furnished by the National Veterinary Services Laboratories (NVSL) for reference.

**2.2.5** An aniline dye stain that will demonstrate intranuclear inclusion bodies (e.g., May Grunwald-Giemsa or Shorr's)

**2.2.6** Guinea pig red blood cells

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**2.2.7** A supply of pretitered porcine parvovirus. The seed will be furnished by the NVSL.

**3. Personnel qualifications/training**

Personnel performing the test must have experience or training in this protocol. This includes knowledge of aseptic biological laboratory techniques and preparation, proper handling and disposal of biological agents, reagents, tissue culture samples, and chemicals. Personnel must also have knowledge of safe operating procedures and policies, as well as training in the operation of the necessary laboratory equipment listed in **Section 2.1**.

**4. Performance of the Test**

**4.1** At least 2 cell culture containers are seeded with each lot of porcine cells to be tested. (A lot shall consist of equal aliquots of cells from pigs in 1 litter only.) The trypsin used to disperse these cells shall be from a pretested negative lot or the trypsin shall have been sterilized with Beta-propiolactone. The cells are incubated at 35°- 37°C until a complete monolayer has formed.

**4.2** After the monolayer has formed, remove the cells either with BPL treated trypsin in ATV or by scraping. The cells from the 2 flasks are pooled and redispersed in fresh growth medium. The cells are reseeded in fresh sterile containers at a 1:1 ratio, unless the manufacturer has a ratio that grows better in their experience. There shall be at least 2 containers to continue the test. Incubate as before in **Section 4.1**.

**4.3** At the time that the cell spread is essentially complete, the cells are removed from the containers as described in **Section 4.1** and redispersed (1 container is reseeded into a sterile fresh container). At this time, the equivalent of 1 container is seeded onto at least 20 Leighton tubes with slides or 10 Tech slides. At this time, at least 10 of these Leighton tubes or 4 Tech slides are inoculated with 0.1 mL of swine parvovirus diluted to contain approximately 100 TCID<sub>50</sub>s per tube. All vessels are incubated as before.

**4.4** On the 2nd day postseeding, at least 2 Leighton tubes or 1 Tech slide from the test cell tubes and 2 Leighton tubes or 1 Tech slide from the positive control tubes are removed and fixed for fluorescent antibody staining. This procedure is repeated on the 3rd, 4th, and 5th day. The slides may be stained every day or may be held at -20°C until all have been fixed.

On the 7th day, the fluid from 1 container is removed and a hemagglutination test is conducted using the 1:2 through 1:32 dilutions. At least 2 Leighton tubes or 1 Tech slide of test cells and 2 positive control Leighton tubes or 1 Tech slide are removed on the 1st day the positive slides show fluorescence. The slides are fixed for aniline dye staining and stained.

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**5. Interpretation of the Test Results**

The cells are satisfactory for freedom of porcine parvovirus if they do not fluoresce specifically after FA staining. The positive control cell shall stain positively.

The fluids taken for the HA test shall not show any hemagglutination. The aniline dye stained coverslips shall not exhibit any intranuclear inclusion bodies. The positive control cells may or may not show inclusions.

**6. Report of Test Results**

Report results of the test(s) as described by standard operating procedures.

**7. References**

Title 9, *Code of Federal Regulations*, section 113.51, U.S. Government Printing Office, Washington, DC.

**8. Summary of Revisions**

**Version . 04**

- The coversheet and contact information have been updated.

**Version .03**

- The Contact information has been updated; however, the Virology Section has elected to keep the same next review date for the document.

**Version .02**

- The Contact information has been updated.

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