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Center for Veterinary Biologics
Testing Protocol

SAM 803

Supplemental Assay Method for Examination of Trypsin Solutions for Porcine Parvovirus Contamination

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1. Introduction

This Supplemental Assay Method (SAM) describes an *in vitro* test method that uses a cell culture system and uses different methods, e.g., immunofluorescence, hemagglutination, and aniline dye staining, to determine whether trypsin contains endogenous porcine parvovirus (PPV).

2. Materials

2.1 Equipment/instrumentation

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

2.1.1 A supply (8 or more) of sterile plastic or glass tissue culture flasks of at least 75-cm^2 surface area each

2.1.2 At least 2 racks 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 Porcine parvovirus susceptible cells, either primary embryonic or a swine cell line, which will grow out in 4 to 5 days

2.2.2 A certified contamination free bovine calf serum

2.2.3 A medium that has been found of sufficient nutriative value to grow a monolayer of primary swine kidney cells in 4 to 6 days. Suggested media are CMRL (Grand Island Biological Company) or Alpha MEM (Flow Laboratories), both supplemented with 1% sodium pyruvate and containing 50 mcg Gentimycin per mL.

2.2.4 Porcine parvovirus fluorescent antibody conjugate

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

2.2.6 Guinea pig red blood cells

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 All operations involving trypsin, until it is inoculated, are conducted at ice bath temperature.

4.2 Five grams of trypsin are put into solution in a sufficient volume to fill the head of a high speed centrifuge. For example, 5 gms in 162.0 mL (a 3.09% solution) will fill the 50 Ti head of a Spinco L-2 Sterile rabbit serum at 2% level may be used to mark the pellet area and provide a cushion.

4.3 The filtered solution is then filled into the centrifuge tubes and centrifuged at 80,000 x g for 1 hour. The supernatant material is decanted and discarded. The area where the pellet would be normally found in each tube is scraped with a small sterile metal spatula into 0.2 to 0.3 mL of sterile distilled water. The material is aspirated with a sterile 10-mL syringe with an attached 6-inch metal cannula and all material is pooled.

4.4 The reconstituted material is divided into 2 equal aliquots and inoculated into 2 flasks containing freshly seeded swine cells. The containers are then incubated until confluent monolayers form (4 to 7 days). Two cell control flasks are set up in parallel.

4.5 After the cells are confluent, the cells in both the inoculated and the control flasks are scraped off with a rubber policeman or removed by pre-tested clean trypsin and the material pooled as to test material or control. Further dispersion of the cells is made by forcing them through a syringe and 20-gauge needle. When it is observed that the cells are well broken apart, they are centrifuged at slow speed and reconstituted in fresh media to original volume and 2 fresh flasks reseeded as well as 10-12 Leighton tubes or 5-6. Tech slides for both inoculated and controls Incubation is as before.

4.6 The 3rd day postseeding and every day thereafter, 2 test coverslips or 1 test Tech slide and 2 control coverslips or 1 Tech slide are stained with anti-porcine parvovirus fluorescent antibody conjugates. This is continued until the flasks are ready to subculture. If typical nuclear staining is observed, the fluid from the inoculated flasks is saved when the subculture is made, and PPV may be further verified by a hemagglutination test.

5. Report of Test Results

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

6. References

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

7. Summary of Revisions

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