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ANIMAL AND PLANT HEALTH INSPECTION SERVICE
NATIONAL VETERINARY SERVICES LABORATORIES
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SAM - 803

9 CFR 113.53	October 14, 1983	Endogenous Viruses
Standard Requirement	Revised	Agent

SUPPLEMENTAL ASSAY METHOD

FOR

EXAMINATION OF TRYPSIN SOLUTIONS

FOR

PORCINE PARVOVIRUS CONTAMINATION

A. SUMMARY

This is 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).

B. MATERIALS

1. Containers

A supply (eight or more) of sterile plastic or glass tissue culture flasks of at least 75 cm^2 surface area each, and at least two (2) racks of sterile Leighton tubes with coverslips or two flats of Tech slides (Miles Laboratories, Elkhart, IN. No endorsement expressed or implied).

2. Cell Culture

Porcine parvovirus susceptible cells either primary embryonic or a swine cell line, that will grow out in four to five days.

3. Serum

A certified contamination free bovine calf serum..

4. Medium

A medium that has been found of sufficient nutritive value to grow a monolayer of primary swine kidney cells in 4 to 6 days. [Suggested media are CMRL (Grand Island Biological Company, Grand Island, NY. No endorsement expressed or implied) or Alpha MEM (Flow Laboratories, McLean, VA. No endorsement expressed or implied), both supplemented with 1% sodium pyruvate and containing 50 meg Gentamycin per ml.}

5. Conjugate

Porcine parvovirus fluorescent antibody conjugate.

6. Stain

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

7. Erythrocytes

Guinea pig RBC's.

C. METHOD

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

2. Five (5) grams of trypsin are put into the 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 the 2% level may be used to mark the pellet area and provide a cushion.

3. The filtered solution is then filled into the centrifuge tubes and centrifuged at 80,000 X g for one (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" metal cannula and all material is pooled.

4. The reconstituted material is divided into two (2) equal aliquots and inoculated into two (2) flasks containing freshly seeded swine cells. The

containers are then incubated until confluent monolayers form (four to seven days). Two cell control flasks are set up in parallel.

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 pretested 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 two fresh flasks reseeded as well as 10 to 12 Leighton tubes or 5 to 6 Tech slides for both inoculated and controls. Incubation is as before.

6. The third day post-seeding and every day thereafter two test coverslips or one test Tech slide and two control coverslips or one control Tech slide are stained with anti-porcine parvovirus fluorescent antibody conjugate. 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.

7. The cells are again passed as described under (5) and the same sequence is carried out as under (6).

8. If the results are equivocal, another passage may be made (5) and the conjugate staining (6) be repeated. Some of the coverslips may be stained for inclusion bodies.

9. Any evidence of viral activity from the trypsin makes it unsatisfactory for use in vaccine production.