

## 4.7 Serum Neutralization

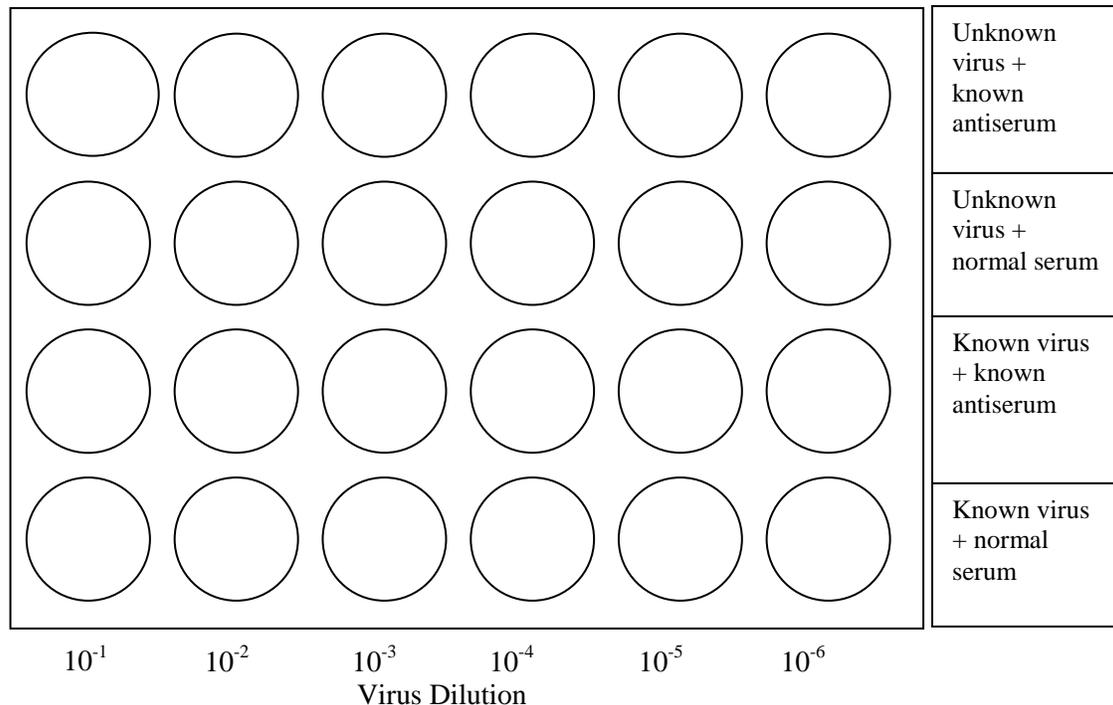
**S**erum neutralization or plaque reduction assays are serological methods used to confirm the identity of an unknown viral isolate. The procedures involve the use of a known dilution of specific neutralizing antiserum mixed with multiple dilutions of the homologous and suspect virus and subsequent observation of the ability of those viruses to produce CPE when inoculated onto a sensitive cell line. Normal serum from the species of animal used to produce the antiserum (usually rabbit or goat) is used as the negative control to account for nonspecific inhibitors of the virus.

### A. Plate Preparation

1. Seed plates (Section 2, 4.3.B “Seeding Procedures for Plates”) with the appropriate cell line (Section 2, 4.2 Selection of Appropriate Cell Lines) 24 to 48 hours before inoculation with virus.
2. Monolayers should be visually healthy and at least 80% confluent at the time of inoculation.

### B. Virus Sample Preparation

1. A dilution of neutralizing antiserum (polyclonal or monoclonal) should be used that allows neutralization of  $10^3$  to  $10^6$  plaque-forming units (PFU) or 50% tissue culture infective dose (TCID<sub>50</sub>) per mL of the homologous virus.
2. Dilute the suspect sample and positive control virus to  $10^{-1}$ ,  $10^{-2}$ ,  $10^{-3}$ ,  $10^{-4}$ ,  $10^{-5}$ , and  $10^{-6}$  in sterile HBSS (Section 2, 4.9.A “Sample Dilution Medium - Hanks Balanced Salt Solution (HBSS)”).
3. Combine equal volumes of each dilution of the suspect sample with the diluted antiserum. Repeat the procedure for a positive control virus. Include negative controls for both the suspect and homologous virus. Incubate for one hour with agitation at the appropriate temperature.
  - a. For IHN, VHSV, and IPNV, incubate at 15°C.
  - b. For SVCV, incubate at 20 to 25°C.
4. Inoculate each of these mixtures onto the cell line in which the suspect virus was isolated as indicated in Diagram 1 below. Incubate at the above temperature for 14 days and observe plates for cytopathic effect (CPE) (see Figures 4.1 to 4.16).
  - a. **Equivalent inhibition of CPE by a specific antiserum for both the suspect and homologous virus, but not for negative controls, provides confirmatory identification of the suspect virus.**
  - b. Alternatively, in a sample mixed with antibody, a titer decrease of less than  $2 \log_{10}$  indicates no neutralization and the suspect sample is considered **negative** for that virus if no problems are identified with the assay.

**Diagram 1.** Diagram for serum neutralization assay using a 24-well plate.

## C. Preparation of Reference Viruses

Where appropriate, positive controls are produced and frozen for use as needed in the serum neutralization assay.

### 1. Thawing of Frozen Viral Isolates

Thaw virus isolates rapidly in lukewarm water, removing the vial just before the last of the ice in the vial has melted.

### 2. Procedure for Producing Reference Viruses

- a. Inoculate viral suspensions onto cell culture flasks containing visibly healthy monolayers of the appropriate cell line. This is done in a manner similar to the tissue inoculation described in Section 2, 4.5.A "Plate Inoculation Procedures for Primary Culture" using an appropriate volume of inoculum for the flask size. If using a 25cm<sup>2</sup> flask, 0.1 mL of viral inoculum is usually sufficient.
  - i. Use EPC cell line for IHNV and SVCV isolates.
  - ii. Use EPC, FHM or BF-2 cell lines for VHSV isolates. During incubation, it is critically important that the pH of the medium remain within the range of 7.4 - 7.8 because cytopathic effects (CPE) of VHSV will not develop in acidic cultures (Batts et al., 1991), probably due to pH-dependent conformational changes in the glycoprotein (Gaudin et al., 1999).

- iii. Use CHSE-214 cell line for IPNV isolates.
- b. To allow for viral adsorption, incubate flasks at the appropriate temperature for one hour with gentle rocking at least every 15 minutes or continuously on a laboratory rocker.
  - i. For IHNV, IPNV, and VHSV, incubate at 15°C.
  - ii. For SVCV, incubate at 20 to 25°C.
- c. Aseptically dispense an appropriate amount of tissue culture media into the flask. For a 25 cm<sup>2</sup> flask this will be approximately 5 mL.
- d. Incubate control sample flasks to allow replication of the viruses.
  - i. For IHNV, IPNV, and VHSV, incubate at 15°C until CPE occurs or for 14 days.
  - ii. For SVCV, incubate at 20 to 25°C until CPE occurs or for 14 days.

**3. Procedure for Harvesting the Virus**

- a. Using aseptic technique, scrape the cell layer from the flask and triturate to break up.
- b. Pour fluid and suspended cells into sterile tubes for centrifugation.
- c. Centrifuge tubes at 4°C for 15 minutes at 2000-3000 times gravity relative centrifugal force (X g).
- d. Use supernatant as positive control virus. Any fluid not needed for the assay may be aliquoted into vials and frozen at -70 C.
- e. Any supernatant that is not frozen or used for the assay must be decontaminated before it is discarded.