Serum Testing Methods and References

Virus Testing
Raw Fetal Bovine Serum is tested for the following adventitious viruses following protocols stated in the Code of Federal Regulations for components of animal origin [CFR Part 113.53c, 1996].

- **Blue Tongue (BT)**
- **Bovine Adenovirus**
- **Bovine Parvovirus (BPV) Type I, Type V**
- **Bovine Respiratory syncytial virus (BRSV)**
- **Bovine viral diarrhea virus (BVDV) [via FA, with not virus testing detected via CPE and HA]**
  - *quantitative BVDV titer is determined prior to processing. Must test “non-detected” after gamma irradiation.
- **Infectious bovine rhinotracheitis (IBR)**
- **Parainfluenza Type 3 (PI3)**
- **Rabies**
- **Reovirus and Vesicular stomatitis virus (VSV)**

The following must provide result of “not detected” to meet specifications:

- **Cytopathic observation (CPE) [CFR 113.46]**
- **Fluorescent antibody staining (FA) [CFR 113.46]**
- **Inclusion body and hemabsorption (HA) [9CFR 113.46]**

Sterility
Sampled and performed by current USP <71> Membrane Filtration Method using tryptic soy broth and fluid thioglycolate incubated at 20°C to 25°C and 30°C to 35°C, respectively for 14 days [21CFR, Part 610.12]

Mycoplasma
FBS is tested for Mycoplasma in compliance with:

- 21 CFR, Part 610.12 - General Biological Products Standards: Sterility
- 9 CFR, Part 113.26 - Detection of viable bacteria and fungi except in live vaccine

Two Independent testing methods are used:
2. Use of ELISA Detection Kit System using immunofluorescence for the control of Mycoplasma bovis and bovigenitalim.

Bacteriophage
Tested via plaque assay using E. coli, utilizing positive controls.

Endotoxin
FBS is tested for endotoxin by USP LAL Gel-Clot method. All other sera types are tested by Quantitative Chromogenic LAL method.

Hemoglobin
Hemoglobin levels in finished serum is an indicator of the quality of the raw material collection and processing systems and overall finished product quality. Tested in accordance with the spectrophotometric method described by Noe and Weeden, which compensates for bilirubin and sample turbidity.

pH
Measured with a standard pH meter. Meters are calibrated daily using standards traceable to the National Institute of Standards Technology using guidelines in the current edition of USP.

Osmolality
Measured with a standardized osmometer, i.e. freezing point depression. Meters are calibrated daily using standards traceable to the National Institute of Standards Technology using guidelines outlined in the current edition of USP.

Total Protein
Measured using gel electrophoresis.

Cloning Efficiency
Each lot of FBS is tested for the support of clonal growth using various cell lines. The cloning efficiency is calculated as follows:

% Cloning Efficiency = (Number of Positive Wells/Total Number of Wells Inoculated) x 100

Biological Performance
Growth promotion and plating efficiency are tested using media (DMEM, MEM, RPMI, etc.) supplemented with FBS at a final concentration of 10%. Concurrent with testing, cultures are examined microscopically for atypical morphology and cytotoxicity.