

§ 16.14

mycostatin (Nystatin) or 2.5 µg/ml of amphotericin B (Fungizone).

(3) *Cell culture procedures.* (i) Both epithelioma papulosum cyprini (EPC) and chinook salmon embryo (CHSE-214) cell lines must be maintained and used in all virus assays. Susceptible, normal appearing, and rapidly dividing cell cultures shall be selected. Penicillin (100 IU/ml), streptomycin (100 µg/ml), and antifungal agents, such as mycostatin/Nystatin (25 IU/ml) or amphotericin B/Fungizone (2.5 µg/ml), are permitted in media used for cell culture and virus assay work.

(ii) Cell cultures shall be seeded and grown, at optimum temperatures, to 80-90 percent confluence in 24-well plates for virus assay work.

(iii) Decant the medium from the required number of 24-well plates of each cell line, and inoculate four replicate wells per cell line with .10 ml per well of each processed sample. When all wells have been inoculated, tilt plates to spread the inocula evenly. Incubate inoculated plates for 1 hour at 15° C. for sample contact. After the 1 hour contact add cell culture medium. Medium shall be buffered or cells incubated so that a pH between 7.4 and 7.8 is maintained. All cell culture assays shall be incubated, without overlays, at 15°C. for 21 days.

(4) *Virus identification by serological methods.* All cell cultures showing cytopathic effects (CPE) must be sub-cultured onto fresh cell cultures. If CPE is observed, determine the presence and identity the virus by serum neutralization, dot blot, enzyme-linked immunosorbent assay, or other equivalent serological technique.

(f) Information concerning the importation requirements of this section and application requirements for designation as a certifying official for purposes of this section may be obtained by contacting: U.S. Department of the Interior, U.S. Fish and Wildlife Service, Division of Fish Hatcheries (820 Arlington Square), 1849 C Street, NW., Washington, DC 20240. Telephone 703-358-1878.

(g) The information collection requirements contained in this part have been approved by the Office of Management and Budget under 44 U.S.C. 3501 *et seq.* and assigned clearance number

50 CFR Ch. I (10-1-02 Edition)

1018-0078. The information is being collected to inform U.S. Customs and USFWS inspectors of the contents, origin, routing, and destination of fish and eggs shipments and to certify that the fish lots were inspected for listed pathogens. The information will be used to protect the health of the fishery resource. Response is required to obtain a benefit.

[58 FR 58979, Nov. 5, 1993, as amended at 65 FR 37063, June 13, 2000]

§ 16.14 Importation of live amphibians or their eggs.

Upon the filing of a written declaration with the District Director of Customs at the port of entry as required under § 14.61, all species of live amphibians or their eggs may be imported, transported, and possessed in captivity, without a permit, for scientific, medical, education, exhibition, or propagating purposes, but no such live amphibians or any progeny or eggs thereof may be released into the wild except by the State wildlife conservation agency having jurisdiction over the area of release or by persons having prior written permission for release from such agency.

§ 16.15 Importation of live reptiles or their eggs.

(a) The importation, transportation, or acquisition is prohibited of any live specimen or egg of the brown tree snake (*Boiga irregularis*): Provided, that the Director shall issue permits authorizing the importation, transportation, and possession of such live snakes or viable eggs under the terms and conditions set forth in § 16.22.

(b) Upon the filing of a written declaration with the District Director of Customs at the port of entry as required under § 14.61, all other species of live reptiles or their eggs may be imported, transported, and possessed in captivity, without a permit, for scientific, medical, educational, exhibitional or propagating purposes, but no such live reptiles or any progeny or eggs thereof may be released into the wild except by the State wildlife conservation agency