Quality Control Testing of IRR Products[1]

(Descriptions of the test methods can be found on page 2)

A. Identity, Purity, and Functional Testing	Live Influenza Viruses	Inactivated Influenza Viruses	Genomic RNA from Influenza Viruses
Bacterial and Fungal Contamination	tested	tested[2]	not tested
Hemagglutination Titer	tested	tested	not tested
Identification and Purity by RT-PCR	tested	tested	tested
Identification by Hemagglutinin Gene Sequencing	tested	not tested	not tested
ID_{50} Assay[3]	tested	not tested	not tested
Mycoplasma Contamination	tested	not tested	not tested

B. Safety Testing[4]	Ferret Antisera to Influenza Viruses	Inactivated Influenza Viruses	Genomic RNA from Influenza Viruses
Screening for Residual Infectious Virus in Eggs	tested[5,6]	tested[6]	tested[6]
Screening for Residual Infectious Virus in Tissue Culture	tested[5,6]	tested[6]	tested[6]
Screening for Residual Viral RNA by RT-PCR	tested[5]	not tested	not tested

[1]The table indicates the standard QC tests that are performed on each major product type available through the Influenza Reagent Resource. Any additional testing of specific items is described on the Certificate of Analysis for each lot, as is the testing of product types not listed here.

[2]Only BPL-inactivated and ether-extracted WHO and Surveillance Antigens are tested for the absence of bacterial and fungal contamination. BPL-inactivated influenza viruses that are provided frozen in cryovials are intended for use as control standards. These products are prepared using sterile technique, but are not tested to confirm sterility.

[3]The specific test employed is dependent on the host and conditions used to propagate the virus. The 50% Infectious Dose (ID_{50}) is the 50% infectious endpoint; that is, the ID_{50} is the dilution of virus that under the conditions of the assay can be expected to infect 50% of the embryonated chicken eggs ($CEID_{50}$) or culture vessels ($TCID_{50}$) inoculated, just as a Lethal Dose 50% (LD_{50}) is expected to kill half of the animals exposed. A reciprocal of the dilution required to yield the ID_{50} provides a measure of the titer (or infectivity) of a virus preparation. See page 2 for additional details.

[4]The specific testing protocol used to confirm that derivatives of influenza viruses are no longer infectious is dependent on both the type of product and the source virus.

[5]Safety testing of Ferret Antisera may involve infectivity assays in embryonated chicken eggs or in tissue culture, screening for the presence of viral RNA by RT-PCR, or both. See the Certificate of Analysis for each lot of a specific item for details.

[6]Screening for residual live virus is carried out in the same host used to propagate the source virus. See page 2 for additional details.

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Quality Control (QC) Test Methodology Descriptions

Bacterial and Fungal Contamination: Several randomly sampled aliquots of the final product were used to inoculate 8 mL ampules of sterile soybean-casein digest medium and fluid thioglycollate medium, which were incubated at 22.5°C ± 2.5°C under aerobic conditions, and 32.5°C ± 2.5°C under anaerobic conditions, respectively. The preparation passed if there was no observable growth in the cultures after 14 days of incubation.

Hemagglutination Titer: Live and inactivated influenza virus preparations were tested in 96 well plates using 0.5% turkey red blood cells. Positive controls for influenza A or B were used. Negative control wells contained uninfected allantoic fluid or tissue culture supernatant with turkey red blood cells only. The specification for the minimum acceptable titer is given on the Certificate of Analysis for each lot.

Hemagglutinin (HA) Gene Sequencing: The entire HA gene was sequenced bidirectionally and the results compared to previously characterized seed stock of the virus (and to published sequences of the source virus if available). The preparation passed if the HA gene sequence was ≥ 99% identical to that of the seed stock of the virus.

Mycoplasma Contamination: Nucleic acid extracted from the test article was tested by PCR. The preparation passed if no mycoplasma DNA was detected.

Screening for Residual Infectious Virus in Eggs: Innocuity of derivatives of egg-grown influenza viruses was demonstrated by serial passage of a standard volume of the test article in 9- to 11-day-old specific pathogen free (SPF) embryonated chicken eggs under conditions identical to those used to propagate the source virus. The positive control used for the assay was a representative egg-grown influenza A or B virus. Diluent specific to the test article was used as a negative control. Allantoic fluid harvested from the second serial passage was tested in a hemagglutination assay using 0.5% turkey red blood cells. The preparation passed if all eggs inoculated with the test article were negative.

Screening for Residual Infectious Virus in Tissue Culture: Innocuity of derivatives of cell-grown influenza viruses was demonstrated by serial passage of a standard volume of the test article in Madin-Darby Canine Kidney (MDCK) cells under conditions identical to those used to propagate the source virus. The positive control used for the assay was a representative tissue culture-grown influenza A or B virus. Diluent specific to the test article was used as a negative control. Cells were evaluated for cytopathic effect (CPE) and culture supernatants harvested from the second serial passage were tested in a hemagglutination assay using 0.5% turkey red blood cells. The preparation passed if all culture wells inoculated with the test article were negative.

Screening for Residual Viral RNA by RT-PCR: Innocuity of convalescent sera raised against cell-grown influenza viruses was demonstrated by testing nucleic acid extracted from the test article qualitatively for the presence or absence of influenza genetic material by RT-PCR. The specific influenza types, subtypes, and/or lineages tested for are listed on the Certificate of Analysis for each lot. The preparation passed if no influenza viral RNA was detected after 45 cycles.

Titer by CEID50 Assay: Serial dilutions of live egg-grown influenza virus preparations were inoculated into SPF embryonated chicken eggs. Negative control eggs were inoculated with diluent only. Virus replication was detected by testing harvested allantoic fluid in a hemagglutination assay, using appropriate controls. The titer was determined by the method of Reed and Muench. The specification for the minimum acceptable titer is given on the Certificate of Analysis for each lot.

Titer by TCID50 Assay: Serial dilutions of live cell-grown influenza virus preparations were inoculated onto MDCK cell cultures. Negative control wells were inoculated with diluent only. Virus replication was detected by testing for the presence of viral antigen in culture supernatants by ELISA, using appropriate controls. The titer was determined by the method of Reed and Muench. The specification for the minimum acceptable titer is given on the Certificate of Analysis for each lot.

Type/Subtype Identification and Purity by RT-PCR: Extracted RNA isolated from allantoic fluid or tissue culture supernatant was tested for the presence or absence of influenza A, influenza A subtypes, influenza B, and/or influenza B lineages qualitatively by RT-PCR. The preparation passed if the designated type and/or subtype or lineage were detected exclusively.