

Certificate of Analysis for NR-3677

Kilbourne F20: A/swine/Nevada/101/1982 (H1N1) Mutant, Low (L) Yield, Animal Isolate

Catalog No. NR-3677

Product Description: Pooled allantoic fluid from specific-pathogen free (SPF) embryonated chicken eggs¹ infected with a low (L) yield mutant of influenza A virus, A/swine/Nevada/101/1982 (H1N1)

Lot^{2,3}: 61982337 Manufacturing Date: 12SEP2013

TEST	SPECIFICATIONS	RESULTS
Identification by Infectivity Using Embryonated Chicken Eggs ¹ Hemagglutination activity using allantoic fluid from infected eggs and 0.5% chicken red blood cells	Positive	Positive
Sequencing of Hemagglutinin and Matrix Coding Regions ⁴ Hemagglutinin (448 nucleotides)	Consistent with	Consistent with
Matrix (598 nucleotides)	swine H1N1 influenza A virus Consistent with swine H1N1 influenza A virus	swine H1N1 influenza A virus Consistent with swine H1N1 influenza A virus
Titer by CEID₅ Assay⁵,6 in Embryonated Chicken Eggs¹	Report results	2.8×10^8 CEID ₅₀ per mL
Sterility (21-day incubation)		
Harpo's HTYE broth ⁷ , 37°C and 26°C, aerobic	No growth	No growth
Trypticase soy broth, 37°C and 26°C, aerobic	No growth	No growth
Sabouraud broth, 37°C and 26°C, aerobic	No growth	No growth
Blood agar, 37°C, aerobic	No growth	No growth
Blood agar, 37°C, anaerobic	No growth	No growth
Thioglycollate broth, 37°C, anaerobic	No growth	No growth
DMEM with 10% FBS, 37°C and 5% CO ₂	No growth	No growth
Mycoplasma Contamination		
Agar and broth culture (14-day incubation at 37°C)	None detected	None detected
DNA detection by PCR of extracted Test Article nucleic acid	None detected	None detected

¹10-day-old SPF Embryonated Chicken Eggs acquired from B&E Eggs, York Springs, Pennsylvania

Date: 04 DEC 2013 Signature: Millard Q. Complex

Title: Technical Manager, BEI Authentication or designee

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²Derived from NIAID Catalog No. V-331-0E5565

³Grown in the allantoic cavity of embryonated chicken eggs¹ for 2 days at 35°C in a humidified chamber

⁴There is no sequence data for influenza A/swine/Nevada/101/1982 (H1N1) in the NCBI database; the sequences obtained for NR-3677 are consistent with those of H1N1 influenza viruses isolated from swine in North America in the late 1970s and early 1980s.

⁵The Chicken Embryo Infectious Dose 50% (CEID₅₀) is the dilution of virus that under the conditions of the assay can be expected to infect 50% of the inoculated embryonated chicken eggs, just as a Lethal Dose 50% (LD₅₀) is expected to kill half of the animals exposed. A reciprocal of the dilution required to yield the CEID₅₀ provides a measure of the infectious titer (or infectivity) of a virus preparation.

⁶2 days at 35°C in a humidified chamber

⁷Atlas, Ronald M. <u>Handbook of Microbiological Media</u>. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.