

Certificate of Analysis for NR-3171

Influenza A Virus, A/Japan/305/57 (H2N2)

Catalog No. NR-3171

This reagent is the tangible property of the U.S. Government.

Product Description: Pooled allantoic fluid from embryonated chicken eggs infected with influenza A virus, A/Japan/305/57 (H2N2).

Lot: V301-032-000 Manufacturing Date: 16AUG1962

TEST	SPECIFICATIONS	RESULTS (AUG2008)
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 Species-Specific Region (~ 700 nucleotides)	Consistent with influenza A virus	Consistent with influenza A virus
Titer by CEID ₅₀ Assay ^{2,3} in Embryonated Chicken Eggs ¹	Report results	8.89 X 10 ⁴ CEID ₅₀ /mL
Functional Activity by RT-PCR Assay ⁴	~ 1030 bp amplicon	~ 1030 bp amplicon
Sterility (21-day incubation) Harpo's HTYE broth ⁵ , 37°C and 26°C, aerobic Trypticase soy broth, 37°C and 26°C, aerobic Sabouraud broth, 37°C and 26°C, aerobic Sheep blood agar, 37°C, aerobic Sheep blood agar, 37°C, anaerobic Thioglycollate broth, 37°C, anaerobic 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) DNA detection by PCR of extracted Test Article nucleic acid	None detected None detected	None detected None detected

¹⁰ to 11-day-old SPF Fertile Chicken Eggs acquired from B&E Eggs, York Springs, Pennsylvania.

Date: 16 SEP 2008 **Signature:** Signature on File

Title: Technical Manager, BEI Authentication or designee

ATCC[®], on behalf of BEI Resources, hereby represents and warrants that the material provided under this certificate has been subjected to the tests and procedures specified and that the results described, along with any other data provided in this certificate, are true and accurate to the best of ATCC[®]'s knowledge.

ATCC® is a trademark of the American Type Culture Collection.

You are authorized to use this product for research use only. It is not intended for human use.

Biodefense and Emerging Infections Research Resources Repository P.O. Box 4137 Manassas, VA 20108-4137 USA

Fax: 703-365-2898

800-359-7370

²The 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.

³48 hours at 35°C in a humidified chamber

⁴BM-M1 and BM-M-1027R primers; Obenauer, J. C., et al. "Large-Scale Sequence Analysis of Avian Influenza Isolates." <u>Science</u> 311 (2006): 1576-1580. PubMed: 16439620.

⁵Atlas, Ronald M. <u>Handbook of Microbiological Media</u>. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.