



**Influenza Reagent
Influenza virus infectious X-327A
NIBSC code: 19/120
Instructions for use
(Version 3.0, Dated 15/07/2019)**

1. INTENDED USE

Reagent 19/120 is prepared from NYMC X-327A (A/Kansas/14/2017 x A/PR/8/34) which was processed for freeze drying in 250µl volumes as described by Campbell, PJ, Journal of Biological Standardisation, 1974, 2,249-267. The derivation and known passage history of X-327A is attached.

2. CAUTION

This preparation is not for administration to humans or animals in the human food chain

The material is not of human or bovine origin. As with all materials of biological origin, this preparation should be regarded as potentially hazardous to health. It should be used and discarded according to your own laboratory's safety procedures. Such safety procedures should include the wearing of protective gloves and avoiding the generation of aerosols. Care should be exercised in opening ampoules or vials, to avoid cuts.

3. UNITAGE

No unitage is assigned to this material

4. CONTENTS

Country of origin of biological material: United Kingdom.
Each ampoule contains 250µl (nominal) of infectious influenza virus as allantoic fluid from SPF embryonated hen's eggs.

5. STORAGE

Store in the dark at -20°C or below

Please note: because of the inherent stability of lyophilized material, NIBSC may ship these materials at ambient temperature.

6. DIRECTIONS FOR OPENING

Vials have a 'flip-up' circular cap. Either on the cap or the collar of the vial, there is an indication of the point at which to lever off the cap. This exposes an area of the stopper through which reconstitution and withdrawal of the preparation can be made using a hypodermic needle and syringe. If use of a pipette is preferred, then fully remove the metal collar using, for example, forceps, taking care to avoid cuts by wearing appropriate gloves. Remove the stopper for access. Care should be taken to prevent loss of the contents.

7. USE OF MATERIAL

Reconstitute the contents of one ampoule of reagent with 250µl of sterile distilled water. Leave for a minimum of 5 minutes before use to allow for complete solution of freeze-dried material. A range of dilutions (e.g. 10⁻³ to 10⁻⁶) should be made in a suitable medium for initial cultivation.

8. STABILITY

Reference Materials should be stored on receipt as indicated on the label.

NIBSC follows the policy of WHO with respect to its reference materials.

9. REFERENCES

NA

10. ACKNOWLEDGEMENTS

NA

11. FURTHER INFORMATION

Further information can be obtained as follows;
This material: enquiries@nibsc.org
WHO Biological Standards:
<http://www.who.int/biologicals/en/>
JCTLM Higher order reference materials:
<http://www.bipm.org/en/committees/jc/jctlm/>
Derivation of International Units:
http://www.nibsc.org/standardisation/international_standards.aspx
Ordering standards from NIBSC:
<http://www.nibsc.org/products/ordering.aspx>
NIBSC Terms & Conditions:
http://www.nibsc.org/terms_and_conditions.aspx

12. CUSTOMER FEEDBACK

Customers are encouraged to provide feedback on the suitability or use of the material provided or other aspects of our service. Please send any comments to enquiries@nibsc.org

13. CITATION

In all publications, including data sheets, in which this material is referenced, it is important that the preparation's title, its status, the NIBSC code number, and the name and address of NIBSC are cited and cited correctly.

14. MATERIAL SAFETY SHEET

Classification in accordance with Directive 2000/54/EC, Regulation (EC) No 1272/2008: Not applicable or not classified

Physical and Chemical properties	
Physical appearance: white powder	Corrosive: No
Stable: Yes	Oxidising: No
Hygroscopic: No	Irritant: No
Flammable: No	Handling: See caution, Section 2
Other (specify):	Live influenza virus
Toxicological properties	
Effects of inhalation:	Likelihood of influenza virus infection
Effects of ingestion:	Not established, avoid ingestion
Effects of skin absorption:	Not established, avoid contact with skin
Suggested First Aid	
Inhalation:	Seek medical advice
Ingestion:	Seek medical advice
Contact with eyes:	Wash with copious amounts of water. Seek medical advice
Contact with skin:	Wash thoroughly with water.
Action on Spillage and Method of Disposal	
Spillage of contents should be taken up with absorbent material wetted with an appropriate virucidal agent. Rinse area with an appropriate virucidal agent followed by water. Absorbent materials used to treat spillage should be treated as biologically hazardous waste.	

15. LIABILITY AND LOSS

In the event that this document is translated into another language, the English language version shall prevail in the event of any inconsistencies between the documents.



Unless expressly stated otherwise by NIBSC, NIBSC's Standard Terms and Conditions for the Supply of Materials (available at http://www.nibsc.org/About_Us/Terms_and_Conditions.aspx or upon request by the Recipient) ("Conditions") apply to the exclusion of all other terms and are hereby incorporated into this document by reference. The Recipient's attention is drawn in particular to the provisions of clause 11 of the Conditions.

16. INFORMATION FOR CUSTOMS USE ONLY

Country of origin for customs purposes*: United Kingdom * Defined as the country where the goods have been produced and/or sufficiently processed to be classed as originating from the country of supply, for example a change of state such as freeze-drying.
Net weight: 0.25g per ampoule
Toxicity Statement: Non-toxic
Veterinary certificate or other statement if applicable.
Attached: No

Derivation of NYMC X-327A

Passage	Lot	Laboratory
E1-E6		unknown
E7	3026015402	CDC, Atlanta, USA
E8-E16		NYMC, New York, USA
E17	E#6387	NYMC, New York, USA
E18	44310	NIBSC, Hertfordshire, UK

Sterility: No visible contamination was detected in a variety of media (tryptose soya broth, thioglycolate broth, Sabouraud's broth and blood agar plates) after 14 days incubation.

The HA and NA sequence of this virus is available at GISAID with the accession number EPI_ISL_364380.



**Derivation of NYMC X-327A High Yield H3N2 Reassortant (5:3)
with A/PR/8/34 PB1, PB2, PA, NP, and M genes and
A/Kansas/14/2017 (3C.3a HA genetic clade) HA, NA NS and genes**

Experiment # 4829 (1/14/2019)
A/Kansas/14/2017 CDC#3026015402
12/14/17 E7(7/12/2018) HA-512

Passages prior to receipt at NYMC -7

Passages at New York Medical College

Passage No.

1

10^{-2}

HA—1:64



Reassortment passage at NYMC

A/Kansas/14/2017 (H3N2) x A/PR/8/34

2

10^{-2} + 10^{-3}

HA—1:2048



3

10^{-1}

+ A/PR/8/34 antisera (as)
A/PR/8/34 HANA antibodies (ab)

HA—256



4

10^{-1}

+ A/PR/8/34 antisera (as)
A/PR/8/34 HANA antibodies (ab)

HA—1:256



5

10^{-3}

+ A/PR/8/34 antisera (as)
A/PR/8/34 HANA antibodies (ab)

HA—1:512



6

10^{-4}

HA—1:512



7

10^{-8}

HA — 1:256



8

10^{-9}

HA—1:256



9

10^{-8}

HA—1:512



10

10^{-5}

HA—1:512

**NYMC X-327A (E7/E10)
E#6387**



HA, NA and NS genes were identified as A/Kansas/14/2017 by RT-PCR/RFLP gene analysis. PB2, PB1, PA, NP, and M genes were identified as A/PR/8/34 by RT-PCR/RFLP analysis.

The HA yield was shown to be 10.8 ug/ml by UPLC analysis. The HA yield for A/Kansas/14/2017 was 8.9 ug/ml by UPLC analysis. HA and NA serological identification A/Kansas/14/2017 by HI and NI tests pending.

SPF eggs were used for all reassortant passages.

All HA titers were tested using chicken red blood cells (cRBC) at room temperature.

Virus seed was shown to be sterile. Sterility testing was performed by streaking the sample on blood agar plates and incubating for 48 hours at 37°C.