



**Influenza Reagent
Influenza Virus Infectious NYMC X-223A
NIBSC code: 13/252
Instructions for use
(Version 4.0, Dated 19/05/2016)**

1. INTENDED USE

Reagent 13/252 is prepared from NYMC X-223A which was processed for freeze drying in 250µl volumes as described by Campbell, P.J. Journal of Biological Standardisation, 1974, 2,249-267. The known passage history of NYMC X-223A 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

DIN ampoules have an 'easy-open' coloured stress point, where the narrow ampoule stem joins the wider ampoule body. Various types of ampoule breaker are available commercially. To open the ampoule, tap the ampoule gently to collect material at the bottom (labelled) end and follow manufactures instructions provided with the ampoule breaker.

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 10⁻⁵) 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: NA
Toxicity Statement: Non-toxic
Veterinary certificate or other statement if applicable.
Attached: No

Passage history of NYMC X-223A (Post mixed infection)

Passage	Lot	Laboratory
E5 (prior to receipt at NYMC)		unknown
E5/E1 – E5/E7		NYMC, New York, USA
E5/E8	E#6034	NYMC, New York, USA
E9	35400	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 on GISAID with the accession number EPI_ISL_215972



**Derivation of NYMC X-223A High Yield H3N2 Reassortant (6:2)
With A/PR/8/34 PA, PB2, PB1, NP, NS and M genes
and A/Texas/50/2012 HA and NA genes**

Experiment #4711 (11/2/12)

A/Texas/50/2012 (H3N2) CDC #: 2012704893 E5 (10/18/12) HA: 256

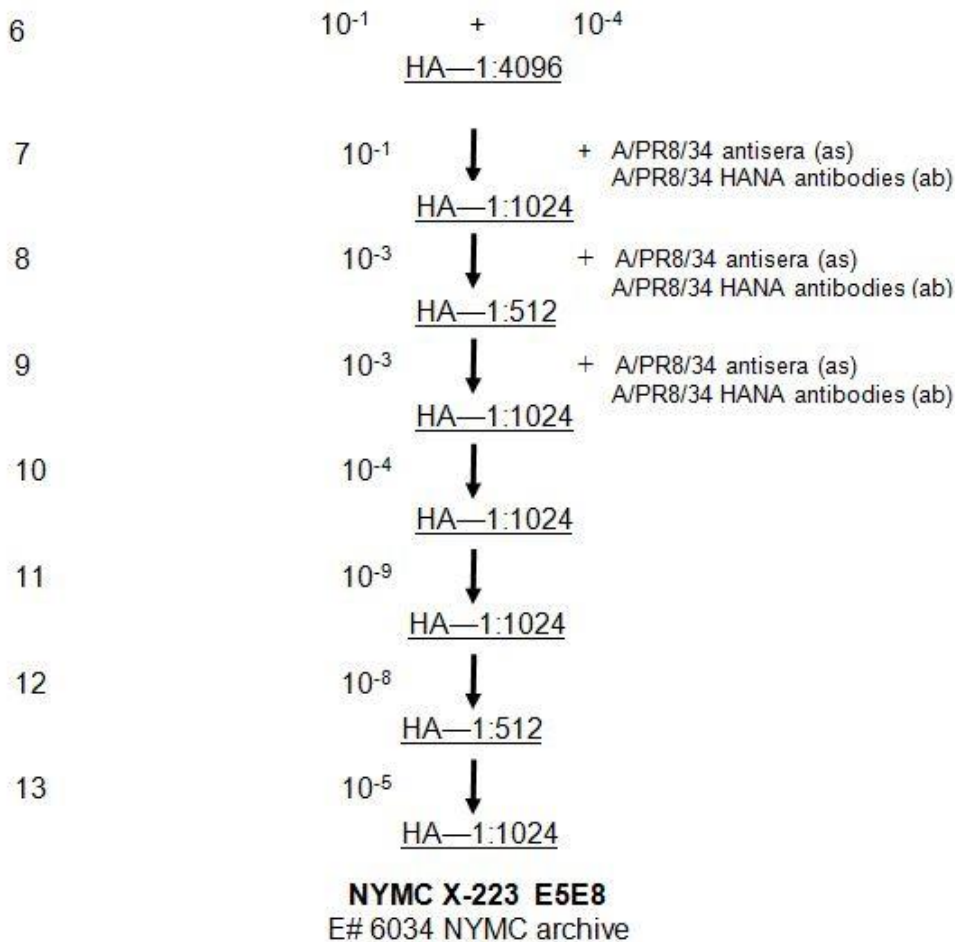
Passage No.

1 to 5

Passages prior to receipt at NYMC (E5)

Reassortment passage at NYMC

A/Texas/50/2012 × A/PR/8/34



HA and NA were identified as A/Texas/50/2012 serologically by HI and NI tests and confirmed by RT-PCR/RFLP analysis. Internal genes PA, PB2, PB1 NP, NS and M were identified as A/PR/8/34 and HA, NA as A/Texas/50/2012 by RT-PCR/RFLP. SPAFAS eggs were used for all reassortant passages. All HA titers were tested using guinea pig red blood cells at room temp. Virus seeds were shown to be sterile by streaking samples on sheep blood agar plates and incubating for 48 hours at 37 degrees C.



12/21/2012

Doris Bucher, Ph.D
Department of Microbiology and Immunology
New York Medical College
Basic Science Building
Valhalla, NY 10595

Dear Dr. Bucher,

We appreciate your submitting influenza reassortants to CDC for analysis. Data from your laboratory and other collaborating laboratories worldwide contribute significantly towards the influenza vaccine recommendations made each year by WHO.

The results we obtained with your reassortants are listed and interpreted below.

Your reassortant was characterized by a "one-way" hemagglutination-inhibition test using post-infection ferret antisera and guinea pig red blood cells (GP).

CDC ID#	Specimen ID#	Results
2013706003	A/HAWAII/22/2012 X-225	CONSISTENT WITH A/HAWAII/22/2012-LIKE (H3N2) PASS
2013706004	A/HAWAII/22/2012 X-225A	CONSISTENT WITH A/HAWAII/22/2012-LIKE (H3N2) PASS
2013706001	A/TEXAS/50/2012 X-223	CONSISTENT WITH A/TEXAS/50/2012-LIKE (H3N2) PASS
2013706002	A/TEXAS/50/2012 X-223A	CONSISTENT WITH A/TEXAS/50/2012-LIKE (H3N2) PASS

Your reassortants have HI reactivity patterns that are consistent with their corresponding wild type viruses.

If you have any questions, please contact us.

Sincerely,

Dr. Xiyan Xu

Team Leader
Virus Reference Team
Virus Surveillance and Diagnosis Branch
Influenza Division, CDC

Dr. Alexander Klimov

Deputy Director
WHO Collaborating Center for Surveillance,
Epidemiology and Control of Influenza
Influenza Division, CDC



DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Centers for Disease Control
and Prevention

1/8/2013

Doris Bucher, Ph.D
Department of Microbiology and Immunology
New York Medical College
Basic Science Building
Valhalla, NY 10595

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We appreciate your submitting influenza reassortants to CDC for analysis. Data from your laboratory and other collaborating laboratories worldwide contribute significantly towards the influenza vaccine recommendations made each year by WHO.

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Your reassortants were characterized by a "two-way" hemagglutination-inhibition test using post-infection ferret antisera and guinea pig red blood cells (GP).

CDC ID#	Specimen ID#	Results
2013706003	A/HAWAII/22/2012 X-225	CONSISTENT WITH A/HAWAII/22/2012-LIKE (H3N2) GP PASS
2013706002	A/TEXAS/50/2012 X-223A	CONSISTENT WITH A/TEXAS/50/2012-LIKE (H3N2) GP PASS

Your reassortants have HI reactivity patterns that are consistent with their corresponding wild type viruses.

If you have any questions, please contact us.

Sincerely,

Dr. Xiyun Xu

Team Leader
Virus Reference Team
Virus Surveillance and Diagnosis Branch
Influenza Division, CDC

Dr. Alexander Klimov

Deputy Director
WHO Collaborating Center for Surveillance,
Epidemiology and Control of Influenza
Influenza Division, CDC



DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Centers for Disease Control
and Prevention

01/09/2013

Doris Bucher, Ph.D
Department of Microbiology and Immunology
New York Medical College
Basic Science Building
Valhalla, NY 10595

Dear Dr. Bucher,

We appreciate your submitting influenza reassortants to CDC for analysis.

The HA and NA genes of your reassortants were sequenced and compared to that of their wild type parental virus A/Texas/50/2012. The results we obtained with your reassortants are listed and interpreted below.

CDC ID#	Specimen ID#	Results
2013706001	A/Texas/50/2012 X223	HA: Ile-226-Asn and Lys-387-Glu NA: No change detected
2013706002	A/Texas/50/2012 X-223A	HA: Ile-226-Asn NA: No change detected

A number of amino acid changes were detected in the HA genes of the reassortants. Further analysis is warranted to better understand the significance of the changes.

If you have any questions, please contact us.

Sincerely,

Dr. Xiyun Xu

Team Leader
Virus Reference Team
Virus Surveillance and Diagnosis Branch
Influenza Division, CDC

Dr. Alexander Klimov

Deputy Director
WHO Collaborating Center for Surveillance,
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Influenza Division, CDC