Medicines & Healthcare products Regulatory Agency

NIBSC Confidence in Biological Medicines

Influenza Reagent
Influenza Virus Infectious NYMC X-299
NIBSC code: 17/162
Instructions for use
(Version 2.0, Dated 23/11/2017)

1. INTENDED USE

Reagent 17/162 is prepared from NYMC X-299 (A/Montana/50/2016 x X-157B) H1N1pdm09 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 NYMC X-299 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^{0}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

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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

Passage history of NYMC X-299

Passage	Lot	Laboratory
E1-E8		New York Medical College, USA
E9	E#6294	New York Medical College, USA
E10	42920	NIBSC, Hertfordshire,UK

Total number of passages post mixed infection: 9

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_281222.

Derivation of NYMC X-299

A/Montana/50/2016 (H1N1pdm09) genetic grp 6B.1 with NYMC X-157B High Yield A H1N1pdm09 Reassortant (6:2) with A/PR/8/34 M, PB1, PB2, PA, NS, and NP genes and A/Montana/50/2016 HA, and NA genes

Exper. # 4796
A/Montana/50/2016
#3025627640
E3 (1/23/17)
HA: 128

Passages at New York Medical College

Passage No.

1 10^{-1}

HA—128

↓

A/Montana/50/2016 (H1N1pdm09) x NYMC X-157B

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

HA—1024

↓

3 10^{-1} + X-157 HANA antisera (as) X-157 HANA antibodies (ab)

HA—64

↓

4 10^{-1} + X-157 HANA antisera (as) X-157 HANA antibodies (ab)

HA—256

↓

5 10^{-1} + X-157 HANA antisera (as) X-157 HANA antibodies (ab)

HA—2048

↓

6 10^{-4}

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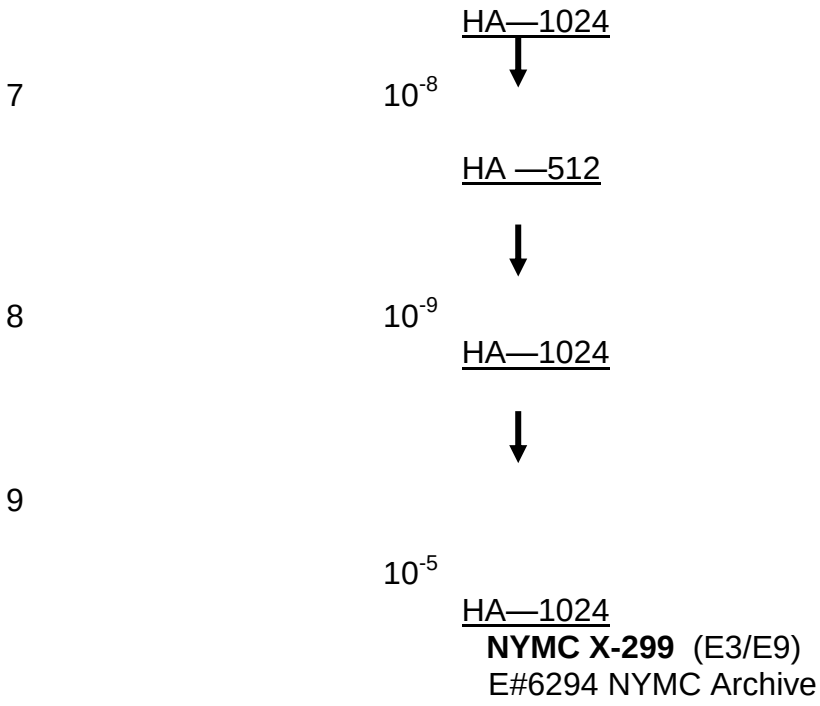

HA Yield by UPLC Analysis (µg HA/ml allantoic fluid)

wt (wild type)	X-299	Fold Increase
4.9	**12.9**	**2.6**

HA and NA genes were identified as A/Montana/50/2016 by RT-PCR/RFLP gene analysis. PB1, PB2, PA, M, NS, and NP genes were identified as A/PR/8/34 by RT-PCR/RFLP analysis.

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.

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Centers for Disease Control and Prevention

07/24/2017

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 submission of influenza reassortant(s) 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.

Your reassortant was antigenically characterized by a "two way" hemagglutination-inhibition (HI) test using a panel of post-infection ferret antisera.

CDC ID#	Specimen ID#	Results
3000628132	A/MONTANA/50/2016 X-299	CONSISTENT WITH A/MONTANA/50/2016; TWO WAY PASS

Your reassortant had HI reactivity patterns that were consistent with the corresponding wild type virus, and it is antigenically similar to A/Michigan/45/2015. Ferret antiserum raised against the A/MONTANA/50/2016 X-299 virus inhibit well the majority of recently circulating viruses in the HI assay. Therefore, it passed the two way test.

The HA and NA genes of your reassortant were sequenced and compared to that of their wild type parental virus A/MONTANA/50/2016. The reassortant virus differed from the parental virus (E2 passage) at amino acid residue 187 in the HA. The parental virus possessed a change of D187A compared to the HA sequence of the original clinical specimen, while the reassortant had a change of D187V. There is no amino acid difference between the reassortant and its parental virus in the NA.

If you have any questions, please contact us.

Sincerely,

Dr. Xiyan Xu

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

Dr. Jacqueline Katz

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

HEMAGGLUTINATION INHIBITION REACTIONS OF INFLUENZA A(H1N1)pdm09 VIRUSES*

DATE TESTED: 7/13/17

		REFERENCE FERRET ANTISERA						
	REFERENCE VIRUSES							
	STRAIN DESIGNATION	**MI/45**	**MI/45**	**MT/50**	**MT/50**	**MT/50 X-299**	**PASSAGE**	**DATE COLL.**
1	A/MICHIGAN/45/2015	**2560**	2560	5120	2560	2560	E3(12/7/15)	9/7/2015
2	A/MICHIGAN/45/2015	1280	**2560**	2560	2560	1280	M1/C3(5/13/16)	9/7/2015
3	A/MONTANA/50/2016	640	1280	**1280**	1280	1280	E4(1/27/17)	10/10/2016
4	A/MONTANA/50/2016	1280	2560	2560	**2560**	2560	C2(1/24/17)	10/10/2016
5	A/MONTANA/50/2016 X-299	1280	1280	2560	2560	**1280**	E3E9	

*A virus is considered consistent with the wild type if it reacts with ferret antisera raised to the reference strain giving an HI titer equal to or within two-fold of the HI titer of the wild type reference strain.

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