Medicines & Healthcare products Regulatory Agency

NIBSC Confidence in Biological Medicines

Influenza Reagent
Influenza Virus Infectious NYMC X-311
NIBSC code: 18/160
Instructions for use
(Version 4.0, Dated 19/10/2018)

1. INTENDED USE

Reagent 18/160 is prepared from NYMC X-311 (A/Brisbane/1/2018 x A/PR/8/34) H3N2 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-311 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

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^{-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.

National Institute for Biological Standards and Control,
Potters Bar, Hertfordshire, EN6 3QG. T +44 (0)1707 641000, nibsc.org
WHO International Laboratory for Biological Standards,
UK Official Medicines Control Laboratory

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-311

Passage	Lot	Laboratory
E1-E8		NYMC, New York, USA
E9	6345	NYMC, New York, USA
E10	43780	NIBSC, Hertfordshire, UK

Number of passages post mixed infection = 8

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.

Derivation of NYMC X-311

A/Brisbane/1/2018 (H3N2) with A/PR/8/34 High Yield A H3N2 3C.2a2 (HA) 3C.2a1a (NA) Reassortant (6:2) with A/PR/8/34 M, NS, PB1, PB2, PA, and NP genes and A/Brisbane/1/2018 HA and NA genes

Exper. # 4815
A/Brisbane/1/2018 (H3N2)
CDC#3000821653
SL/1801428-1
22/2/2018
E4
HA 128

Passages at New York Medical College

Passage No.

1 10^{-2}

HA—1:32

↓

A/Brisbane/1/2018 (H3N2) x A/PR/8/34

2 10^{-2} + 10^{-4}

HA—1:4096

↓

3 10^{-1} + A/PR/8/34 antisera (as) A/PR/8/34 HANA antibodies (ab)

HA—1:64

↓

4 10^{-1} + A/PR/8/34 antisera (as) A/PR/8/34 HANA antibodies (ab)

HA-1:512

↓

5 10^{-3} + A/PR/8/34 antisera (as) A/PR/8/34 HANA antibodies (ab)

HA—1:128

↓

6 10^{-4}

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HA—1:256

7 10^{-8}

HA — 1:512

8 10^{-8}

HA—1:256

9 10^{-5}

HA— 1:1024

NYMC X-311 (E4/E9)
E#6345 NYMC Archive

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

wt (wild type)	X-311	Fold Increase
3.6	**8.6**	**2.4**

HA and NA genes were identified as A/Brisbane/1/2018 by RT-PCR/RFLP. M, PB1, PB2, PA, NS and NP were identified as A/PR/8/34 by RT-PCR/RFLP.

SPF eggs were used for all reassortant passages.

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.

The HA and NA sequence of this virus is available on GISAID with the accession number EPI_ISL_330356

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Centers for Disease Control and Prevention

10/18/2018

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.

The results we obtained with your specimen(s) are listed and interpreted below.

CDC ID#	Specimen ID#	Results
3000628152	A/BRISBANE/1/2018 X-311	CONSISTENT WITH A/BRISBANE/1/2018; TWO WAY PASS
3000628153	A/BRISBANE/1/2018 X-311A	CONSISTENT WITH A/BRISBANE/1/2018; TWO WAY PASS
3000628152	A/BRISBANE/1/2018 X-311	CONSISTENT WITH A/SWITZERLAND/8060/2017; TWO WAY PASS
3000628153	A/BRISBANE/1/2018 X-311A	INCONSISTENT WITH A/SWITZERLAND/8060/2017; TWO WAY FAIL

Your reassortants were antigenically characterized by a "two-way" Hemagglutination Inhibition test (HI) using post infection ferret antisera. Ferret antiserum raised against the wild type parental virus A/Brisbane/1/2018 well inhibited both reassortants (within two-fold of HI titers compared with the homologous virus). Similarly, antisera raised against the A/Brisbane/1/2018 X-311 and A/Brisbane/1/2018 X-311A reassortant viruses well inhibited the parental virus. Therefore, both reassortant viruses are antigenically similar to the parental virus and pass the two-way test as A/Brisbane/1/2018-like (as previously reported to you on 8/20/18). Based on antigenic characterization by HI, egg-propagated A/Brisbane/1/2018 and A/Brisbane/1/2018 X-311 can be considered A/Switzerland/8060/2017-like. Ferret antisera raised against A/Brisbane/1/2018 X-311A poorly inhibited A/Switzerland/8060/2017 (greater than 8-fold reduction in HI titer) compared with the homologous virus. Therefore, A/Brisbane/1/2018 X-311A is not considered A/Switzerland/8060/2017-like.

The HA and NA genes of your reassortants were sequenced and compared to that of the wild type parental virus A/Brisbane/1/2018 (previously reported to you on 8/20/18) and to A/Switzerland/8060/2017. The HA gene of A/Brisbane/1/2018

X-311 has two amino acid changes, S96N and A212T, compared to A/Switzerland/8060/2017. The HA gene of A/Brisbane/1/2018 X-311A has three amino acid changes, S96N, T203I, and D408G, compared to A/Switzerland/8060/2017. Compared to the NA genes of A/Switzerland/8060/2017, both A/Brisbane/1/2018 X-311 and A/Brisbane/1/2018 X-311A have amino acid changes at V143A and V194I. 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 H3 VIRUSES
WITH 20nM OSELTAMIVIR, 4 HA UNITS/ 50 MICROLITERS
DATE TESTED: 10/17/18

REFERENCE VIRUSES	HA GROUP	EGG SZ/8060	EGG AS/01	EGG X-311 AS/01	EGG X-311A AS/01	PASSAGE	DATE COLL.
1 A SWITZERLAND/8060/17	3C.2a2	**2560**	2560	1280	<20	E5/E2(10/1/18)	
2 A/BRISBANE/01/18	3C.2a2	5120	**5120**	2560	80	E4/E2(5/11/18)	1/2/2018
TEST VIRUSES							
3 A/BRISBANE/1/2018 X-311	3C.2a2	2560	2560	**2560**	80	E4E9	
4 A/BRISBANE/1/2018 X-311A	3C.2a2	5120	5120	2560	**160**	E4E9	

QUALITY CONTROL:

TEST	RESULTS
HA GENETIC CLADE	3C.2a2
EXCLUSIVITY PCR*	H3 POSITIVE ONLY

*This PCR test determines qualitatively the presence or absence of influenza A H3, A H1pdm09, and Bs