Medicines & Healthcare products Regulatory Agency

NIBSC Confidence in Biological Medicines

Influenza Reagent
Influenza Virus Infectious NYMC BX-63A
NIBSC code: 17/126
Instructions for use
(Version 2.0, Dated 23/11/2017)

1. INTENDED USE

Reagent 17/126 is prepared from NYMC BX-63A (B/Arizona/10/2015 x NYMC BX-46) 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 BX-63A 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

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

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 BX-63A

Passage	Lot	Laboratory
E1-E9		New York Medical College, USA
E10	E#6276	New York Medical College, USA
E10/E1	42780	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_268551.

Derivation of NYMC BX-63A

B/Arizona/10/2015 (Yamagata lineage) - like High Yield Reassortant (1:2:5) B/Lee:B/Panama:B/Arizona

With B/Lee/40 NP gene; B/Panama/45/90 PB2, NS genes; B/Arizona/10/2015 PB1, PA, HA, NA and M genes

Exper. #4792 10/18/16
B/Arizona/10/2015 (Yamagata lineage) CDC ID# 3000411271 E4 (8/16/2016) HA:16
NYMC BX-46: Hybrid strain with B/Panama/45/90 PB1, PB2, PA, NS and B/Lee/40 HA, NP, NA and M genes

Passage No.

1 to 4 Passages prior to receipt at NYMC (E4)

Passage at NYMC

1 pre-reassortment passage

B/Arizona/10/2015 X NYMC BX-46

2 10^{-2} + $1/3 \times 10^{-3}$

HA—1:512

3 10^{-3} ↓ + B/Lee/40 HANA antibodies (ab) B/Lee/40 NA antibodies (ab)

HA—1:128

4 10^{-3} ↓ + B/Lee/40 HANA ab B/Lee/40 NA ab

HA— 1:128

5 10^{-3} ↓ + B/Lee/40 HANA B/Lee/40 NA ab

HA—1:128

6 10^{-4} ↓

HA—1:256

7 10^{-4} ↓

HA—1:256

8 10^{-8} ↓

HA—1:256

9 10^{-8} ↓

HA—1:1024

10 10^{-5} ↓

HA—1:512

NYMC BX-63A (E4:E10)
E# 6276 NYMC archive

HA, NA, PB1, PA and M genes were identified as B/Arizona/10/2015, NP gene as B/Lee/40, and PB2 and NS genes as B/Panama/45/90 by RT-PCR/RFLP analysis.
SPAFAS eggs were used for all passages.
HA titers were performed using chicken 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 ºC. The sterility test is not performed according to a method of the USP <71> / Ph. Eur. 2.6.1 / 21 CFR 610.12.

UPLC—HA quant. ug/ml allantoic fluid

Influenza B Reassortant	**HA ug/mL**
Yamagata Lineage	
B/Arizona/10/2015	3.4
B/Arizona/10/2015 Passage 10	7.2
NYMC BX-63 (B/Arizona/10/2015)	8
NYMC BX-63A (B/Arizona/10/2015)	10

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Centers for Disease Control
and Prevention

03/17/2017

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
3000628126	B/ARIZONA/10/2015 BX-63A	CONSISTENT WITH B/ARIZONA/10/2015; TWO WAY PASS

Your reassortant had HI reactivity patterns that were consistent with the corresponding wild type virus, and it is antigenically similar to B/PHUKET/3073/2013 virus. Ferret antiserum raised against the B/ARIZONA/10/2015 BX-63A 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 B/ARIZONA/10/2015. The reassortant virus has a number of amino acid changes at residue K162E, N197S and A/T199T in the HA, compared with that of the wild type B/ARIZONA/10/2015. There is no amino acid difference between the reassortant and its parental virus in 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 TYPE B YAMAGATA LINEAGE VIRUSES*								
DATE TESTED: 3/9/2017								
REFERENCE ANTIGENS		REFERENCE FERRET ANTISERA						
	STRAIN DESIGNATION	PHU/3073	PHU/3073	AZ/10	AZ/10	AZ/10 BX-63A	PASSAGE	DATE COLL.
1	B/PHUKET/3073/2013	**640**	320	640	320	2560	E4/E2(4/7/15)	11/21/2013
2	B/PHUKET/3073/2013	640	**640**	640	320	2560	C2/C2(1/13/17))	11/21/2013
3	B/ARIZONA/10/15	320	320	**640**	160	1280	E4(8/16/16)	11/2/2015
4	B/ARIZONA/10/15	640	320	320	**320**	1280	C2(8/15/16)	11/2/2015
5	B/ARIZONA/10/15 BX-63A	640	320	640	160	**2560**	E4E10	

*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.