Medicines & Healthcare products Regulatory Agency

NIBSC Confidence in Biological Medicines

Influenza Reagent
Influenza Virus Infectious NYMC BX-53C
NIBSC code: 15/212
Instructions for use
(Version 2.0, Dated 02/06/2016)

1. INTENDED USE

Reagent 15/212 is prepared from NYMC BX-53C which was processed for freeze drying in 250µl volumes as described by Campbell, PJ, Journal of Biological Standardisation, 1974, 2,249-267. The known passage history of NYMC BX-53C 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: NA
Toxicity Statement: Non-toxic
Veterinary certificate or other statement if applicable**.** **Attached:** No

Passage history of NYMC BX-53C (Post mixed infection)

Passage	Lot	Laboratory
E1-E5		NYMC, New York, USA
E6	E#6070	NYMC, New York, USA
E7	39400	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_220927

Derivation of NYMC BX-53C

B/Texas/02/2013 (Victoria lineage) - like High Yield Reassortant (2:6) With B/Panama/45/90 PB1 and PA

Exper. # 4734 10/11/13
B/Texas/02/2013 (Victoria lineage) CDC#2013705942 E5 (4/16/13) HA:64
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 5 Passages prior to receipt at NYMC (E5)

Passage at NYMC

6 pre-reassortment passage

B/Texas/02/2013 X NYMC BX-46

7 10^{-2} + 2.5 X 10^{-3}

HA—1:1024

↓

8 10^{-3} + B/Lee/40 HANA antibodies (ab)
B/Lee/40 NA antibodies (ab)

HA—1:32

↓

9 10^{-3} + B/Lee/40 HANA ab
B/Lee/40 NA ab

HA— 1:512

↓

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

10	10^{-8}	HA—1:512
11	10^{-8}	HA—1:1024
12	10^{-5}	HA—1:1024
13	10^{-5}	HA—1:1024

NYMC BX-53C (E5:E6-13)
E#6070 NYMC archive

HA, NA, PB2, NP, M and NS genes were identified as B/Texas/02/2013, and PB1 and PA 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.

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Centers for Disease Control and Prevention

2/12/2014

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 result we obtained with your reassortant is listed and interpreted below.

Your reassortant was characterized by a "two-way" hemagglutination-inhibition test using post-infection ferret antisera.

CDC ID#	Specimen ID#	Results
2014701174	B/TEXAS/02/2013 BX-53C	CONSISTENT WITH B/TEXAS/02/2013; PASS

Your reassortant has an HI reactivity pattern that is consistent with its corresponding wild type virus.

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. Nancy Cox

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

HEMAGGLUTINATION INHIBITION REACTIONS OF INFLUENZA TYPE B VICTORIA LINEAGE VIRUSES*						
TWO-WAY TEST						
TESTED 2/11/2014						
STRAIN DESIGNATION	REFERENCE FERRET ANTISERA					
			BX-53C		DATE	
REFERENCE ANTIGENS	BRI/60	TX/02	TX/02	PASSAGE	COLLECTED	
1 B/BRISBANE/60/2008	**320**	640	1280	E4/E4(6/19/12)	8/4/2008	
2 B/TEXAS/02/2013	320	**1280**	2560	E5(4/16/13)	1/9/2013	
TEST ANTIGEN						
3 B/TEXAS/02/2013 BX-53C	320	640	**2560**	E5E8	REASS	

*A reassortant is considered consistent with the wild type if its HI titer is equal to or within a two-fold difference to the homologous HI titer of the wild type reference virus.

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Centers for Disease Control and Prevention

02/14/2014

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 reassortant were sequenced and compared to those of its egg propagated wild type parental virus B/TEXAS/02/2013. The results we obtained with your reassortant are listed and interpreted below.

CDC ID#	Specimen ID#	Results
2014701174	B/TEXAS/02/2013 BX-53C E5E8	HA: Ala-127-Thr, Ile/Thr-199-Thr NA: No change detected

Although two amino acid change were detected in the HA gene, the reassortant virus has two-way HI reactivity patterns that are consistent with its corresponding wild type virus, B/TEXAS/02/2013. Pass.

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. Nancy Cox

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