



**Influenza Reagent  
Influenza Virus Infectious NYMC BX-69A  
NIBSC code: 17/256  
Instructions for use  
(Version 2.0, Dated 04/04/2018)**

**1. INTENDED USE**

Reagent 17/256 is prepared from NYMC BX-69A (B/Maryland/15/2016 x NYMC BX-46) B-Victoria lineage 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-69A 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**

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<sup>-3</sup> to 10<sup>-6</sup>) 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](mailto: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](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](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](mailto: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.



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**16. INFORMATION FOR CUSTOMS USE ONLY**

<b>Country of origin for customs purposes*:</b> 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.
<b>Net weight:</b> 0.25g per ampoule
<b>Toxicity Statement:</b> Non-toxic
<b>Veterinary certificate or other statement</b> if applicable.
<b>Attached:</b> No

**Derivation of NYMC BX-69A**

Passage	Lot	Laboratory
E1-E10		NYMC, New York, USA
E11	6305	NYMC, New York, USA
E12	43230	NIBSC, Hertfordshire, UK

Number of passages post mixed infection = 11

**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\_305349.



## Derivation of NYMC BX-69A

**B/Maryland/15/2016 (Victoria lineage) - like High Yield Reassortant (1:1:6)  
B/Lee:B/Panama:B/Maryland  
with B/Lee/40 NP gene; B/Panama/45/90 PB2 gene; B/Maryland/15/2016 PB1,  
PA, HA, NA, M, NS genes**

Exper. # 4802 6/02/17

B/Maryland/15/2016(Victoria lineage) CDC# 3025628112 E3 (2/21/2017) 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 3

Passages prior to receipt at NYMC (E3)

### Passage at NYMC

1

pre-reassortment passage

### B/Maryland/15/2016 X NYMC BX-46

2

$10^{-3}$  +  $5 \times 10^{-4}$

HA—1:512

3

$10^{-3}$

↓ + B/Lee/40 HANA antibodies (ab)  
B/Lee/40 NA antibodies (ab)

HA—1:256

4

$10^{-3}$

↓ + B/Lee/40 HANA ab  
B/Lee/40 NA ab

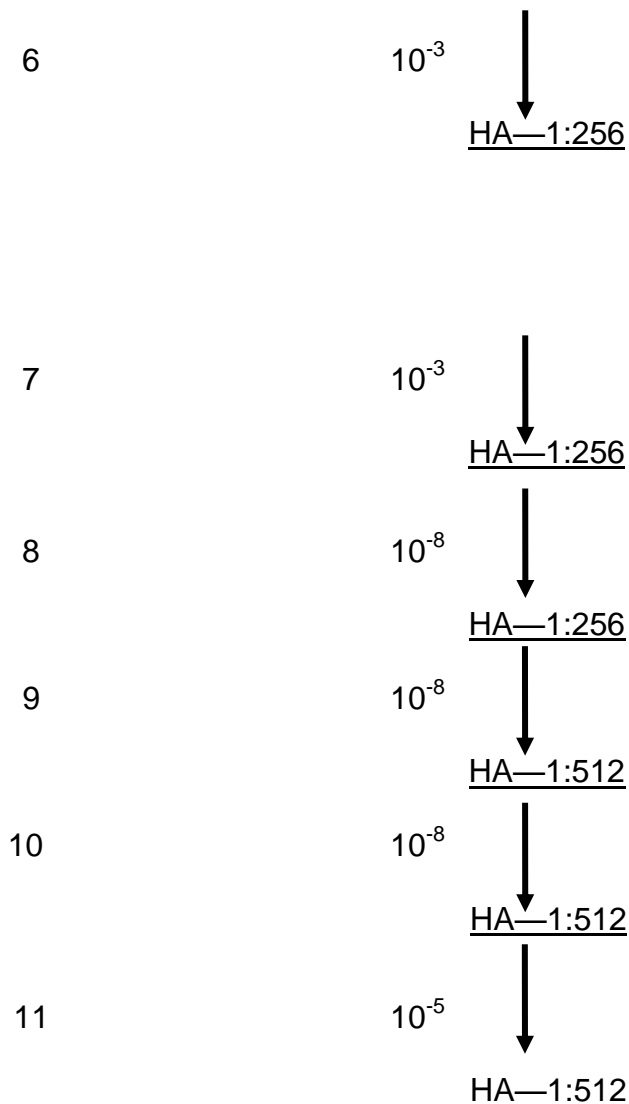
HA— 1:512~1024

5

$10^{-3}$

↓ + B/Lee/40 HANA ab  
B/Lee/40 NA ab

HA—1:128~512



**NYMC BX-69A (E3:E11)**  
E#6305 NYMC archive

HA and NA identified as B/Maryland/15/2016 by RT-PCR/RFLP analysis of HA and NA genes. RT-PCR/RFLP analysis also identified NP as B/Lee/40; PB2 as B/Panama/45/90; PB1, PA, M, NS (in addition to HA and NA) as B/Maryland/15/2016.

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 °C. 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

11/15/2017

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.

CDC ID#	Specimen ID#	Results
3000628134	B/MARYLAND/15/2016 BX-69	CONSISTENT WITH B/MARYLAND/15/2016; TWO WAY PASS
3000628135	B/MARYLAND/15/2016 BX-69A	CONSISTENT WITH B/MARYLAND/15/2016; TWO WAY PASS

Your reassortants have HI reactivity patterns that are consistent with the corresponding wild type virus B/Maryland/15/2016 which has two amino acid deletions at positions 162 and 163 in the HA. Therefore, they are antigenically similar to the parental virus and pass the two way test. On the other hand, ferret antiserum raised against B/Brisbane/60/2008, the current vaccine strain, poorly inhibit the B/Maryland/15/2016 and the two high yielding reassortants, indicating the parental double deletion variant virus and these two reassortants are antigenically distinct from the vaccine virus. In a supplemental evaluation of the reassortants, we tested a small number of circulating viruses for their reactivity with ferret antisera raised against the two reassortants; ferret antiserum raised against B/MARYLAND/15/2016 BX-69A well inhibit (a  $\leq 4$ -fold reduction in titer compared to homologous virus titer) the majority of the double deletion viruses tested. Whereas ferret antiserum to B/MARYLAND/15/2016 BX-69 well inhibit a smaller proportion of the double deletion viruses tested.

The HA and NA genes of your reassortants were sequenced and compared to that of the wild type parental virus B/Maryland/15/2016. There are no amino acid difference between both reassortant and the parental virus in the HA and 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											
Date Tested: 11/6/2017			Reference Antiserum								
			MI/45	HK/4801	PHU/3073	BRI/60	CO/6	MD/15	MD/15 BX-69	MD/15 BX-69A	
	Reference Antigen	Subtype/Lineage	A	B	C	D	E	F	G	H	Passage
1	A/Michigan/45/2015	H1N1pdm09	2560	<10	<10	<10	<10	<10	<10	<10	E3(12/17/15)
2	A/HongKong/4801/14	H3N2	<10	640	<10	<10	<10	<10	<10	<10	E5/E2(9/9/16)
3	B/Phuket/3073/2013	B/Yam	<10	<10	320	20	<10	<10	<10	<10	E4/E2(10/3/17)
4	B/Brisbane/60/2008	B/Vic	<10	<10	<10	640	160	160	320	80	E4/E4(4/4/16)
5	B/Colorado/6/2017	B/Vic	<10	<10	<10	160	320	320	320	160	E5(7/13/17)
6	B/Maryland/15/2016	B/Vic	<10	<10	<10	160	640	640	1280	640	E4(2/28/17)
<b>Test Antigens</b>											
7	B/MARYLAND/15/2016 BX-69	B/Vic	<10	<10	<10	160	640	640	640	320	E3/E12(7/26/17)
8	B/MARYLAND/15/2016 BX-69A	B/Vic	<10	<10	<10	160	640	640	1280	640	E3/E11(7/26/17)
	Reference Antiserum	LOT#	SPECIES	PASSAGE							
A	A/Michigan/45/2015	2017-091,092	FERRET	E3(12/7/15)							
B	A/HongKong/4801/14	2016-025	FERRET	E5/E2(4/9/15)							
C	B/Phuket/3073/2013	2015-077	FERRET	E4/E2(4/7/15)							
D	B/Brisbane/60/2008	2013-029, 030	FERRET	E4/E4 (6/19/12)							
E	B/Colorado/6/2017	2017-095	FERRET	E5(7/13/17)							
F	B/Maryland/15/2016	2017-041	FERRET	E4(2/28/17)							
G	B/MARYLAND/15/2016 BX-69	2017-109	FERRET	E3E12							
H	B/MARYLAND/15/2016 BX-69A	2017-110	FERRET	E3E11							