



**Influenza Reagent  
Influenza Virus Infectious NYMC BX-69  
NIBSC code: 17/252  
Instructions for use  
(Version 2.0, Dated 04/04/2018)**

### 1. INTENDED USE

Reagent 17/252 is prepared from NYMC BX-69 (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-69 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:<br>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.<br>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<br>* 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-69**

| Passage | Lot   | Laboratory               |
|---------|-------|--------------------------|
| E1-E11  |       | NYMC, New York, USA      |
| E12     | 6304  | NYMC, New York, USA      |
| E13     | 43220 | NIBSC, Hertfordshire, UK |

Number of passages post mixed infection = 12

**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\_304702.



## Derivation of NYMC BX-69

**B/Maryland/15/2016 (Victoria lineage) - like High Yield Reassortant (1:2:5)  
B/Lee:B/Panama:B/Maryland  
with B/Lee/40 NP gene; B/Panama/45/90 PB1, PB2 genes; B/Maryland/15/2016  
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

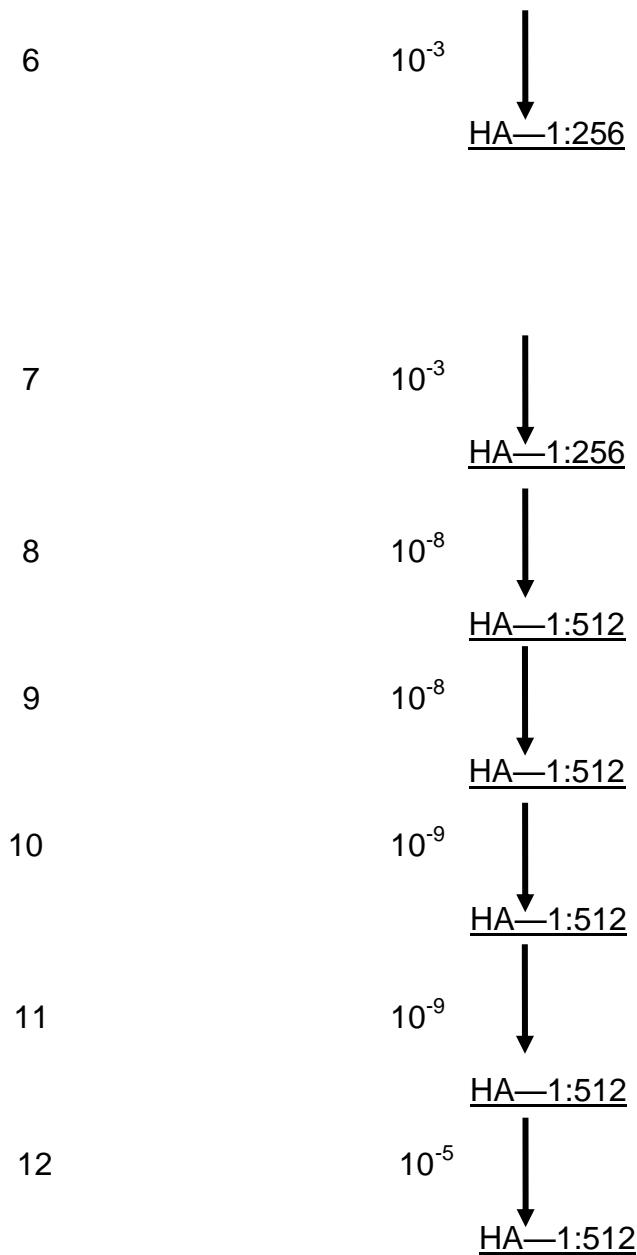
HA— 1:1024

5

$10^{-3}$

↓ +B/Lee/40 HANA ab  
B/Lee/40 NA ab

HA—1:128



**NYMC BX-69 (E3:E12)**  
E#6304 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; PB1, PB2 as B/Panama/45/90; 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           |          |        |      |       |             |              |                 |