Medicines & Healthcare products Regulatory Agency

NIBSC Confidence in Biological Medicines

Influenza Reagent
Influenza Virus Infectious NIB-93
NIBSC code: 15/154
Instructions for use
(Version 2.0, Dated 29/09/2015)

1. INTENDED USE

Reagent 15/154 is prepared from NIB-93 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 NIB-93 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 NIB-93 (Post mixed infection)

Passage	Lot	Laboratory
E1-E6		NIBSC, Hertfordshire, UK
E7	40620	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.

Derivation of NIB-93

A/Hong Kong/7127/2014 (H3N2)-like High Growth Reassortant

Strain: A/Hong Kong/7127/2014 (H3N2)
Received from CRICK #150057, E4
Passage undertaken at NIBSC #40400, E5

Mixed Infection: A/Hong Kong/7127/2014 (10^{-1}) x IVR-145 (10^{-5})
HA Titre: 512

↓

1st Antiserum passage: Inoculum 10^{-2} with IVR-145 antiserum
HA Titre: 512

↓

2nd Antiserum passage: Inoculum 10^{-3} with IVR-145 antiserum
HA Titre: 256

↓

3rd Antiserum passage: Inoculum 10^{-2} with IVR-145 antiserum
HA Titre: 512

↓

4th passage: Inoculum 10^{-8}
HA Titre: 512

↓

5th passage: Inoculum 10^{-8}
HA Titre: 512

↓

6th passage: Inoculum 10^{-9}
HA Titre: 512

↓

7th passage: Inoculum 10^{-5}
HA Titre: 512
Lot: 40620

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

Total number of passages since mixed infection= E7
SPF eggs were used for all passages.
RT-PCR/RFLP analysis indicates that NIB-93 has HA and NA genes from A/Hong Kong/7127/2014 and NP, NS, PB1, PB2, PA and MX genes from IVR -145 making it a 6:2 reassortant.

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 Francis Crick Institute, Mill Hill Laboratory,
The Ridgeway, Mill Hill, London NW7 1AA. UK
info@crick.ac.uk www.crick.ac.uk

THE FRANCIS CRICK INSTITUTE

Monday, 03 August 2015

We have the 2-way results for the HI results for NIB-93 (based on A/Hong Kong/7127/2014).

Both the antiserum you raised against A/Hong Kong/7127/2014 and the one raised here recognised NIB-93 in an HI test using guinea pig red blood cells done in the presence of 20nM Oseltamivir at titres equal to the titres of the antisera for the homologous virus A/Hong Kong/7127/2014; the antiserum you raised against NIB-93 recognised A/Hong Kong/7127/2014 in the HI test at the same titre of the antiserum for NIB-93. Therefore NIB-93 is antigenically indistinguishable from its parent A/Hong Kong/7127/2014.

The results are below.

Antigenic analyses of influenza A(H3N2) viruses (Guinea Pig RBC with 20nM Oseltamivir) 2015-07-24

Viruses	Collection Date	Passage History	A/HK 7127/14 F11/15	A/HK 7127/14 NIB F52/15	NIB-93 A/HK/7127/14) NIB F53/15
Genetic group					
REFERENCE VIRUSES					
A/Hong Kong/7127/2014		E5	1280	640	640
NIB-93 (A/Hong Kong/7127/2014)		E7	1280	640	640

I hope that you find the results useful.

With best wishes,

Digitally signed by John William McCauley
Date: 2015.08.03 15:43:48 +01'00'

John McCauley BSc PhD
Director, Crick Worldwide Influenza Centre.

The Francis Crick Institute, Mill Hill Laboratory,
The Ridgeway, Mill Hill, London NW7 1AA. UK
info@crick.ac.uk www.crick.ac.uk

THE FRANCIS CRICK INSTITUTE

Monday, 28 September 2015

We have more 2-way results for the HI results for NIB-93 (based on A/Hong Kong/7127/2014).

To extend the results I sent in my letter of 3rd August, an antiserum raised against A/Hong Kong/4801/2014 recognised NIB-93 in an HI test using guinea pig red blood cells done in the presence of 20nM Oseltamivir at a titre 2-fold reduced over the titre of the antiserum for the homologous virus A/Hong Kong/4801/2014; the antiserum you raised against NIB-93 recognised A/Hong Kong/4801/2014 in the HI test at a titre 2-fold higher than its titres for NIB-93. This antiserum also recognised A/Hong Kong/7127/2014 as before, at the same titre as it recognised NIB-93, and the antiserum raised against A/Hong Kong/7127/2014 recognised NIB-93 at a titre within 2-fold of the titre of the homologous titre of the antiserum.

Therefore, NIB-93 is antigenically indistinguishable from its parent A/Hong Kong/7127/2014, as previously described, and it is also antigenically like A/Hong Kong/4801/2014.

The results are below.

Antigenic analyses of influenza A(H3N2) viruses (Guinea Pig RBC with 20nM Oseltamivir) 2015-08-13

Viruses	Collection Date	Passage History	A/HK 4801/14 F12/15	A/HK 7127/14 F11/15	NIB-93 (A/HK/7127/14) NIB F53/15
A/Hong Kong/4801/2014	2014-02-26	E6E1 Isolate 1	320	1280	1280
A/Hong Kong/7127/2014	2014-07-29	E6/E1	320	1280	640
NIB-93 (A/Hong Kong/7127/2014)		E7	160	640	640

I hope that you find the results useful.

With best wishes,

Digitally signed by John William McCauley
Date: 2015.09.28 16:22:18 +01'00'

John McCauley BSc PhD
Director, Crick Worldwide Influenza Centre.