Medicines & Healthcare products Regulatory Agency

NIBSC Confidence in Biological Medicines

Influenza Reagent
Influenza Virus Infectious NIB-103
NIBSC code: 17/184
Instructions for use
(Version 2.0, Dated 23/11/2017)

1. INTENDED USE

Reagent 17/184 is prepared from NIB-103 (A/Norway/3806/2016 x IVR-145) (H3N2) 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 NIB-103 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 NIB-103 (post mixed infection)

Passage	Lot	Laboratory
E1-E6		NIBSC, Hertfordshire, UK
E7	42610	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_281392.

Derivation of NIB-103

A/Norway/3806/2016 x IVR-145 (H3N2)-like High Growth Reassortant

Strain: A/Norway/3806/2016 (H3N2)
Received from CRICK 162860 1s2c25, E7
Passage undertaken at NIBSC #42480, E8

Mixed Infection: A/Norway/3806/2016 (10^{-2}) x IVR-145 (10^{-4})
HA Titre: 2048

↓

1st Antiserum passage: Inoculum 10^{-5} with IVR-145 antiserum
HA Titre: 512

↓

2nd Antiserum passage: Inoculum 10^{-4} with IVR-145 antiserum
HA Titre: 512

↓

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

↓

4th Antiserum passage: Inoculum 10^{-2} with IVR-145 antiserum
HA Titre: 1024

↓

5th passage: Inoculum 10^{-9}
HA Titre: 512

↓

6th passage: Inoculum 10^{-7}
HA Titre: 256

↓

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

Lot: 42610

Total number of passages since mixed infection= E7

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

SPF eggs were used for all passages.

RT-PCR/RFLP analysis indicates that NIB-103 has HA, NA, NP, NS, PB2, PA and PB1 genes from A/Norway/3806/2016 and the MX gene from A/PR/8/34, making it a 1:7 reassortant.

Medicines & Healthcare products Regulatory Agency

NIBSC Confidence in Biological Medicines

The Crick Worldwide Influenza Centre,
The Francis Crick Institute,
1 Midland Road, London NW1 1AT. UK
info@crick.ac.uk, www.crick.ac.uk

THE FRANCIS CRICK INSTITUTE

Monday, 04 September 2017

NIB-103 (A/Norway/3806/2016)

Your high yield/growth reassortant virus NIB-103 derived from A/Norway/3806/2016 has now been analysed in a 2-way test and its HA and NA gene sequences determined.

In HI assays using guinea pig red blood cells in the presence of oseltamivir the post infection antiserum raised against egg-propagated cultivar of A/Norway/3806/2016 (F45/16) recognised NIB-103 with a titre equal to the titre of the antiserum for the homologous virus. The post infection antiserum raised against egg-propagated cultivar of A/Hong Kong/4801/2014 (F12/15) recognised NIB-103 with a titre at a titre 2-fold lower than the titre of the antiserum for the homologous virus. Antiserum raised against NIB-103 (NIB F13/17) recognised against the egg-propagated cultivar of A/Norway/3806/0216 at the same titre as it recognised the reassortant virus, and the antiserum raised against NIB-103 also recognised against the egg-propagated cultivar of A/Hong Kong/4801/2016 at a titre equal to the titre of the antiserum for the reassortant virus. The results of the test are shown in the annex below.

The gene sequence of the HA and NA genes of NIB-103 was determined and compared with the cell culture-propagated and egg-propagated cultivars of A/Norway/3806/2016. Alignments are attached. You will see that the HA gene sequence of NIB-103 differed from the HA gene sequence of the egg-propagated cultivar of A/Norway/3806 (Is2 c25) at residue 203 of HA1 (T203I). The HA gene sequences of both the egg-propagated cultivar of A/Norway/3806/2016 and the resassortant NIB-103 also differed from the cell culture-propagated cultivar of A/Norway/3806/2016 at two positions: those associated with egg-adaptation T160K in HA1 (resulting in the loss of the glycosylation site at residue 158 of HA1, and L194P in HA1, the second common site of egg-adaptation in 3C.2a viruses. There were no changes in seen the NA sequences.

These results indicate that NIB-103 can be considered as antigenically like its parent egg-propagated A/Norway/48012/2016 and also antigenically like the prototype virus A/Hong Kong/4801/2014.

I hope that you find the results useful.

With best wishes,
Yours sincerely,

John McCauley
Director, WHO Collaborating Centre for Reference and Research on Influenza
The Francis Crick Institute.

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

Annex 1. HI analysis of NIB-103.

Antigenic analyses of influenza A(H3N2) viruses (Guinea Pig RBC with 20nM Oseltamivir) 2017-08-11

WIC number	Viruses	Other information		Collection date	Passage history	A/HK 4801/14	A/Norway 3806/16	NIB-103 (A/Nor/3806/16)
			Passage history			Egg	Egg	Egg
			Ferret number			F12/15	NIB F45/16	NIB F13/17
			Genetic group			3C.2a	3C.2a1	3C.2a1
	REFERENCE VIRUSES							
	A/Hong Kong/4801/2014	isolate 1	3C.2a	2014-02-26	E6/E2	320	160	1280
	TEST VIRUSES							
	A/Norway/3806/2016	isolate 1 clone 25	3C.2a1	2016-16-06	E8	160	160	1280
	NIB-103 (A/Norway/3806/2016)				Ex	160	160	1280

Assay HI (Guinea Pig RBC with 20nM Oseltamivir)

Vaccine SH 2016 NH2016-17

RBC Guinea Pig

Virus Influenza A(H3N2)

Date 2017-08-11