



**Influenza Reagent  
Influenza Virus Infectious NIB-96  
NIBSC code: 16/360  
Instructions for use  
(Version 1.0, Dated 14/03/2017)**

**1. INTENDED USE**

Reagent 16/360 is prepared from NIB-96 (A/Slovenia/2903/2015 x X-157) (H1N1pdm09) 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-96 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**

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<sup>-3</sup> to 10<sup>-5</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.

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](http://www.nibsc.org/About_Us/Terms_and_Conditions.aspx) or upon request by the Recipient) ("Conditions") apply to the exclusion of all other



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**

<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

**Passage history of NIB-96 (post mixed infection)**

Passage	Lot	Laboratory
E1-E6		NIBSC, Hertfordshire, UK
E7	41920	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\_253574.



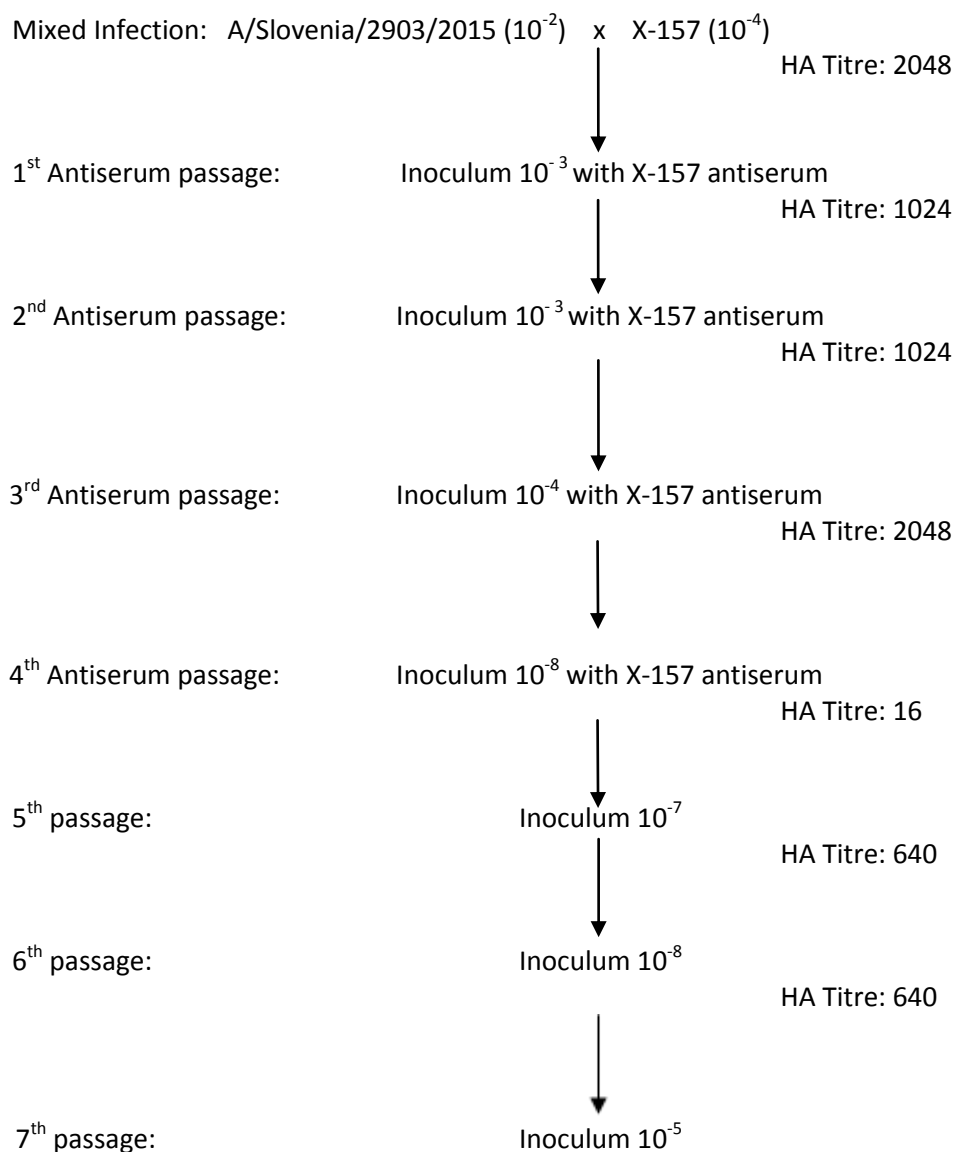
## Derivation of NIB-96

### A/Slovenia/2903/2015 (H1N1)-like High Growth Reassortant

Strain: A/Slovenia/2903/2015 (H1N1)

Received from CRICK clone 56 E3

Passage undertaken at NIBSC #41730, E4





HA Titre: 320

Lot: 41920

Total number of passages since mixed infection= E7

SPF eggs were used for all passages.

RT-PCR/RFLP analysis indicates that NIB-96 has HA and NA genes from A/Slovenia/2903/2015 and NP, NS, PB1, PB2 and MX genes from X-157. The PA gene is mixed.



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Wednesday, 08 March 2017

This is to confirm that NIB-96 was tested again in two sets of 2-way HI tests on 13<sup>th</sup> and 16<sup>th</sup> February 2017 and passed both 2-way tests. The results were the same in both tests.

The post infection antiserum raised against egg-propagated cultivar of A/Slovenia/2903/2015 (F02/16) recognised NIB-96 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/Michigan/45/2015 (NIB 42/16) recognised NIB-96 with a titre within 2-fold of the titre of the antiserum for the homologous virus, the titre of the antiserum raised against A/Michigan/45/2015 recognising NIB-96 at a titre 2-fold higher than the titre for the homologous virus. Antiserum raised against NIB-96 (NIB F08/16) recognised against the egg-propagated cultivar of A/Slovenia/2903/2015 at the same titre as it recognised the homologous virus, and the antiserum raised against NIB-96 (NIB F08/16) recognised against the egg-propagated cultivar of A/Michigan/45/2015 at a titre 2-fold lower than the titre of the antiserum for the homologous virus. The results of one of the two tests are shown below.

The gene sequence of the HA gene of NIB-96 was determined again and reanalysed and compared with A/Slovenia/2903/2015 and A/Michigan/45/2015. A PDF of an alignment is attached. You will see that as previously NIB-96 had an identical HA gene sequence to its parent A/Slovenia/2903/2015 but differed from the HA gene sequence at two positions: residue 271 of HA1 in which A/Slovenia/2903/2015 had a Glutamine residue and A/Michigan/45/2015 had a Proline, and residue 18 of HA2 A/Slovenia/2903/2015 was Valine but position 18 of HA2 for A/Michigan/45/2015 showed a mixed sequence.

WHO International Laboratory for Biological Standards,  
UK Official Medicines Control Laboratory



## Antigenic analyses of influenza A(H1N1)pdm09 viruses (2017-02-13 KC)

Viruses	Other information	Genetic group	Collection date	Passage history	NIB-96		
					A/Slov 2903/2015	A/Mich 45/15	(A/Slov) 2903/2015
Passage history					Egg	Egg	Egg
Ferret number					F02/16 <sup>-1</sup>	NIB 42/16 <sup>-1</sup>	NIB F08/16 <sup>-1</sup>
Genetic group					6B.1	6B.1	6B.1
<b>REFERENCE VIRUSES</b>							
A/Slovenia/2903/2015	clone 37	6B.1	2015-10-26	E4/E1	5120	2560	5120
A/Michigan/45/2015		6B.1	2015-09-07	E3/E2	2560	1280	2560
<b>TEST VIRUSES</b>							
NIB-96 (A/Slovenia/2903/2015)		6B.1		E7/E1	5120	2560	5120

Assay HI (Turkey RBC)

RBC Turkey

Virus A(H1N1)pdm09

\* Superscripts refer to antiserum properties (< relates to the lowest dilution of antiserum used)

<sup>1</sup> < = <40

I hope that you find the results useful.

With best wishes,  
Yours sincerely,

John McCauley.

