



**Influenza Reagent
Influenza Virus Infectious CNIC-1701
NIBSC code: 17/156
Instructions for use
(Version 2.0, Dated 23/11/2017)**

1. INTENDED USE

Reagent 17/156 is prepared from CNIC-1701 (A/Yunnan-Wuhua/SWL1938/2016 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 CNIC-1701 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. 10⁻³ 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



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 CNIC-1701 (post mixed infection)

Passage	Lot	Laboratory
E1-E7		CNIC, Beijing, China
E8		CNIC, Beijing, China
E9	42910	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_277728.



Derivation of CNIC-1701

A/Yunnan-Wuhua/SWL1938/2016-like High Growth Reassortant

Strain: A/Yunnan-Wuhua/SWL1938/2016

Passage undertaken at CNIC

Mixed Infection: A/Yunnan-Wuhua/SWL1938/2016 (10^{-2}) X X-157 (10^{-6})

HA Titer: 1024



1st Antiserum passage: Inoculum 10^{-1} with X-157 HA & NA antiserum

HA Titer: 256



2nd Antiserum passage: Inoculum 10^{-3} with X-157 antiserum

HA Titer: 2048



3rd passage: Inoculum 10^{-7}

HA Titer: 1024

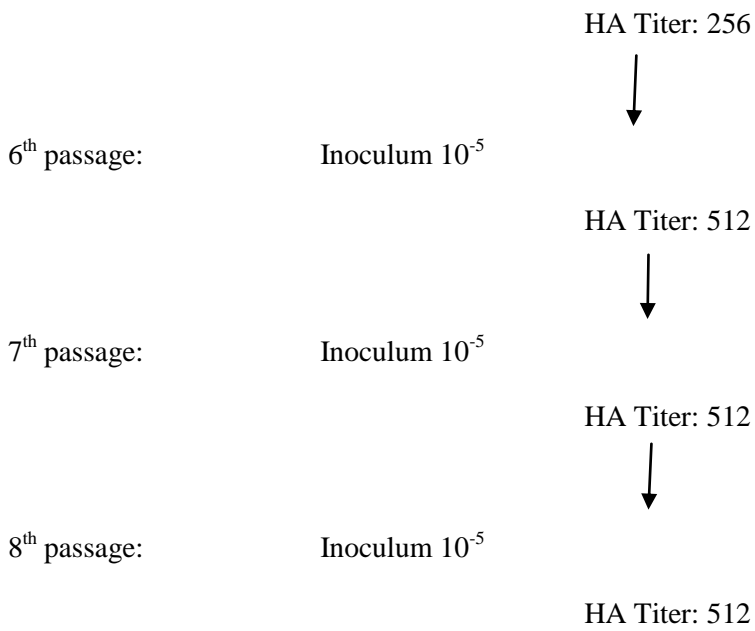


4th passage: Inoculum 10^{-8}

HA Titer: 1024



5th passage: Inoculum 10^{-5}



Total number of passages since mixed infection=E8

SPF eggs were used for all passages.

RT-PCR/RFLP and sequencing analysis indicates that CNIC-1701 has HA and NA genes from A/Yunnan-Wuhua/SWL1938/2016 and M, NP, PB1, PB2, PA and NS genes from A/PR/8/34 (X-157) making it a 6:2 reassortant. An amino acid change at position L208I and D239N was detected in the HA gene.

CNIC-1701 HIGH GROWTH REASSORTANT REPORT

The reassortant was characterized by a “two-way” hemagglutination-inhibition test using post-infection ferret antisera raised against both egg and cell isolates of wild type virus and the HGR.

The results obtained with the reassortant are listed and interpreted below.

Specimen ID#	Results
A/Yunnan-Wuhua/SWL1938/2016 CNIC-1701	A/Yunnan-Wuhua/SWL1938/2016-LIKE; TWO-WAY PASS

Although amino acid changes at position L208I and D239N was detected in the HA gene, the reassortant virus has HI reactivity pattern that was consistent with its corresponding wild type virus, therefore, it **passed** the two-way test.

HEMAGGLUTINATION INHIBITION REACTIONS OF INFLUENZA TYPE A H1N1pdm VIRUSES TWO-WAY TEST

TESTED 15/06/2017

REFERENCE ANTIGEN ^o	FERRET ANTISERA													DATA COLL.
	SC/S WL1 ^o	CA/7 ^o	Texas/ 77 ^o	JL-CY/ 1182 ^o	SH-JD /1970 ^o	SH-JD /1970 ^o	MI/45 ^o	MI/45 ^o	YN-WH/ 1938 ^o	YN-WH/ 1938 ^o	CNIC- 1701 ^o	PASSAGE HISTORY ^o		
A/sichuan/SWL1/2009 ^o	2560 ^o	2560 ^o	640 ^o	2560 ^o	160 ^o	640 ^o	640 ^o	2560 ^o	640 ^o	2560 ^o	2560 ^o	2560 ^o	2009/5/10.	
A/California/7/2009C ^o	2560 ^o	2560 ^o	640 ^o	2560 ^o	320 ^o	1280 ^o	1280 ^o	2560 ^o	1280 ^o	2560 ^o	2560 ^o	C3+C3 ^o	2009/4/9.	
A/Texas/77/2009 ^o	640 ^o	160 ^o	1280 ^o	1280 ^o	160 ^o	320 ^o	80 ^o	320 ^o	320 ^o	320 ^o	1280 ^o	2560 ^o	2009/10/6.	
A/jilin-chaoyang/SWL1 182/2014 ^o	2560 ^o	2560 ^o	640 ^o	2560 ^o	640 ^o	640 ^o	1280 ^o	2560 ^o	1280 ^o	2560 ^o	2560 ^o	E4 ^o	2014/3/6.	
A/shanghai-jiading/SW L1970/2015E ^o	2560 ^o	1280 ^o	320 ^o	2560 ^o	320 ^o	640 ^o	640 ^o	2560 ^o	640 ^o	2560 ^o	1280 ^o	E4 ^o	2015/12/6.	
A/shanghai-jiading/SW L1970/2015C ^o	640 ^o	640 ^o	320 ^o	2560 ^o	640 ^o	1280 ^o	640 ^o	2560 ^o	640 ^o	2560 ^o	2560 ^o	C3 ^o	2015/12/6.	
A/Michigan/45/2015E ^o	2560 ^o	2560 ^o	320 ^o	2560 ^o	320 ^o	640 ^o	640 ^o	2560 ^o	640 ^o	2560 ^o	2560 ^o	E4+1 ^o	2015/9/7.	
A/Michigan/45/2015C ^o	2560 ^o	1280 ^o	160 ^o	2560 ^o	320 ^o	320 ^o	640 ^o	1280 ^o	640 ^o	2560 ^o	2560 ^o	M1/C2+ C2 ^o	2015/9/7.	
A/yunnan-wuhua/SWL 1938/2016E ^o	2560 ^o	1280 ^o	160 ^o	2560 ^o	320 ^o	640 ^o	640 ^o	1280 ^o	640 ^o	2560 ^o	2560 ^o	E1+3 ^o	2016/12/14.	
A/yunnan-wuhua/SWL 1938/2016C ^o	2560 ^o	2560 ^o	320 ^o	2560 ^o	640 ^o	2560 ^o	2560 ^o	2560 ^o	2560 ^o	2560 ^o	2560 ^o	C1+1 ^o	2016/12/14.	
CNIC-1701 ^o	2560 ^o	2560 ^o	160 ^o	2560 ^o	320 ^o	640 ^o	640 ^o	2560 ^o	640 ^o	2560 ^o	2560 ^o	E2/E8 ^o		