Medicines & Healthcare products Regulatory Agency

NIBSC Confidence in Biological Medicines

**Influenza Reagent**
**Influenza virus infectious IVR-175**
**NIBSC code: 14/232**
**Instructions for use**
**(Version 2.0, Dated 22/03/2016)**

## 1. INTENDED USE

Reagent 14/232 is prepared from IVR-175 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 IVR-175 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.<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.
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

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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<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. |
| **Net weight:** NA |
| **Toxicity Statement:** Non-toxic |
| **Veterinary certificate or other statement** if applicable**.**<br>**Attached:** No |

**Passage history of IVR-175 (Post mixed infection)**

| Passage | Lot | Laboratory |
|---|---|---|
| E1-E4 | | BioCSL, Australia |
| E5 | VI-1596 | BioCSL, Australia |
| E6 | 39900 | 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_207650

## Derivation of IVR-175

## A/South Australia/55/2014 – like High Growth Reassortant

A/South Australia/55/2014 (IVR-175, Lot VI-1596) is a H3N2 high growth reassortant influenza virus.

### PREPARATION

The preparation of A/South Australia/55/2014 (IVR-175, Lot VI-1596) high growth reassortant influenza virus was conducted in R&D Influenza Operations Department at bioCSL.

The high yielding parent strain used was A/Puerto Rico/8/34.

### MATERIALS

The following materials of biological origin were used during the preparation of high growth reassortant IVR-175:

**Virus Isolate:** The virus isolate was obtained from the WHO Collaborating Centre for Reference & Research on Influenza, Melbourne (WHO-CC).

Supply details are:

A/South Australia/55/2014

| | |
|---|---|
| WHO-CC Laboratory number: | 1407041 |
| Passages prior to receipt at WHO-CC: | N/A |
| Passages undertaken in WHO-CC: | 5 |

**Eggs:** Specific Pathogen Free (SPF) Premium Plus eggs were used for all passages at bioCSL.

**Antiserum:** Trypsin-periodate treated sheep hyperimmune antiserum Lot# AS367, Sub-lot # 4830, raised against influenza virus A/Puerto Rico/8/34 .

The antiserum was derived from sheep born and raised in Australia.

*Note on Transmissible Spongiform Encephalopathies (TSEs):*

Australia and New Zealand have been declared TSE free in accordance with OIE guidelines. Detailed information on Australia's animal health status can be obtaind from the following Animal Health Australia website link: http://www.animalhealthaustralia.com.au/programs/biosecurity

The trypsin used is 10x solution of gamma irradiated porcine pancreatic trypsin;

Invitrogen / Gibco Cat # 15090046, Lot No. 1142809

**PASSAGE HISTORY:**

| | | |
|---|---|---|
| *Mixed infection passage:* | A/South Australia/55/2014 wild type virus @$10^{-3}$ x A/Puerto Rico/8/34 (H1N1) @$10^{-3}$ | HA titre >=1576 |
| | ↓ | |
| *1st Antiserum Passage* | Inoculum @ $10^{-3}$ with antiserum to A/Puerto Rico/8/34 (H1N1) | HA titre = ND* |
| | ↓ | |
| *2nd Antiserum Passage* | Inoculum @ $10^{-3}$ with antiserum to A/Puerto Rico/8/34 (H1N1) | HA titre = 331 |
| | ↓ | |
| *1st Limit Dilution Passage* | Inoculum @ $10^{-8}$ | HA titre = 686 |
| | ↓ | |
| *2nd Limit Dilution Passage* | Inoculum @ $10^{-9}$ | HA titre = 422 |
| | ↓ | |
| *Preparation of IVR-175* | *Lot VI-1596* Inoculum @ $10^{-5}$ | Mean HA titre = 648 |

*ND = Not detected

Total number of passages post mixed infection = 5

Total number of passages since this virus was received from an approved laboratory = 6

HA titres were determined using chicken red blood cells at room temperature.

## TESTING OF A/SOUTH AUSTRALIA/55/2014 INFLUENZA VIRUS SEED LOT (IVR-175, LOT VI-1596)

| Test | Result | | |
|---|---|---|---|
| Sterility (in accordance with EP/BP/USP) | Pass | | |
| Antigenicity | Seed lot VI-1596 (IVR-175) has a HI reactivity pattern that is consistent with the wild type A/South Australia/55/2014 virus.<br><br>Refer to WHO-CC Influenza Virus Seedlot Identity Test Report (attached) | | |
| Genotype (by real time RT-PCR) | **6 : 2 (A/Puerto Rico/8/34 : A/South Australia/55/2014) Reassortant**<br>A/Puerto Rico/8/34 PB1, PB2, PA, NP, Matrix and NS genes were detected.<br>A/South Australia/55/2014 (wild type virus) H3 and N2 genes were detected. | | |
| | **Gene** | **A/Puerto Rico/8/34** | **A/South Australia/55/2014** |
| | **H3** | | √ |
| | **N2** | | √ |
| | **H1** | X | |
| | **N1** | X | |
| | **PB1** | √ | NT |
| | **PB2** | √ | NT |
| | **PA** | √ | NT |
| | **NP** | √ | NT |
| | **M** | √ | NT |
| | **NS** | √ | NT |
| Infectivity EID50 ($log_{10}$/0.2mL) | 7.64 | | |
| Appearance (Electron Microscopy) | The following morphologies were reported (in order of abundance):<br>Whole virus present. Small spheres, small kidneys, short filaments, medium spheres, medium irregular particles, medium filaments. | | |

√ - positive by PCR X - negative by PCR NT – Not Tested