



**Influenza Reagent  
Influenza Virus Infectious  
A/Sydney/5/2021 (H1N1) SAN-013  
NIBSC code: 22/142  
Instructions for use  
(Version 3.0, Dated 03/03/2023)**

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**1. INTENDED USE**

Reagent 22/142 is prepared from SAN-013 (A/Sydney/5/2021 (H1N1) x X-157) which was processed in 250µl volumes as liquid stock. The known passage history of SAN-013 is attached.

**2. CAUTION**

**The material is not of human or bovine origin. This preparation is not for administration to humans or animals**

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 vial contains 250µl (nominal) of infectious influenza virus as allantoic fluid from SPF embryonated hen's eggs.

**5. STORAGE**

Store in the dark at -70°C or below.  
Material type: Liquid – will be shipped according to the storage and shipping conditions of the product

**6. DIRECTIONS FOR OPENING**

Vials have a screw cap; an internal stopper may also be present. The cap should be removed by turning anti-clockwise. Care should be taken to prevent loss of the contents. Please note: If a stopper is present on removal of the cap, the stopper should remain in the vial or be removed with the cap.

**7. USE OF MATERIAL**

Ready to use.

**8. STABILITY**

Reference materials are held at NIBSC within assured, temperature-controlled storage facilities. 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](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

**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

| <b>Physical and Chemical properties</b>   |   |
|---|---|
| Physical appearance:<br>Clear liquid  | Corrosive: No   |
| Stable: Yes   | Oxidising: No   |
| Hygroscopic: No   | Irritant: No  |
| Flammable: No   | Handling: See caution, Section 2                        |
| Other Live influenza virus<br>(specify):  |   |
| <b>Toxicological properties</b>   |   |
| 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                |
| <b>Suggested First Aid</b>  |   |
| 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.                             |
| <b>Action on Spillage and Method of Disposal</b>  |   |
| Spillage of ampoule contents should be taken up with absorbent material wetted with an appropriate disinfectant. Rinse area with an appropriate disinfectant followed by water. Absorbent materials used to treat spillage should be treated as biological 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<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. |
| <b>Net weight:</b> 0.25g per vial.  |
| <b>Toxicity Statement:</b> Non-toxic  |
| <b>Veterinary certificate or other statement</b> if applicable.<br><b>Attached:</b> No  |

Passage history of SAN-013 (H1N1)

| Cumulative number of passages | Passage numbers at each stage | Lot         | Laboratory       |
|-------------------------------|-------------------------------|-------------|------------------|
| E1-E3                         | E3                            | SL10044716  | VIDRL, Australia |
| E4-E6                         | E3/E3                         | unknown     | Sanofi, USA      |
| E7-E15                        | E3/E3/E9                      | SP-2022-013 | Sanofi, USA      |
| E16                           | E3/E3/E9/E1                   | 47040       | NIBSC, 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 at GISAID with the accession number EPI\_ISL\_14134125.



**sanofi**

*Derivation of SAN-013*

*A/Sydney/5/2021 High Growth Reassortant*

*A/Sydney/5/2021 (SAN-013) is an H1N1 high growth reassortant influenza virus*

A/Sydney/5/2021 (SAN-013) is an H1N1 high growth reassortant influenza virus was conducted in Sanofi Flu Reassortant Lab, department Bacterial and Viral Technology at Sanofi, US.

Sanofi Lot No.: A/Sydney/5/2021 SAN-013 (Lot # SP-2022-013)

**Wildtype Virus:**

A/Sydney/5/2021 (the virus isolate was obtained from VIDRL)

VIDRL Lot #: SL10044716

Passages prior to receipt from VIDRL: 3

Passages prior to reassortant co-infection: 3

**Donor Virus:**

The high yielding parent donor virus, X-157 (A/New York/55/2004 x PR8, HA and NA external genes from A/New York/55/2004, and all 6 internal genes from A/Puerto Rico/8/1934) was used.

**Eggs:**

Specific Pathogen Free (SPF) premium eggs were used for all passages.

**Antiserum:**

Rabbit antisera raised against influenza reassortant virus X-157 was used in the process.



### Passage History

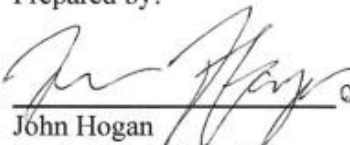
|   |  |  |                                 |
|---|--|--|---------------------------------|
| 1 | Co-infection passage                   | A/Sydney/5/2021 (H1N1) wild type virus @ $10^{-4}$<br>x X-157 (H3N2) @ $10^{-4}$ | HA titer<br>GP:10240            |
|   |  | ↓  |                                 |
| 2 | 1 <sup>st</sup> antiserum passage      | Inoculum @ 1:20 with X-157 HANA<br>antibodies                                    | HA titer<br>GP:1280             |
|   |  | ↓  |                                 |
| 3 | 2 <sup>nd</sup> antiserum passage      | Inoculum @ 1:20 with X-157 HANA<br>antibodies                                    | HA titer<br>GP:640              |
|   |  | ↓  |                                 |
| 4 | 3 <sup>rd</sup> antiserum passage      | Inoculum @ 1:20 with X-157 HANA<br>antibodies                                    | HA titer<br>GP:320              |
|   |  | ↓  |                                 |
| 5 | 4 <sup>th</sup> antiserum passage      | Inoculum @ 1:20 with X-157 HANA<br>antibodies                                    | HA titer<br>GP:1280             |
|   |  | ↓  |                                 |
| 6 | 1 <sup>st</sup> Limit dilution passage | Inoculum @ $10^{-6}$   | HA titer<br>GP:640              |
|   |  | ↓  |                                 |
| 7 | 2 <sup>nd</sup> Limit dilution passage | Inoculum @ $10^{-9}$   | HA titer<br>GP:5120             |
|   |  | ↓  |                                 |
| 8 | 3 <sup>rd</sup> Limit dilution passage | Inoculum @ $10^{-8}$   | HA titer<br>GP:2560<br>CH:10240 |
|   |  | ↓  |                                 |
| 9 | Final amplification                    | Inoculum @ $10^{-5}$   | HA titer<br>GP:5120<br>CH:20480 |



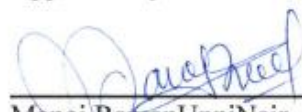
**Testing of A/Sydney/5/2021 SAN-013**

| Test   | Results  |                                 |                 |
|--|--|---------------------------------|-----------------|
| Sterility  | No growth on Thioglycolate and SCD broth after 7 days  |                                 |                 |
| Infectivity  | 9.20 EID <sub>50</sub> / mL  |                                 |                 |
| Gene Ratio<br>Determined by qPCR<br>and confirmed by<br>NGS.   | 5:3 reassortant<br>HA, NA, and NP genes from A/Sydney/5/2021<br>Internal genes PB2, PB1, PA, M, and NS from X-157. |                                 |                 |
|  | Gene   | A/Puerto Rico/8/1934<br>(X-157) | A/Sydney/5/2021 |
|  | HA   |                                 | +               |
|  | NA   |                                 | +               |
|  | PB2  | +                               |                 |
|  | PB1  | +                               |                 |
|  | PA   | +                               |                 |
|  | NP   |                                 | +               |
|  | M  | +                               |                 |
|  | NS   | +                               |                 |
| Passages prior to receipt from VIDRL = 3   |  |                                 |                 |
| Total number of passages post co-infection = 8   |  |                                 |                 |
| Final HA titer for A/Sydney/5/2021 SAN-013 = CH: 20480, GP: 5120   |  |                                 |                 |
| HA titers were determined using 0.5% chicken and/or 1.0% guinea pig red blood cells at room temperature. |  |                                 |                 |
| HA-HPLC showed 3.8x increase compared to the original wildtype virus                                     |  |                                 |                 |

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