



**Influenza Reagent**  
**Influenza Virus Infectious SAN-018**  
**(A/Maryland/02/2021) (H3N2)**  
**NIBSC code: 23/116**  
**Instructions for use**  
**(Version 1.0, Dated 12/05/2023)**

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**1. INTENDED USE**

Reagent 23/116 is prepared from SAN-018 (H3N2), a reassortant of A/Maryland/02/2021 (H3N2) and A/Puerto Rico/8/34 (H1N1), which was processed in 250µl volumes as liquid stock. The derivation and known passage history of 23/116 are 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 -70°C or below.

**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 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

| <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 (specify): Live influenza virus          |  |
| <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                      |
| The material is not of human or bovine origin. |  |
| Contact with eyes:                             |  |
| Contact with skin:                             |  |
|  |  |
|  |  |

**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 ampoule  |
| <b>Toxicity Statement:</b> Non-toxic  |
| <b>Veterinary certificate or other statement if applicable.</b>   |
| <b>Attached:</b> No   |

Passage history of SAN-018 (H3N2)

| Cumulative number of passages | Passage numbers at each stage | Lot         | Laboratory  |
|-------------------------------|-------------------------------|-------------|-------------|
| E6                            | E6                            | 3001586464  | CDC, USA    |
| E17                           | E6/E11                        | SP-2022-018 | Sanofi, USA |
| E18                           | E6/E11/E1                     | 47480*      | MHRA, UK    |

\* The HA titre of this virus using 0.7% Guinea Pig Red blood cells is 256. The infectious titre is unknown.

**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 are available at GISAID with the accession number EPI\_ISL\_17592645.



*Derivation of SAN-018*

*A/Maryland/02/2021 High Growth Reassortant*

*A/Maryland/02/2021 (SAN-018) is an H3N2 high growth reassortant influenza virus*

A/Maryland/02/2021 (SAN-018) is an H3N2 high growth reassortant influenza virus developed in Sanofi Flu Reassortant Lab, department Bacterial and Viral Technology at Sanofi, US.

Sanofi Lot #: SP-2022-018

**Wildtype Virus:**

A/Maryland/02/2021 (the virus isolate was obtained from the CDC)

CDC Lot #: 3001586464

Passages prior to receipt from CDC: 6

Passages prior to reassortant co-infection: 7

**Donor Virus:**

A/Puerto Rico/8/1934 (SP Lot # Batch 2)

**Eggs:**

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

**Antiserum:**

Rabbit antisera raised against influenza virus A/Puerto Rico/8/1934 was used in the process.



**sanofi**

**Passage History**

|   |  |  |                       |
|---|--|--|-----------------------|
|   | wt Amplification                       | A/Maryland/02/2021 (H3N2) wt virus amplified from source @ $10^{-2}$ dilution                            | HA titer<br>GP: 1280  |
|   |  | ↓  |                       |
|   | Co-infection passage                   | A/Maryland/02/2021 (H3N2) wild type virus direct harvest fluid x A/Puerto Rico/8/1934 (H1N1) @ $10^{-5}$ | HA titer<br>GP:1280   |
|   |  | ↓  |                       |
| 1 | 1 <sup>st</sup> antiserum passage      | Inoculum with A/Puerto Rico/8/1934 HANA antibodies @ 1:50 dilution                                       | HA titer<br>GP:1280   |
|   |  | ↓  |                       |
| 2 | 2 <sup>nd</sup> antiserum passage      | Inoculum with A/Puerto Rico/8/1934 HANA antibodies @ 1:50 dilution                                       | HA titer<br>GP:10     |
|   |  | ↓  |                       |
| 3 | 3 <sup>rd</sup> antiserum passage      | Inoculum with A/Puerto Rico/8/1934 HANA antibodies @ 1:50 dilution                                       | HA titer<br>GP:2560   |
|   |  | ↓  |                       |
| 4 | 4 <sup>th</sup> antiserum passage      | Inoculum with A/Puerto Rico/8/1934 HANA antibodies @ 1:20 dilution                                       | HA titer<br>GP:640    |
|   |  | ↓  |                       |
| 5 | 5 <sup>th</sup> antiserum passage      | Inoculum with A/Puerto Rico/8/1934 HANA antibodies @ 1:20 dilution                                       | HA titer<br>GP:640    |
|   |  | ↓  |                       |
| 6 | 1 <sup>st</sup> Limit dilution passage | Inoculum @ $10^{-5}$ dilution  | HA titer<br>GP:2560   |
|   |  | ↓  |                       |
| 7 | 2 <sup>nd</sup> Limit dilution passage | Inoculum @ $10^{-7}$   | HA titer<br>GP:10240  |
|   |  | ↓  |                       |
| 8 | 3 <sup>rd</sup> Limit dilution passage | Inoculum @ $10^{-6}$   | HA titer<br>GP:5120   |
|   |  | ↓  |                       |
| 9 | Final amplification                    | Inoculum @ $10^{-4}$   | HA titer<br>GP: 10240 |

Passages prior to receipt from the CDC = 6

Total number of passages post co-infection = 9



**Testing of A/Maryland/02/2021 SAN-018**

| Test   | Results  |                      |                    |
|--|--|----------------------|--------------------|
| Sterility  | No growth on Thioglycolate Medium and Trypticase Soy Broth after 10 days.  |                      |                    |
| Infectivity  | 10 <sup>8.24</sup> EID <sub>50</sub> / mL  |                      |                    |
| Gene Ratio<br>Determined by qPCR and confirmed by NGS. Additionally, HA and NA of SAN-018 confirmed to be 100% identical to wt based on NGS. | 6:2 reassortant<br>HA and NA genes from A/Maryland/02/2021.<br>Internal genes PB1, PB2, PA, NP, M, and NS from A/Puerto Rico/8/1934. |                      |                    |
|  | Gene   | A/Puerto Rico/8/1934 | A/Maryland/02/2021 |
|  | HA   |                      | +                  |
|  | NA   |                      | +                  |
|  | PB2  | +                    |                    |
|  | PB1  | +                    |                    |
|  | PA   | +                    |                    |
|  | NP   | +                    |                    |
|  | M  | +                    |                    |
|  | NS   | +                    |                    |
| Final HA titer for A/Maryland/02/2021 SAN-018 = 10240  |  |                      |                    |
| HA titers were determined using 1.0% guinea pig red blood cells at room temperature.   |  |                      |                    |
| HA-HPLC showed 2.1x increase compared to the original wildtype virus   |  |                      |                    |

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