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## Proposed 3rd International Standard for Interferon alpha 2b

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NOTE:
This document has been prepared for the purpose of inviting comments and suggestions on the proposals contained therein, which will then be considered by the Expert Committee on Biological Standardization (ECBS). Comments MUST be received by **5 October 2020** and should be addressed to the World Health Organization, 1211 Geneva 27, Switzerland, attention: Technologies, Standards and Norms (TSN). Comments may also be submitted electronically to the Responsible Officer: **Dr Ivana Knezevic** at email: knezevici@who.int.

## Summary

The WHO Second International Standard (IS) for Interferon alpha 2b (IFN-α2b), 95/566, was established at the 51st meeting of the WHO Expert Committee on Biological Standardisation (25 – 29 Oct 1999). There has been consistent demand for this standard since then and stocks of the IS are now running low and a replacement standard will be required soon. We therefore assessed the suitability of an IFN-α2b preparation, coded 95/656, which was previously included in the international collaborative study for Interferon alpha. Bioassay data generated in the previous collaborative study for 95/656 was examined to determine a potency value, and this was verified using recent in-house studies which have also demonstrated that the candidate preparation 95/656 is highly stable when stored at the recommended storage temperature of -20$^0$C. It is therefore proposed that the preparation 95/656 be accepted as the WHO 3rd IS for IFN-α2b with an assigned value for IFN-α2b activity of 24,000IU/ampoule.

## Introduction

IFN-α2b was originally licensed in 1986, and is currently approved, either as a monotherapy or in combination with other drugs (e.g. ribavirin, cytarabine), for the treatment of a number of diseases including chronic hepatitis B, chronic hepatitis C, hairy cell leukaemia, chronic myelogenous leukaemia, multiple myeloma, follicular lymphoma, carcinoid tumour, malignant melanoma.

IFN-α2b is a pleiotropic cytokine regulating different cellular functions, which signals through the type1 IFN heterodimeric receptor complex comprising of the IFNAR1 and IFNAR2 subunits, which are expressed on many different cell types (1). Binding of IFN-α2b to the IFNAR1/2 receptor triggers a complex sequence of intracellular events that give rise to various cellular responses, mediated through the Janus Kinase (JAK) / Signal transducers and activators of transcription (STAT) signalling pathway. IFN-α2b activation of the JAK/STAT signalling cascade leads to the formation of the IFN stimulated gene factor 3 (ISGF3) complex which translocates to the nucleus where it binds to the Interferon Stimulated Response Element (ISRE) resulting in the transcription of numerous Interferon stimulated genes (ISGs) (2, 3). This results in a variety of cellular responses including antiviral, antiproliferative, antitumour and immunomodulatory activity (4), some, or all of these may contribute to the therapeutic effects shown by IFN-α2b.

Over the years, the clinical use of IFN-α2b has declined significantly in major economies with approval of the pegylated form, listed also as a WHO essential medicine, particularly for Hepatitis C treatment (typically in combination with ribavirin and an approved protease inhibitor), due to its longer half-life in the circulation requiring less frequent administration. The significant side effects of IFN-α2b and the introduction of direct acting antiviral agents and newer cancer treatments (e.g. Tyrosine kinase inhibitors) have also contributed to a decline in the therapeutic use of IFN-α2b. However, interest in new applications (off-label) for IFN-α2b in the treatment of certain ophthalmic disorders, e.g. ocular surface squamous neoplasia (5), and more recently as a potential treatment for COVID-19 have been reported (6).

The current 2nd International Standard (IS) for IFN-α2b (95/566) containing a highly purified clinical grade IFN-α2b protein expressed in E.coli was established by the WHO Expert Committee

on Biological Standardisation (ECBS) in 1999. It was assigned a unitage of 70,000IU per ampoule, based on the results from a large international collaborative study on human Interferon alpha (WHO/BS99.1911) involving 93 participants from 29 countries, in which a variety of assays including antiviral assays, antiproliferative assays, immunoassays and various other biological assays (e.g. immunomodulatory, gene expression) were used to measure the potency of different IFNα preparations (7, 8).

The 2nd IS for IFN-α2b has proved suitable as the primary biological reference standard for IFN-α2b and has supported the calibration of secondary bioactivity standards. It is used by manufacturers and control testing laboratories for calibrating in vitro bioactivity assays for IFN-α2b products and in the control of marketed products. Stocks of this standard are now running low and a replacement is required for maintaining IFN-α2b product safety and efficacy. Currently there are approximately 400 ampoules of the IS in stock, and an average of 126 are distributed per year.

A proposal for the requirement of a replacement for the 2nd IS for IFN-α2b was endorsed by the WHO ECBS in October 2019 (WHO/BS/2019.2378). It was proposed that the lyophilised candidate preparation 95/656 included in the previous collaborative study could potentially serve as a replacement standard subject to a) the preparation demonstrating suitable stability and b) calculation of potency estimates of 95/656 relative to the current 2nd IS (95/566), thereby maintaining continuity of the IU. This would be based on data from the assays used in the previous collaborative study, together with supporting data from evaluation in a reporter gene assay (RGA) at NIBSC. A similar strategy for a replacement IS was used previously for the establishment of the WHO 2nd IS for human tumour necrosis factor alpha (BS/03.1981).

## Aims of the study

The purpose of this study was to assess the suitability of a candidate WHO 3rd International Standard for human IFN-α2b, followed by assignment of a unitage for activity through the following steps:

- Assessment of the stability of the candidate preparation 95/656 by assessing thermal degradation samples from recent in-house studies using a reporter gene assay.
- Calculation of a potency estimate for a candidate preparation of IFN-α2b (95/656) relative to the 2nd IS, 95/566 using assay data from laboratories that had included both these preparations in a previous collaborative study for interferon alpha.
- Verification of the potency of the candidate preparation 95/656 relative to the 2nd IS from recent in-house studies using a reporter gene assay.

## Materials and Methods

Two preparations of clinical grade recombinant human sequence IFN-α2b expressed in E.coli were donated to the WHO and processed at NIBSC in 1995. The bulk protein was dissolved in 4L of pyrogen free six-salt phosphate buffered saline pH 7.0, containing 0.6% human serum albumin, and 1ml of each solution containing a predicted mass of IFN-α2b (calculated from the dilution of

the bulk material of known protein content determined by the manufacturer) distributed into 3,700 ampoules according to WHO recommendations in place at the time (WHO Technical report series 800, 1990, Annex4).

Following lyophilization and secondary desiccation, the ampoules were sealed under dry nitrogen by heat fusion of the glass and stored at -20$^0$C in the dark at NIBSC, as the custodian laboratory. A number of the filled ampoules were weighed prior to lyophilization, and the filling variation was expressed as the coefficient of variation by weight. Residual moisture was calculated using the coulometric Karl-Fischer method (MitsubishiCA100), and the headspace oxygen content, which is a measure of ampoule integrity, was determined by frequency modulated spectroscopy (Lighthouse FMS-760). The characteristics of both preparations which were included in the previous WHO international collaborative study for IFN-α undertaken between 1996 and 1998 are shown in Table 1.

**Table 1. Study materials: Mean fill weights, residual moisture and headspace oxygen content**

| Ampoule Code | Date filled | Study Code | Predicted IFN-α2b content | Mean Fill Weight g (cv%) | Mean residual moisture % (cv%) | Mean Headspace Oxygen % (cv%) |
|---|---|---|---|---|---|---|
| 95/566[1] (2nd IS) | 29/6/95 | D | 500ng | 1.008 (0.17) n = 72 | 0.211 (1.13) n = 3 | 0.66 (176.77) n = 12 |
| 95/656[1,2] | 21/9/95 | P | 250ng | 1.006 (0.35) n = 81 | 0.185 (10.91) n = 3 | 0.45 (93.29) n = 12 |

[1]Custodian laboratory: NIBSC; [2]Current stock: 3,500 ampoules

# Study strategy

The strategy involved potency evaluation for the candidate preparation 95/656 relative to the 2nd IS 95/566 considering data from antiviral assays from the previous study for interferon alpha together with recent data generated in-house from reporter gene assays to verify the potency values. To further substantiate this approach the potency of an additional preparation, the 2nd IS for human leukocyte IFN-α 94/784, which was also included in the previous study, was calculated relative to 95/566, using previous study data as well as recent reporter gene assay data.

# Previous collaborative study data

In the previous WHO collaborative study for IFN-α, data from a variety of different assay types were submitted, however, the assignment of units to the different IFN-α preparations was based on data from antiviral assays only, which comprised the majority of assay types contributed to the study. Traditional antiviral assays are based on reduction of cytopathic effect, in which the antiviral activity of interferon is measured by its ability to inhibit virus induced cytopathology. Such assays use different combinations of cell types and virus in which cells, seeded into microtiter plates, are

incubated with titrations of IFN preparations followed by incubation with virus and measurement of cell viability using live cell stains such as crystal violet or by assessing metabolic activity using formazan dyes e.g. MTT/MTS. A total of 24 laboratories evaluated both IFN-α2b preparations 95/656 and 95/566 using various antiviral assays, and the data from these laboratories was used to determine the geometric mean (GM) potency estimates for the candidate preparation 95/656 relative to 95/566 the 2nd IS for IFN-α2b. The potency of an additional preparation 94/784, the 2nd IS for human leukocyte IFN-α, was also determined by calculating its potency relative to 95/566 using data from 73 laboratories that had evaluated these preparations in a variety of antiviral assays.

# Recent in-house studies using a reporter gene assay

A reporter gene assay utilising the HEK 293P cell line which harbours a transfected DNA plasmid containing the interferon stimulated response element (ISRE) promoter, linked to the gene encoding secreted embryonic alkaline phosphatase (SEAP) was used in recent in-house studies (9). It has been shown previously that the HEK293P/ISRE-SEAP cell line is sensitive to Type 1 interferons, and following treatment with IFN-α2b, releases SEAP into the culture medium in a dose dependant manner which can be quantified using the substrate para-nitrophenyl phosphate (pNPP), (9,10). For the RGAs HEK 293P/ISRE-SEAP cells seeded into 96 well microtiter plates were incubated for 24hrs at $37^0$C followed by further incubation for 48hrs with appropriate titrations of the IFN-α preparations. The cell supernatants were then removed and transferred into new microtitre plates followed by addition of pNPP substrate for 1 hr at room temperature in the dark prior to measuring the absorbance at 405nm using a microplate reader.

A total of ten independent HEK293P/ISRE-SEAP reporter gene assays were conducted, in which titrations of the candidate preparation 95/656 and the 2nd IS 95/566 were included across multiple plates which allowed testing of replicate titrations of the preparations in different positions on the plates. Each assay consisted of three plates, with titration curves for each preparation in duplicate wells on every plate, and the preparations were replicated a minimum of three times in each assay. Additionally, a preparation 94/784, the 2nd IS for human leukocyte IFN-α, which was also included in the previous collaborative study, was assessed in a total of five assays in a similar manner.

# Stability studies

### Accelerated degradation studies

In order to assess the stability of the candidate preparation 95/656, ampoules stored at $-70^0$C, $-20^0$C, $+4^0$C, $+20^0$C, $+37^0$C for approximately 24 years were assayed in the HEK 293P/ISRE-SEAP RGA at NIBSC. Although samples were also stored at $+45^0$C, it was not possible to reconstitute these samples after prolonged storage at this temperature. A total of three assays were performed, each consisting of the different temperature degradation samples replicated over three plates. The potencies of the samples stored at different temperatures were expressed relative to the baseline samples stored at $-70^0$C.

### Stability after reconstitution

Samples of the candidate preparation were tested in the HEK 293P/ISRE-SEAP RGA following reconstitution and storage for 1 day or 1 week at either 4°C or room temperature, and after a series of freeze thaw cycles. Three assays were performed for the storage following reconstitution samples and two assays peformed for the freeze thaw samples, each assay consisting of the samples replicated over three plates. The potencies of the samples following reconstitution were expressed relative to freshly reconstituted ampoules of the candidate preparation.

## Statistical analysis

Individual laboratory potency estimates from the previous collaborative study report were taken from Appendix Tables A7.1a and A7.1b (WHO/BS99.1911) and used to determine the relative potency estimates reported here. Estimates of potency relative to IS 95/566 from recent in-house reporter gene assays were calculated using CombiStats v6.0 (11) with a sigmoid curves model and a log transformation of the assay response. Instances where the weighted correlation coefficient was less than 0.99, or where the ratio of fitted slopes was outside the range 0.90-1.25, were considered invalid and no estimates were used in these cases (one assay out of ten analysed was found invalid using these criteria).

Results from all valid assays were combined as geometric means. Variability between assays and between laboratories has been expressed using geometric coefficients of variation (GCV = $\{10^{s}-1\}\times100\%$ where $s$ is the standard deviation of the log10 transformed estimates).

For the analysis of results obtained for accelerated thermal degradation samples, relative potencies were used to fit an Arrhenius equation relating degradation rate to absolute temperature assuming first-order decay (12) and hence predict the degradation rate when stored at -20 °C.

## Results

### Potency calculations using previous collaborative study data

In the previous WHO collaborative study for IFN-α, testing of two different preparations of IFN-α2b (95/566 & 95/656) was carried out in 24 laboratories using a variety of antiviral assay systems (Appendix - Table taken from WHO/BS99.1911). This enabled an estimated potency for the preparation 95/656 relative to the 2$^{nd}$ IS 95/566 to be calculated using the data contained in the original ECBS collaborative study report (Table 2). Using data derived from these laboratories, the geometric mean (GM) potency for preparation 95/656 was calculated as 24,149IU, with 95% confidence interval (CI) 21,074 – 27,673IU, relative to the 2$^{nd}$ IS 95/566 in antiviral assays, with a geometric coefficient of variation (GCV) of 38.1% (Table 3).

To give further support to the calculated potency estimate derived for the candidate preparation 95/656 from the previous collaborative study data, the estimated potency of an additional preparation, 94/784, the 2$^{nd}$ IS for human leukocyte IFN-α, was derived relative to 95/566,

calculated using data from 73 laboratories that had previously evaluated these preparations in the same antiviral assays. For 94/784 a GM estimated potency of 10,860IU, with 95% CI 9,700 – 12,160IU, was derived relative to 95/566, with a GCV of 62.3% (Table 3). This value is consistent with the potency of 11,000IU assigned to this preparation when it was established as the 2nd IS for human leukocyte IFN-α.

**Table 2.**

**Individual laboratory geometric mean estimates taken from Appendix Table A7.1b of the previous WHO collaborative study report for establishment of IFN-α standards: Potency of preparation D (code 95/566) relative to preparation P (code 95/656) and potency estimates (IU) derived for preparation P relative to preparation D.**

| Cell type | Virus | Lab code | D/P | P (IU) |
|---|---|---|---|---|
| 2D9 | EMC | 7 | 2.62 | 26,675 |
| A549 | EMC | 60 | 2.69 | 25,974 |
| A549 | EMC | 10 | 1.56 | 45,013 |
| A549 | EMC | 15 | 3.15 | 22,223 |
| A549 | EMC | 9 | 3.15 | 22,192 |
| A549 | EMC | 56 | 3.43 | 20,431 |
| A549 | EMC | 18 | 2.89 | 24,240 |
| A549 | VSV | 10 | 2.79 | 25,049 |
| FL | SIN | 59 | 2.44 | 28,717 |
| FL | SIN | 94 | 2.88 | 24,269 |
| FL | SIN | 104 | 3.53 | 19,834 |
| FL | SIN | 35 | 3.30 | 21,211 |
| FIB | EMC | 60 | 2.96 | 23,657 |
| FIB | VSV | 97 | 1.66 | 42,256 |
| Hep2 | VSV | 25 | 4.13 | 16,942 |
| HeLa | EMC | 66 | 2.09 | 33,543 |
| MDBK | VSV | 68 | 1.57 | 44,692 |
| MDBK | VSV | 43 | 2.79 | 25,055 |
| MDBK | VSV | 53 | 3.56 | 19,649 |
| WISH | SIN | 71 | 2.61 | 26,772 |
| WISH | VSV | 102 | 5.19 | 13,488 |
| WISH | VSV | 40 | 2.62 | 26,693 |
| WISH | VSV | 71 | 5.76 | 12,159 |
| WISH | VSV | 8 | 3.71 | 18,882 |

Cell types: 2D9 human glioblastoma cell line; A549 human lung carcinoma cell line; FL human amnion-derived cell line; FIB human fibroblast; Hep2 human larynx carcinoma cell line; HeLa human cervix carcinoma cell line; MDBK Madin-Darby bovine kidney cell line; WISH human amniotic cell line. Viruses: EMC encephalomyocarditis virus; VSV vesicular stomatitis virus; SIN sindbis virus.

**Table 3. Potency estimates calculated from previous collaborative study data**

| Antiviral assays | 95/656 relative to IS 95/566 | | 94/784 relative to IS 95/566 | |
|---|---|---|---|---|
| Estimate GM (IU) | 24,149 | | 10,860 | |
| 95% CI | 21,074 | 27,673 | 9,700 | 12,160 |
| GCV (%) | 38.1 | | 62.3 | |
| N | 24 | | 73 | |

**Results from recent in-house studies**

Using data from recent RGAs conducted at NIBSC to evaluate the preparations 95/566, 95/656 and 94/784, a GM potency for 95/656 of 23,243IU, with 95% CI 22,501 – 24,010 and GCV of 5.5%, was calculated relative to the 2nd IS for IFN-α2b, 95/566. The GM potency of the additional preparation 94/784, the 2nd IS for human leukocyte IFN-α, was also derived relative to 95/566, and a potency of 11,938IU with 95% CI 9,700 – 12,160 and GCV of 3.6%, calculated (Table 4). The low GCV values calculated for 95/656 and 94/784 relative to 95/566, reflect the high precision (low variability) obtained in these assays. The potency values calculated for both preparations from the RGAs are in good agreement with the estimates calculated from the antiviral assays in the previous collaborative study, corroborating the estimates derived for these preparations from the collaborative study data.

**Table 4. Potency estimates calculated from recent reporter gene assay data**

| Reporter Gene Assays | 95/656 relative to IS 95/566 | | 94/784 relative to IS 95/566 | |
|---|---|---|---|---|
| Overall GM (IU) | 23,243 | | 11,938 | |
| 95% CI | 22,501 | 24,010 | 11,662 | 12,222 |
| GCV (%) | 5.5 | | 3.6 | |
| N | 9 | | 5 | |

**Stability studies**

Stability of the candidate preparation 95/656, was assessed in accelerated thermal degradation (ATD) studies after storage for approximately 24 years in three independent RGA assays. The GM potency estimates relative to the sample stored at -70$^0$C, are shown in Table 5. Comparison of the sample stored at -20$^0$C with the sample stored at -70$^0$C gave a relative potency estimate not significantly different from 1.0, indicating that no relative loss in activity could be detected after 24 years of storage at this temperature. The ATD data showed a good fit to the Arrhenius model for calculating predicted yearly losses of activity and a predicted yearly loss of 0.008% at the recommended storage temperature of -20$^0$C was calculated, demonstrating that 95/656 is highly stable (Table 5). These results are in excellent agreement with a previous study which assessed the thermal stability of the 2nd IS for IFN-α2b, 95/566 where the potencies of ampouled preparations that were stored at different temperatures for 9 years were evaluated using the same reporter gene assay system (13).

**Table 5. Relative potencies of ATD samples relative to samples stored at -70$^0$C and yearly predicted loss in activity**

| Storage Temperature $^0$C | GM Relative Potency | | | Predicted loss per year % |
|---|---|---|---|---|
| | 95% LCL | Estimate | 95% UCL | |
| -20 | 0.965 | 1.002 | 1.040 | 0.008 |
| 4 | 0.905 | 0.944 | 0.986 | 0.139 |
| 20 | 0.805 | 0.854 | 0.907 | 0.729 |
| 37 | 0.383 | 0.426 | 0.474 | 3.468 |

**Stability after reconstitution**

Samples of the candidate standard 95/656 were reconstituted and stored at 4°C or room temperature for either 1 day or 1 week. The reconstitutions were timed to allow all samples to be assayed concurrently against a freshly reconstituted ampoule. The GM potencies for all samples expressed relative to a freshly reconstituted sample are summarised in Table 6. Results indicated that there was a small loss in potency of the reconstituted candidate standard on storage at either 4°C or room temperature.

**Table 6: Relative potencies of reconstituted and stored samples as % of a freshly reconstituted ampoule of 95/656**

| Temperature (°C) | Time (Days) | 95% LCL | GM relative potency (%) | 95% UCL |
|---|---|---|---|---|
| +4 | 1 | 91.5 | 94.8 | 98.2 |
| +4 | 7 | 89.1 | 92.0 | 94.9 |
| Room temperature | 1 | 89.2 | 92.3 | 95.5 |
| Room temperature | 7 | 85.5 | 87.8 | 90.1 |

Samples of the candidate standard 95/656 were reconstituted and subjected to a series of freeze-thaw cycles (1 up to 4). They were then assayed concurrently with a freshly reconstituted ampoule. The GM potencies for all samples expressed relative to a freshly reconstituted sample are summarised in Table 7. The results showed that there was no evidence of any significant loss in potency of this preparation with up to 3 repeated freeze-thaw cycles.

**Table 7: Relative potencies of freeze-thaw samples as % of a freshly reconstituted ampoule of 95/656**

| Number of freeze/thaw cycles | 95% LCL | GM relative potency (%) | 95% UCL |
|---|---|---|---|
| 1x | 87.7 | 95.7 | 104.4 |
| 2x | 89.9 | 97.4 | 105.5 |
| 3x | 91.2 | 98.5 | 106.5 |
| 4x | 86.7 | 92.5 | 98.8 |

Based on the stability data obtained after reconstitution, the candidate standard 95/656 should be stored frozen in aliquots post-reconstitution, to minimise any loss in potency of the preparation.

# Discussion

Traditionally the activity of IFNs has been assayed by determining the protection of cells from the cytopathic effect of certain viruses, the preference for antiviral assays was due in part to this being the activity that initially defined these molecules. Antiviral assay systems are, however, time consuming, labour intensive involving numerous handling steps, are inherently variable, and can show significant variation in results depending on the cell/virus combinations used in different assay systems with particular IFN-α preparations (8). Safety issues associated with the use of viruses in these assays also require consideration. These issues have led to the development of alternative methods such as RGAs to assess the biological activity of interferons. RGAs tend to show lower within assay and between assay variation giving improved precision and reproducibility (13), this aspect together with their added convenience and ease of use has led to their widespread use for the measurement of bioactivity of many proteins including interferons.

In this study, we employed the RGA to derive potency values for the proposed standard mainly for two reasons. Firstly, there was considerable data already available from antiviral assays (used in the previous WHO collaborative study for IFN-α), and secondly, RGAs due to their many advantages, are gaining momentum and the use of the HEK 293P/ISRE-SEAP RGA for the assessment of IFN-α2b preparations has been shown to give very similar results to those from an antiviral assay (13).

The candidate preparation 95/656 was not assigned a potency in the original collaborative study, however, using data from the laboratories that had evaluated this preparation together with the 2$^{nd}$ IS for IFN-α2b 95/566 in antiviral assays, an estimated GM potency for 95/656 relative to 95/566 of 24,149IU was calculated. We found that the potency values calculated for the candidate preparation 95/656 relative to 95/566 in recent RGAs concur with the potency estimates calculated using antiviral assay data from the previous collaborative study (Fig 1), giving a GM potency of 23,243IU. The agreement in potency estimates between data of the antiviral assays from the previous collaborative study and the recent RGA data is not surprising given that similar observations were shown previously when comparing data using the same RGA system with an antiviral assay (13).

To substantiate the approach used in this study, the potency of an additional preparation included in the previous collaborative study (94/784), was also determined. The estimated potency for 94/784 relative to 95/566 derived from the previous study data and from recent RGA data were in good agreement with values of 10,860IU and 11,938IU respectively, providing further assurance to the reliability of the potency estimates derived in this study. These values are also consistent with the assigned unitage for 94/784 of 11,000IU.

The potency data and high stability demonstrated for 95/656 indicates it is suitable as the replacement for the 2$^{nd}$ International Standard for IFN-α2b. There are currently 3,500 ampoules of

95/656 in stock. It is recommended that a value of 24,000IU/ampoule is assigned to preparation 95/656 in continuity with the IU assigned to the current 2nd IS for IFN-α2b.

## Conclusion and proposal

The findings of this study have shown that the candidate IFN-α2b preparation 95/656 is suitable to serve as a replacement for the 2nd International Standard for IFN-α2b for assessing potency of IFN-α2b preparations. It is proposed that the candidate preparation 95/656 be accepted as the WHO 3rd International Standard for IFN-α2b with an assigned potency of 24,000IU/ampoule, following exhaustion of ampoules of the 2nd IS for IFN-α2b.

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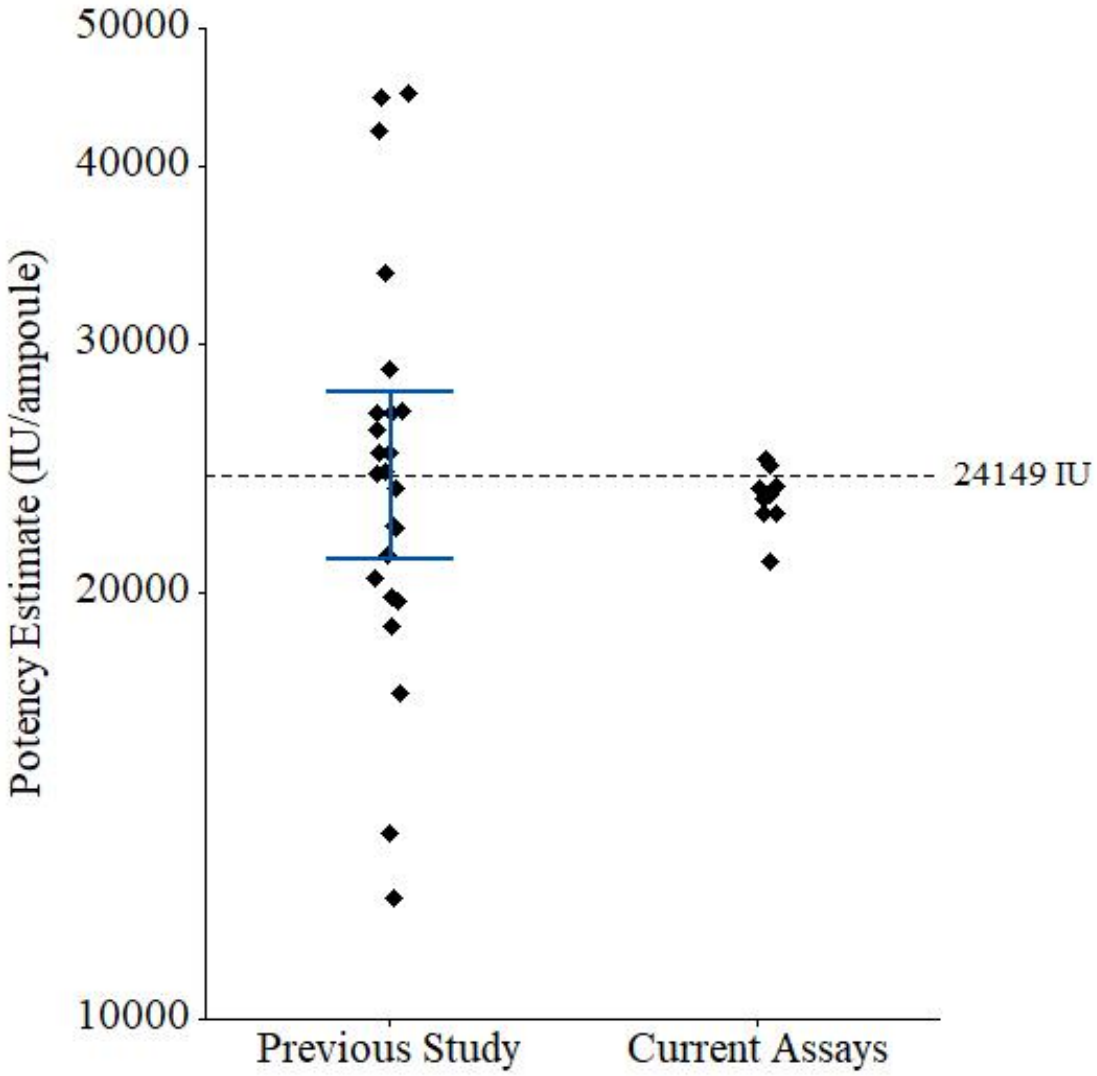
**Figure 1. Potency estimates for 95/656 calculated relative to IS 95/566**

Plotted points from previous study are individual laboratory geometric means (N=24); points from current assays are individual assay estimates (N=9); reference line at 24149 IU indicates geometric mean estimate from previous study with 95% CI



# Appendix

**Individual laboratory potency data taken from Appendix Table A7.1b of the previous WHO collaborative study report for establishment of IFN-α standards: Geometric mean of potency estimates in antiviral assays for preparations D (code 95/566) and P (code 95/656) in terms of preparation A (code 69/19)**

| Cell type | Virus | Lab code | D/A | P/A |
|---|---|---|---|---|
| 2D9 | EMC | 7 | 7.61 | 2.9 |
| A549 | EMC | 60 | 11.75 | 4.36 |
| A549 | EMC | 10 | 7.76 | 4.99 |
| A549 | EMC | 15 | 11.56 | 3.67 |
| A549 | EMC | 9 | 16.56 | 5.25 |
| A549 | EMC | 56 | 18.33 | 5.35 |
| A549 | EMC | 18 | 14.41 | 4.99 |
| A549 | VSV | 10 | 14.28 | 5.11 |
| FL | SIN | 59 | 16.6 | 6.81 |
| FL | SIN | 94 | 22.7 | 7.87 |
| FL | SIN | 104 | 21.74 | 6.16 |
| FL | SIN | 35 | 15.61 | 4.73 |
| FIB | EMC | 60 | 35.3 | 11.93 |
| FIB | VSV | 97 | 28.94 | 17.47 |
| Hep2 | VSV | 25 | 6.9 | 1.67 |
| HeLa | EMC | 66 | 12.73 | 6.1 |
| MDBK | VSV | 68 | 5.2 | 3.32 |
| MDBK | VSV | 43 | 20.73 | 7.42 |
| MDBK | VSV | 53 | 14.25 | 4.0 |
| WISH | SIN | 71 | 42.28 | 16.17 |
| WISH | VSV | 102 | 10.12 | 1.95 |
| WISH | VSV | 40 | 16.18 | 6.17 |
| WISH | VSV | 71 | 13.99 | 2.43 |
| WISH | VSV | 8 | 3.04 | 0.82 |

Medicines & Healthcare products Regulatory Agency

NIBSC Confidence in Biological Medicines

**WHO International Standard**
**Interferon Alpha 2b (Human rDNA derived)**
**NIBSC code: 95/656**
**Instructions for use**
**(Version 1.00, Dated )**

## 1. INTENDED USE

This preparation is the 3rd WHO International Standard for human interferon alpha 2b (IFN-alpha 2b). This preparation replaces the 2nd International Standard for interferon alpha 2b coded 95/566. It is intended for use as the primary biological reference standard in bioassays for IFN-alpha 2b.

## 2. CAUTION

**This preparation is not for administration to humans or animals in the human food chain**.

The preparation contains material of human origin, and either the final product or the source materials, from which it is derived, have been tested and found negative for HBsAg, anti-HIV and HCV RNA. 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

24,000 International Units per ampoule

## 4. CONTENTS

Country of origin of biological material: United Kingdom.
Each ampoule contains a freeze-dried residue comprising, under an atmosphere of nitrogen:

Interferon alpha 2b, approximately 250 ng
6-salt phosphate buffered saline pH 7.0
6.0 mg human serum albumin

The Interferon alpha 2b protein was expressed in E. coli.

## 5. STORAGE

For economy of use, it is recommended that the final solution be sub-divided into several small aliquots and stored at -40°C or below. Avoid repeated thawing/freezing. Unopened ampoules should be stored at -20°C.
**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

No attempt should be made to weigh out any portion of the freeze-dried material prior to reconstitution

Dissolve the total contents of the ampoule in 0.5ml of sterile distilled water and transfer to a sterile container. Rinse the ampoule with about 0.4ml of sterile distilled water and add to the first solution. Make up the total volume to 1.0ml with sterile distilled water. The final solution will contain IFN alpha 2b at a concentration of 24,000 International Units per ml. Use carrier protein where dilution is required. It is recommended that initial dilutions, i.e. 1:10, 1:100, are either made in cell culture medium containing - 5%v/v –10%v/v calf serum or in phosphate-buffered saline, pH 7.0-7.4, containing 0.3%v/v bovine casein to prevent adsorption of IFN to container surfaces.

## 8. STABILITY

Reference materials are held at NIBSC within assured, temperature-controlled storage facilities and they should be stored on receipt as indicated on the label. It is the policy of WHO not to assign an expiry date to their international reference materials. Accelerated degradation studies have indicated that this material is suitably stable, when stored at -20°C or below, for the assigned values to remain valid until the material is withdrawn or replaced. These studies have also shown that the material is suitably stable for shipment at ambient temperature without any effect on the assigned values. Once reconstituted, diluted or aliquoted, users should determine the stability of the material according to their own method of preparation, storage and use. Users who have data supporting any deterioration in the characteristics of any reference preparation are encouraged to contact NIBSC.

## 9. REFERENCES

Report on Proposed 3rd International Standard for Interferon-alpha 2b WHO/BS/

Meager, A, Gaines Das, R, Zoon K. and Mire-Sluis, A. (2001) Establishment of new and replacement World Health Organisation International Biological Standards for human interferon alpha and omega. Journal of Immunological Methods, 257, 17-33.

This standard was produced under WHO guidelines as cited in the WHO Technical Reports Series 800, 1990, annex 4.

## 10. ACKNOWLEDGEMENTS

N/A

## 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

National Institute for Biological Standards and Control,
Potters Bar, Hertfordshire, EN6 3QG. T +44 (0)1707 641000, nibsc.org
WHO International Laboratory for Biological Standards,
UK Official Medicines Control Laboratory

World Health Organization

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**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: Freeze-dried white powder | Corrosive: No |
| Stable: Yes | Oxidising: No |
| Hygroscopic: Yes | Irritant: No |
| Flammable: No | Handling: See caution, Section 2 |
| Other (specify): | Contains material of human origin |
| **Toxicological properties** | |
| Effects of inhalation: | Not established, avoid inhalation |
| 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 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 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<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**: 1g |
| **Toxicity Statement**: Toxicity not assessed |
| **Veterinary certificate or other statement** if applicable.<br>**Attached**: No |

**17. CERTIFICATE OF ANALYSIS**
NIBSC does not provide a Certificate of Analysis for WHO Biological Reference Materials because they are internationally recognised primary reference materials fully described in the instructions for use. The reference materials are established according to the WHO Recommendations for the preparation, characterization and establishment of international and other biological reference standards http://www.who.int/bloodproducts/publications/TRS932Annex2_Inter_biolefstandardsrev2004.pdf (revised 2004). They are officially endorsed by the WHO Expert Committee on Biological Standardization (ECBS) based on the report of the international collaborative study which established their suitability for the intended use.

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World Health Organization