



**Non WHO Reference Material
Human Serum Proteins (other than Immunoglobulins)
NIBSC code: 74/520
Instructions for use
(Version 6.0, Dated 30/01/2013)**

This material is not for in vitro diagnostic use.

1. INTENDED USE

By 1967, when standards for human serum immunoglobulins were being prepared, it was becoming increasingly recognized that standards for other serum proteins would be useful and that a single pooled preparation of serum could serve as the standard for each of the proteins of interest.

It was expected that most or all of these proteins would be most stable in a freeze-dried serum, but it was recognized that the turbidity of the reconstituted product would render it less than suitable for nephelometry.

In 1974 a single batch of 20 litres of human serum was obtained from donors in the UK, and in collaboration with the International Union of Immunological Societies was prepared for freeze-drying. Four litres of this were freeze-dried and secondarily desiccated, and code-labelled 74/520. In 1975, as an interim measure, this preparation was established as the 1st British Standard for Human Serum (other than immunoglobulins), 74/520 until such time as an international collaborative study was made to establish the validity of the assay of the protein components, as well as their stability.

In 1978 the 1st International Reference Preparation (IRP) of six human serum proteins was established by WHO. These proteins are albumin, alpha-antitrypsin, alpha-2-macroglobulin, caeruloplasmin, complement C3 and transferrin. The 1st IRP was freeze-dried from a pool of serum from 85 donors from Atlanta, Georgia, USA. A number of protein components of the IRP and of other preparations, including the 1st British Standard for Human Serum Proteins (other than immunoglobulins), 74/520 were cross-calibrated in an international study (Reimer et al., 1978). Hence when the 1st IRP was established by WHO in 1978, there were adequate data to allow the re-calibration of the 1st British Standard in terms of the 1st IRP, in respect of three of the proteins, alpha-antitrypsin, caeruloplasmin, and transferrin. Data were deemed insufficient to calibrate the potency of albumin and of alpha-2 macroglobulin in the 1st British Standard in terms of the 1st IRP.

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

Establishment of the 1st British Standard: Unitage originally assigned

In 1975 preparation 74/520 was established as the 1st British Standard for Human Serum Proteins (other than immunoglobulins). It was originally assigned a unitage such that 1 Unit of activity of each of the twelve specified proteins was present in 0.7740 milligrams of the freeze-dried powder.

Since there are for all practical purposes 77.40 mg of powder in each ampoule of 74/520, there were originally 100 units of activity of each of the

proteins in each ampoule. However, this figure was later revised for the five proteins specified by the 1st IRP.

Revised unitage of the 1st British Standard

The present position results from re-calibration in 1978 of certain proteins in the 1st British Standard, in terms of the new IRP. The present position is as follows:

i) Seven protein components in the 1st British Standard did not form part of the International Collaborative study. The unitage originally assigned to these seven proteins in the 1st British Standard remains unaltered. The units are British Units and there are as yet no International Units for the following seven proteins:

Alpha-1-acid glycoprotein	100 'British Units' per ampoule
Alpha-1-antichymotrypsin	"
Alpha-2-HS glycoprotein	"
GC Globulin	"
Haemopexin	"
Haptoglobin	"
Pre-albumin	"

ii) The international unitage of 3 protein components in the 1st British Standard has been estimated in terms of the 1st IRP. The revised unitage of these proteins is as follows:

ALPHA-1-ANTITRYPSIN

One International Unit of alpha-1-antitrypsin activity is present in 0.9197 mg of the freeze-dried ampoule contents. Dissolve the total contents of the ampoule. The solution will contain 84.16 International Units of alpha-1-antitrypsin activity.

CAERULOPLASMIN

One International Unit of caeruloplasmin activity is present in 1.0801 mg of the freeze-dried ampoule contents. Dissolve the total contents of the ampoule. The solution will contain 71.66 International Units of caeruloplasmin activity.

TRANSFERRIN

One International Unit of transferrin activity is present in 0.7928 mg of the freeze-dried ampoule contents. Dissolve the total contents of the ampoule. The solution will contain 97.63 International Units of transferrin activity.

The albumin and alpha-2-macroglobulin components of the 1st British Standard were studied in the same International Collaborative Study but the data were considered to be statistically insufficient to express their potency in International Units. At least in the immediate future the 1st British standard cannot be used to express the potency of albumin and alpha-2-macroglobulin in International Units.

There is some doubt whether the stability of the third component of complement (C3) in the 1st British Standard is sufficient to allow 74/520 to be used as a standard for C3. The International Collaborative Study estimated that in the ampoules of 74/520 examined, there was a mean of 76.32 International Units of C3 in each ampoule of the 1st British Standard. However, it is advised that 74/520 should not at present be used as a standard for the third component of complement.

"A note on a principle used in calibration of the British Standard for alpha-1-antitrypsin, caeruloplasmin and transferrin".

Both the 1st International Reference Preparation and the 1st British Standard 74/520 are accepted for calibration of these three proteins, so for these the British Standard must be calibrated in terms of the IRP.

It should be noted that the methods recommended for reconstitution of these two standards differ. For the British Standard the normal recommendation for most other WHO standards has been followed, i.e. that the total contents of one ampoule should be taken to contain 84.16 International Units of alpha-1-



antitrypsin, 71.66 International Units of caeruloplasmin and 97.63 International Units of transferrin and that a solution of the total contents of the ampoule will contain these numbers of units of these proteins. For the International Preparation for these proteins, the WHO recommendation deviates from their usual practice and states that 1ml of distilled water should be added to the contents of the ampoule and that the resulting reconstituted product should be taken to have 100 International Units per ml. Since the contents of an ampoule of 74/520, reconstituted with one ml of distilled water will have a volume greater than 1.0 ml (approximately 1.06 ml) (Rowe et al 1970), a factor including this increase in volume must be introduced into the calibration of the 1st British Standard in terms of the 1st International Reference Preparation for these three proteins. This factor has been assumed to be 1.06 and this is the value used to calculate the figures shown above for the potencies of these three proteins contained in each ampoule of the 1st British Standard for Human Serum Proteins (other than immunoglobulins), 74/520.

NOTE No attempt should be made to weigh out any portion of the freeze-dried material.
The total contents of an ampoule should be reconstituted with distilled water.

4. CONTENTS

Country of origin of biological material: United Kingdom.
Citratid plasma was provided by courtesy of the North London Regional Transfusion Centre of the National Blood Transfusion Service. The plasma came from healthy adult donors in London and each sample was separately frozen. In the National Institute for Biological Standards and Control (NIBSC) part of each sample was tested by radioimmunoassay for freedom from hepatitis B associated antigen. The individual samples of plasma were then sent to the Wellcome Research Laboratories where they were thawed and processed. A pool of 82 samples was made and clotted at room temperature by the addition of calcium chloride to a final concentration of approximately 0.014 molar. Clots were removed and the serum was held at 4°C overnight. Next day it was filtered through balston microfibre filter tubes and Sartorius filtration membranes of average pore diameter 0.45 micrometre. The first six litres to be filtered were sent at 4°C to NIBSC. Four litres of this batch were used to prepare the 1st British Standard for Human Serum Proteins (other than immunoglobulins), 74/520.

Ampoules of the freeze-dried preparation were tested at NIBSC. The dry weight of contents, estimated individually on 6 ampoules was 77.40mg (range 77.25-77.52 mg \pm 0.18%). The residual moisture content was estimated on 6 ampoules by heating over P₂O₅ at 56°C for five hours at a pressure of 0.05 mm of mercury. Losses varied between 0.45% and 0.58% with a mean of 0.51% of the weight of the ampoule contents. The oxygen concentration in the gas in 3 ampoules was determined and the mean found to be 0.11%. No bacterial growth was seen after 5 days incubation of broth inoculated with ampoule contents.

IMMUNOCHEMICAL ACTIVITY OF STANDARD

A preliminary examination of preparation 74/520 was originally undertaken in NIBSC and by Dr. W Becker in Behringwerke, Germany by Laurell and/or rocket electrophoresis. The proteins named above, excluding C3 were found to be present in reasonable concentration in the pooled serum before freeze-drying and to be present in the freeze-dried serum in about the same concentration. No gross change in electrophoretic pattern of these proteins was found to have occurred during the freeze-drying procedure.

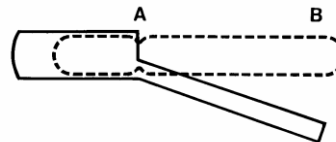
5. STORAGE

Unopened ampoules should be stored at -20°C or below in the dark.

Please note: because of the inherent stability of lyophilized material, NIBSC may ship these materials at ambient temperature.

6. DIRECTIONS FOR OPENING

Tap the ampoule gently to collect the material at the bottom (labelled end). Ensure ampoule is scored all round at the narrow part of the neck, with a diamond or tungsten carbide tipped glass knife file or other suitable implement before attempting to open. Place the ampoule in the ampoule opener, positioning the score at position 'A'; shown in the diagram below. Surround the ampoule with cloth or layers of tissue paper. Grip the ampoule and holder in the hand and squeeze at point 'B'. The ampoule will snap open. Take care to avoid cuts and projectile glass fragments that enter eyes. Take care that no material is lost from the ampoule and that no glass falls into the ampoule.



Side view of ampoule opening device containing an ampoule positioned ready to open. 'A' is the score mark and 'B' the point of applied pressure.

7. USE OF MATERIAL

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

Immediately before use the contents of one ampoule of the standard should be reconstituted by the addition of 1.0 ml of distilled water. The powder should dissolve readily on standing at room temperature, to yield a slightly turbid solution.

8. STABILITY

It is the policy of WHO not to assign an expiry date to its reference materials. They remain valid with the assigned potency and status until withdrawn or amended. NIBSC follows the policy of WHO with respect to its reference materials.

Reference materials are held at NIBSC within assured, temperature-controlled storage facilities. Reference Materials should be stored on receipt as indicated on the label. For information specific to a particular biological standard, contact NIBSC.

Users who have data supporting any deterioration in the characteristics of any reference preparation are encouraged to contact NIBSC.

9. REFERENCES

Campbell, P. J (1974) J. Biol. Stand. 2, 259., Reimer, C. B et al (1978) J. Biol. Stand. 6, 133.
Rowe, D.S, Anderson, S.G and Grab, B. (1970) Bull. W.H.O., 42, 535.

10. ACKNOWLEDGEMENTS

It is a pleasure to acknowledge the advice or technical collaboration of a number of colleagues from other establishments including Mrs I. Batty, Dr W. Becker, Dr T. E Cleghorn, Dr T. D Davies, Prof. J. R Hobbs, Dr M.E Mackay, Dr W. d'A, Maycock, Mr L. Vallet and Dr P. D Weston. We are most grateful to the staff of the North London Blood Transfusion Service who collected the blood.

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: Lyophilisate	Corrosive: No
Stable: Yes	Oxidising: No
Hygroscopic: No	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 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 * 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: 0.08g
Toxicity Statement: Toxicity not assessed
Veterinary certificate or other statement if applicable. Attached: No