Medicines & Healthcare products Regulatory Agency

NIBSC Confidence in Biological Medicines

Non WHO Reference Material
Primary Billiary Cirrhosis Serum, Human
NIBSC code: 67/183
Instructions for use
(Version 3.0, Dated 15/04/2008)

This material is not for in vitro diagnostic use.

1. INTENDED USE

Patients suffering from primary biliary cirrhosis frequently form non-organ specific antibodies to mitochondria (Donlach et al., 1966, Walker et al., 1965) and other auto-immune antibodies can be present in their sera, usually at a lower titre (Holborow et al., 1963, Paronetto et al., 1961). In order to provide a reference material against which to assay anti-mitochondrial antibodies, a single sample of primary biliary cirrhosis serum has been ampouled and freeze-dried and examined for suitability to serve as a Standard. Techniques of immunofluorescence and complement fixation have been used in this examination, but it is not yet certain that these two techniques measure quite the same activities.

The source material from which the Standard was prepared was serum taken from a female patient with primary biliary cirrhosis. It was supplied by courtesy of Professor Sheila Sherlock at the Royal Free Hospital, London.

The serum contained particulate matter and it was therefore centrifuged at 6,5000 G for 15 minutes. The supernatant was removed aseptically and a sample cultured aerobically and anaerobically for microorganisms. Growth was absent after 48 hours incubation. The serum was then diluted 1 part in 20 in veronal-buffered saline with added calcium and magnesium at pH 7.0 and clarified by filtration through a coarse, sterile membrane (Millipore) filter. The diluted serum was filled into glass ampoules on the 19th September 1968, frozen in liquid nitrogen and freeze dried. On the 24th September, 1968, the ampoules were fitted with plastic capillary-leak plugs and the serum was dried to constant weight by secondary desiccation over phosphorous pentoxide at a vacuum of 0.03 torr. On the 3rd October 1968 ampoules were filled with pure dry nitrogen and sealed by fusion of the glass. They were then tested for the presence of leaks and have since been stored at
–20^{o}C in the dark. The ampoules were coded 67/183.

During filling, 80 ampoules, out of a total of just over 2000 were tested for wet weight of contents. The mean weight was 1.020 gm (range ± 1.3%). Dry weight of the freeze-dried material was estimated in 3 ampoules. The mean weight was 14.26 mg (range ± 1.78%). Moisture content of the freeze dried powder was estimated in 3 ampoules by weighing each ampoule before and after 'complete' desiccation of the contents at 56^{o}C. The average percentage loss in weight for the three ampoules was 0.52% (range 0.41-0.69%). Mass spectroscopic examination of the gas in each of 3 ampoules of the Standard showed a mean oxygen content of 0.68% (range 0.65-0.72%).

2. CAUTION

<u>This preparation is not for administration to humans or animals in the human food chain</u>.

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

100 units/ampoule

4. CONTENTS

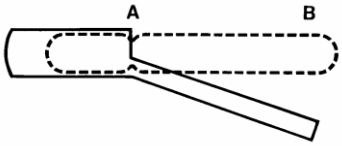
Country of origin of biological material: United Kingdom.

5. STORAGE

Store unopened ampoules at -20^{o}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

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

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

Ampoule contents were reconstituted by adding 1 ml of distilled water. They were then examined for 4 types of activity, (1) fixation to cytoplasmic components (by immunofluorescence tests), (2) fixation to nuclear components (by immunofluorescence tests), (3) complement fixation reactions and (4) rheumatoid factor activity. The first two activities were studied on rat liver, complement fixation with rat kidney, and rheumatoid factor activity was titrated against coated sheep red blood cells. Ampoule contents held at -20^{o}C and also material held at +37^{o}C for two years eleven months were both titrated to study stability of the material.

Complement fixation tests

These were carried out by the classic technique using homogenate of normal rat kidney as antigen and incubating at + 37^{o}C for 30 minutes at each stage. The titration end-point was read as that dilution of serum which produced 50% haemolysis of test red cells (table 1).

Immunofluorescence tests

Unfixed frozen cryostat sections of normal rat liver were cut to 6 µ thickness. These were treated for 30 minutes at room temperature with serial dilutions of 67/183 in Coon's saline*. The sections were then washed in Coon's saline and treated with Fluorochrome-labelled Antibody to Human Immunoglobulins, MRC Research Standard A, 68/45, at a concentration of 27 units per ml. Sections were incubated with conjugate for 30 minutes at room temperature. At the end of this time they were washed and examined microscopically for fluorescence. Fluorescent staining of either nuclei or cytoplasm was recorded in a system of scoring ranging from maximal brightness (4+) to no staining (zero). The titration end-point was taken to be 2+, which represented staining estimated by eye as 50% of maximal brightness. Appropriate controls were included. Results on liver cells are given in Table II but there was approximately the same titre of staining of kidney tubules.

Rheumatoid factor activity

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

This was estimated by means of the Rose-Waaler (sheep cell agglutination) test. The primary biliary cirrhosis serum (67/183 was titrated against sheep red cells sensitised with amboceptor, MRC Research Standard A, 66/236, for Rabbit Antibody to Sheep Red Cells, at a concentration of a quarter of a minimal agglutinating dose. Tests were incubated for 18 hours at +4°C. Appropriate controls were included. Tests were read microscopically and the titration end-points was taken to be 1+ agglutination in a sequence of scoring ranging through visual agglutination, 2+, 1+, (1+), weak, 0 (Table III).

*Formula for Coon's buffer

Sodium barbitone	20.6 gm
Sodium chloride	85.0 gm
N/1 hydrochloric acid	80.6 ml
Distilled water to	5.0 litres

Final pH 7.2
The buffer is diluted with an equal volume of distilled water before use.

As far as the above results go, the preparation 67/183 appeared suitable as a quantitative reference material for non-organ specific fluorescent staining and/or complement-fixing antibody found in patients with primary biliary cirrhosis and certain other diseases. The end-points of titrations were suitably for reading and the antibody activity appeared to be stable on storage. The material 67/183 was established as MRC Research Standard A, 67/183, for Primary Biliary Cirrhosis Serum and given a unitage such that one unit is contained in 0.1426 mg of the freeze-dried powder and for all practical purposes, each ampoule contains 100 units of activity.
In using the Standard no attempt should be made to weigh out ant portions of the freeze-dried material. Instead, the contents of each ampoule should be completely dissolved by adding 1.0 ml of distilled water and the inner wall of the ampoule, together with the partially reconstituted material, should be repeatedly washed out into a container by the use of a suitable diluent. The total volume of the reconstituted, diluted material should be precisely estimated.

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.
The freeze dried preparation 67/183 which had been held at –20°C rapidly dissolved in 1 ml of distilled water to give a clear yellow solution. Material which had been held at +37°C for approximately 3 years also dissolved in the same volume of distilled water at room temperature but took one or two minutes longer to dissolve than the –20°C material although solution was finally complete. No deposit was present after the reconstituted serum had been centrifuged at 2,500 G for 10 minutes. Loss of potency was not demonstrated in the +37°C preparation in either complement-fixation or immunofluorescence tests. Tests for rheumatoid factor indicated that this activity was absent from the +37°C material. The potency of rheumatoid factor was low in the –20°C material, approximately 30 International Units per ml and tests carried out on the 4th January, 1972 (Table III) indicated that not even two I.Us per ml of rheumatoid factor antibody was present in the reconstituted, undiluted +37°C material.

9. REFERENCES

Donlach, Deborah, Roitt, I.M., Walker, J.G., and Sherlock Sheila (1966). Tissue antibodies in primary biliary cirrhosis, active chronic (lupoid) hepatitis, cryptogenic cirrhosis and other liver diseases and their implications. J. Exp. Immunol., 1, 237

Holborow, E.J., Asherson, G.L, Johnson, G.D., Barnes, R.D.S., Carmichael, D.S (1963).
Antinuclear factor and other antibodies in blood and liver diseases. Brit. Med. J., I, 656.

Paronetto, F., Schaffner, F. and Popper, H. (1961). Immunocytochemical reaction of serum of patients with hepatic diseases with hepatic structures. Proc. Soc. Exp. Biol., 106, 216.

Walker, J.G, Donlach, D., Roitt, J.M., and Sherlock Sheila. (1965). Serological tests in diagnosis of primary biliary cirrhosis. Lancet, 1, 827.

10. ACKNOWLEDGEMENTS

We should like to express thanks to Professor S. Sherlock for supplying the serum from which the standard was prepared and to Dr D. Donlach and Miss B. Pandit for their help in the preliminary examination of materials.

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.

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

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: 0.01426g
Toxicity Statement: Toxicity not assessed
Veterinary certificate or other statement if applicable**.** **Attached:** No See attached Tables I, II and III

Tables I, II and III

Percentage potency, relative to that of -20°C material, of 67/183 which had been stored at +37°C for 2 years 11 months.

Table I Complement fixation tests

Date examined	Storage temperature °C	Complement fixing activity	
		Titre	% potency relative to -20°C material
14th December 1971*	-20 +37	120 40	100 33.3
16th December 1971*	-20 -20 +37 +37	120} 140 160} 160 160	100 114 114
17th December 1971*	-20 -20 +37 +37	160}140 120} 160 160	100 114 114
20th December 1971*	-20 -20 +37	120 160 160	100 133 133

Titration end-point = 50% haemolysis

*These results were the first in the titration series and it seemed likely that there was some imbalance of the test system on this occasion.

Table II Immunofluorescence tests on liver cells

(a) Cytoplasmic staining

Date examined	Storage temperature	Activity of antibody demonstrated by Immunofluorescence		
		Titre (2+ end-point)	% potency (2+ end-point) relative to -20°C material	Titre (end-point of trace staining)
6th January 1972	-20 +37	12.5 12.5	100 100	80 100
10th January 1972	-20 -20 +37 +37 +37	9 7 9 24 9	}100 } 112.5 300 112.5	24 16 24 64 24

(b) Nuclear staining

The material tested on 6th January 1972 was used at a maximal concentration of 1:2. No nuclear staining was detected. As a control to this MRC Research Standard A, 66/233, for Anti-Nuclear Factor Serum (Homogeneous) Human, gave a 2+ end-point in this system, on both days, at a concentration of 12.5 units per ml.

Table III Rheumatoid factor agglutination tests

Date Examined	Storage Temperature °C	Rheumatoid factor activity	
		Titre	Potency (I.U./ml)*
20th December 1971	-20 -20 +37 +37	15 15 <10 <10	31 31 <20 <20
29th December 1971	-20 -20 +37 +37	20 15 <10 <10	71.4 54 <36 <36
4th January 1972	-20°C -20°C +37°C +37°C	16 16 <1 <1	31 31 <2 <2

Titration end-point = 1 + agglutination

*The titres of the 1st British Standard for Rheumatoid Arthritis Serum diluted from 500 units per ml, were on 20th December 1971, 1:240, on 29th December 1971, 1:140 and on 4th January 1972, 1:256.