Medicines & Healthcare products Regulatory Agency

NIBSC Confidence in Biological Medicines

Non WHO Reference Material
Anti-Nuclear Factor (Homogenous) Serum
NIBSC code: W1064
Instructions for use
(Version 2.0, Dated 10/04/2008)

This material is not for in vitro diagnostic use.

1. INTENDED USE

This material was previously distributed by Sanquin Diagnostic Services, the Netherlands, and is the same as that distributed under NIBSC code 66/233. With effect from 1st February 2007, the National Institute for Biological Standards and Control (NIBSC), Potters Bar, UK, is the custodian and distributor of this material.

The package insert from Sanquin is attached.

2. CAUTION

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

This preparation contains material of human origin. The ampouled material has been tested and found negative for anti-HIV, and HCV RNA by PCR, but **positive for HBsAg**. 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 IU/ampoule.

4. CONTENTS

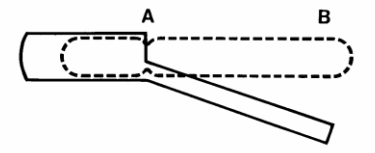
Country of origin of biological material: United Kingdom.

5. STORAGE

Store unopened ampoules at -20°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

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

See accompanying details from Sanquin.

8. STABILITY

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 They remain valid with the assigned potency and status until withdrawn or amended.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

See accompanying details.

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

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 that is potentially infectious		

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

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

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

WHO 1064

W. H. O.

WORLD HEALTH ORGANISATION
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THE USE OF THE WHO INTERNATIONAL REFERENCE PREPARATION FOR ANTINUCLEAR FACTOR 66/233 (HOMOGENEOUS) IN ASSAYING ANTINUCLEAR ANTIBODIES.

INTERNATIONAL UNION OF IMMUNOLOGICAL SOCIETIES
UNION INTERNATIONALE DES ASSOCIATIONS D'IMMUNOLOGIE

STANDARDISATION COMMITTEE

THE USE OF THE WHO INTERNATIONAL REFERENCE PREPARATION FOR ANTINUCLEAR FACTOR 66/233 * IN ASSAYING ANTINUCLEAR ANTIBODIES

Immunofluorescent tests for antinuclear antibodies (ANA, previously known as antinuclear factor - ANF) are widely employed in the routine investigation of patients with connective tissue disease. The test procedure has not been standardised and various methods are employed which have considerable differences in sensitivity. Factors affecting sensitivity include the choice of tissues providing nuclear antigen, the dilution of patients' sera employed for screening, the staining and washing procedures, physicochemical and immunochemical properties of the fluorescent conjugate and optical and spectral features of the fluorescent microscope employed. Thus, it is not possible to compare directly results obtained in different laboratories either in terms of positivity or when expressed as titres.

In an international collaborative study[1] it was established that when the results of ANA tests were expressed in relation to a reference preparation satisfactory inter-laboratory comparability may be achieved, especially when local methods rather than an imposed standard procedure were employed. Serum 66/233 was established as the first International Reference Preparation of Anti-Nuclear Factor Serum in 1970. 100 IU of activity was assigned to the contents of each ampoule. Routine clinical tests should therefore be reported in International Units by comparison with a laboratory reference preparation calibrated against the

International Reference Preparation. General adoption of a calibrated reference preparation would also enhance the value of research reports concerned with the significance of ANA.

PRINCIPLE

The reference preparation is titrated by the local method using precisely the same conditions and material employed for routine tests. The titre obtained is expressed in International Units per ml, and this provides a factor for conversion of results obtained on test sera.

METHOD

1 Dissolve the contents of the ampoule in exactly 1 ml of the diluent used in the tests (usually phosphate-buffered saline - PBS). This gives a solution containing 100 IU/ml The error due to the volume of the lyophilised material may be ignored for practical purposes.

2 Make a series of doubling dilutions in PBS and carry out the routine indirect immunofluorescent procedure.

3 Determine the titration end-point showing weak nuclear immunofluorescence, i.e. the highest dilution giving minimal nuclear staining that is distinguishable from a negative reaction

4 Calculate the potency in IU/ml at the end-point.

5 Express results obtained on test sera in IU/ml by multiplying the reciprocal of their dilution at their titration end-point by the potency of the reference preparation at its end-point.

EXAMPLES - based on titration of serum from the same patient (X) in two laboratories, A and B, using the Reference Preparation

LABORATORY A

Titration of Reference Preparation 66/233 :-

Dilution of solution containing 100 IU/ml

1:10	1:20	1:40	1:80	1:160	1:320	1:640
++	++	+	+	weak	-	-

i.e. End-point = 1:160 = $\frac{1}{160}$ x $\frac{100}{1}$ IU per ml = 0.625 IU/ml

Titration of serum from Patient X :-

Dilution of serum

1:10	1:40	1:160	1:640	1:2560
++	+	+	weak	-

i.e. End-point = 1:640 = 640 x 0.625 IU/ml = 400 IU/ml.

LABORATORY B

Titration of Reference Preparation 66/233 :-

Dilution of solution containing 100 IU/ml

1:10	1:20	1:40	1:80	1:160	1:320	1:640
++	+	weak	-	-	-	-

i.e. End-point = 1:40 = $\frac{1}{40}$ x $\frac{100}{1}$ IU per ml = 2.5 IU/ml

Titration of serum from Patient X :-

Dilution of serum				
1:10	1:40	1:160	1:640	1:2560
+	+	weak	-	-

i.e. End-point = 1:160 = 160 x 2.5 IU/ml = 400 IU/ml

Thus, although the test procedure employed in Laboratory A is four times more sensitive than that in Laboratory B, the antinuclear activity of serum from Patient X is found to be the same in both laboratories when recorded in International Units per ml.

The sensitivity of a particular system should remain constant and a local reference ANA-containing control serum may be established in order to provide internal day-to-day consistency. However, when any change is made in the test procedure (for example use of tissue from a different species, or a microscope objective of different numerical aperture), the sensitivity must be re-assessed.

The clinical interpretation of unitage[2] is shown below :-

International Units/ml	Interpretation
6	very low
25	low
100	moderate
400	high
1600	very high

1 ANDERSON S.G., ADDISON I.E., & DIXON H.G. 1971
Antinuclear-factor serum (homogeneous): An international collaborative study of the proposed research standard 66/233
Ann. N.Y. Acad. Sci. 177, 337-345

2 G.D. JOHNSON, SHIREEN CHANTLER, IRENE BATTY, & E.J. HOLBOROW, 1978. Use and Abuse of International Reference Preparations in Immunofluorescence
In 'Laboratory and Clinical Standardisation in Rheumatoid Arthritis (Part 1)', ed D.C. Dumonde and M.W. Steward.
M.T. Publications, Lancaster.

G.D. JOHNSON

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or

The Director, National Institute for Biological Standards and Control, Holly Hill, Hampstead, London, NW3 6RB, England.

will supply the International Reference preparation on request.