Medicines & Healthcare products Regulatory Agency

NIBSC Confidence in Biological Medicines

Working Standard
Anti-D for assuring operator and test performance
NIBSC code: 98/540
Instructions for use
(Version 1.0, Dated 22/03/2022)

This material is not for in vitro diagnostic use

1. INTENDED USE

This material replaces anti-D preparation 07/304. Anti-D preparation 98/540 can be used as an internal quality control to assure the sensitivity of routine antibody screening methods, to assess operator competence in performing and reading antiglobulin tests, to assure the efficacy of cell washing prior to the addition of the antiglobulin reagent in spin-tube antiglobulin tests, and as an internal control for antibody titrations.
This material has been prepared and assessed by the UKBTS/NIBSC Standing Advisory Group for Immunohaematology. The reconstituted material is intended as an internal quality control being used at different dilutions:
- at a 1 in 20 dilution as an 'assurance' dilution
- at a 1 in 20 dilution to assure cell washing efficacy
- at a 1 in 40 dilution as a 'monitoring' dilution.
- as a series of doubling dilutions [1 in 2, 1 in 4 etc.] as an internal quality control for antibody titrations.

2. CAUTION

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. This preparation is not for administration to humans or animals

3. UNITAGE

Reconstituted 98/540 has an anti-D content of 1.8 IU/ml determined by Cambridge BTC.

4. CONTENTS

Country of origin of biological material: United Kingdom.
Each ampoule of anti-D 98/540 contains the freeze-dried residue of 1 mL of pooled anti-D. Constituent donations were provided by the UK Blood Transfusion Service.

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

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

Please see attached instruction sheets for usage.

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.

NIBSC follows the policy of WHO with respect to its reference materials.

9. REFERENCES

N/A

10. ACKNOWLEDGEMENTS

We thank the following for evaluating preparation 98/540:
Michelle Weston, NBS Reagents Liverpool; Chris Elliot, South Tees Hospital and Richard Knowles, RCI Birmingham.

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: Classified under CLP

Physical and Chemical properties			
Physical appearance: Lyophilisate		Corrosive:	No
Stable:	Yes	Oxidising:	No
Hygroscopic:	No	Irritant:	Unknown
Flammable:	No	Handling: See caution, Section 2	
Other (specify):	Contains human plasma		

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

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

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 * 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

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7. Use of Material

a) Reconstitution

Add 1.0 mL deionised/distilled water to the opened ampoule. Allow to stand for at least 5 minutes at room temperature with periodic agitation to allow for complete reconstitution of the entire contents.

b) Preparation of dilutions for use

Using the reconstituted contents from one or more ampoules the following can be prepared -

- a 1 in 20 [1 + 19 volumes] dilution in phosphate buffered saline [PBS; 15mM, pH 7.0]
- a 1 in 40 [1 + 39 volumes] dilution in phosphate buffered saline [PBS; 15mM, pH 7.0]
- doubling dilutions from 1 in 2 in phosphate buffered saline [PBS; 15mM, pH 7.0]

Dispense aliquots of suitable volume of the 1 in 20 and 1 in 40 dilutions into containers appropriate for subsequent use. Stopper and store at -20°C or below, pending use. Where automated sampling equipment is routinely in use, it may be advantageous to use containers which could be placed directly on the sampler.

c) Use to assure satisfactory sensitivity of the antibody detection test

Use as an 'assurance' internal quality control for antibody screening methods.

Aliquots of the 1 in 20 dilution can be included as an internal quality control with each series of routine antibody screening tests and should give unequivocal positive reactions (Table 1). If another weak antibody control is used daily, this dilution can be used on regular occasions to assure the continued sensitivity of the routine technique. Users of very sensitive antibody screening methods may wish to use a greater dilution as an internal quality control but this dilution needs to be assessed by the user.

Table 1 Percentage positive reactions (≥1+) using 1 in 20 and 1 in 40 dilutions of reconstituted 98/540 obtained in three test centres using various antibody detection methods.

Dilution of 98/540	Percentage of positive reactions (≥1+) by direct and anti-globulin test methods									
	Liss IAT		BioRAD IAT		BioRAD Neutral		Biovue IAT		Grifols	
	R_1R_1	R_2R_2	R_1R_1	R_2R_2	R_1R_1	R_2R_2	R_1R_1	R_2R_2	R_0r (Saline)	R_0r (DG Gel Sol)
1 in 20	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**
1 in 40	**100**	**100**	**83**	**100**	**100**	**100**	**100**	**100**	**50**	**100**

d) Use to monitor continued satisfactory performance of the test and test operator

Use as an internal quality control to monitor continued satisfactory performance of the test system and operator.

The 1 in 40 dilution may not be detected unequivocally in each series of tests (Table 1) but by testing aliquots of this dilution over time and monitoring the strength of reaction, changes in performance can be detected and appropriate action taken. This dilution can also be used to ensure continued user competence in performing the indirect antiglobulin test for antibody detection.

This internal data monitoring can enable changes in performance to be detected and appropriate action implemented before such changes become significant to the test.

Users of sensitive antibody screening methods may wish to use a monitoring dilution greater than 1 in 40 to assure consistency of performance.

e) Use to assure efficacy of cell washing

A 1 in 20 dilution made in AB plasma can be used as an internal quality control to assure that in methods that require a cell washing step prior to addition of the antiglobulin reagent this has been adequately performed. This can either be done as a separate QC procedure or as part of the daily control of the system in use.

f) Use as an internal quality control for antibody titrations

The reconstituted material can be used for this purpose by making doubling dilutions, in PBS at pH 7.0, using the same procedure as that used for the test titration and processing the two in parallel. The expected titration for the method used has to be assigned by each testing laboratory based on a number of tests performed using the local standard technique and performed by several workers.