



DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Centers for Disease Control
and Prevention (CDC)
Atlanta GA 30333

PR3-ANCA HUMAN REFERENCE SERUM #16

Product Package Insert Catalogue #IS2721 Lot #07-0002

INTERNATIONAL UNION OF IMMUNOLOGIC SOCIETIES - CENTERS FOR DISEASE CONTROL
REFERENCE SERUM FOR ANTI-PROTEINASE 3 ANTIBODIES (PR3-ANCA)

Intended Use

For in vitro immunodiagnostic use as a reference human serum giving a positive response in PR3-ANCA ELISA tests. The obtained responses are due to antibodies in serum to native proteinase 3.

Description of Reference Serum

Plasma from a single donor was defibrinated by addition of CaCl_2 (2mM) and thrombin (100 NHI-U/ml), allowed to clot 1.5h at 37°C followed by -20°C over night, thawed, centrifuged, filtered and frozen at -70°C until lyophilization. Volumes of 0.50 ml were dispensed into borosilicate vaccine vials, freeze-dried, and sealed with butyl rubber stoppers while still under reduced pressure. Vials are stored at the CDC at -20°C.

The vial contents were negative for HIV antibodies, negative for hepatitis B DNA, surface antigen and core antibodies, and negative for hepatitis C virus RNA and antibodies.

Reconstitution and Storage

Store the freeze-dried material at -20°C until use. To reconstitute, the contents should first be shaken to the bottom by tapping of the upper end. Before the stopper is removed, the vacuum should be broken by insertion of a hypodermic needle through the rubber stopper. Accurately 0.50 ml of distilled water should then be added, and the vial restoppered. [This should be by volumetric pipette, by weight (to 3 decimal places) or by accuracy checked automatic pipette.] The freeze-dried powder should dissolve readily with gentle swirling (avoid foam). Allow to stand for at least 1 h before use and store at 4°C until use, not later than 24 h after reconstitution. Although not recommended, the reconstituted material will withstand at least 8 weekly freeze-thaws without loss of activity. If future use of reconstituted material is contemplated, portions of the undiluted material sufficient for a single use should be stored at -70°C and discarded after use.

Anti-PR3 Content

This international standard should be considered to contain 100 International Units / ml, i.e. it should give a titre of 100 IU / ml in any PR3-ANCA ELISA it may be analysed. The standard should be used for establishing a PR3-ANCA laboratory standard in both the direct ELISA and the capture ELISA analysis. Any local method may be used as long as the result is expressed in relation to the standard and obtained titres presented as IU / ml. Six expert laboratories participated in selecting the plasma for the standard. One plasma was selected out of 16 candidates. The criteria was that it should give a typical C-ANCA pattern, be specific for Proteinase 3 without reactivity with other ANCA antigens such as myeloperoxidase, azurocidin, elastase, lactoferrin, cathepsin G, BPI or lysozyme. It should neither contain any RF IgA or IgM. It was selected to give an intermediate strength in the direct PR3-ANCA ELISA.

Suggested procedure for Standardization of Quality Control Reagents

Since the amount of IUIS-CDC reference preparation is limited, it should be used to calibrate secondary standards which can be run each day along with other samples being analyzed.

In selecting a secondary standard, serum (available in plentiful supply) containing anti-proteinase 3 antibodies of similar specificity and resulting in a similar dilution curve should be obtained. The titer need not be similar.

To calibrate a secondary standard:

1. Reconstitute ampoule as described above.
2. Prepare dilutions between 1/50 and 1/5000 of the IUIS-CDC reference serum and the secondary standard. One of the dilutions should be the recommended sample dilution of the test as this corresponds to 100 IU/ml of the IUIS-CDC reference. It is recommended to compare several potential sera for secondary standard as the shape of the dilution curve might differ between patient samples.
3. Carry out the routine test procedure on the dilutions.
4. Choose the secondary standard with a dilution curve most similar to IUIS-CDC reference serum. Titrate the dilution of the chosen secondary standard until it overlaps with the IUIS-CDC serum. The IUIS-CDC reference serum shall give a signal of 100 IU / ml at the recommended sample dilution of the test.
5. Calculate the relative potency for several dilutions along the dilution curve. Relative potency shall give a ratio as close to 1.0 as possible.

$$\text{Relative potency} = \frac{\text{IU/ml of secondary standard}}{\text{IU/ml of IUIS-CDC serum}}$$

If the secondary standard is stable, the relative potency obtained should not change. It is recommended to perform at least an annual comparison with the IUIS-CDC reference serum and adjust the secondary standard if needed.

Generally, in the day to day performance of the test, serial dilutions of the secondary standard are run. A single dilution, unless it is run at or near its end point, may not reveal major changes in test performances.

The reference sera can also be used to determine the most suitable or sensitive method, kit, or reagents, to compare one's results with that of the laboratories which initially evaluated the reference serum, and as a common reference for interlaboratory comparability.

Consensus evaluation results on the IUIS-CDC preparation are given above (PR3-ANCA antibody content) for comparison to your own results.

Caution

This serum was found to be negative for markers of hepatitis-B, hepatitis C and HIV. Since no test method can offer complete assurance that these or other infectious agents are absent, this serum should be handled at the Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen (Centers for Disease Control, National Institutes of Health. Biosafety in Microbiological and Biomedical Laboratories, 1st Edition, 1984, 11-13).

Supplementary Information: Detailed description of the preparation, analysis, reagents, antigens, and individual reference laboratory results are in a supplementary publication that is under preparation.

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