



DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

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

**IUIS – CENTERS FOR DISEASE CONTROL REFERENCE REAGENT
FOR HUMAN REFERENCE SERUM FOR CITRULLINATED PEPTIDE/PROTEIN
ANTIBODIES (ACPA)**

Product Package Insert Catalogue IS2723 Lot 08-0202 DOM: 06/2008

Human ACPA serum

Intended Use

For in vitro immunodiagnostic use as a reference human serum in solid phase enzyme immunoassays. The obtained responses are due to antibodies in serum directed against citrullinated peptides/proteins.

Description of Reference Reagent

Plasma from a single donor was defibrinated by addition of CaCl₂ (2mM) and thrombin (100 NHI-U/mL), allowed to clot 1.5 hr at 37⁰ C and stored over night at -20⁰ C, thawed, centrifuged and filtered, stored at -70⁰ C until freeze-drying. Volumes of 0.50 mL were dispensed in borosilicate vaccine vials, freeze-dried and sealed with butyl rubber stoppers while still under reduced pressure. Vials are stored at CDC at -20⁰ C. Evaluation of randomly selected vials in 10 different reference laboratories resulted in consistent values and identical to the original activity within experimental error.

The vial contents were negative for HIV antibodies, negative for Hepatitis B surface antigen and core antibodies and negative for Hepatitis C antibodies. Plasma was donated by Euro-Diagnostica in Arnhem, The Netherlands.

Reconstitution and Storage

Store the freeze-dried material at -20⁰ 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. Precisely 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 4⁰ C until use no longer than 24 h after reconstitution. Although not recommended, the reconstituted material will withstand at least 4 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 -70⁰ C and discarded after use.

Antibody Content

This international standard should be considered to contain 100 International Units / mL, i.e. it should give a level of 100 IU / mL in any ACPA ELISA it may be analyzed. The standard should be used for establishing an ACPA laboratory standard ELISA analysis. Any local method may be used as long as the result is expressed in relation to the standard and obtained levels presented as IU/mL. The plasma was selected to give an intermediate strength in the ACPA ELISA.

Suggested procedure for the 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 selection of a secondary standard, serum (available in plentiful supply) containing ACPA antibodies of similar specificity and resulting in a similar dilution curve should be obtained. The level needs not to be similar.

To calibrate a secondary standard:

1. Reconstitute ampoule as described above.
2. Prepare a serial dilution of the IUIS/CDC reference serum and secondary standard. The first dilution should be the recommended sample dilution of the test as this corresponds to 100 IU/mL of the IUIS/CDC reference. Pipette standard serum in duplicate. 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. 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 should give a ratio as close to 1.0 as possible.

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

If the secondary standard is stable, the relative potency 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. These reference reagents can also be used to determine the most suitable or sensitive method, kit or reagents; to compare one's test results with that of the laboratories which initially evaluated the reference reagents and as a common reference for interlaboratory comparability.

Caution

This serum was found to be negative for markers of Hepatitis B, Hepatitis C and HIV. Since no test methods 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 and individual reference laboratory results are in a supplementary publication that is under construction.

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