DEPARTMENT OF HEALTH & HUMAN SERVICES Public Health Service

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

ARTHRITIS FOUNDATION – CENTERS FOR DISEASE CONTROL REFERENCE REAGENT FOR IgG ANTI-CARDIOLIPIN ASSAY AGAINST EPITOPE ON BETA-2 GPI

Product Package Insert Catalogue IS2717 Lot 09-xxxx 10/20/09

Human IgG Anti-Cardiolipin Monoclonal Antibody
HCAL 1.1 μg/ml

Intended Use

For in vitro immunodiagnostic use as an external control in solid phase enzyme immunoassays detecting beta 2-glycoprotein 1 (β_2 GPI) dependent IgG anti-cardiolipin (aCL) antibodies.

Description of Reference Reagent

HCAL is a chimeric antibody with human γ1 constant regions and variable regions of WBCAL-1, a mouse monoclonal antibody derived from an anti-phospholipid syndrome (APS) prone mouse which has a specificity similar to that of aCL in sera from humans with APS. HCAL is continuously secreted by a transfected mouse myeloma cell line into the culture supernatant, producing a continuous supply of the antibody. [1] HCAL with a protein concentration of 2.2μg/ml was obtained from Drs. K. Ichikawa and T. Koike at the Hokkaido Univ. School of Medicine in Sappora, Japan. It was diluted to a protein concentration of 1.1μg/ml with 10mM Tris buffered saline, pH 7.4 with 1% BSA. Volumes of 0.50 ml were dispensed in borosilicate vaccine vials, freeze-dried, and sealed with butyl rubber stoppers while still under reduced pressure. The vial contents were sterile as determined by U.S.P. approved methods of sterility testing. Vials are stored at the CDC at –20°C. The mean dry weight and SD of material in 13 randomly selected vials after freeze-drying was 7.01 ± 1.0 mg. Further evaluation of rehydrated vials colorimetrically and by HCAL ELISA revealed a significant proportion of vials had reduced HCAL levels. These vials were removed and the remaining vials were rehydrated and pooled. The pooled material was re-distributed into new vials which were evaluated for HCAL activity by ELISA in three different reference laboratories. The HCAL values on the new lot of vials was found to be consistent between vials and identical to the original activity within experimental error. Therefore, the values for Antibody Content (see below) were retained for the new lot.

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. Precisely 0.50ml of distilled water should then be added, and the vial restoppered. The freeze-dried powder should dissolve readily with gentle swirling (avoid form). Allow to stand at least 30 minutes before use and store at 4°C until use, not later than 24 h after reconstitution. Note: Contents of vials that have been stored at high temperatures may not completely go into solution. These vials should be discarded. 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 store at –70°C and discarded after use.

Antibody Content

In the presence of β_2GPI, HCAL bound to the wells of cardiolipin-coated microtiter plates in a dose-dependent manner and reacted with β_2GPI on oxygenated polystyrene plates. Eight international reference laboratories using a variety of IgG Anti-cardiolipin ELISA procedures, both in-house and commercial kits, tested HCAL at the dilution used when routinely testing samples. The variability of anti-cardiolipin is well known, therefore it is recommended that results be reported in protein concentration of μg/ml or ng/ml. However, when expressed in GPL units, a median value of 14 GPL units with a modified range of 8 – 26 GPL units was reported with OD values ranging from 0.190 to 0.579. Four of the laboratories ran HCAL at a 1:10 dilution (110 ng/ml) without further dilution. A median value of 93 GPL units with a range of 77 – 119 GPL units was reported. When expressed in OD values, the range was 0.891 to 2.532. Seven laboratories also ran HCAL in their IgG anti-β_2-GPI assay. A median concentration of 20 arbitrary units (AU) with a modified range of 17 – 43 AU and OD range of 0.398 to 1.187 were reported.

Suggested procedure for Standardization of Quality Control Reagents

Monoclonal antibodies can provide an external control of the technical variations both between various kits as well as in day to day assays. Since the amount of the reference preparation is limited, it should be used to calibrate secondary standards that can be run each day along with other samples being analyzed. The monoclonal antibody should be serially diluted to produce a standard curve. Based on these results, it is recommended that 2 different concentrations, one with results expected in the cut off range and the other in the medium positivity range be selected for use as controls.

To calibrate a secondary standard (i.e., a positive control to be included in subsequent routine test runs):

1. Reconstitute ampule as described above.

2. Prepare doubling dilution of the reference reagent and the secondary standard from 1:10 to 1:1280.

3. Carry out the routine test procedure on the dilutions.

4. Determine the points in the cutoff and medium positivity range.

5. Relative potency = $\frac{\text{Reciprocal of endpoint dilution of secondary standard}}{\text{Reciprocal of endpoint dilution of monoclonal antibody}}$

If the secondary standard is stable, the relative potency obtained should not change. Any changes in day to day performance of the secondary standard are more likely due to changes in test performance. Generally, in the day to day performance of the test, several 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 test performances.

These reference reagents can also be used to determine the most suitable or sensitive method, kit or reagents; to compare lot to lot changes; as a common reference for interlaboratory comparability; and for comparison of results with that of the laboratories which initially evaluated the reference reagent.

Reference:

1. Ichikawa K, Tsutsumi A, Atsumi T, Matsuura E, Kobayashi S, Hughes GRV, Khamashta M, Koike T. A chimeric antibody with the human γ1 constant region as a putative standard for assays to detect IgG β_2-glycoprotein I-dependent anticardiolipin and anti-β_2-glycoprotein I antibodies. Arthritis Rheum 1999; 42:2461-70.

ANA Reference Materials Laboratory
c/o Robert Vogt, Mailstop F-19
Centers for Disease Control and Prevention (CDC)
Atlanta, GA 30333
Email: rfv1@CDC.GOV
WWW.AutoAb.org