DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

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

ANA HUMAN REFERENCE SERUM #1

Product Package Insert Catalogue #IS2072 Lot #98-0026 2/24/82 (rev. 12/05)

ARTHRITIS FOUNDATION - CENTERS FOR DISEASE CONTROL REFERENCE SERUM FOR FLUORESCENT ANTINUCLEAR ANTIBODY (HOMOGENEOUS/RIM PATTERN)

Intended Use

For in vitro immunodiagnostic use as a reference human serum giving a homongeneous/rim pattern in the fluorescent antinulcear antibody (FANA) test. The homogeneous/rim pattern obtained is due to antibodies to native DNA present in this serum.

Description of Reference Serum

Citrated plasma from a single donor was made 0.01 M $CaCl_2$ and 0.013 M epsilon-amino-n-caproic acid, allowed to clot at 4°C overnight, centrifuged, 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 mean dry weight and SD of material in 14 randomly selected vials after freeze-drying was 7.5 ± 2.2 mg; The residual water content was less than 1%.

The vial contents were sterile as determined by U.S.P. approved methods of sterility testing, negative for hepatitis-B surface antigen by radioimmunoassay, negative for antibody to HIV by Western blot, and free of rheumatoid factors as determined by latex agglutination.

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 0.50 ml of distilled water should then be added, and the vial restoppered. 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.

FANA Content

This serum gives a homogeneous/rim FANA pattern due to high levels of antibodies to native DNA. Fifteen reference laboratories using fluorescein-labelled polyvalent (all Ig classes) α-human Ig reagents and a variety of substrates (mouse liver, mouse kidney, and the Hela and Hep-2 human cell lines) obtained a median titer of 1:320 with a modified range of 1:120 to 1:128.

Suggested procedure for Standardization of Quality Control Reagents

The sensitivity of the FANA test is influenced by the choice of tissue substrate, the staining procedure, the fluorescein conjugate, the microscope, and the observer. Changes in any of these in the day to day performance of the test may be detected by including a stable positive control (and negative control) serum in each run.

Since the amount of AF/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. The validity of any secondary standard depends on its having the same specificity as the primary AF/CDC standard (anti-nDNA, anti-Sm, etc.). The specificity can be determined by EIA, double immunodiffusion (anti-nRNP, anti-Sm, anti-SSB/La) or by analysis for anti-nDNA (Farr assay, millipore filter assay, Crithidia test).

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 dilutions of the AF-CDC reference serum and the secondary standard from 1:10 to 1:1280.
3. Carry out the routine test procedure on the dilutions.
4. Determine the nuclear fluorescence end point showing minimal recognizable staining.
5. Relative potency = $\frac{\text{reciprocal of end point dilution of secondary standard}}{\text{reciprocal of end point dilution of AF-CDC serum}}$

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

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.

In selecting a secondary standard, a serum (available in plentiful supply) containing anti-nuclear antibody of similar specificity and resulting in a similar staining pattern should be obtained. The titer need not be similar. This should be stored undiluted in aliquots sufficient for one run (store at -20°C or below).

These 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 AF-CDC preparation are given above (FANA content) for comparison to your own results.

Caution

This serum was found to be negative for hepatitis-B surface antigen, hepatitis C and HIV antibody. 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

The reference for a general description of the AF-CDC reference sera is: Tan EM, Fritzler MJ, McDougal JS, McDuffie FC, Nakamura RM, Reichlin M, Reimer CB, Sharp GC, Schur PH, Wilson MR: Reference Sera for Antinuclear Antibodies. Arthritis and Rheumatism 25:1003-1005, 1982. Detailed descriptions of the preparation, analysis, reagents, antigens, substrates, and individual reference laboratory results are in a supplementary publication available upon request:

AF-CDC ANA Reference Lab
Immunology Branch, 17-3040, A25
Centers for Disease Control and Prevention (CDC)
Atlanta, GA 30333

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ARTHRITIS FOUNDATION – CENTERS FOR DISEASE CONTROL REFERENCE SERUM FOR HUMAN ANTIBODIES TO NATIVE DNA

Intended Use

For in vitro immunodiagnostic use as a reference human serum having high levels of antibodies to native DNA.

Description of Reference Serum

Citrated plasma from a single donor was made 0.01 M CaCl2 and 0.013 M epsilon-amino-n-caproic acid, allowed to clot at 4°C overnight, centrifuged, 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 mean dry weight and SD of material in 14 randomly selected vials after freeze-drying was 7.5 ± 2.2 mg.

The vial contents were sterile as determined by U.S.P. approved methods of sterility testing, negative for hepatitis-B surface antigen by radioimmunoassay, negative for antibody to HIV by Western blot, and free of rheumatoid factors as determined by latex agglutination.

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 0.50 ml of distilled water should then be added, and the vial restoppered. 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.

α-Native DNA Antibody Content

Eleven international reference laboratories evaluated this reagent and found high levels of a-nDNA. Methods used included the crithidia indirect immunofluorescence assay, the Farr assay, EIA and Western Blot. Results, including median titer and modified ranges, are indicated below. Several labs also reported the presence of α-histone. This serum is negative for antibodies against Sm, U1-RNP, SS-A/Ro, SS-B/La, Scl-70, Jo-1, PM-Scl and cardiolipin.

Assay	Median	Range
Crithidia (titer)	1:640	1:160-1:1280
Farr (IU)	81 IU	72 – 117 IU
Farr (% DNA bound)	90%	

Suggested Procedure for Standardization of Quality Control Reagents

Since the amount of AF/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. The validity of any secondary standard depends on its having the same specificity as the primary AF/CDC standard (anti-nDNA, anti-Sm, etc.). The specificity can be determined by EIA, Western blot, double immunodiffusion (anti-nRNP, anti-Sm, anti-SSB/La) or by analysis for anti-nDNA (Farr assay, millipore filter assay, Crithidia test).

To confirm the specificity of a secondary standard in double diffusion or counterimmuno-electrophoresis:

1. Reconstitute the ampule as described above.

2. Use both undiluted (neat) and 1:4 dilutions of the AF-CDC sera and the secondary standard.

3. Arrange antibody wells such that the secondary standards (neat and 1:4) are each next to an undiluted and 1:4 dilution of the AF-CDC serum.

4. Run the routine test procedure using nuclear extract antigen.

5. Lines of identity should be obtained without extra lines or ambiguous reactions.

To calibrate a secondary standard (double diffusion, counterimmunoelectrophoresis):

1. Reconstitute the ampule as described above.

2. Prepare undiluted and serial four-fold dilutions up to 1:256 of the secondary standard and AF-CDC serum.

3. Run the routine test on all dilutions.

4. Relative potency = $\frac{\text{reciprocal of end point dilution of secondary standard}}{\text{reciprocal of end point dilution of AF-CDC serum}}$

If the secondary standard is stable, the relative potency obtained should not change when periodically recalibrated. Any changes in day to day results with the secondary standard are more likely due to changes in test performance. Titer results may be particularly useful in assessing lot to lot changes in antigen preparations and for comparison of your results with the laboratories which initially evaluated these sera (see antibody content above).

Caution

This serum was found to be negative for hepatitis-B surface antigen, hepatitis C and HIV antibody. 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

The references for a general description of the AF-CDC reference sera are: Tan EM, et al. Reference Sera for Antinuclear Antibodies I. Arthritis Rheum 25:1003-1005,1982. Smolen JS et al. Reference Sera for Antinuclear Antibodies II. Arthritis Rheum 40: 413-418,1997. Detailed descriptions of the preparation, analysis, reagents, antigens, substrates, and individual reference laboratory results are in a supplementary publication available upon request:

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