U. S. Department of Commerce
Malcolm Baldrige
Secretary

National Bureau of Standards
Ernest Ambler, Director

National Bureau of Standards

Certificate

Reference Material 8430

Aspartate Aminotransferase (AST) (E.C.2.6.1.1.)-Human Erythrocyte Source

This Reference Material (RM) is intended for clinical laboratory use in evaluating and improving the precision of aspartate aminotransferase (AST) assays. It is supplied as a set of three bottles of a freeze-dried preparation. At present, enzymes are measured on a method-dependent basis rather than on an accuracy basis. Therefore this characterized material is being issued as a Reference Material rather than as a Standard Reference Material. The catalytic activity concentration is given as a recommended value, and is not certified by NBS.

Recommended Value: The mean catalytic activity concentration of AST in RM 8430 is 96.1 ± 2.6 U/L (International enzyme units per liter), where U is the micromoles of substrate converted per minute. This concentration and uncertainty is equivalent to 1602 ± 43 nanokatals per liter (1.0 U equals 16.67 nkat). The reported concentration is a consensus value, i.e., the mean of results from an interlaboratory study in which ten laboratories participated. The uncertainty is two standard deviations of the mean and includes an allowance for observed vial-to-vial variability.

AST Method: The catalytic concentration of AST in RM 8430 was measured by coupling oxalacetate production with reduced nicotinamide adenine dinucleotide (NAD) and malate dehydrogenase. The catalytic activity of AST is determined by measurements of the rate of nicotinamide adenine dinucleotide (NADH) oxidation (1,2,3). The method used in the interlaboratory study is based on the International Federation of Clinical Chemistry (IFCC) approved recommendations (1985) (3) but with clarifications described by Bowers et al. (4).

Source of Material: The highly purified cytoplasmic AST enzyme was prepared in lyophilized form from human erythrocytes by Dade/Baxter Travenol Inc., Miami, Florida. The material was donated to the National Reference System for the Clinical Laboratory (NRSCL) by that organization. NRSCL Council requested that NBS coordinate the interlaboratory study, evaluate the results, and distribute the characterized material.

Preparation of RM 8430: The procedure for the preparation of erythrocyte AST from outdated whole human blood was adopted from Rej, et al., (5) which included fractionation by ammonium sulfate, organic extraction, ion exchange, and dialysis. The final product was lyophilized.

The overall coordination of the technical measurements was performed by G.N. Bowers Jr., Hartford Hospital, Hartford, CT, and J.J. Edwards, NBS Organic Analytical Research Division.

The statistical analysis of the data was performed by R.C. Paule of the NBS National Measurement Laboratory.

The support aspects concerning the issuance of this Reference Material were coordinated through the Office of Standard Reference Materials by R. Alvarez.

June 2, 1987
Gaithersburg, MD 20899

Stanley D. Rasberry, Chief
Office of Standard Reference Materials

NOTICE AND WARNINGS TO USERS

-- All human blood-based products should be handled in accordance with good laboratory practices using appropriate precautions (6).

--RM 8430 IS INTENDED FOR "IN VITRO" DIAGNOSTIC USE ONLY!

RM 8430 is a lyophilized preparation and is to be reconstituted as described under "Instructions for Use".

--Hepatitis: HANDLE AS IF CAPABLE OF TRANSMITTING HEPATITIS!

Although this product was tested with a Food and Drug Administration approved method and found negative for the presence of Hepatitis B Surface Antigen, no known test method can offer assurance that products derived from human blood will not transmit infectious agents.

--HTLV-III: The raw material for this pool was prepared prior to the existence of an FDA approved test for HTLV-III antibody. HTLV-III antibody testing of in-vitro diagnostic products by reagents designed for the testing of human blood is considered inappropriate, and no valid conclusion can be drawn from such testing results.

INSTRUCTIONS FOR STORAGE AND USE

HANDLE AS IF CAPABLE OF TRANSMITTING HEPATITIS!

STORAGE: RM 8430 should be stored in a refrigerator at a temperature between 2 and 8 °C. It should not be frozen nor exposed to sunlight or ultraviolet radiation. Under the recommended storage conditions, RM 8430 is expected to be stable for at least two years. This material will be monitored and should the concentration change significantly, purchasers will be notified by NBS. RM 8430 should not be used after two years from date of purchase.

USE: Reconstitute as follows: Tap bottle before carefully removing stopper to avoid possible loss of particles. Use a Type 1, Class A volumetric transfer pipet or other dispenser of known accuracy to slowly add 2.00 ± 0.02 mL of distilled or demineralized water at 20 to 25 °C to the sides of the bottle while continually turning the bottle. Replace stopper, gently swirl bottle two or three times at 5-min intervals. DO NOT SHAKE VIGOROUSLY. Strong shaking will produce frothing. Wait an additional 15 min after reconstitution. If material is not used at that time, store between 2 and 8 °C until ready for use--within 2 hours after reconstitution. However, the Centers for Disease Control, National Institutes of Health reports that reconstituted material is stable for 8 hours.

References:

1. Karmen, A. A Note on the Spectrophotometric Assay of Glutamic-Oxalacetic Transaminase in Human Blood Serum, J. Clin. Invest. 34, 131-133 (1955).

2. Expert Panel on Enzymes (IFCC), Provisional recommendation on IFCC methods for the measurement of catalytic concentrations of enzymes. Part 2. IFCC method for aspartate aminotransferase. Clin. Chim. Acta 80, 21F-22F (1977).

3. Bergmeyer, H.U., Hørder, M., and Rej, R. Approved recommendations on IFCC methods for the measurement of catalytic concentrations of enzymes. IFCC method for aspartate aminotransferase. J. Clin. Chem. Clin Biochem. 24, 481-510 (1986).

4. Bowers, G.N. Jr., Edwards, G.C., Edwards, J.J., Paule, R.C., Greenberg, N., Hørder, M., Koedam, J.C., McComb, R.B., Syed, D., Miller, W.G., Miller, R.R. Jr., Rej. R., Siest. G., Schiele, F., and Whitner, V. Aspartate Aminotransferase Reference Material-NBS-RM #8430: The measurement of its catalytic activity concentration by the meticulous use of the IFCC Reference Method for AST within an interactive network of experienced enzyme standardization laboratories. To be published in Clin. Chem. 33, xx-xx (1987).

5. Rej, R., Vanderline, R.E., and Fasce, C.F. Jr., An L-Aspartate: 2-oxoglutarate aminotransferase reference material from human erythrocytes: preparation and characterization. Clin. Chem. 18, 374-383 (1972).

6. Centers for Disease Control/National Institutes of Health Manual, "Biosafety in Microbiological and Biomedical Laboratories", 1984.

Cooperative analyses were provided by the following laboratories, listed alphabetically, through the courtesy of the individuals listed.

1. Centers for Disease Control, Clinical Biochemistry Atlanta, GA 30333, V.S. Whitner.

2. Centre de Medecine Preventive de Vandoeuvre-les-Nancy Laboratoire de Biologie Clinique, 54501 Vandoeuvre-les-Nancy-Cedex, France, G. Siest and F. Schiele.

3. Eastman Kodak Co., Clin. Prod. Div., Rochester, NY 14650, N. Greenberg, L.A. Mauck, and R.J. Ferris.

4. Institute of Clinical Chemistry, Odense University Hospital, DK-5000, Odense C, Denmark, M. Hørder.

5. Hartford Hospital, Clin. Chem. Div. Hartford, CT 06115, G.N. Bowers Jr., R.B. McComb, and D. Syed.

6. Virginia Commonwealth University, Medical College of Virginia, Dept. of Pathology, Richmond, VA 23298, W.G. Miller.

7. National Bureau of Standards, Org. Anal. Res. Div., Gaithersburg, MD 20899, J.J. Edwards (Coordinator) and M.C. Kline.

8. Rijkinstituut voor volksgezondheid en milieuhygiene, Lab. for Clin. Chem. and Hematology, Bilthoven, The Netherlands, J.C. Koedam.

9. Technicon Instruments Corporation, Reference and Evaluation Laboratory, Tarrytown, NY 10591, R. Miller and C. Peters.

10. Wadsworth Center for Laboratories and Research, New York State Department of Health, Albany, NY 12201, C.S. Norton and R. Rej.