4392-310.07
40

NBSIR 74-548(R)

Quarterly Report of In-Vitro Diagnostic Products

FDA Contract No 74-58 (0)

R. Schaffer

Analytical Chemistry Division
Institute for Materials Research
National Bureau of Standards
Washington, D. C. 20234

August 1974
Interim Report for Period
April -- June 1974

Prepared for

Bureau of Medical Devices and Diagnostic Products
Food and Drug Administration
Rockville, Maryland 20852

NBSIR 74-548

QUARTERLY REPORT OF IN-VITRO DIAGNOSTIC PRODUCTS FDA CONTRACT NO 74-58 (0)

R. Schaffer

Analytical Chemistry Division
Institute for Materials Research
National Bureau of Standards
Washington, D. C. 20234

August 1974
Interim Report for Period
April -- June 1974

Prepared for
Bureau of Medical Devices and
Diagnostic Products
Food and Drug Administration
Rockville, Maryland 20852

U. S. DEPARTMENT OF COMMERCE, Frederick B. Dent, Secretary

NATIONAL BUREAU OF STANDARDS, Richard W. Roberts, Director

Quarterly Report of In-Vitro Diagnostic Products
FDA Contract No 74-58(0)

Quarterly Report April--June 1974
to
Food and Drug Administration
by the
National Bureau of Standards

Progress toward the goal of establishing the accuracy of several clinical reference methods is reported. These are reference methods for serum glucose, sodium, potassium, lithium, magnesium, and chloride, and for blood lead. Progress is reviewed on the development of or use of base-line methods for assigning high accuracy target values to samples needed for the study. The progress made by expert teams for the several reference methods is reported.

TASK 1a: SERUM GLUCOSE

Base-line Methods

Preparations of U-^{13}C-D-glucose and 6,6-^{2}H-D-glucose were carefully purified. The two isotope-labeled sugars and normal D-glucose (NBS-SRM 917)* were separately converted into crystalline 1,2:5,6-di-O-isopropylidene-D-glucofuranose, and purified. (This derivative, unlike glucose itself, is suited to gas chromatography-mass spectroscopy [GC-MS].) A series of mixtures composed of a variety of known proportions of either of the isotopic derivatives with the normal glucose derivative were prepared for use in calibrating GC-MS isotope-dilution measurements. To examine for evidence of isotope effects that may occur during the course of preparing that derivative from the isotopic sugars, a series of mixtures having known proportions of either of the labeled glucoses and the non-labeled glucose were converted into the di-isopropylidene derivative for testing for evidence of alterations of isotopic proportions from that predicted from the starting materials. Different known amounts of either of the labeled glucoses were added to

*Available from National Bureau of Standards
Office of Standard Reference Materials
Washington, D. C. 20234

aliquots of one serum containing glucose, and the isotopic mixture of glucoses was isolated from these sera as the di-isopropylidene derivative. The precision and accuracy of the isotopic dilution-mass spectrometry (ID-MS) method of measurement for the original glucose content of the serum will be determined from the data obtained in the last cited series. Use of the two isotopic glucoses for this same kind of measurement is to provide added assurance for the applicability of ID-MS as a base-line method for glucose.

A gas chromatograph--mass spectrometer system for application to problems in organic isotope dilution mass spectrometry has been designed. The major components of the system are a Varian 2740* gas chromatograph, a Varian MAT CH7-A* mass spectrometer and a Hewlett-Packard 9830 programmable calculator. The gas chromatograph is capable of dual column operation and has flame ionization detectors to allow comparison of effluent peak profiles with the total ionization trace obtained from the mass spectrometer.

The mass spectrometer is capable of operation in electron impact and chemical ionization modes. Dual sets of electronics allow rapid switching between the two modes without refocusing the ion beam. A multiple ion selection (MIS) unit allows the monitoring of eight (8) mass-to-charge (m/e) ratios simultaneously, a feature of importance for isotope dilution analyses of multiply labeled compounds. Switching between m/e values is accomplished magnetically, avoiding the refocusing problems and mass range limitations of accelerating voltage alternation.

An interface is being designed which will multiplex the analog signal from the MIS unit of the mass spectrometer and feed the data through a voltage-to-frequency converter to the programmable calculator. Software is being written for the analysis of the data obtained.

Experts Team

The AACC Standards Committee's Subcommittee on a Glucose Reference Method (Dr. R. Schaffer, Chairman) serves as the

*brand name waiver

Experts Team. Subcommittee member, Dr. Jane Neese, undertook the clinical development of the protocol for the hexokinase plus glucose 6-phosphate dehydrogenase method on which the experts team chose to concentrate its attention as the candidate reference method. Neese's work, performed at the Center for Disease Control (CDC), was reviewed at the meeting of the Experts Team held in Washington, 28 March 1974. Subsequent to that meeting, reagents and samples were prepared for a first-round performance of the candidate method; this was initiated in early June 1974. The assembled data, which looks encouragingly good, is undergoing statistical examination (at the time of this writing).

TASK 1b: SODIUM, POTASSIUM, LITHIUM, MAGNESIUM, AND CHLORIDE

Base-line Methods

Portions of analytical reports pertaining to base-line determinations are reproduced as illustrations of isotope-dilution applications.

Lithium in Blood Serum:--A 5 gram sample was taken from each of six ampoules for each of the three lots of serum. The samples were spiked with ^{6}Li, ^{41}K, ^{26}Mg and ^{44}Ca solutions. The serum was decomposed by adding HNO_3 and $HClO_4$, and heating. The samples were then evaporated to dryness, taken up in H_2O and the four elements, Li, K, Mg, and Ca were separated using cation exchange columns. The Li and K were eluted with 0.5 mol/liter HCl and the Ca was eluted with 6 mol/liter HCl. The fractions were evaporated to dryness, heated with a small amount of HNO_3 to destroy organic matter, and the Li and K were converted back to the chloride by adding a small volume of HCl.

A 6-inch radius of curvature 60° analyzer tube instrument was used for the isotopic ratio measurement. The mass spectrometric technique for lithium is similar to a tantalum triple-filament procedure developed for potassium analysis. The estimated lithium sample size per analysis is approximately

5 μg. Isotopic compositional analyses for each serum concentration level gave $^{6}Li/^{7}Li$ ratios that were experimentally identical to the isotopic ratio of the natural reference standard. The isotopic abundances (atom %) for ^{6}Li and ^{7}Li of the serum samples are 7.4555 and 92.5445, respectively. Concentration values for a single analysis of each of six serum subsamples at three different concentration levels, are given in table 1.

The data has been corrected for an average blank of three nanograms which is insignificant with respect to the total amount of lithium in each subsample. The indicated uncertainty is a 95% limit of error for a single analysis and is based upon a careful study of $^{6}Li/^{7}Li$ measurements for the tantalum triple-filament technique. This study has revealed that the ability to reproduce the same isotopic fractionation between analyses limits the precision of the ratio measurement to 0.25%-0.50%. The larger uncertainty is being used as the 95% limit of error for the concentration measurements.

Table 1. Concentration of Lithium in Blood Serum

Sample	µg/g
1-2	6.8168
1-170	6.7832
1-235	6.8095
1-287	6.8088
1-356	6.8039
1-443	6.7877
Average =	6.816 ± 0.034
3-64	13.331
3-116	13.364
3-184	13.366
3-256	13.357
3-401	13.363
3-495	13.378
Average =	13.36 ± 0.07
5-63	20.058
5-96	20.005
5-266	20.072
5-320	20.002
5-398	20.020
5-500	20.020
Average =	20.03 ± 0.10

Chloride in Blood Serum:--The samples consisted of six randomly selected ampoules from each of the three lots of serum. Each sample was treated as follows:

A 1 gram sample was spiked with ^{37}Cl and, after equilibration, the protein was precipitated by ammonium molybdate solution. The chloride in the "protein-free" filtrate was precipitated as silver chloride which was removed by centrifuging the solution. The AgCl was then dissolved in concentrated ammonium hydroxide solution so that 1 ml of solution contained 5 mg Cl.

Two different mass spectrometers were used for chlorine isotopic ratio measurements. One instrument was dedicted to blanks and enriched ^{37}Cl samples and the other to spiked samples and the natural isotopic standard. This arrangement is absolutely essential in order to avoid serious memory problems caused by analysis of enriched and natural chlorine ratios using a single instrument. In addition, the maximum range in isotopic ratio without removing and thoroughly cleaning the source was restricted to 3%.

Isotopic composition analyses, made for each lot of serum, were found to be identical to the absolute isotopic ratio of the natural standard. Concentration values for a single analysis of each sample of the three lots of serum are given in table 2. A blank correction of 1.5 μg which is less than 0.03% of the total amount of chlorine present in a 1 g sample has been made. The indicated uncertainty is an estimated 95% limit of error and is slightly larger than the 95% confidence limits for a single determination ($t\sigma$) calculated from the concentration data. The additional allowance is made for possible error contributions from background signals, trace impurities and loss of chlorine during the sample mounting procedure.

Table 2. Concentration of Chlorine in Blood Serum

Sample	µg/g
1-1	3173.30
1-2	3175.44
1-3	3177.06
1-4	3184.18
1-5	3172.43
1-6	3174.83
Average	3176.2 ± 15.9
3-1	3554.27
3-2	3554.17
3-3	3552.50
3-4	3548.84
3-5	3541.98
3-6	3542.83
Average	3549.1 ± 17.7
5-1	3948.37
5-2	3956.44
5-3	3942.33
5-4	3945.62
5-5	3951.22
5-6	3946.53
Average	3948.4 ± 19.7

For base-line sodium values, gravimetry and activation analysis are being applied, but the results of these efforts are not yet completed.

Experts Team

On 21 March 1974, the Experts Team (Drs. George N. Bowers, Jr., Bradley E. Copeland, Denis O. Rodgerson, and James M. White) assembled at NBS. The team selected Dr. Bowers as chairman. Base-line methods, candidate reference clinical methods and accuracy goals were discussed. NBS would proceed with its work on base-line methods for the electrolytes. Individual members of the Expert Team would take the principal lead in working with the NBS coordinator to prepare drafts of candidate reference protocols for assigned electrolytes. Recommendations for cooperating laboratories were discussed, and lists of proposed laboratories to work with the reference protocols were prepared.

TASK 1c: LEAD IN BLOOD

Base-line Methods

The quality of isotope dilution-mass spectrometric analyses for lead at all concentration levels, including trace levels, was established in previous programs involving analysis for this element, and hence the method is ideal for providing base-line values for the samples studied for the candidate reference method. Samples are being prepared from animals receiving lead acetate in their diets and from other animals whose blood lead content is at very low levels as proved by similar application of ID-MS.

Experts Team

This team was organized prior to begining of the FDA-NBS interagency agreement. Eight experts in lead analysis were invited to participate on the team. Initially, the chairman was Dr. Philip D. LaFleur, who is now Acting Chief of the

Analytical Chemistry Division; Mr. Donald A. Becker is now leading the effort. The results of two rounds of analyses were needed before the experts team was able to concentrate on the development of a single method as the candidate clinical reference method. Details of a procedure for a proposed reference method were discussed at the Experts Team meeting and then integrated in a candidate protocol. This protocol was distributed to all members of the experts team for comment and criticism.

NBS-114A (REV. 7-73)

U.S. DEPT. OF COMM. BIBLIOGRAPHIC DATA SHEET	1. PUBLICATION OR REPORT NO. NBSIR 74-548	2. Gov't Accession No.	3. Recipient's Accession No.

4. TITLE AND SUBTITLE

Quarterly Report of In-Vitro Diagnostic Products
FDA Contract No 74-58(0)

5. Publication Date August 1974

6. Performing Organization Code

7. AUTHOR(S) Robert Schaffer

8. Performing Organ. Report No.

9. PERFORMING ORGANIZATION NAME AND ADDRESS

NATIONAL BUREAU OF STANDARDS
DEPARTMENT OF COMMERCE
WASHINGTON, D.C. 20234

10. Project/Task/Work Unit No.

11. Contract/Grant No.

12. Sponsoring Organization Name and Complete Address *(Street, City, State, ZIP)*

Bureau of Medical Devices and Diagnostic Products
Attn: Dr. Eloise Eavenson, Rm. 330 HFK-200
Food and Drug Administration
1901 Chapman Avenue, Rockville, Maryland 20852

13. Type of Report & Period Covered Interim April-June 1974

14. Sponsoring Agency Code

15. SUPPLEMENTARY NOTES

16. ABSTRACT *(A 200-word or less factual summary of most significant information. If document includes a significant bibliography or literature survey, mention it here.)*

Progress toward the goal of establishing the accuracy of several clinical reference methods is reported. These are reference methods for serum glucose, sodium, potassium, lithium, magnesium, and chloride, and for blood lead. Progress is reviewed on the development of or use of base-line methods for assigning high accuracy target values to samples needed for the study. The progress made by expert teams for the several reference methods is reported.

17. KEY WORDS *(six to twelve entries; alphabetical order; capitalize only the first letter of the first key word unless a proper name; separated by semicolons)*

Clinical chemical accuracy; clinical chemical analysis; electrolytes in serum; glucose in serum; lead in blood; reference clinical methods.

18. AVAILABILITY

☐ Unlimited

☒ For Official Distribution. Do Not Release to NTIS

☐ Order From Sup. of Doc., U.S. Government Printing Office Washington, D.C. 20402, SD Cat. No. C13

☐ Order From National Technical Information Service (NTIS) Springfield, Virginia 22151

19. SECURITY CLASS (THIS REPORT)	21. NO. OF PAGES
UNCLASSIFIED	9
20. SECURITY CLASS (THIS PAGE)	**22. Price**
UNCLASSIFIED	


AMERICAN REVOLUTION BICENTENNIAL
1776-1976