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Silver Spring, Maryland 20910

[illegible]

INTRODUCTION

This report describes work performed at the National Bureau of Standards from October 1978 through March 1979 in the continuation of project initiated under [illegible] 74-58(0). The previous report was for the period October 1977 through September 1978.

Task 1a. GLUCOSE IN SERUM

A. Glucose in WHO Serum by the Paramenoperiodate/ID-MS Method

This new method for ID/MS was carried out on samples of the WHO reference serum pool. In order to study the precision of the method, duplicate samples were tested, and in order to observe whether the time allowed for equilibration between the labeled and unlabeled molecules of glucose would influence the results the duplicate samples were allowed to stand at room temperature with the added glucose-U-^{13}C for either 2, 6, or 20 hours before continuing with the procedure. The results are given in Task 1a, Table 1. The interesting fact emerged that the glucose levels were found to be larger with the increase of time allowed for equilibration.

It was later learned that, by use of the reference method, CDC had also found increases in the glucose concentrations in their -20 °C-stored serum pools if the serum was allowed longer periods of time at room temperature, up to two or three days, before proceeding with the method (instead of allowing the serum to stand at room temperature for one hour). CDC then found a maximum increase in glucose of about 1 to 2 percent. But only serum stored at -20 °C for a long time showed this effect; newly frozen serum does not show it. CDC is studying the temperature of storage as a possible control parameter to overcome the problem.

Task 1a, Table 1. Glucose Levels in [illegible] Samples by the [illegible] ID/MS Method, Measured After [illegible] Equilibration Times

Equilibration Time Hours	Aliquot Number	Glucose ([illegible]/L) Day 1	Day 2	Day 3	Glucose Average [illegible]
2	1	(905.8)[a]	([illegible].2)[a]		961.[illegible]
	2	959.3	9[illegible].8		
	3	961.8	[illegible].6		
6	4	954.7	965.1	956.1	9[illegible]
	5	961.2	964.[illegible]		
	6	961.8	962.0		
24	7	958.2	964.4	960.8	9[illegible]
	8	967.1	966.2		
	9	965.2	967.4		
	10	966.5	965.2		

[a]Outlier not included in average.

From these observations, it appears necessary to exert further control over the time of sampling from the already initially necessary for the reference and definitive methods to be properly compared.

B. Comparative Study of Diacetone Glucose and Butanediaconate Acetate Glucose ID/MS Methods

New serum samples containing glucose at 5 different concentration levels were received for this study from the CDC. The testing pattern to be followed is to analyze 2 aliquots of each of the 5 pools once per week for 5 weeks. (The need for additional analyses would be decided after these results could be studied.) The CDC would also run the reference method at the same times. Samples of the five pools were to be stored at -50° and for analysis kept at room temperature for a total of 22±2 hours before the analyses were to proceed.

At NBS, each week, a number of vials from each of the pool were combined into separate pools, sufficient in volume for the aliquots needed for both ID/MS methods. Aliquots were taken between 1 to 2 hours after the vials of serum had been allowed to thaw and mixed and combined. The labeled glucose for each method was then added. For the remainder of the 20-plus hours, the aliquots were left at room temperature. Then the individual procedures were begun. The study was begun in the latter part of [illegible] results were available in time for this report.

Task 1b. LITHIUM, MAGNESIUM, SODIUM, POTASSIUM AND CHLORIDE

A. Lithium: The NBS-260 is in preparation.

B. Magnesium: The magnesium gluconate·$2H_2O$ SRM has now been certified. The program for developing a reference method for magnesium needs to be reviewed.

C. Sodium: Work is completed.

D. Potassium: The NBS-260 is being printed.

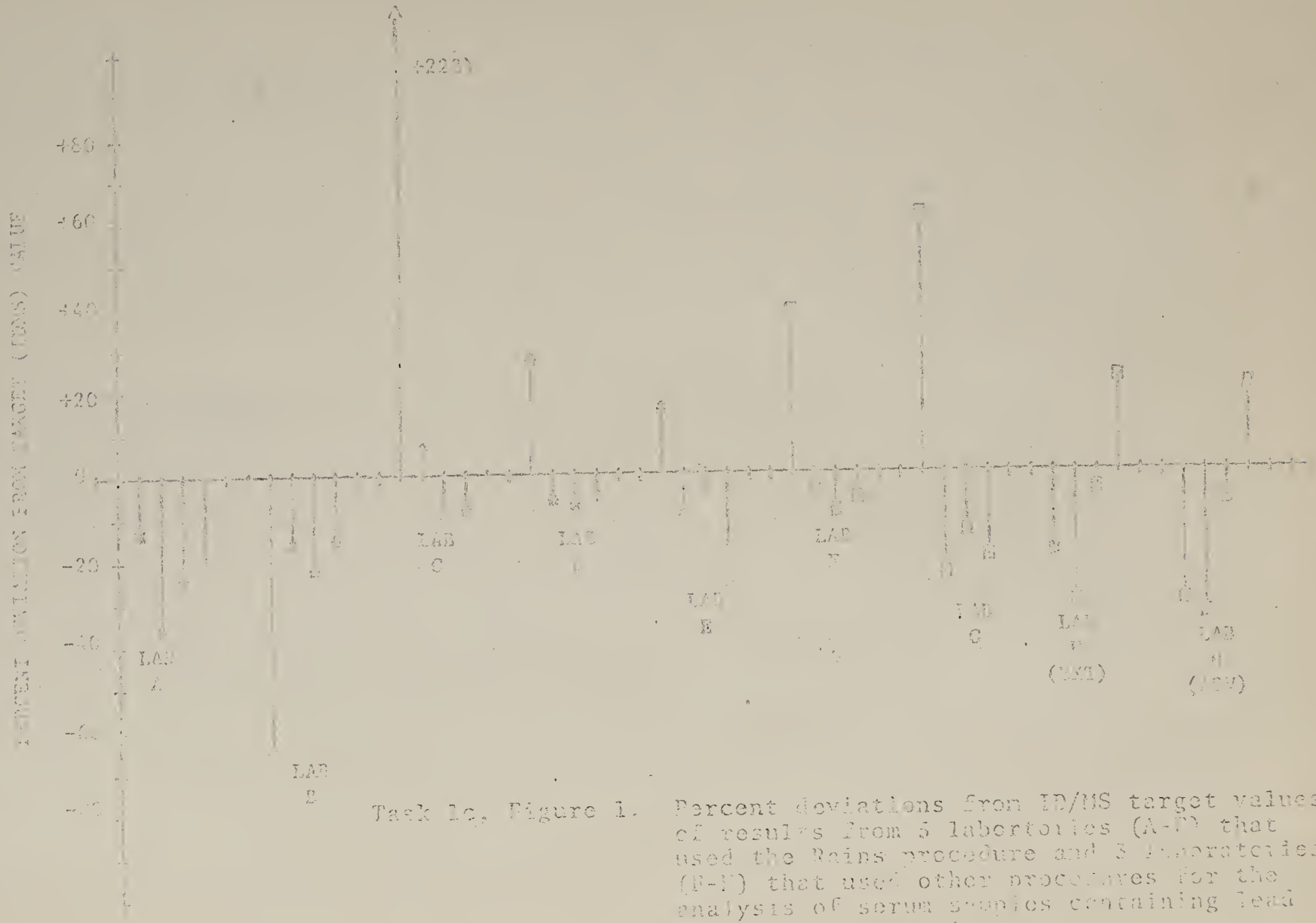
E. Chloride: The NBS-260 is undergoing final review.

Task 1c. LEAD (Pb) IN BLOOD

Isotope dilution-mass spectrometric measurements were completed for samples of the porcine blood. These values were used as target values for comparison of data from the five laboratories reporting results using the Dains procedure. In addition, three other laboratories using a variety of techniques reported values for comparison.

Results were reported to the laboratories in correspondence sent in March 1979 with data plotted graphically as percent deviation from the ID/MS target value. These are shown in Task 1c, figure 1. The sequence of analytical results are, from left to right, the low, medium, medium-high, and high levels of lead in blood. More deviation from the target value was seen in the low-level values, as expected. There appears to be a slight overall negative bias for the method, with the low value estimates being higher than the ID/MS target value. The ID/MS values are summarized in Task 1c, Table 1.

The criteria established by the CDC Proficiency Testing Division for acceptable results in blood lead analysis are as follows: when the target value is greater than 40 µg/100 mL, the acceptable range is defined as the mean ±15 percent, when the target value is equal to or less than 40 µg/100 mL,



Task 1c, Figure 1. Percent deviations from ID/MS target values of results from 5 laboratories (A-E) that used the Rains procedure and 3 laboratories (F-H) that used other procedures for the analysis of serum samples containing lead at four concentrations.

Task 1c, Table 1. Pb in [illegible] Interlaboratory Comparison, 1978

Level	ID/MS µg/g $\bar{x} \pm SD$	ID/MS µg/100ml [illegible]	Lab* - Average Value Reported A	[illegible]	C	D	E	F	G	H	H'
[illegible]	.0[illegible]±.0[illegible]	[illegible].34	3.8	[illegible]	14	[illegible].5	5.0	5.[illegible]	[illegible]	5.5	3.0
Med	.340±.007	36.[illegible]	25	30.[illegible]	[illegible]3	34	32.[illegible]	[illegible]	27.5	26.7	23.7
Med-High	.488±.00[illegible]	51.59	39	[illegible].3	47	48	[illegible]	[illegible]7	44.5	[illegible]	[illegible].1
High	.8[illegible]±.0[illegible]3	85.97	65	73.[illegible]	79	8[illegible]	63.9	8[illegible]	69	10[illegible].4	102.3

*Labs A-E used [illegible] method.

Lab F used a micro method.

Lab G used tantalum ribbon.

Lab H used extraction.

Lab H' used ASV.

the acceptable range is the mean value ±6 µg/100 mL. Results from the 1976 exercise were compared to the CDC criteria and are shown in Task 1c, Table 2.

The exercise showed the need for continued work on method development and experience with the techniques involved.

Task 1c, Table 2

ID/MS level µg/100 mL	"Acceptable Range" by CDC Definition	No. Laboratories Meeting CDC Criteria	
		Rains' Method	"Other" Methods
4.34	0 - 10.3	4/5	4/4
36.01	30.01 - 42.01	4/5	1/4
51.59	43.9 - 59.3	5/5	4/4
85.37	75.0 - 98.1	3/5	1/4

Consistent differences in reported high lead levels in human blood were noted when the Rains' method and the chelation extraction procedure of Berman were compared. It was thought that the differences may be explained by the existence of a lead-binding protein in lead-exposed workers as described in the paper by Raghaven and Conick (Proc. Soc. Exper. Biol. Med. 155: 164, 1977). To resolve this interesting difference, we are trying to obtain blood samples from lead-

exposed workers. These samples will then be analyzed in an inter-comparison protocol that will require all samples to be analyzed by the graphite-furnace and chelation extraction methods within the same week that ID/MS values are obtained. We feel this validation of the graphite-furnace method will be a necessary step in establishing the utility of the procedure as a reference method for human serum samples.

Task Id. URIC ACID IN SERUM

A. Uric Acid in the WHO Reference Serum by ID/MS

Nine vials, randomly selected from the serum pool, were thawed, mixed, and combined. Eight weighed aliquots of this combined sample were analyzed. Two independent stock solutions of $^{15}N_2$-uric acid were prepared. Four of the samples were treated with one stock solution, and the remaining samples were treated with the other. The ratio of labeled to unlabeled uric acid in the mixtures ranged between 7 to 1 and 10 to 1. Calibration mixtures composed of the labeled uric acid and of Uric Acid, SRM 913 were also prepared in this range.

The isotope-enriched serum samples and standard mixtures were similarly treated to ethylate the uric acid. The same two tetraethyl uric acid isomers were isolated from each reaction mixture. GC/MS was used to determine the observed isotopic ratios in each standard mixture and unknown. Intensity ratios were measured at m/z 282 and 280 in a medium resolution mass spectrometer that employs magnetic field switching and is equipped for multiple ion monitoring.

Measurements were made in duplicate on each of two days. Sample concentrations were calculated by linear interpolation between the intensity ratios of two standards that closely bracketed the isotopic composition of the

sample and were run immediately before and after that sample. The individual values in Task 1d, Table 1 are averages of the daily duplicate measurements. Each RSD is based on 7 average values. The averages for Sample 8 are significantly different from the others, and have been therefore excluded from the calculations. The correspondence of the values for Isomers A and C is very good.

B. Uric Acid in the Serum Pools Used in Interlaboratory Exercise IV

The results we obtained are shown in Task 1d, Table 2. Only the values of the averages for the 4 measurements on an aliquot in a set (2 on one day and 2 again on a second day) are shown. The pairs of measurements were within our 1% agreement requirement. Unfortunately the agreement between the results for isomers A and C is not as good as we would like to have for a definitive method. We believe we know its cause. Therefore we have requested additional samples of IE-IV for further analyses.

We received from the CDC the average values obtained in IE-IV. Our values and those values are shown in Task 1d, Table 3.

Task 1d, Table 1. Uric Acid in mg/g in the NBS Serum Pool by ID/MS.

	Isomer A			Isomer C		
Sample	Run 1	Run 2	Average	Run 1	Run 2	Average
1	.045078	.044859	.044968	.045011	.045033	.045022
2	.044538	.044726	.044632	.044743	.044767	.044755
3	.044643	.044820	.044731	.044709	.044754	.044732
4	.045142	.045247	.045194	.045215	.045230	.045223
5	.045004	.044852	.044928	.044905	.044764	.044835
6	.044866	.044841	.044853	.044819	.044792	.044806
7	.044906	.044720	.044813	.044975	.045019	.044997
8	.047649	.047378	.047514	.048401	.048461	.048431
Average (excluding #8)			.044893			.044911
Deviation of #8			+5.89%			+7.84%
PSD (excluding #8)			0.40%			0.42%

Difference Between C & A Averages = 0.06%

Average of C & A (excluding #8) = .044902

Average corrected for purity of Uric Acid SRM = .044767

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* This concentration of uric acid per gram of the NBS serum corresponds to 45.34 mg/l or 0.2727 mmol/L. The specific gravity of the serum was 1.0042 g/L.

Task 1d Table 2. ID/MS of the Interlaboratory Exercise IV Serum Pools. Results on the A- and C-Isomers of Tetrachyluric Acid.

Isomer A (in mg/g)

Set	Pool 3	Pool 4	Pool 1	Pool 5	Pool 2
1	0.023379	0.035920	0.041015	0.055775	0.100520
2	0.023091	0.035461	0.042408	0.055406	0.099981
3	0.023646	0.035515	0.041321	0.055176	0.098382
4	0.023713	0.035409	0.041601	0.05 997	0.099071
5	0.023166	0.034862	0.040897	0.054808	0.098654
Average	0.023409	0.035433	0.041388	0.055210	0.099320
RSD (%)	1.24	1.04	1.44	0.57	0.92

Isomer C (in mg/g)

Set	Pool 3	Pool 4	Pool 1	Pool 5	Pool 2
1	0.022964	0.035165	0.040954	0.054673	0.098615
2	0.023013	0.035219	0.042013	0.055282	0.099195
3	0.023302	0.035207	0.041187	0.055052	0.098061
4	0.023485	0.035382	0.041365	0.055033	0.098875
5	0.023317	0.034952	0.040977	0.054985	0.098615
Average	0.023215	0.035185	0.041299	0.054985	0.098672
RSD (%)	0.94	0.44	1.01	0.46	0.43

Task 1d, Table 3. Average Values of A Isomer and the C Isomer Obtained by the ID/MS and Reference Method.

Pool	A Isomer (mg/100 g)	C Isomer (mg/100 g)	IE-IV (mg/100 g)
3	2.5409	2.3215	2.39
4	3.5433	3.5185	3.59
1	4.1378	4.1295	4.59
5	5.5210	5.4985	5.56
2	9.9520	9.8672	9.98

Task 1e. CHOLESTEROL

A manuscript on the definitive method, to be submitted for publication in Clinical Chemistry, is undergoing final examination by the authors before being entered in the Bureau's prepublication review process. A preprint of the manuscript will be submitted to the Food and Drug Administration at the completion of its processing at NBS.

Task 1f. SERUM IRON

No work has been done during this period.

Task 1g. TRIGLYCERIDES

No work was done during this period.

Task 1h. UREA

Ratio Measurements

Provision was made for cooling the probe of the mass spectrometer for the purpose of better controlling the volatilization of the urea when the probe is inserted into the mass spectrometer for the measurement. However, this effected only a small improvement and it was realized that for the ratio measurement of the quality desired, it would be necessary to use a derivative of urea and GC/MS rather than direct probe insertion. The urea would be

isolated as we have been doing; then the derivative would be made. A search for suitable derivatives was begun.

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August 197[illegible]

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DEPARTMENT OF COMMERCE
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