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Screening Trace Elements and Electrolytes in Serum by Inductively-Coupled Plasma Emission Spectrometry

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This rapid, accurate procedure for trace elements and electrolytes in serum requires little sample preparation: to 1 mL of serum a single reagent is added that contains trichloroacetic and hydrochloric acids for protein precipitation, hydroxylamine sulfate for iron reduction, and yttrium as the internal standard. After centrifugation, the supernates are directly analyzed for Na, K, Mg, Ca, P_i, Fe, Cu, and Zn by inductively-coupled plasma emission spectrometry. The CVs were respectively 7.9%, 8.4%, 8.6%, 10.0%, 9.0%, 9.4%, 9.0%, and 9.0% for five assays of National Institute of Standards and Technology Standard Reference Material (SRM) no. 1598, Bovine Serum. Analytical recoveries ranged from 92% to 107% for both SRM 1598 and commercial control serum.

Full evaluation of trace-element and electrolyte concentrations in serum is often difficult. Clinical chemical techniques for doing so are diverse, with colorimetry, atomic absorption spectrophotometry, and flame emission spectrometry predominating. Measuring a complete "panel" of trace elements and electrolytes in serum that includes Na, K, Ca, Mg, P_i, Cl^-, HCO_3^{2-}, Fe, Cu, and Zn would require many separate analyses with commonly available methods, and a relatively large sample. In addition, the laboratory would have to prepare and maintain multiple reagent systems. Although flame atomic absorption spectrophotometry can be used in analysis for most of these elements (*1*), separate analyses would be required for each component, with concomitant instrument optimizations and sample consumption.

Significant diagnostic and economic benefits can result when concentrations of multiple trace elements and electrolytes in a specimen are measured simultaneously. Moreover, for screening tests it would be desirable to quantify multiple analytes with little additional expense and sample. If the results of the screen can be easily manipulated by a microprocessor-controlled instrument, indices such as the Ca^{2+}/Mg^{2+} ratio and the Ca^{2+}/P_i ratio, which may be useful in clinical studies, can be automatically produced.

Inductively-coupled plasma emission spectrometry (ICP) can meet these requirements for Na, K, Ca, Mg, P_i, Fe, Cu, and Zn (*2–5*).[2] Such instruments are sensitive and accurate, have a high throughput, are very versatile, and of course several elements can be determined at once. The procedure described here for measuring trace elements and electrolytes in serum has been successfully applied to specimens in a clinical diagnostic veterinary toxicology laboratory for longer than six months.

The protocol for trace elements and electrolytes in serum we describe here evolved from our earlier (unpublished) procedure in which serum was diluted with Triton X-100 detergent and analyzed directly by ICP. We desired to improve this early method by (*a*) reducing fouling of nebulizer and torch by protein deposits; (*b*) extending the number of elements in the screen; (*c*) producing values for iron in serum that correspond to those by classical clinical chemistry methods; and (*d*) decreasing interference from hemolysis in the determination of iron.

Materials and Methods

Apparatus and Reagents

Equipment: We analyzed the samples with an inductively-coupled argon plasma spectrometer (Applied Research Laboratories, Dearborn, MI; Model 3520 sequential ICP, equipped with a Meinhardt TR-30-A3 concentric nebulizer). We introduced the samples to the plasma at about 0.5 mL/min by using a peristaltic pump (Gilson, Middleton, WI; Model Minipuls 2) with 0.79 mm (i.d.) manifold tubing and an ISIS autosampler (ISCO, Inc., Lincoln, NE). Emission wavelengths monitored and photomultiplier settings were as follows: P, 178.283 nm and 120; Zn, 213.856 nm and 115; Fe, 259.940 nm and 105; Mg, 279.080 nm and 100; Ca, 317.933 nm and 50; Cu, 324.754 nm and 95; Y (internal standard), 371.030 nm and 80; Na, 589.592 nm and 70; and K, 766.488 nm and 110. After an 80-s delay, the signals were integrated twice for 4 s each in the ratio intensity mode for the analyte signal vs the yttrium internal standard signal. Background correction and spectral overlap correction were not used.

Protein-precipitation/reducing/internal standard (PPI) solution and reference blank solution: Into a 1000-mL volumetric flask containing 400 mL of reagent-grade water, add 70 mL of a 6.1 mol/L solution of trichloroacetic acid (Sigma Chemical Co., St. Louis, MO) and dissolve in 35 g of hydroxylamine sulfate (Alfa Products, Danvers, MA). Carefully add 140 mL of 38% (concd.) hydrochloric acid ("Instra-Analyzed"; J.T. Baker, Inc., Phillipsburg, NJ), and mix well. After cooling to room temperature, dilute to 1000 mL and mix thoroughly. Decant exactly 100 mL of the solution into an acid-washed bottle; label this solution "reference blank." To the remaining 900 mL of solution, add 12.0 mL of 1000 mg/L yttrium stock solution, and mix thoroughly. Transfer this to a previously acid-washed bottle, and label this solution "PPI solution." When either of these solutions is exhausted, the other should be discarded and new solutions of each prepared to ensure good blank matching.

Standard solution: Weigh 149.1 ± 1.5 mg of dried high-purity KCl and 1.1689 ± 0.006 g of high-purity NaCl (Johnson Matthey, Seabrook, NH; Grade 1) into a 100-mL volumetric flask. Add 5.00 mL each of 1000 mg/L stock atomic absorption standard solutions of Cu, Mg, Fe, and Zn (Fisher Scientific Co., Fair Lawn, NJ), and 20 mL each of

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[2] Nonstandard abbreviations: ICP, inductively-coupled plasma emission spectrometry; "PPI solution," protein-precipitation/reducing/internal standard solution; NIST, National Institute of Standards and Technology; SRM, Standard Reference Material; and TIBC, total iron-binding capacity.

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1000 mg/L stock atomic absorption standard solutions of Ca and P. With intermittent mixing, dilute with reagent-grade water to 100.0 mL. The final concentrations will be 50 mg/L for Cu, Mg, Fe, and Zn; 200 mg/L for Ca and P; 20 mmol/L for K; and 200 mmol/L for Na.

Calibrators: The blank is 3.0 mL of the reference blank solution plus 1.0 mL of reagent-grade water; the zero standard, 3.0 mL of the PPI solution plus 1.0 mL of reagent-grade water; and the calibration standard, 3.0 mL of the PPI solution plus 1.0 mL of the standard solution. Calibration solutions should be prepared freshly for each analytical batch.

Reference materials: We used National Institute of Standards and Technology (NIST) Standard Reference Material (SRM) no. 1598, Inorganic Constituents in Bovine Serum, and Fisher Diagnostics' Serachem Clin-Chem Control Serum (human) Level I (lot no. N0280069).

Procedures

Sample preparation: Set the delay time so that the sample is introduced into the nebulizer for about 10 s before signal integration starts. Set the pump rate so that the entire volume of sample (about 3 mL) is consumed during the analytical run. The exact values will depend on the geometry of the autosampler and the timing of the ICP program.

Pipet 3.0 mL of the PPI solution into a clean 13 × 100 mm test tube. Add 1.0 mL of the serum sample, cover, and vortex-mix thoroughly. Centrifuge (1500 × *g*, 10 min), check that the supernate is clear, and transfer it to a clean labeled test tube. Load the tube into the autosampler of the ICP.

Calibrate the ICP at the start of each run by using the blank, zero standard, and calibration standard, in that sequence. At regular intervals in the run, analyze the zero standard and calibration standard to verify that the calibration has not drifted during the course of analysis.

Hemolyzed sample/iron interference study: An aqueous 30 g/L solution of bovine hemoglobin was added to pooled bovine serum (both from Sigma Chemical Co.) to give final concentrations of 0, 100, 300, 1000, and 3000 mg/L. We then analyzed each such fortified serum in triplicate. The aqueous solution of hemoglobin was assayed for free iron by protein ultrafiltration (Centrifree micropartition devices; Amicon Divn., W. R. Grace and Co.), followed by ICP analysis of the filtrate.

Recovery studies: We analyzed the NIST SRM 1598 five times for Na, K, Mg, Ca, P_i, Fe, Cu,and Zn. We also used the Fisher Serachem Level I serum as a control for routine analysis during six months—a total of 28 determinations.

Table 2. Effect of Hemoglobin on Fe Determination

	Fe, mg/L				
Hemoglobin added	0	100	300	1000	3000
Fe added[a]	0	0.35	1.02	3.41	10.2
Mean Fe found (n = 3)	1.79	1.82	1.91	2.20	2.96

[a] Hemoglobin contains 3.4 g of Fe per kilogram.

Results

Detection limits for the procedure, calculated as 3× the SD of the background signal, are: Na, 0.2 mmol/L; Mg, 0.7 mg/L; P_i, 1.0 mg/L; K, 0.04 mmol/L; Ca, 0.1 mg/L; Fe, 0.03 mg/L; Cu, 0.07 mg/L; and Zn, 0.001 mg/L. Results of the five analyses of NIST SRM 1598 are listed in Table 1. Generally good agreement as well as narrow standard deviations are found for most NIST-certified element concentrations (Mg, K, Cu, and Zn) and for NIST-published but not certified element concentrations (Na and Ca). NIST does not report a phosphorus concentration for this reference material.

Analysis for iron by our protocol accounted for only 62% of the NIST-certified value for iron. This result was expected because of the analytical methods used by NIST to produce a value for total Fe: whole-sample neutron activation analysis and flame atomic absorption analysis (*6*). Selective protein-precipitating procedures such as ours, which result in assay only of transferrin and free iron residues (*7*) and not precipitable iron, predictably will yield lower iron concentrations than methods involving digestion or whole-sample analysis for total Fe.

By Student's *t*-test at the 95% confidence level, the difference between the trace elements and electrolytes screen results and the certified values for Mg, K, Cu,and Zn were not significant. The concentration for Na differed by 4.5% from the NIST-reported value. The concentration for Ca by the trace elements and electrolytes screen, 0.09 μg/g, was the same as the NIST-reported value.

Pooled bovine serum was fortified with bovine hemoglobin to ascertain the rejection of hemoglobin iron by the trace elements and electrolytes screen protocol; the results are summarized in Table 2. Free iron in the reagent hemoglobin, <0.040 mg/kg, had no measurable effect on any other constituent. Linear regression of iron found (y) vs hemoglobin iron added (x) gave a line with slope of 0.115 (r^2 = 0.998), which shows that 88.5% of the hemoglobin iron was rejected. Evidently, the amounts of hemolysis caused by ordinary handling of blood specimens will not affect results by this procedure, and moderate hemolysis will not cause serious error in the values for iron. For example, a visibly pink serum containing 450 mg of hemo-

Table 1. ICP Trace Elements and Electrolytes Screen Results for NIST SRM 1598, Inorganic Constituents in Bovine Serum

	Na	K	Mg	Ca	P_i	Fe	Cu	Zn
	mmol/L				μg/g			
Our mean (n = 5)	140	4.98	20.9	0.09	60.1	1.59	0.77	0.89
SD	11.1	0.42	1.8	0.009	5.43	0.15	0.07	0.08
CV, %	7.9	8.4	8.6	10.0	9.0	9.4	9.0	9.0
NIST mean value	(134)[a]	5.16	20.0	(0.09)[a]		2.55	0.72	0.89
SD		0.13	0.4			0.10	0.04	0.06

[a] NIST reported-but-not-certified value given in parentheses.

Table 3. ICP Trace Elements and Electrolytes Screen Results for Commercial Control Serum[a]

	Na	K	Mg	Ca	P_i	Fe	Cu	Zn
	mmol/L				μg/g			
Our mean (n = 28)	146	4.11	20.7	92.3	30.0	1.77	1.16	1.31
SD	4.3	0.14	3.6	2.9	1.2	0.14	0.08	0.63
CV, %	2.9	3.4	17	3.1	4.0	7.9	6.9	48
Reference value[b]	145	4.1	22.5	91	30.5	1.78		
SD	9	0.9	7.3	12	5.5	0.39		

[a] Fisher Diagnostics Serachem Clin Chem Control Serum (human) Level 1 (lot N0280069). [b] Mean of all certified values for the various methods employed by the manufacturer.

globin per liter will affect the serum iron value by no more than 10%. These results suggest that Fe determination by the described protocol is resistant to a positive bias for hemolyzed samples found in other assays that do not involve hemoglobin removal.

We analyzed commercially prepared control sera during six months to gauge the overall accuracy and precision of the trace element and electrolyte screen. Table 3 summarizes the results for 28 analyses of a lot of this material, which is certified for Na, K, Mg, Ca, P_i, and Fe. Commercial certified concentrations are generally determined for various methods and instruments, varying greatly from the method described here. The reference values in Table 3 are the means and SDs of these various values. For good reasons, these materials are not certified for Cu and Zn. However, these results for trace elements and electrolytes screen are included for completeness. All of the results obtained by the trace elements and electrolytes screen protocol are within ±1 SD of the reference value. The CV for the Cu result is an acceptable 6.9% over the different analytical runs. The CV for Zn appears abnormally high, 48%; we traced this to the presence of zinc in the septum caps on the serum bottles by analyzing leachates of the septa. Otherwise, there was no significant difference between the trace elements and electrolytes screen results and the reference values at the 95% confidence level.

Discussion

Table 1 shows that NIST SRM 1598 Bovine Serum values for Na, K, Mg, Ca, Cu, and Zn compare well with the values we obtained with the present ICP procedure. NIST Bovine Serum values are determined by whole-sample analysis techniques (*6*). It is apparent from the data in Table 1 that the reduction and protein precipitation before ICP analysis effectively releases these elements from any protein carriers or enzymes. The trace elements and electrolytes screen, analogous to other protein-precipitating methods (*8*), yields values for inorganic phosphate (P_i) rather than for total phosphorus. This is supported by the agreement (30.0 vs 30.5 mg/L) with the certified value for SeraChem control serum, which is certified for phosphomolybdate colorimetry.

The resistance to hemoglobin contamination, a potential problem in analysis for iron in serum, is a significant aspect of this protocol. In addition, it is important to note from Table 3 that in the trace element and electrolyte screen procedure, the Fe value (1.77 ± 0.07 mg/L, n = 28) agrees well with the certified value for the commercial control serum (1.78 ± 0.39 mg/L). The commercial control material is certified for reduction and release of iron from transferrin and protein precipitation, followed by analysis for iron. This helps to verify that the low Fe recovery for NIST Bovine Serum SRM probably results from the difference in sample preparation and presentation during certification analysis and suggests that NIST serum may contain precipitable iron. These results confirm that the ICP screen is valid for analysis for iron in serum.

Analysis for magnesium by use of colorimetric procedures involving calmagite requires sequestering of interfering Ca and other divalent metals in the procedure. We have noted a distinct positive bias in magnesium concentrations of commercial control sera certified by colorimetry, and we speculate that this could be caused by interference from residual divalent cations. Normally, the concentration of Mg in serum is only 25-fold the detection limit for this protocol, which could explain the CV being higher than desired. The data in Tables 1 and 3 lead us to think that this may only be a problem in analysis for Mg.

As with any trace-element procedure, contamination control is a major concern. Careful selection of blood and serum containers is required for application of this protocol, as underlined by the Zn contamination by rubber septa. We have tested the water and 0.1 mol/L acid leachates of common "red-top" tubes (various vendors) and have noted large contamination with zinc and small contamination with iron. We have also observed zinc contamination from gray septum caps. We recommend the use of "royal blue top," no-additive, blood-collection Vacutainer Tubes (Becton Dickinson no. 6526) for trace-element determinations.

This protocol has been used routinely for over six months. During that time, the method has exhibited the statistical control and ruggedness required for routine clinical diagnostic analysis. This is in great part due to the yttrium internal standard contained in the PPI solution. Internal standardization allows a measure of "forgiveness" to routine instrumental analysis problems and contaminated reagents, as well as acting as a flag for dilution error, because its emission intensity is a sample-to-sample constant. The method can be performed with 0.5 mL of serum if larger CVs can be tolerated. Use of a simultaneous ICP spectrometer rather than the sequential type used here could allow routine analysis of smaller samples and greater productivity.

The primary clinical value of the method in our routine use has been in analysis for copper and zinc in serum. Diagnostic interpretation of values for potassium and sodium would require separate determinations of chloride and bicarbonate. The total iron-binding capacity of a serum sample can be determined by the standard TIBC treatment of the serum with Fe(III) followed by precipitation with magnesium carbonate before the ICP protocol. In the ICP procedure described, the simultaneous acquisition of values for serum calcium, magnesium, and inorganic phosphate can be important as a clinical chemistry panel analysis.

The resistance of the procedure to hemoglobin contamination can be valuable when samples are hemolyzed. Additional study is required to directly compare the performance of this ICP procedure with that of the highly developed and widely used conventional methods.

In summary, this method yields eight results for the cost of one sample preparation and one method preparation, helps minimize error through the use of an internal standard, and produces quality results with a high degree of statistical control.

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