Trace Element Analysis in Hair: Factors Determining Accuracy, Precision, and Reliability

Dean A. Bass, PhD, Darrell Hickok, David Quig, PhD, Karen Urek, BS, MT(ASCP)

Abstract

Trace element analysis in biological samples has improved significantly over the last 40 years. Improvements in instrumentation such as inductively coupled plasma-mass spectrometry and microwave digestion have resulted in improved precision, accuracy, reliability, and detection limits. The analysis of human scalp hair has benefited significantly from these improvements. A recent article in the *Journal of the American Medical Association* found significant inter-laboratory variation amongst several laboratories performing trace metal hair testing. It concluded that standardization was necessary to improve inter-laboratory comparability, and an accompanying commentary described the characteristics of a laboratory that should be used in performing hair analysis. The objective of this study is to demonstrate that good laboratory practices will generate precise, accurate, and reliable results. A method for establishing reference ranges and specific data on an analytical method will also be presented. The use of prescribed clinical quality control, including method validation, proficiency testing, split sampling, and good laboratory practices clearly demonstrates that measuring trace elements in hair can be analytically valid.

(*Altern Med Rev* 2001;6(5):472-481)

Introduction

Scalp hair has been used as early as 1929 to assess human systemic levels of elements.[1] Hair is widely accepted for assessing toxic element exposures and measured by most clinical laboratories capable of making trace element measurements. Using hair to assess essential elements is more controversial, yet researchers have found many correlations of essential elements to diseases, metabolic disorders, environmental exposures, and nutritional status.[2-14] Compared to other types of clinical specimens, hair has different uses and even advantages over blood or urine. While urine and blood tend to show current or recent body status, hair represents a longer time frame, potentially years. Elements also occur in hair at higher levels, allowing for more sensitive and, because of the higher levels, more analytically accurate results. Hair is easier and

Dean A. Bass, PhD – Technical Director, Doctors Data Inc., St. Charles, IL
Correspondence address: 3755 Illinois Ave., St. Charles, IL 60174; E-mail: dbass@doctorsdata.com

Darrell Hickok – CEO, Doctors Data Inc., St. Charles, IL

David Quig, PhD – VP Scientific Support, Doctors Data Inc., St. Charles, IL

Karen Urek, BS, MT(ASCP) – VP Operations, Doctors Data Inc., St. Charles, IL

safer to collect, ship, and store than blood or urine and the analysis is less expensive. This makes hair an excellent choice in certain situations and as a screening tool. Other challenges that must be addressed when measuring trace elements in hair include external contamination, lack of standardization, and analytical accuracy.

Making accurate and precise measurement in trace metal analysis of hair samples is important to the validity and usefulness of the test. Literature reviews well characterize analytical considerations and limitations of trace metal analysis in a variety of clinical samples.[15-19] Biological trace element analysis has improved significantly over the last 40 years. Improvements in instrumentation have resulted in improved precision, accuracy, reliability, and detection limits. The analysis of human scalp hair has benefited significantly from these improvements.

A recent article by Seidel et al[20] found significant inter-laboratory variation and concluded that standardization was necessary. An accompanying commentary by Steindel and Howanitz[21] stated, "A sample for hair analysis is best sent first to a laboratory that can validate its certification or accreditation for performing the test and that reports test characteristics, such as the hair-washing procedure, digestion techniques, recovery rates for the elements, internal quality control performance over time, and the minimum detection limits for each element." Puchyr et al reported such specific test characteristics for 36 elements in hair.[22] Rodushkin and Axelsson also reported on 71 elements in hair and nails using high-resolution inductively coupled plasma-mass spectrometry (ICP-MS).[23]

The objective of this paper is to demonstrate that good laboratory practices and validated methodology will generate precise, accurate, and reliable results. Variations in hair cleaning procedures, analytical methodology, and types of quality control performance assessments will be reviewed. A method for establishing reference ranges and data on analytical variability and accuracy will also be presented. The data presented in this paper follow the guidelines of the Clinical Laboratory Improvement Act (CLIA) and the National Committee for Clinical Laboratory Standards (NCCLS) for test method validation and quality assurance.

Pre-analytical Variables

One issue often raised with trace metal analysis of hair is the potential for contamination from external sources. Shampoo has been shown by LeBlanc, Dumas, and Lefebvre[24] not to have a significant impact on hair element levels for most elements. Unpublished results in the authors' laboratory confirm these findings. However, several hair preparation products contain metals that will significantly impact the levels of metals in hair. Most notably is selenium in shampoos that contain selenium sulfide, and lead in hair dyes that contain lead acetate.

Hair collection protocols[25] recommend clean hair that has not been dyed, permed, bleached, or straightened for three months and that only the newest hair growth be sampled. This procedure is designed to prevent major sources of contamination, which include the environment (water, grease, oil, dust, dirt, air) and hair treatments (dyes, perms, and other chemical treatments). However, these protocols are not always adhered to or contamination occurs in spite of these protocols.

Cleaning procedures should be employed by laboratories to remove exogenous contaminants. An ideal washing procedure would remove only external contaminants and leave endogenous elements intact. A variety of cleaning procedures are used and their efficacy may vary based on the nature of the contaminant and the element.

Most researchers who study trace metals in hair report the method they use for cleaning hair prior to analysis. Numerous authors have conducted studies of hair washing

procedures.[26-32] Other authors have used radioactive labeling,[33] scanning electron microscopy,[34] and other methods[35-43] to assess the efficacy of removal of exogenous and endogenous elements from hair using various cleaning procedures. A recent study that assessed lead and mercury in the hair of President Andrew Jackson[44] used Triton X100, water and ethanol, and sonication, and showed that endogenous lead was not appreciably removed by this washing procedure.

Studies by the International Atomic Energy Agency (IAEA) examined different washing procedures as well, and found that even in cases where endogenous elements were removed it was not to the extent that would render the sample unusable. They concluded that while there are many variables associated with the washing procedure, including incomplete removal of exogenous contamination or partial removal of endogenous elements, representative measurements could be made if standardized washing procedures were employed.[45,46]

A standardized cutting and washing procedure has been described in detail elsewhere.[22] The hair specimen is cut and washed using a modified method developed by the IAEA.[44] The hair is first cut into approximately 0.125-inch (0.3-cm) pieces and mixed to allow a representative sub-sampling of the hair specimen. After cutting, each sample is washed four times with a 1:200 V/V dilution of Triton X-100. The samples are then rinsed with acetone and allowed to drain. This is followed by three rinses with de-ionized water (18-MW) and two rinses with acetone. The samples are then dried in an oven at 75± 5 °C.

The accuracy and reproducibility of this procedure has been documented in other studies[45,46] and in studies looking at blind split samples, results from identical twins, and repeat testing from the same individual. The expected reproducibility from such procedures is presented later in this paper. As indicated in the literature cited in this section, variation from this methodology may result in different measured element levels.

Trace Metal Analysis (Digestion and Analysis)

Analytical methodology choices can also introduce biases and variability, which can result from volatilization losses during digestion, contamination during digestion or analysis, or from interferences during analysis. Microwave digestion has been recommended as the sample preparation method most suitable for standardization. In addition, ICP-MS is the best technique for ultra-trace, multi-element analysis.[47,48] To assure accurate and precise measurements, it is important to characterize and validate the method used. The most important parameters in method validation are precision, accuracy, and detection limits. Table 1 shows the precision, accuracy, and detection limits for a method using temperature-controlled microwave digestion, followed by analysis using ICP-MS (Elan 5000, Perkin Elmer, Danbury, CT). As shown in this table, spike recovery values were generally between 90-110 percent, which is within expected ICP-MS performance. Table 1 also shows good agreement for all elements when compared to a certified reference material (CRM). Only chromium, nickel, and arsenic showed significant deviation from the certified values. However, spike recoveries (Table 1) and testing another CRM (Table 2), indicate more accurate measures are being made.

The digestion method utilizes 0.2 g of washed hair samples accurately weighed into 50-mL disposable polypropylene centrifuge tubes. Concentrated trace-metal-grade nitric acid (3 mL) is added and the tubes are capped and placed in a microwave oven (MDS 2000 and MDS 2100; CEM Corporation, Matthews, NC). The digestion sequences are controlled by temperature through use of a fiber optic temperature probe placed in one of the samples. The process causes a refluxing of the

Table 1. Analytical Characteristics

Element	Precision (%RSD)		Accuracy			MDL µg/g
	Within Run	Between Run	Measured Value	Certified Value	SR (%)	
Li	11	16			91	0.003
Be					101	0.01
B	8	24			97	0.1
Na	3.0	3.8	268	266	90	1
Mg	4.2	6.2	94	105	95	7
Al	8.6	20	9.2	13.3	91	1
P	13	20	207	(184)	104	30
S			42300	(46900)	94	2000
K	12	15	5.3	(11.8)		3
Ca	5	7.1	1022	1090	90	90
Cr	13	15	2.88	4.77	107	0.04
Ti	10	17				0.2
V	7.3	8.1	0.062	(0.069)	97	0.005
Mn	4.3	6.3	2.5	2.94	99	0.05
Fe	9.7	11				2
Co	7.2	8.4	0.095	0.135	92	0.002
Ni	9.7	12	2.11	3.17	96	0.02
Cu	4.1	5.7	22	23	92	0.3
Zn	3.3	6.2	192	189	91	6
Ge	14	18				0.003
As	7.1	8.6	0.78	0.59	116	0.02
Se	7.2	8.5	0.61	0.58	120	0.8
Rb	8.9	9.0				0.003
Sr	6.3	7.2	4.22	4.19	98	0.07
Zr	4.1	5.6			104	0.03
Mo	6.2	7.8	0.39	(0.58)	105	0.006
Ag	8.0	11	0.30	(0.35)		0.02
Cd	9.4	15	0.091	0.095	100	0.02
Sb	6.6	11				0.02
I	9.0	12				0.7
Ba	9.1	9.6	5.54	5.41	96	0.1
Au	7.7	11				0.004
Hg	8.5	10	2.18	2.16	123	0.07
Pb	4.5	4.9	7.77	7.72	94	0.2
Bi	6.5	8.0			95	0.005
U	6.8	6.3			90	0.003

SR = spike recovery MDL = method detection limit
RSD = relative standard deviation
Certified value is for human hair control CRM GBW 09101, Shanghai Institute of Nuclear Research Academia Sinica, P.O. Box 8204, Shanghai 201849, China.

sample, with no visible loss of liquid volume. The resulting digestate is a clear liquid with a yellow tint. After digestion an internal standard mixture is added, and the samples are diluted to a final volume of 50 mL. Recovery of all elements, including volatile elements such as mercury, was verified by CRMs and spike recoveries as shown in Tables 1 and 2.

This method, described in greater detail elsewhere, was found to have the best reliability, as other methods may have a potential loss of analytes, contamination of sample, or incomplete digestion.[22] Because ICP-MS has better sensitivity than other techniques for most elements, if other analysis techniques are used poorer detection limits and poorer reproducibility at lower concentrations would be expected.[49]

Standardization is an important aspect of any clinical test. It has been identified as a problem by Seidel et al,[20] and Ryabukhin[45,46] states that standardization of the hair washing step is critical to obtaining reproducible results. Standardization of sample washing protocols between laboratories should certainly allow better comparability among laboratories using quality

Table 2. Accuracy for China Hair CRM GBW 07601

Element	Certified Value (μg/g)	Measured Value (μg/g)
Chromium	0.37	0.37
Nickel	0.83	0.73
Arsenic	0.28	0.27

analytical methodology. While this would be the ideal, variations in philosophy, the expense of re-engineering laboratory protocols, and laboratory politics may make the setting of standards not acceptable to all laboratories. However, if such a step were accepted by regulatory agencies, as suggested by Steindel and Howantiz,[21] inter-laboratory comparability would be improved.

Quality Assessment

The data shown in Table 1 are typical of the type of information collected in an initial method development and method validation. Once the analytical parameters are determined from the method development, and the method is determined suitable for making routine measurements, controls must be put in place to assure continued quality and to better monitor potential sources of errors. Controls used include pre- and post-digestion in-house controls, and CRMs. In addition, split sampling, proficiency testing, and external audits are important parts of assuring quality, both internally and externally. All of the quality tools described here are part of the method validation and quality control steps prescribed by CLIA and NCCLS.[50]

Certified reference materials are often the best tools for assessing accuracy. Several certified reference materials are available for hair. These include two from China (GBW 09101, 30 elements; and GBW 07601, 60 elements), two from the IAEA (IAEA-085, IAEA-086, both 9 elements), and one from Japan. In addition to using CRMs, in-house controls may be used to assess accuracy and precision. A control comprised of digested hair allows monitoring of between-run variations in the instrument, while a control comprised of finely cut homogenized hair allows monitoring of run-to-run variation in the digestion procedure. Together these controls provide a good means of assessing accuracy and precision. Because in-house control values are not certified, some effort must be made to "certify" these values. This can be done by spiking the control with a known quantity of analyte and measuring the recovery, and by measuring the control in conjunction with a CRM. In addition, the control or other samples can be sent to other quality laboratories and results can be compared.

Split sampling is useful in assessing precision and accuracy. Sending the same sample to the same laboratory at different times is useful in assessing reproducibility. Results of such blind split sampling are shown in Table 3. For assessing accuracy, part of a sample is analyzed in-house and part is sent to another laboratory. Results of samples tested in the authors' laboratory and in another laboratory are summarized in Table 4. This particular study was conducted by a university research laboratory and was submitted blind to our laboratory.

An important aspect of split testing is sample homogenization. When trying to homogenize samples for inter-laboratory comparisons, or preparing CRMs, organizations often powder the hair sample to assure reproducible sub-sampling. This has been shown to provide a more homogenous sample. However, the sample is different than a normal hair specimen and may have different characteristics.

While appropriately identified by Seidel et al[20] as an added variable to the results,

Table 3. Repeated Testing Results of an Individual Sample Submitted at Different Times (in µg/g)

Element	Report 1	Report 2	Report 3	% RSD
Al	20	21	23	7.1
Sb	0.064	0.069	0.046	20
As	<0.01	0.013	<0.01	
Be	<0.002	<0.002	<0.002	
Bi	0.286	0.270	0.262	4.5
Cd	0.379	0.373	0.399	3.5
Pb	6.2	6.3	6.7	4.1
Hg	1.06	1.01	1.00	3.1
Ni	3.39	3.32	3.66	5.2
Pt	<0.001	<0.001	<0.001	
Ag	0.18	0.20	0.13	21
Tl	<0.001	<0.001	0.002	
Th	<0.001	<0.001	<0.001	
Sn	1.6	1.4	1.6	7.7
U	0.099	0.091	0.105	7.2
Ca	2216	2137	2465	7.5
Mg	464	806	487	33
Na	1195	969	1324	15
K	2	5	2	58
Cu	95	90	100	5.3
Zn	276	266	281	2.8
Fe	19	21	18	7.9
Mn	2.23	2.10	3.36	27
Cr	0.86	0.77	0.70	10
Co	4.48	4.37	4.69	3.6
V	0.17	0.18	0.17	3.3
Mo	0.064	0.068	0.089	18
B	1.8	1.74	1.47	11
I	0.7	0.6	0.7	11
Li	0.207	0.185	0.221	8.9
P	167	171	160	3.4
Se	1.39	1.42	1.19	9.1
Sr	4.79	4.66	4.90	2.5
S	38500	39800	40800	2.8
Ba	29.4	26.4	28.2	5.5
Ge	0.017	0.017	0.021	13
Rb	0.028	0.026	0.026	4.2
Ti	<0.1	<0.1	<0.1	
Zr	0.175	0.275	0.137	36

RSD = relative standard deviation

Table 4. Results of Split Samples from Two Laboratories (in µg/g)

Element	Lab #1	Lab #2
Lead	6.6	6.85
Mercury	0.56	0.51
Cadmium	0.335	0.33
Silver	0.31	0.42
Barium	8.04	8.62
Arsenic	0.03	< 0.04
Antimony	0.095	0.041
Aluminum	30	32.5
Bismuth	0.057	0.012
Nickel	7.60	7.68
Lithium	< 0.007	< 0.03
Sodium	52	83
Potassium	< 2.0	< 0.05
Phosphorus	182	169
Boron	0.14	0.3
Calcium	938	878
Magnesium	82	68
Vanadium	0.107	0.050
Chromium	0.71	<0.30
Iron	38	36.1
Manganese	2.34	2.21
Copper	27	28.9
Zinc	492	481
Molybdenum	0.041	0.035
Strontium	6.97	6.31
Selenium	1.18	0.7
Germanium	0.12	< 0.001
Cobalt	0.178	0.17

we have found the homogenization procedure they described to be sufficient to demonstrate acceptable precision and accuracy for measuring trace metal in hair. While variations in results occasionally occur, split sampling results generally confirm acceptable analytical precision and

accuracy; i.e., representative results of an individual's relative trace element levels are attained.

Typical results are shown in Tables 3 and 4. Variations shown may be the results of variations in the hair sample. When variations occur with between-laboratory split sampling, other measures of accuracy are assessed to confirm accuracy or quantify potential biases. As shown in these tables, precise and accurate measurement of hair can be made.

Some researchers requiring accurate and reliable trace element testing in hair also develop their own reference material. One such user[51] obtained a large sample of hair, homogenized it, and had it tested at numerous laboratories. This approach produced results similar to that described by Seidel et al.[20] The researcher concluded some laboratories were of better quality and the poorer quality laboratories were eliminated from consideration. The accuracy of the better laboratories was confirmed with continued testing.

Proficiency testing is often used to allow laboratories to assess and improve performance. It also serves as an independent assessment of accuracy. Unfortunately, few proficiency testing programs exist for hair. The National Public Health Institute of Quebec (INSPQ) Toxicology Center (Laboratory) conducts trace metal proficiency programs for blood and urine and, on a rotating basis, other clinical matrices such as hair (studies with hair conducted in 1997, 1999, and 2001). The study is an ICP-MS inter-laboratory comparison for hair and found significantly different results than did Seidel et al.[20] There were some key differences, however; for example INSPQ asked for volunteer laboratories to participate, they looked at more laboratories, they used a homogenized hair sample, and all the laboratories used ICP-MS. The results showed much lower inter-laboratory variability, and support the thesis that trace elements can be accurately measured in hair.

External assessment is often used to evaluate the quality of laboratories, often in the form of audits for purposes of certification. Audits typically look at all aspects of quality control described in this paper, with the intent of assessing whether a laboratory can make an accurate measurement of the clinical specimen of interest. Although certification is not available for many types of testing, including hair, as laboratories follow proper laboratory procedures and quality control programs, such certifications should be attainable when they become available.

Reference Ranges and Interpretation

Once a measurement is made, what does it mean? Is 5 mcg/g mercury in hair high or low? The determination of reference ranges is an important part of providing this interpretation. Guidelines for determining reference ranges are available from NCCLS.[52] Druyan et al described a detailed method for determining references ranges in hair using results from a large patient population.[14] Because of potential bias in using a general patient population, a smaller, physician-defined "Healthy American Population Study" was used to verify the ranges established with Druyan's patient population. Druyan's work further compared these ranges to literature values and ranges established by other laboratories.

For non-essential or potentially toxic elements with a single-tailed distribution, the reference limit is that seen at the 95th percentile. The designation of an expected range (as opposed to reference limit) is taken to be 68 percent of the reference population. For physiologically essential analytes, this range covers the 16th through 84th percentiles.[53] Individuals outside this limit may or may not have a problem that warrants attention. Clinicians making diagnoses and designing treatment plans based on test results should interpret the data in terms of a patient's history, medical

condition, physical examination, diet, lifestyle, and environment, as well as other laboratory data.

Conclusion

Published research demonstrates that testing of trace metals in hair can be done with accuracy and precision. The work by Seidel correctly identified the need for standardization. The interest and use of hair testing they identified should call for more proficiency testing programs and better CRMs. The lack of inter-laboratory comparability is not supportive of the conclusion that hair testing is not valid, especially when it was inferred that some of the laboratories compared were unscrupulous and others had method-related differences. Like the findings of Walsh, Seidel could have drawn the conclusion that some laboratories do not perform quality work.

If hair can be reliably measured and is useful in screening for trace element status, why is its use so controversial? Why did Seidel come to the conclusion that hair testing is invalid when their data does not support it? The authors believe a general distrust of the utilization of hair for analyzing the presence of trace metals exists among some individuals who may feel some laboratories or doctors misuse or over-interpret the results of hair element analysis. The key to obtaining reliable, accurate, and precise hair element analysis, as with any other laboratory test, is to ensure that the laboratory of choice is appropriately licensed and employs standardized and documented procedures such as those described in this article.

References

1. Althausen TL, Gunther L. Acute arsenic poisoning: a report of seven cases and a study of arsenic excretion with especial reference to hair. *JAMA* 1929;92:2002-2006.
2. LeClair JA, Quig DW. Mineral status, toxic metal exposure and children's behaviour. *J Orthomolecular Med* 2001;16:13-32.
3. Werbach MR. Lab methods for nutritional evaluation. In: *Nutritional Influences on Illness*, 2nd ed. Tarzana, CA: Third Line Press; 1993:642-654.
4. Foo SC, Khoo NY, Heng A, et al. Metals in hair as biological indices for exposure. *Int Arch Occup Environ Health* 1993;65:S83-S86.
5. Bosque MA, Domingo JL, Llobet JM, Corbella J. Cadmium in hair of school children living in Tarragona Province, Spain. Relationship to age, sex, and environmental factors. *Biol Trace Elem Res* 1991;28:147-155.
6. Man AC, Zheng YH, Mak PK. Trace elements in scalp hair of professional drivers and university teachers in Hong Kong. *Biol Trace Elem Res* 1996;53:241-247.
7. Huang BX, Lin SQ, Chen SY, et al. Hair chromium levels in patients with vascular diseases. *Biol Trace Elem Res* 1991;29:133-137.
8. Donma O, Gunbey S, Tas MA, Donma MM. Zinc, copper, and magnesium concentrations in hair of children from southeastern Turkey. *Biol Trace Elem Res* 1990;24:39-47.
9. Cavdar AO, Bahceci M, Akar N, et al. Maternal hair zinc concentration in neural tube defects in Turkey. *Biol Trace Elem Res* 1991;30:81-85.
10. Aharoni A, Tesler B, Paltieli Y, et al. Hair chromium content of women with gestational diabetes compared with nondiabetic pregnant women. *Am J Clin Nutr* 1992;55:104-107.
11. Suzuki T, Hongo T, Yoshinaga J, et al. The hair-organ relationship in mercury concentration in contemporary Japanese. *Arch Environ Health* 1993;48;221-229.
12. Chattopadhyay PK, Joshi HC, Samaddar KR. Hair cadmium level of smoker and non-smoker human volunteers in and around Calcutta City. *Bull Environ Contam Toxicol* 1990;45:177-180.
13. Katz SA, Chatt A. The significance of hair analysis. In: *Hair Analysis: Applications in the Biomedical and Environmental Sciences*. New York, NY: VCH Publishers; 1988:105-109.
14. Druyan ME, Bass D, Puchyr R, et al. Determination of reference ranges for elements in human scalp hair. *Biol Trace Elem Res* 1998;62:183-197.
15. Berman E. *Toxic Metals and Their Analysis.* London: Heyden & Son, Ltd.; 1980.

16. Prasad AS. *Trace Elements and Iron in Human Metabolism*. New York; Plenum Medical Book Company; 1978.
17. Gossel TA, Bricker JD. *Principles of Toxicology*. New York: Raven Press; 1984.
18. Versieck J, Cornelis R. Normal levels of trace elements in human blood, plasma, or serum. *Anal Chim Acta* 1980;116:217-254.
19. Versieck J. Trace elements in human body fluids and tissues. *Crit Rev Clin Lab Sci* 1985;22:97-184.
20. Seidel S, Kreutzer R, Smith D, et al. Assessment of commercial laboratories performing hair mineral analysis. *JAMA* 2001;285:67-72.
21. Steindel SJ, Howanitz PJ. The uncertainty of hair analysis for trace metals. *JAMA* 2001;285:83-85.
22. Puchyr RF, Bass DA, Gajewski R, et al. Preparation of hair for measurement of elements by inductively coupled plasma-mass spectrometry (ICP-MS). *Biol Trace Elem Res* 1998;62:167-182.
23. Rodushkin I, Axelsson MD. Application of double focusing sector field ICP-MS for multielemental characterization of human hair and nails. Part I. Analytical methodology. *Sci Total Environ* 2000;250:83-100.
24. LeBlanc A, Dumas P, Lefebvre, L. Trace element content of commercial shampoos: impact on trace element levels in hair. *Sci Total Environ* 1999;229:121-124.
25. Doctor's Data Inc., Hair Kit Collection Instructions 2001.
26. Airey D. Total mercury concentrations in human hair from 13 countries in relation to fish consumption and location. *Sci Total Environ* 1983;31:157-180.
27. Assarian GS, Oberleas D. Effect of washing procedures on trace-element content of hair. *Clin Chem* 1977;23:1771-1772.
28. Salmela S, Vuori E, Kilpio JO. The effect of washing procedures on trace element content of human hair. *Anal Chim Acta* 1981;125:131-137.
29. Chat A, Sajjad M, DeSilva KN, Secord CA. Health-related monitoring of trace element pollutants using nuclear techniques. *IAEA-TECDOC-330*, International Atomic Energy Agency, Vienna 1985:33-49.
30. Suzuki T, Hongo T, Morita M, Yamamoto R. Elemental contamination of Japanese women's hair from historical samples. *Sci Total Environ* 1984;39:81-91.
31. Pankhurst CA, Pate BD. Trace elements in hair. *Rev Anal Chem* 1979;4:111-235.
32. Hilderbrand DC, White DH. Trace-element analysis in hair: an evaluation. *Clin Chem* 1974;20:148-151.
33. Buckley RA, Dreosti IE. Radioisotopic studies concerning the efficacy of standard washing procedures for the cleansing of hair before zinc analysis. *Am J Clin Nutr* 1984;40:840-846.
34. Niculescu T, Dumitri R, Botha C, et al. Relationship between lead concentration in hair and occupational exposure. *Brit J Indust Med* 1983;40:67-70.
35. Mikasa H, Suzuki Y, Fujii N, Nishiyama K. Adsorption and elution of metals on hair. *Biol Trace Elem Res* 1988;16:59-66.
36. Kopito LE, Shwachman H. Lead in human scalp hair: some factors affecting its variability. *J Invest Dermatol* 1975;64:342-348.
37. Chattopadhyay A, Roberts TM, Jervis RE. Scalp hair as a monitor of community exposure to lead. *Arch Environ Health* 1977;32:226-236.
38. Dang HS, Jaiswal DD, Mehta U, Deshpande A. Trace element changes in hair during pregnancy: preliminary study. *Sci Total Environ* 1983;31:187-192.
39. Jervis RE, Tiefenbach B, Chattopadhyay A. Scalp hair as a monitor of exposure to environmental pollutants. *J Radioanal Chem* 1977;37:751-760.
40. Ellis KJ, Yasumura S, Cohn SH. Hair cadmium content: is it a biological indicator of the body burden of cadmium for the occupationally exposed worker? *Am J Ind Med* 1981;2:323-330.
41. Kollmer WE. The significance of cadmium in hair. The influence of a recommended washing procedure on the cadmium content of hair of rats experimentally exposed to cadmium. *Sci Total Environ* 1983;27:251-259.
42. Ryan DE, Holzbecher J, Stuart CD. Trace elements in scalp-hair of persons with multiple sclerosis and of normal individuals. *Clin Chem* 1978;24:1996-2000.
43. Das HA, Dejkumhang,MM, Herer RFM, et al. Instrumental Neutron Activation Analysis of Human Hair and Related Experiments on Washing and Leaching, *Report ECN-107* Netherlands Energy Research Foundation, Petten; 1981.

44. Deppisch LM, Centeno JA, Gemmel DJ, Torres NL. Andrew Jackson's exposure to mercury and lead: poisoned president? *JAMA* 1999;282:569-571.
45. Ryabukin YS. Activation analysis of hair as an indicator of contamination of man by environmental trace element pollutants. *IAEA Report* IAEA/RL/50, Vienna; 1978.
46. Ryabukhin YS. Nuclear-based methods for the analysis of trace element pollutants in human hair. *J Radioanal Chem* 1980;60:7-30.
47. Montaser A, Golightly DW. *Inductively Coupled Plasma in Analytical Atomic Spectroscopy*. New York, NY: NCA Publishers; 1987:338:361.
48. Thomas R. Instrument and applications: money to burn. *Today's Chemist at Work* 1999;8:42-48.
49. Miekeley N, Dias Carneiro MT, da Silveira, CL. How reliable are human hair reference intervals for trace elements? *Sci Total Environ* 1998;218:9-17.
50. NCCLS, *Internal Quality Control Testing: Principles and Definitions, Approved Guideline*, Doc. C24-A, 11, 6, 1996.
51. Walsh WJ. Commercial hair analysis: science or scam? Letter. *JAMA* 1986;255:2603-2604.
52. NCCLS, *How to Define and Determine Reference Intervals in the Clinical Laboratory, Approved Guideline*, DOC C28-A, 15, 4, 1995.
53. Daniel WW. *Biostatistics: A Foundation for Analysis in the Health Sciences, 3rd ed., Wiley Series in Probability and Mathematical Statistics-Applied*. New York, NY: John Wiley & Sons; 1983.