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

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Abstract-
Over the past 40 years, the measurement of trace elements in biological samples has advanced dramatically. Precision, accuracy, dependability, and detection limits have all improved as a result of advancements in technology, including inductively coupled plasma-mass spectrometry and microwave digestion. These advancements have had a considerable positive impact on the analysis of human scalp hair. Significant inter-laboratory variation was discovered among multiple laboratories performing trace metal hair testing, according to a recent report in the Journal of the American Medical Association. It came to the conclusion that standardisation was required to enhance inter-laboratory comparability, and a commentary that went along with it outlined the qualities of a laboratory that should be employed for conducting hair analysis. The goal of this study is to show that using proper laboratory procedures will result in results that are exact, accurate, and trustworthy a procedure for setting reference ranges additionally detailed information on an analytical technique will be offered. The implementation of recommended clinical quality control procedures, such as technique validation, proficiency testing, split sampling, and acceptable laboratory practices, amply supports the possibility of analytical validity for the measurement of trace elements in hair.

Introduction

Since 1929, human systemic amounts of elements have been evaluated using scalp hair(1,2). The majority of clinical laboratories equipped to analyze trace elements use hair, which is generally recognized for determining harmful element exposures. Although there is considerable debate about the use of hair to measure vital elements, studies have shown that several essential elements are correlated with illnesses, metabolic abnormalities, exposure to the environment, and nutritional status. Hair has varied applications and even advantages over blood or urine when compared to other kinds of clinical specimens. Hair represents a longer time range, possibly years, whereas urine and blood typically show current or recent body status(3,4). Higher levels of the elements also exist in hair, allowing for more analytically precise results and enabling outcomes that are more sensitive.

Hair is less expensive to analyze easier, safer, and more conveniently collected, sent, and stored than blood or urine(5). This makes hair a great option in some circumstances and as a screening tool. When evaluating trace elements in hair, external contamination, a lack of uniformity, and analytical precision are further issues that must be taken into consideration.

The validity and use of the test depend on taking exact measurements when analyzing hair samples for trace metals. The analytical concerns and restrictions of trace metal detection in various clinical samples are thoroughly described in literature reviews. Over the past 40 years, biological trace element analysis has advanced dramatically. As a result of instrumentation advancements, precision, accuracy, reliability, and detection limits have all improved.

These advancements have had a considerable positive impact on the analysis of human scalp hair. Seidel et al. concluded that standardization was required after finding significant inter-laboratory variation in a recent article. The accompanying commentary by Steindel and Howanitz stated, "A sample for hair analysis is best sent first to a laboratory that can validate its certification or accreditation for per- forming 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." Such precise test characteristics for 36 components in hair were reported by Puchyr et al. Additionally, Rodushkin and Axelsson used high-resolution inductively coupled plasma mass spectrometry (ICP-MS)to report on 71 components in hair and nails.

The purpose of this work is to show that reliable findings may be produced using proven methods and excellent laboratory techniques. We'll go through several analytical approaches, hair cleaning techniques, and quality control performance evaluation types a process for data on analytical variability and precision as well as the establishment of reference ranges will be presented. For test method validation and quality assurance, the data in this publication adhere to the standards set out by the National Committee for Clinical Laboratory Standards (NCCLS) and Clinical Laboratory Improvements Act (CLIA)(6,7).

Pre-analysis Parameters

The possibility of contamination from outside sources is one problem that is frequently brought up with trace metal analysis of hair. LeBlanc, Dumas, and Lefebvre have demonstrated that shampoo has little to no effect on the levels of the majority of components in hair. These findings are supported by unpublished findings from the authors' laboratory. The levels of metals in hair will be considerably impacted by the presence of metals in a number of hair preparation products. The two that stand out the most are lead in hair dyes that include lead acetate and selenium in shampoos that contain selenium sulfide.

According to hair collection procedures, only the most recent hair growth should be tested, along with clean hair that hasn't been straightened, bleached, coloured, or permed in three months(8). are prevented by this technique. Despite these precautions, contamination still happens or these protocols are not always followed.

Laboratories should use cleaning processes to get rid of external pollutants. An ideal washing process would preserve endogenous components while removing only exterior pollutants. Various cleaning techniques are employed, and depending on the circumstances, their efficacy may change the element and the tainant. The method used to clean hair before to analysis is disclosed by the majority of researchers who examine trace metals in hair(9,10)Procedure other writers have evaluated the effectiveness of removing exogenous and endogenous components from hair using various cleaning techniques utilizing radioactive tagging, scanning electron microscopy, and other techniques. Using Triton X100, water and ethanol, and sonication, a recent study that examined the presence of lead and mercury in President Andrew Jackson's hair found that these washing techniques did not significantly reduce endogenous lead levels.

The International Atomic Energy Agency (IAEA) has conducted studies on various washing techniques and discovered that even when endogenous materials were eliminated, the sample was not rendered useless as a result(11). They came to the conclusion that, despite the fact that the washing process involves numerous factors, such as the incomplete removal of exogenous contamination or the partial removal of endogenous materials, representative measurements could be made provided standardised washing protocols were applied.

There have been other places where a conventional cutting and cleaning process has been thoroughly explained. The hair is first cut into around 0.125-inch (0.3-cm) pieces and combined to provide a representative sub-sample of the hair specimen. The hair is then cleaned using a modified process created by the IAEA(12). Each sample is sliced, then washed four times with Triton X-100 diluted to 1:200 V/V. The samples are then given an acetone rinse before being left to drain. This is followed by two rinses with acetone and three rinses with de-ionized water (18-MW). After that,the samples are dried in an oven set to 75 + 5 °C.

Studies looking at data from identical twins, blind split samples, and repeat testing from the same person have all shown that this approach is accurate and reproducible(13,14). In a later section of this study, the expected reproducibility from such approaches is described. The literature cited in this section indicates that deviating from this methodology may lead to different measures at the element level.

Digestion and Analysis of Trace Metals

The choice of analytical methods can also bring biases and unpredictability, which might be from contamination during digestion or analysis, losses in volatilization during digestion, or interferences during analysis. The sample preparation technique best suited for standardization has been suggested as microwave digestion. Additionally, the best method for ultra-trace, multi-element analysis is ICP-MS(15,17).It's crucial to describe and validate the procedure being used if you want to guarantee accurate and exact readings. Precision, accuracy, and detection limits are the three most significant method validation characteristics(18,21).

The precision, accuracy, and detection limits for a method involving temperature-controlled microwave digestion and ICP-MS analysis (22,25)(Elan 5000, Perkin Elmer, Danbury, CT) are displayed in Table 1. The spike recovery values in this table are generally between 90 and 110 percent, which is within the range of ICP-MS performance that is expected. When compared to certified reference material (CRM), Table 1 also demonstrates good agreement for all aspects. The only elements that significantly deviated from the verified values were chromium, nickel, and arsenic. Spike recoveries (Table 1) and using a different CRM, however, show that more accurate measurements are being taken.the procedure that causes the sample to reflux but causes no discernible loss of liquid volume. The end product, called digestate, is a clear liquid with a hint of yellow. The samples are diluted to a final volume of 50 mL after digestion, and an internal standard mixture is then added. By using CRMS and spike recoveries, which are displayed in Tables 1 and 2, it was confirmed that all elements had been recovered, even volatile ones like mercury.

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

The spike recovery values in this table are generally between 90 and 110 percent, which is within the range of ICP-MS performance that is expected. When compared to certified reference material (CRM), Table 1 also demonstrates good agreement for all aspects. The only elements that significantly deviated from the verified values were chromium, nickel, and arsenic(26,27)

Spike recoveries (Table 1) and using a different CRM, however, show that more accurate measurements are being taken.For the digesting procedure, 50-mL disposable polypropylene centrifuge tubes are filled with 0.2 g of correctly weighed washed hair samples. The tubes are filled with concentrated trace-metal-grade nitric acid (3 ml), sealed, and put in a microwave (MDS 2000 and MDS 2100; CEM Corporation, Matthews, NC). Temperature is used to control the digestion sequences using a fiber optic temperature probe inserted into one of the sample tubes.

Table 2-Accuracy for China Hair CRW 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

The procedure that causes the sample to reflux but causes no discernible loss of liquid volume. The end product, called digestate, is a clear liquid with a hint of yellow. The samples are diluted to a final volume of 50 mL after digestion, and an internal standard mixture is then added. By using CRMS and spike recoveries, which are displayed in Tables 1 and 2, it was confirmed that all elements had been recovered, even volatile ones like mercury analytical Method Since other procedures could result in the loss of analytes, contamination of the sample, or insufficient digestion, it was determined that this method, which is described in further detail elsewhere, had the highest level of reliability. If alternative analysis techniques were utilized, they would result in inferior detection limits and poorer reproducibility at lower concentrations because ICP-MS has better sensitivity than other techniques for most elements normal 49 Any clinical test must adhere to certain standards. Seidel et al. noted it as a concern, and Ryabukhin 5.46 claims that standardization of the hairwashing procedure is essential for attaining repeatable results. Standardization of sample washing procedures between labs could undoubtedly improve comparability between labs employing quality while this is the goal, not all laboratories may agree with the standards set because of differences in philosophies, the cost of reengineering laboratory methods, and politics within the lab. However, interlaboratory comparability would be improved if such a step were adopted by regulatory bodies, as stated by Steindel and Howantiz.

Quality Evaluation

The information displayed in Table 1 is typical of the kind of data that was gathered throughout the first method development and technique validation. Controls must be put in place to ensure continuing quality and to better monitor potential sources of errors when the analytical parameters from the method development have been obtained and the method has been found to be adequate for performing routine measurements. Pre- and post-digestion in-house controls, as well as CRMS, are used as controls(28,29). Additionally, split sampling, proficiency testing, and external audits are crucial components of both internal and external quality assurance.

The method validation and quality control procedures outlined by CLIA and NCCLS include all of the quality tools that are presented in this article.(30,31)

The finest resources for determining correctness are frequently certified reference texts. There are numerous approved reference resources accessible for hair. One from Japan, two from the IAEA (IAEA-085, IAEA-086, both 9 elements), two from China (GBW 09101, 30 elements, and GBW 07601, 60 elements), and two from the IAEA. In-house controls and CRMs can both be used to evaluate precision and accuracy. While a control made of finely chopped, homogenized hair enables for monitoring of run-to-run differences in the digestion process(33, 34)a control made of digested hair provides for monitoring of between-run variations in the instrument.Together, these controls offer a useful method for evaluating precision and accuracy. Since internal control values are not verified, some effort must be done to "certify" these values. This can be achieved by evaluating the recovery after spiking the control with a given amount of analyte, as well as by measuring the control alongside a CRM. Additionally, results from other high-quality laboratories that received the control sample or additional samples can be compared.

Split sampling can be used to evaluate accuracy and precision(35,36). The same sample can be sent to the same lab multiple times to evaluate repeatability. Table 3 displays the results of such a blind split sampling.Part of a sample is evaluated internally while another part is submitted to a lab for analysis in order to determine accuracy. Table 4 lists the outcomes of samples examined in the authors' lab and a different lab. This specific investigation was carried out by a university research lab and submitted to our laboratory undercover.

Sample homogenization is crucial to split testing. Organizations frequently powder the hair sample to ensure reproducible sub-sampling when attempting to homogenize samples for inter-laboratory comparisons or for creating CRMs. There is evidence that this produces a more homogeneous sample. The sample, however, differs from a typical hair sample and can have various properties. Although Seidel correctly noted it at an al20 and assigned a variable to the accuracy, i.e., attaining results that are indicative of a person's relative trace element levels. The typical outcomes are displayed in Tables 3 and 4.

Table : 3 Repeated testing result Of an individual sample submitted.

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

Table : 4 result of split samples From two laboratories (in µg/g) At different time (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

The variances that are visible can be the outcome of variations in the hair sample. when differences in between-laboratory split sampling arise. To verify accuracy or quantify potential biases, additional accuracy metrics are evaluated. These tables demonstrate that it is possible to measure hair precisely and accurately

Some researchers create their own reference materials in order to conduct accurate and trustworthy trace element tests on human hair. One such user gathered a significant amount of hair, homogenized it, and sent it to various laboratories for analysis. This strategy yielded outcomes similar to those mentioned by Seidel et al. The researcher came to the conclusion that some laboratories were of higher quality, and those of lower quality were disqualified from consideration. With more testing, the superior laboratories' accuracy was confirmed.

Testing for proficiency is frequently used in all laboratories to evaluate performance and improve it. It also acts as a third-party verification of accuracy. Unfortunately, there aren't many professional testing programs for hair. The Toxicology Center (Laboratory) of the National Public Health Institute of Quebec (INSPQ) conducts trace metal proficiency programs for blood and urine as well as, on a rotating basis, various clinical matrices like

hair (studies with hair were carried out in 1997, 1999, and 2001). In contrast to Seidel et al., the study's inter-laboratory ICP-
MS comparison for hair produced noticeably different findings.There were some significant contrasts, though; for instance, INSPQ requested the participation of volunteer laboratories. they examined more labs, using a homogenized hair sample, and examined all the labs use ICP-MS. The findings supported the idea that trace elements may be precisely detected in hair because there was a significant decrease in inter-laboratory variability.

External assessment is frequently used to assess the caliber of laboratories, frequently in the form of audits for certification purposes. To determine if a laboratory can accurately measure the clinical specimen of interest, audits normally examine all components of quality control discussed in this study. As long as laboratories adhere to standard laboratory practices and quality control protocols, certification for various types of testing, including hair, should be attainable when it becomes available.

Ranges of Reference and Interpretation

Once the measurement has been taken. What does that imply? Is a mercury level of 5 mcg/g in hair high or low? A crucial step in offering this interpretation is determining the reference ranges. The NCCLS offers instructions for establishing reference ranges. Druyan et al. provided a comprehensive method for determining hair reference ranges based on data from a sizable patient group. The ranges established with Druyan's patient population were validated using a narrower, physician-defined "Healthy American Population Study" because to the potential bias in employing a broad patient population. Druyan's research then compared these ranges to values found in the literature and ranges set by other laboratories.

The reference limit is that seen at the 95th percentile for non-essential or possibly hazardous components with a single-tailed distribution. An expected range is defined as 68 percent of the reference population (as opposed to the reference limit). This range covers the 16th through 84th percentiles for physiologically necessary analytes. Individuals who are outside of this range might or might not have an issue that needs to be addressed.Clinicians should consider a patient's history, medical condition, physical examination, nutrition, lifestyle, and environment when establishing diagnosis and developing treatment strategies based on test results.

Conclusion

Published studies show that testing for trace metals in hair may be done precisely and accurately. The necessity for standardization was correctly recognised by Seidel's work. They should demand more proficiency testing programs and better CRMS in light of the interest and utilization of hair testing they discovered.When it is implied that some of the facilities being compared were unethical and others had method-related differences, the lack of inter-laboratory comparability does not support the assertion that hair testing is invalid. Walsh's findings and Seidel's findings both support the notion that some laboratories provide subpar work.

Why is hair measurement so contentious if it can be used to accurately screen for trace element status? Why, given that the evidence does not support it, did Seidel come to the conclusion that hair testing is invalid? According to the authors, there is a general mistrust of the use of hair for detecting the presence of trace metals among some people who may assume that some labs or physicians abuse or incorrectly interpret the results of hair element analysis. As with any other laboratory test, the key to achieving dependable, accurate, and precise hair element analysis is to make sure that the laboratory of choice is properly licensed and uses standardized and documented techniques like those outlined in this article.

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