SPECIMEN PREPARATION IN THE LABORATORY

---- David L. Watts, Ph.D., Director Of Research -----

The question concerning sample preparation pertaining to washing or not washing samples prior to analysis has arisen recently. We have visited this subject many times over the years and will now briefly share our comments in the Newsletter. TEI has performed a number of studies on washed and unwashed hair samples. Our results agreed with our published studies, in that there was found a significant removal of endogenous elements with different washing techniques. For instance, sodium and potassium, which are lightly bound within the hair were affected the most. As much as in some cases over 90 percent of the sodium and potassium in the hair were removed by washing. Other elements were affected as well to a lesser degree.

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Inter-laboratory comparison studies have revealed a good agreement between labs when sample preparation was the same (unwashed). Good agreement is also noted on results from a standard reference material between laboratories where the sample is not subjected to the various laboratory wash techniques. However, a significant inter-laboratory deviation is found on some elements from washed and unwashed samples, most notably, sodium and potassium. There was a two-fold decrease of sodium on washed samples and a ten-fold decrease in potassium values while washing procedures as well influenced other elements. Other independent research has shown the same conclusions.

Chittleborough tabulated twenty-four treatment techniques and found that many preparatory treatments removed significant fractions of endogenous elements. His studies support a no-wash policy of hair samples for hair trace element analysis. *Science of the Total Environment.* 14, 1, 53-75, 1980.

Different washing procedures remove different contaminants as well as different proportions of trace elements from hair samples. Washing procedures are therefore a major source of inconsistencies among laboratories. *Hair: A Diagnostic Tool to Complement Blood Serum and Urine. Vol., 202. 1271-1273. Dec. 1978*

The different pre-analytical treatments of the hair samples could be responsible for the lack of agreement on trace element levels. Some mineral levels in samples treated by different washing procedures showed significant differences depending upon the technique used. *The use of hair as a biopsy material for trace elements in the body. Katz, S. American Lab. Feb. 1979.*

Buckley et al reported that no washing procedure effectively removed all exogenous minerals, but all treatments extracted varying proportions of the endogenous minerals. Further it has been found that absorption of elements from the cleaning solutions can occur depending upon the length of time the hair is exposed to the solution. *Radiosotopic studies concerning the efficacy of standard washing procedures for the cleansing of hair before zinc analysis. Buckley, RA, Chem, HCA, Dresoti, IE. The American J. of Clin. Nutr. 40, 840-846, 1984.*

No washing procedure reported could completely remove all external trace elements and therefore, it has been suggested that for lead, cadmium, and arsenic there is no purpose in attempting to cleanse hair samples. Data for other elements are quite varied depending upon the type of washing procedure and the mineral in question. Studies of various washing techniques imply that test results from different laboratories may not be comparable, even if the same washing procedure is used for different time periods. *Taylor, A. Usefulness of measurements of trace elements in hair. Ann. Clin. Biochem. 23, 364-378, 1986.*

It is well known that washing techniques vary from laboratory to laboratory. Published research studies into the many different wash techniques still in use today, whether a standard wash technique or a modified variation of an older technique, verify the inadequacies and inconsistencies found from lab to lab. In fact, as reported above, minor deviations in specimen exposure time to just one wash cycle alone can contribute to variation in final test results. In addition, we are aware of no published study that shows that any of the various wash techniques can insure removal of only the exogenous elements found on the hair while not adversely affecting the endogenous levels.

Our continuing research supports the view that analyte levels found in unwashed hair samples provide a better metabolic indication of nutritional status, neuro-endocrine activity and the nutritional interrelationships of an individual.

Since various washing techniques remove significant amounts of endogenous elements, thereby invalidating the clinical significance of some of the elements tested, Trace Elements has adopted a no-wash policy in its preparation of hair samples in order to preserve the important endogenous elements. Our continuing research supports the view that analyte levels found in unwashed hair samples provides a better metabolic indication of nutritional status, neuro-endocrine activity and the nutritional interrelationships of an individual. In quoting from Chittleborough's report, many researchers attitude "is that each hair sample as collected, has a unique character". "Hair is a unique biological material which reflects the biomedical and environmental history of the individual."

Further, our research as well as the research of other investigators has shown that shampoo treatments have little effect upon test outcome. One study, using volunteers analyzed the hair before and after hair washing using selected shampoos and conditioners. "While some shampoos showed high levels of certain elements, the degree of contamination on the hair was found to be negligible. Only one shampoo tested, formulated with selenium sulfide, was found to seriously contaminate the hair." Leblanc, A., Dumas, P., Lefebvre, and L. Trace element content of commercial shampoos: impact on trace element levels in hair. Sci Total Environ 1999, May 7, 229 (1-2): 121-4

We are not saying that to wash or not to wash is right or wrong, as this issue has been debated for over thirty years with still no consensus among researchers. However, at the very least, each laboratory should provide clinician's with results based upon their own test methods from which the reference intervals were established. Appropriately, many or most laboratories that wash samples avoid placing clinical significance on most elements affected by their washing procedure.