Laboratory protocol for the analysis of serum or plasma zinc by flame atomic absorption spectrophotometry

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Preparation of sample diluent

The sample diluent which is used to make all standard curves and diluted samples is prepared by adding 8.34 ml of ARISTAR grade concentrated hydrochloric acid to a 2 litre volumetric flask and making up to the mark with milliQ water. (NB I used n-butanol in the past to enhance the sensitivity but because our current Atomic Absorption Spectrophotometer (AAS) is very sensitive, I have opted to exclude this component).

Preparation of standard curve

A three point standard curve is used for the determination of zinc in blood serum. First a stock solution at a concentration of 10 ppm zinc is prepared by adding 1mL from a commercial 1000 ppm Zn Standard to a 1 liter volumetric flask and made up to the mark with milliQ water. Next, 2.5 mL of HPLC grade glycerol is added to three 100 mL volumetric flasks. From the 10 ppm stock solution, three aliquots of 1.0, 2.0, and 4.0 mL are taken and each placed in one of the 100 mL volumetric flasks. Then the sample diluent is added to each flask and made up to the mark. The three flasks will now have concentrations of 0.1 ppm, 0.2 ppm, and 0.4 ppm of zinc. The glycerol is added to each flask in an attempt to match the matrix of the serum used in the controls and samples.

Preparation of serum samples and controls for analyses

A 1:10 dilution of sample or control to diluent is first carried out in order to prepare the samples and controls for analyses. To accomplish this dilution, take 0.25 mL of the sample or control and pipette into a Sarstedt trace-element free tube (part number given elsewhere in this document). Then add 2.25 mL of sample diluent to the tube. Cap the tube and mix on a vortex machine.

Note: It pays to prepare all the samples and controls first before analyses. If possible try to allow the prepared standards, controls, and samples to reach room temperature prior to analyses because temperature has a major effect on viscosity. Hence, by following this protocol, inconsistencies in the results will be minimized.

Analytical method

When doing the analysis on the AAS use the sample diluent as the blank. After the blank, analyze the standards to build the standard curve. Once the standard curve is established, then it is advisable to analyze 2 – 4 diluted pooled serum samples. The zinc concentrations of these pooled serum samples are almost always lower than expected. If this occurs, then recalibrate using the top standard of 0.4 ppm. This has the effect of lowering the standard curve. After this adjustment, the correct values are always obtained for all the controls. Normally, the analytical sequence is as follows: analysis of one control (such as UTAK) followed by an internal lab control, and then 10 prepared serum/plasma samples. Next, recalibrate with the top standard of 0.4 ppm again, and repeat the analytical sequence until all the prepared samples have been analyzed.