A129 Metal Ions as Forensically-Relevant Inhibitors of PCR-Based DNA Testing

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The goal of this presentation is to introduce metal ions as relevant PCR inhibitors associated with human skeletal remains to the forensic DNA community. The data presented includes sources of metal ions, their expected concentrations in forensic samples, as well as demonstrate the effects of these inhibitors on the genetic results obtained from samples amplified using PCR-based DNA amplification kits. Attendees will be given discrete examples of inhibition phenomena which can occur as a result of interactions between metal ions and DNA or PCR reagents required for testing.

This presentation will impact the forensic science community by expanding the pool of known, forensically-relevant PCR inhibitors associated with human skeletal remains to include metals commonly found in bone samples that have been exposed to the environment or to material culture. The ability to identify samples that contain inhibitors or DNA profiles, demonstrating inhibition will aid decision-making capabilities of analysts and provide a foundation for future research.

Bone samples obtained from human skeletal remains are considered one of the most challenging sample types in the laboratory. Laborious sample processing is required and it frequently yields incomplete genetic results or amplification failure. While this may be caused by and is often attributed to DNA which has been damaged, it can also occur as a result of PCR inhibition caused by the co-purification of metal ions with DNA.

Cadaver bone was obtained through the Willed Body Program at the University of North Texas Health Science Center and its metal content was determined by inductively coupled plasma-mass spectrometry (ICP-MS) by the Department of Chemistry at the University of North Texas. Elemental analysis was performed in solution mode ICP-MS to determine the concentration of analytes, including isotopes: ^{27}Al, ^{43}Ca, ^{63}Cu, ^{57}Fe, ^{60}Ni, and ^{208}Pb. ICP-MS measurements were performed on a -MS System with Autosampler. Acid digestion was used for sample preparation; approximately 100 mg of each sample was weighed out into a polypropylene test tube and dissolved in 2mL of ultra-pure concentrated HNO_3, and then diluted with 100mL of 1% HNO_3. For major and trace metal analysis, dilution factors vary between 20 and 100. All samples were measured in triplicates for statistical analysis.

In order to investigate metal ions as PCR inhibitors, a dilution series was created using certified analytical standards for aluminum (Al), calcium (Ca), copper (Cu), iron (Fe), nickel (Ni), and lead (Pb) in solution. These standards were initially diluted to approximately 21 mM and the pH adjusted between three and five with 3 M NH_4OH and 1 M HCl; subsequent serial dilutions were prepared with DNase/RNase-free distilled water. Final sample solutions were prepared, containing 1ng of control DNA and sufficient metal to yield 7.5mM, 1.25mM, 0.2mM, 0.03 mM, 0.006 mM, and 0.001mM in the 25µL PCR reaction. Duplicate samples were amplified using the AmpFℓSTR® Identifiler® Plus (Applied Biosystems) multiplex system and a HS System. Fragment analysis was conducted via capillary electrophoresis on a genetic analyzer using 10-second injections. STR profiles, including individual allele peak heights and areas, were obtained using ID software and a 50 RFU allele detection threshold. Amplification success was determined by the average allele count, expressed as a percent of the expected number of alleles for the control DNA profile and the effect of inhibitor on the quality of the genetic results was determined by regression analysis and the calculation of Pearson's correlation coefficients.

The elemental analysis revealed isotope concentrations (µg/g) in the cadaver bone were as follows: ^{27}Al, 473-4; ^{63}Cu, 146-5; ^{57}Fe, 366-27; ^{60}Ni, 0.5-0.1; and ^{208}Pb, 165-3. The PCR inhibition studies demonstrated the effective inhibitory concentrations (mM) for amplification was: 0.001-0.006 for Al; 0.2-1.25 for Ca; 0.2-1.25 for Cu; 0.2-1.25 for Fe; 0.2-1.25 for Ni; and, Pb, 1.25-7.5. The effective inhibitory concentrations (mM) for the HS System were: 0.006-0.03 for Al; 0.2-1.25 for Ca; 1.25-7.5 for Cu; 0.2-1.25 for Fe; 0.2-1.25 for Ni; and for Pb 1.25-7.5.

Skeletal Remains, PCR Inhibition, Metal Ions

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