B58 Use and Detection of Ribosomal Inactivating Proteins (RIPs) as Surrogates for Active Toxins Via Immuno-Precipitation and Matrix-Assisted Laser Desorption-Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF/MS)

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After attending this presentation, attendees will understand the forensic issues associated with Ribosomal Inactivating Proteins (RIP), the purpose of a surrogate for validation of detection assays, and the significance of detection using immune-precipitation and Matrix-Assisted Laser Desorption/Ionization-Time-Of-Flight/Mass Spectrometry (MALDI-TOF/MS).

This presentation will impact the forensic science community by providing a rapid, cost-effective, and sensitive method for the detection of RIP in various food matrices thought to be targets during a biocrime. Due to RIP toxins' lethality, availability, ease of isolation, and lack of treatment, there is a critical need for continued development of RIP countermeasures.

Naturally produced RIPs, such as ricin and saporin, can be highly lethal when ingested. Due to the availability or ease of production of these toxins, their use is attractive in the commissioning of a biocrime or use as a tactical bioweapon. Therefore, it is necessary to test complex matrices, such as food or drink, for protein toxins in a rapid, cost-effective manner. Researchers have developed an MS-based assay that involves capture of ricin from milk, apple juice, serum, and saliva using magnetic beads and testing of the toxin's enzymatic activity; however, many protein toxins are tightly regulated select agents making it difficult for many laboratories to test new methodologies or perform basic research. A surrogate, non-toxic protein that works in the immuno-capture step and mimics ricin's activity should be capable of use in validating the detection assay with additional matrices in order to test the effects of pH and viscosity. If successful with ricin, this approach could be used to safely test variety of toxins for adulteration of different food stuffs and drinks.

The first goal of this study was to demonstrate the efficacy of a surrogate protein as a toxin substitute for validation and matrix testing. This study used a MALDI-TOF/MS assay developed by the Centers of Disease Control and Prevention (CDC) to test and optimize detection of a surrogate RIP protein, Uracil DNA Glycosylase (UNG). UNG, which removes a single uracil from an AUAT stem-loop, yields a similar DNA product as a RIP-II toxin, while by-passing the safety concerns of active, purified toxins. Next, the validity of this assay concept as a detection and activity method was confirmed by testing complex matrices with a wide pH range and texture complexity, such as a sports drink, ketchup, salad dressing, dry coffee creamer, mustard, and sweet potato and banana baby foods. Lastly, this study determined the lower Limit Of Detection (LOD) of this assay by reducing the UNG concentration in water.

AmpErase® UNG and its target substrate, ric12aUtA, proved suitable surrogates for ricin and its substrate target since this enzyme has similar RIP activity and DNA target as ricin. The cleaved substrate resulted in a loss of 94 Da, which was measured with MALDI-TOF/MS. Ratios of the cleaved product, corresponding to the m/z 3,535 peak, and the uncleaved product, seen at m/z 3,630, were used to estimate the efficiency of the process in the various matrices. A cleavage efficiency of ≥90% was measured for water, the sports drink, ketchup, salad dressing, and coffee creamer. Very viscous matrices (mustard, sweet potato baby food, and banana baby food) had very low to zero cleavage efficiencies (46.8±31.4%, 16.6±8.3%, and 0.0%, respectively). The LOD for this assay was reduced from 1-5pmol (CDC's method) to 0.18-1.8pmol of the surrogate per reaction. Various incubation times were also tested in an effort to optimize the assay when protein concentrations were very small. Overnight incubation of water was 32.1±0.01% efficient, while three- to four-hour incubation was 10.7±0.01% efficient. This indicates that a longer incubation time could be used when surrogate concentration is very low.

Ribosomal Inactivating Protein, MALDI-TOF, Validation