H100 Species Identification of Small Skeletal Fragments Using Protein Radioimmunoassay (pRIA)

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This presentation will demonstrate how protein radioimmunoassay can be utilized in the forensic analysis of small skeletal fragments to distinguish human from non-human animal and determine non-human animal species.

This presentation will make the forensic community aware how protein radioimmunoassay (pRIA) can be used in the analysis of small bone fragments to identify human vs. non-human origin and animal species.

With increased awareness of the potential for positive identification through molecular analysis of skeletal remains, fragmentary evidence is frequently submitted for forensic examination. Prior to DNA analysis it is usually desirable to determine if remains are of human origin and if not, on occasion, to determine the species represented. When specimens are too small or taphonomically compromised for conventional morphological diagnosis, investigators can use SEM/EDS to help determine if bone and/or tooth is represented. Histological examination may reveal a distinct non-human pattern but usually is not diagnostic for human since the human histological pattern is shared with some non-human animals. In addition, preparation of histological sections may preclude DNA analysis in cases involving very small particles. In such cases, protein radioimmunoassay can be utilized to distinguish human from non-human animal and to identify the species. In addition to identification of modern, well-preserved specimens, pRIA has been shown to make correct determinations on cremated specimens and on archaeological specimens as ancient as 11,000 years.

Analysis by pRIA consists of three phases: extraction of sample bone material from the specimen, isolation of the protein, and pRIA to identify species.

Extraction of the sample material consists first of removing and discarding the outer one-two mm of the surfaces to remove any significant external protein contamination.

Protein is isolated by placing 20-200 mg of bone matter into a 10 ml vaccutainer and capping it. A syringe is then utilized to first add a solution of 1M EDTA and then to create a partial vacuum within the vaccutainer. Subsequently, the solution is gently shaken and rotated for two to five days at room temperature to dissolve the bone.

The pRIA species identification is obtained using solid phase double antibody radioimmunoassay. The EDTA protein extract is placed in polystyrene microtiter plates to allow the protein to bind to the plate (thus in a “solid phase”). Various samples of antisera (raised in rabbits) of albumins or sera of various species are then added to the wells containing the extracted solid phase protein (antigen), allowing the antibody to bind to the antigen. After cleansing, radioactive (iodine-125) antibody of rabbit gamma globulin (produced in donkeys) is added and radioactivity of the wells is quantified in a scintillation counter. Species is determined through evaluation of the measure of radioactivity uptake which represents the extent of binding of species specific antisera.

As a demonstration of the applicability of this technique, six bone samples were submitted for blind analysis. Each weighed less than 1.8 grams and was too small for morphological determination of species. Three of these samples had been removed from known human remains and three from known non-human animal bones. In addition, one of the non-human specimens was known to represented a deer (Odocoileus virginianus). pRIA analysis correctly differentiated the human from nonhuman samples and correctly identified the known deer sample, given the choice of five possible non-human animals (cow, deer, dog, goat and pig) using 200 mg or less of each specimen.

pRIA, Bone Fragments, Species Identification

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