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**Standard for Protein Serology Methods for Taxonomic
Identification in Wildlife Forensics**



Standard for Protein Serology Methods for Taxonomic Identification in Wildlife Forensics

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Foreword

This document addresses the need in wildlife forensics to have a method outlining taxonomic identification using serology. Specifically, this document addresses serology testing using isoelectric focusing on an enzyme system. It can be used in a number of capacities including electrophoresis, counter immunoelectrophoresis (CIEP) and isoelectric focusing (IEF).

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This document was revised, prepared, and finalized as a standard by the Wildlife Forensics Consensus Body of the AAFS Standards Board. The draft of the first edition of this standard was developed by the Wildlife Forensic Biology Subcommittee of the Organization of Scientific Area Committees (OSAC) for Forensic Science.

Questions, comments, and suggestions for the improvement of this document can be sent to AAFS-ASB Secretariat, asb@aafs.org or 410 N 21st Street, Colorado Springs, CO 80904.

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DRAFT

Standard for Protein Serology Methods for Taxonomic Identification in Wildlife Forensics

1 Scope

This document provides the requirements for general protein serology methods for taxonomic identification used in the laboratory. This standard requires a laboratory to have protocols that address the serology methods used, the method validation process, and the statistical analysis and interpretation of serology results generated by a method. This document also addresses the use of quality control samples (positive, negative), the use of reference materials, and the analysis of serology data if a control fails. The document explains how differences in expressed proteins can be used to identify animals at family or species level using a suite of serology methods.

2 Normative References

There are no normative reference documents. Annex A, Bibliography, contains informative references.

3 Terms and Definitions

For purposes of this document, the following definitions and acronyms apply.

3.1 control

A sample of known properties, run in parallel with experimental, reference, or evidence samples that is used to demonstrate that a method is working correctly.

3.2 electrophoresis

A technique used to separate macromolecules based on size.

NOTE Charged molecules (e.g., proteins, DNA and RNA) migrate towards an oppositely charged pole through a sieving matrix, which permits a size-dependent separation.

3.3 family

The level of taxonomic classification that generally defines groups of genera.

3.4 genus

The level of taxonomic classification that defines a group of related species.

3.5 isoelectric focusing IEF

A method of separating amphoteric molecules with minor differences in isoelectric points (pI).

NOTE IEF can be used with several different enzyme systems including Phosphoglucose Isomerase (PGI), Erythrocyte Acid Phosphatase (EAP), Superoxide Dismutase (SOD) or Tetrazolium Oxidase (TO), Albumin (in

conjunction with Western Blot), Mannose Phosphate Isomerase (MPI), Carbonic Anhydrase I (CAI) and Hemoglobin (Hb).

3.6

PhastSystem™^a

A semi-automated workstation designed for horizontal electrophoresis and isoelectric focusing of proteins.

3.7

reference material

A biological specimen, material or substance of known identity and verified properties, or data derived from them.

3.8

serology

The detection or taxonomic identification of body tissues and fluids, through the use of chemical reagents and the reaction of an antibody to an antigen.

3.9

species

The fundamental unit of taxonomic classification.

NOTE There is no singular species definition in biology. Essentially the term denotes a group of organisms with a unique shared evolutionary lineage.

3.10

taxonomic identification

An analysis to establish the classification of an organism to family, genus, species, etc.

NOTE This analysis is based on class characters diagnostic for the taxonomic level in question.

4 General Protein Serology Methods for Taxonomic Identification

4.1 General Laboratory

4.1.1 Documented protocols covering serology testing shall include:

- a) all serology analysis methods, including necessary reagents, routinely used in the laboratory;

NOTE Serology methods include: Counter Immunelectrophoresis (CIEP), Phosphate Glucose Isomerase (PGI), Albumin, Erythrocyte Acid Phosphatase (EAP), Superoxide Dismutase (SOD) or Tetrazolium Oxidase (TO), Mannose Phosphate Isomerase (MPI), Hemoglobin (hb), Carbonic Anhydrase I (CAI), Identifying fish species with IEF PhastSystem, Immunodiffusion (Ouchterlony).

- b) the method validation process;

- c) all interpretation of serology results generated in the laboratory.

4.1.2 The laboratory shall follow specific enzyme system user manuals for:

- a) installation;

- b) operation;
- c) evaluation and presentation of data; and
- d) maintenance.

NOTE Isoelectric focusing (IEF) can be used with several different enzyme systems.

4.2 Quality Control

4.2.1 A positive control shall be run alongside unknown samples.

4.2.2 A negative control shall be run alongside unknown samples.

NOTE The edge of the comb serves as the negative control for the PhastSystem.

4.3 Analysis of Results

4.3.1 If a positive control fails, the data shall not be used for reporting.

4.3.2 If the negative control yields a product, the data shall not be used for reporting.

4.4 Taxonomic Identification

4.4.1 Taxonomic identification based protein serology methods shall be supported by comparisons as defined by the laboratory protocols:

- a) to reference material that represent the species of interest, and
- b) closely related species.

NOTE Additional support, if considered relevant, can be obtained by the analyst to include the following:

- a) the biogeography, ecological characteristics, life history, or taxonomic characters of the species of interest or
- b) citations of published isoelectric points for species not commonly analyzed in the laboratory.

4.4.2 If a species-level identification is not supported by comparison to laboratory reference materials, results shall be reported at a higher taxonomic level, such as genus and family.

Annex A (informative)

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