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Wildlife Forensics—Protein Serology Method for Taxonomic Identification

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Academy Standards Board
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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).

The draft of this standard was developed by the Wildlife Forensics Subcommittee of the Organization of Scientific Area Committees. It was prepared and finalized as a standard by the Wildlife Forensics Consensus Body of the ASB.

All hyperlinks and web addresses shown in this document are current as of the publication date of this standard.

Keywords: *electrophoresis, immunoelectrophoresis, isoelectric focusing, protein, serology, taxonomic identification*

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Wildlife Forensics—Protein Serology Method for Taxonomic Identification

1 Scope

This document addresses the protocols required for general protein serology methods for taxonomic identification routinely used in the laboratory. These protocols include: Serology methods routinely used in the laboratory, the validation process, and statistical analysis and interpretation of serology results generated in the laboratory. This document also covers the use of quality controls (positive, negative and comparison samples) and the analysis of results if controls fail. The document explains how differences in expressed proteins can be used to identify animals at family and/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
controls
Samples of known types, run in parallel with experimental, reference, or evidence samples that are used to demonstrate that a procedure is working correctly.

3.2
electrophoresis
A technique used in laboratories to separate macromolecules based on size. 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). 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
immunodiffusion
Any of several techniques for obtaining a precipitate between an antibody and its specific antigen by letting both antibody and antigen migrate through the gel from separate wells to form an area of precipitation (also known as ouchterlony double immunodiffusion).

3.7
PhastGel™ IEF[1]
PhastGel IEF media are precast homogeneous or gradient polyacrylamide gels containing Pharmalyte as carrier ampholyte.

3.8
PhastSystem™ [1]
Is a self-contained electrophoresis unit, that consists of a separation-control unit for system control.

3.9
positive control
An analytical control sample that is used to determine if a test performed properly. This control consists of the test reagents and a known sample that will provide a positive response in the test.

3.10
reference materials
Biological specimens, materials or substances of known identity and verified properties, or data derived from them.

3.11
reference samples
Samples of known origin collected for purposes of comparison to samples of unknown origin.

3.12
serology
The detection and/or taxonomic identification of body tissues and fluids, through the use of chemical reagents and antibody/antigen reactions.

3.13
species
The fundamental unit of taxonomic classification. There is no singular species definition in biology. Essentially the term denotes a group of organisms with a unique shared evolutionary lineage.

3.14
taxonomic identification
Analysis to establish the classification of an organism to family, genus, species, etc. This analysis is based on class characters diagnostic for the taxonomic level in question.

[1] This term is used as an example only, and does not constitute an endorsement of this product by the AAFS Standards Board.

4 General Protein Serology Methods for Taxonomic Identification

4.1 General Laboratory

4.1.1 Protocols covering serology testing shall include:

a) All serology analysis methods routinely used in the laboratory;

EXAMPLES Counter Immunoelectrophoresis (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 validation process;

c) reagents used; and

d) all analysis and 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 Controls

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 the positive control fails, the data shall not be used for interpretation.

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

4.4 Taxonomic Identification

4.4.1 Taxonomic identification based on serological and isoelectric focusing data shall be supported by comparisons as defined by the laboratory SOP(s):

a) to validated reference samples that represent the species of interest, and

b) closely related species.

4.4.2 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.

b) Citations of published isoelectric points for species not commonly analyzed in the laboratory.

4.4.3 If species-level identifications are not supported by comparison to laboratory reference materials, results shall be reported at a higher taxonomic level.

Annex A
(informative)

Bibliography

This is not meant to be an all-inclusive list as the group recognizes other publications on this subject may exist. At the time this standard was drafted, these were the publications available for reference. Additionally, any mention of a particular software tool or vendor as part of this bibliography is purely incidental, and any inclusion does not imply endorsement.

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