A48 Generating DNA Profiles From Immunochromatographic Cards Using LCN Methodology

Reena Roy, PhD, Penn State University, Forensic Science Program, 325 Whitmore Lab, University Park, PA 16802; and Tam Ho, MPS, Penn State University, Forensic Science Program, 107 Whitmore Laboratory, University Park, PA 16802*

After attending this presentation, attendees performing DNA analysis will learn the optimum use of evidence which is limited in size.

This presentation will impact the forensic science community by providing a method which allows forensic scientists to identify the body fluid as well as the donor of the body fluid.

Biological fluids such as blood, saliva, and semen encountered at crime scenes are valuable sources of physical evidence in forensic casework since it can be used in the identification of an individual via DNA analysis. However, some of these evidence samples may not originate from humans or be mixtures of body fluids between humans and animals. Currently, various presumptive tests are available for detection of body fluids such as blood, saliva, semen and other body fluids. Once the presence of a certain body fluid is indicated on a piece of evidence confirmation of the nature of the stains are performed. Several confirmatory tests are available in the forensic science community for determining the species of origin of a biological stain. Species identification of a biological fluid usually relies on the binding of an antibody to a corresponding antigen, resulting in a visual precipitation**.** These human specificity tests include the use of antibodies, and allow the scientist to determine if the stains are of human in origin. Each assay employs slightly different working mechanism. One type of confirmatory test, which is available to scientists, employs immunochromatographic assay on membrane strips.

The one-step immunochromatographic assays used to identify human body fluids such as blood, semen, and saliva are now widely used in the field of forensic science. These devices have been validated by many scientists and have been confirmed to be reliable, sensitive and specific for forensic casework. These tests are used replacing the traditional methods, such as cross-over electrophoresis, which can be time consuming. These monoclonal and polyclonal antibody based tests come in the form of one inclusive single device, similar to a pregnancy test, and provide results within ten minutes and are quite sensitive. In addition, these devices are small and easily portable so that the assays can be performed at the crime scene. The procedures for these tests are fairly simple to follow, and the results are very easy to interpret. Immunochromatographic tests thus provide a convenient and versatile method in forensic casework.

The goal of this research was to obtain DNA profiles from immunochromatographic test devices which have already yielded positive or negative results with body fluids such as blood and saliva using Polymerase Chain Reaction method (PCR). The present research involved body fluid samples from 14 male and four female donors. Three different immunochromatographic cards for the identification of human blood and one for the identification of human saliva were used to confirm the presence of human blood and human alpha amylase. Two of these cards used for confirmation of human blood are also known to react with blood from ferret.

Each body fluid was detected using the appropriate immunochromatographic card. The used cards were kept at room temperature for various lengths of time. The membranes were removed at the end of the designated times and the entire strip was extracted using a low copy number (LCN) extraction procedure. The extracted DNA was purified and concentrated using a Microcon® 100 device, and quantified using the Applied Biosystems (AB) Quantifiler™ kit on the AB 7500 Real Time PCR System. The extracted DNA was amplified using a reduced amplification volume and higher PCR cycle numbers for both the AB AmpFlSTR® Identifiler™ and AmpFlSTR® Yfiler™ kits.

While the best results were obtained when membranes were extracted at the end of one week, it was possible to obtain complete STR DNA profiles from most of the cards which were stored at room temperature for almost three months. These profiles were consistent with the profiles obtained from the donors' reference samples. Figure 1 is the Y-STR DNA profile obtained from a RSID-Blood immunochromatographic card. It is consistent with the DNA profile obtained from the donor's reference buccal swab (Figure 2). DNA profile was obtained from all of the three types of immunochromatographic cards used in this study to confirm the presence of human blood and from one type of device which detects human alpha amylase.

Given these results, when evidence samples are limited in size an analyst may confirm the presence of a blood and saliva and identify the donor of a particular body fluid by performing DNA analysis using only the immunochromatographic cards. This procedure could also be used should there be a question about the presence of ferret blood at the crime scene. These immunochromatographic cards can be used to determine the identity of the human donor by performing DNA analysis from the same cards.

 ** Presenting Author*

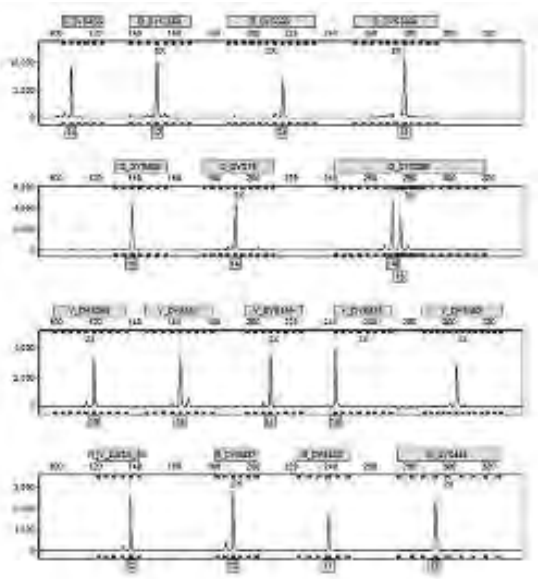

Figure 1: Y-STR DNA profile generated from a two month-old RSID™-Blood Card

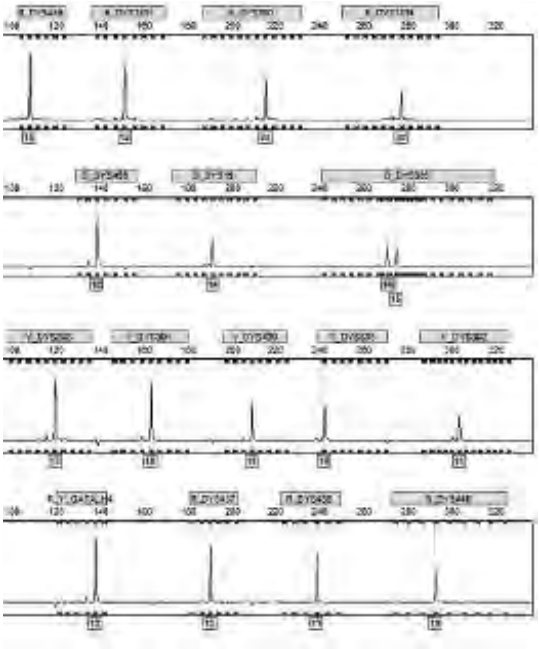

Figure 2: Y-STR DNA profile generated from the donor's reference buccal swab

DNA, LCN Methodology, STR DNA

 ** Presenting Author*