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Validating Expert Systems: Examples with the FSS-i3™ Expert Systems Software

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INTRODUCTION

Validation of new techniques in a forensic laboratory is a critical component of a laboratory's quality assurance program, helps ensure accurate results and demonstrates the reliability of the test method for forensic casework. Recommended standards for the forensic community were adopted by the FBI Director pursuant to the DNA Identification Act of 1994 (1) and have addressed validation and its definition (see inset) (2). These standards apply to crime laboratories that submit DNA results to the FBI's National DNA Index System or accept certain federal grants. Since publication of these standards, forensic laboratories have been performing mostly internal validation of chemistry kits and instruments, while manufacturers of these kits and instruments typically have performed developmental validation. Many forensic laboratories have assisted in the developmental validation of different methods, but usually the forensic community relies on the manufacturer to compile and publish the developmental validation.

Validation is a process by which a procedure is evaluated to determine its efficacy and reliability for forensic casework analysis and includes:

1. Developmental validation is the acquisition of test data and determination of conditions and limitations of a new or novel DNA methodology for use on forensic samples.
2. Internal validation is an accumulation of test data within the laboratory to demonstrate that established methods and procedures perform as expected in the laboratory.

Validation definitions published by the DNA Advisory Board (2).

DEVELOPMENTAL VALIDATION OF EXPERT SYSTEMS

Validation of an expert system is an important component of a laboratory's quality assurance program. In contrast to validation of new techniques in a forensic laboratory as required by the DNA Advisory Board (2), a sponsoring laboratory, and not the manufacturer, must perform the developmental validation (3). The system, or complete set, consists of the software version of the expert system (e.g., FSS-i3™ Expert Systems Software, version 4.2, with GeneMapper® *ID*, version 3.2.1), along with the chemistry (e.g., PowerPlex® 16 System) and the instrumentation with the corresponding version of data collection software (e.g., Applied Biosystems 3130*xl* Genetic Analyzer, Data Collection Software, version 3.0).

At this time, the developmental validation of four complete sets has been approved for use in the National DNA Index System (NDIS; see Table 1) (personal correspondence with Douglas Hares, Ph.D., NDIS Custodian). For the sponsoring laboratory to perform the developmental validation, it must follow Section 8 of the Quality Assurance Standards for Convicted Offender DNA Databasing Laboratories (2) and any other CODIS requirements for acceptability of the data. Further, the laboratory shall use employees of their laboratory who are not provided by or affiliated with the expert system vendor. The process of performing developmental validation of an expert system is an excellent exercise for any team. Whereas many analysts have stated that they are waiting for others to perform the developmental validation of the complete set in which they are interested or own in their laboratory, they may want to reconsider the wait. The collection of required challenges is reasonable; the required challenges encourage the analyst to thoroughly evaluate the software and understand the software's rule firings, and in the process, the analyst becomes the expert.

The goal of the National Institute of Justice's Expert System Testbed (NEST) Project was to evaluate, not validate, different expert systems to educate managers and scientists in the forensic community and provide ample information so each one is a more informed consumer of the software systems. Even though it was not the

Table 1. Complete Sets Where Developmental Validation has been Performed, Submitted and Approved by the NDIS Board (Updated July 2007).

Expert System and Version(s)	Manufacturer	Instrument Platform(s)	Kit(s)
FSS-i³™ version 4.0.2 using GeneMapper® *ID* version 3.2	The Forensic Science Service and Applied Biosystems	Applied Biosystems 3700 (Data Collection Software version 3.1.1)	Identifiler®
FSS-i³™ version 4.1.3 using GeneMapper® *ID* version 3.2	The Forensic Science Service and Applied Biosystems	Applied Biosystems 3130*xl* (Data Collection Software version 3.0)	Identifiler®
TrueAllele® version 2.7.348	Cybergenetics	ABI PRISM® 3100 (Data Collection Software version 1.1)	Profiler Plus™ and COFiler®
TrueAllele® version 2.9	Cybergenetics	ABI PRISM® 3100 (Data Collection Software version 1.1)	Profiler Plus™ and COfiler®

goal of the NEST Project to conduct a thorough validation of the expert systems, the steps taken to evaluate the different systems were the same. The NEST Project Team evaluated three expert systems using four different chemistry kits on a multicapillary instrument (for a total of 12 complete sets), an evaluation of more than 45,000 individual allele calls. Obviously, we gained a lot of experience through this process.

All combinations of DNA-typing kits and instrument platforms are considered different when performing validation of an expert system. An ABI PRISM® 310, 3100 or 3100-*Avant*™ Genetic Analyzer, Applied Biosystems 3130 Genetic Analyzer and Applied Biosystems 3730 DNA Analyzer are all considered different instrument platforms. In a recent survey of the NDIS-participating laboratories, 15 different sets (i.e., chemistry and instrumentation) will need to go through developmental validation. This number does not correspond to the different complete sets (i.e., the three components: version of expert system software, chemistry and instrumentation). If one laboratory chose to use the 3130*xl* Genetic Analyzer, PowerPlex® 16 and FSS-i³™ Expert Systems Software, and another laboratory chose the same instrument and chemistry but a different expert system, each of these complete sets must go through a unique developmental validation study.

Developmental validation of the expert system and its accompanying chemistry and instrument is not as difficult as the term may infer, but it is an excellent exercise to understand and define the parameters of the expert system for your laboratory. The analyst becomes the expert when performing developmental validation. The individual performing the developmental validation will understand the software and its different features at a more advanced level than if he or she just performed the internal validation or only became a user of the expert system. Further, he or she will better understand the rule firings during the analyses, required optimization processes and defined marker ranges.

When performing developmental validation for an expert system for NDIS, a unique set of 200 samples for calibration of the system and a set of 1,000 unique samples analyzed with the current genotyping system for concordance are to be used.

Different observed results, or challenges, need to be evaluated by the system in the validation process (3): stutter, locus peak amplitude imbalance (Figure 1), artifacts [pullup/bleedthrough, shoulders (+A and –A), spikes], peaks [tri-allelic, mixture, contamination (Figure 2)], off-ladder alleles [microvariant allele (Figure 3) above or below ladder] and missing

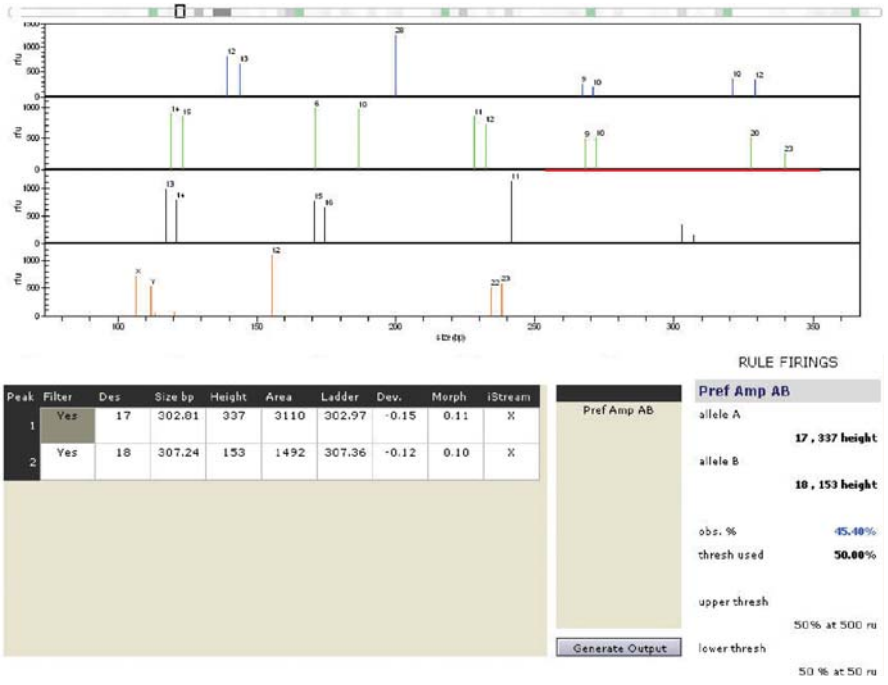

Figure 1. Locus peak amplitude imbalance. The challenge presented in this sample is locus peak amplitude imbalance. The peak height ratio is set at 50%; the FSS-i³™ Expert Systems Software has accurately identified the results in D18S51 (highlighted by the red bar above the locus) to have a peak height ratio, or Preferential Amplification AB (Pref Amp AB), below the set point.

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loci. The minimum number of five examples required for each challenge is practical and easy to find.

There are different things to consider when preparing for validation. For example, identify and retain samples that have demonstrated these different challenges. Consider transferring amplicons of the identified samples with challenges into another plate and analyze together. Thus, many challenges will be in a single run.

This course of action was executed with a data set of 288 samples processed together on several plates instead of dozens of plates. In so doing, this sample set produced 19 tri-allelic peaks, 157 off-ladder alleles (102 microvariant alleles and 55 above or below the allelic ladders), as well as stutter, locus peak amplitude imbalance and artifacts. Implementing this procedure, or one similar to it, may aid the analyst in obtaining the requisite challenges for developmental validation.

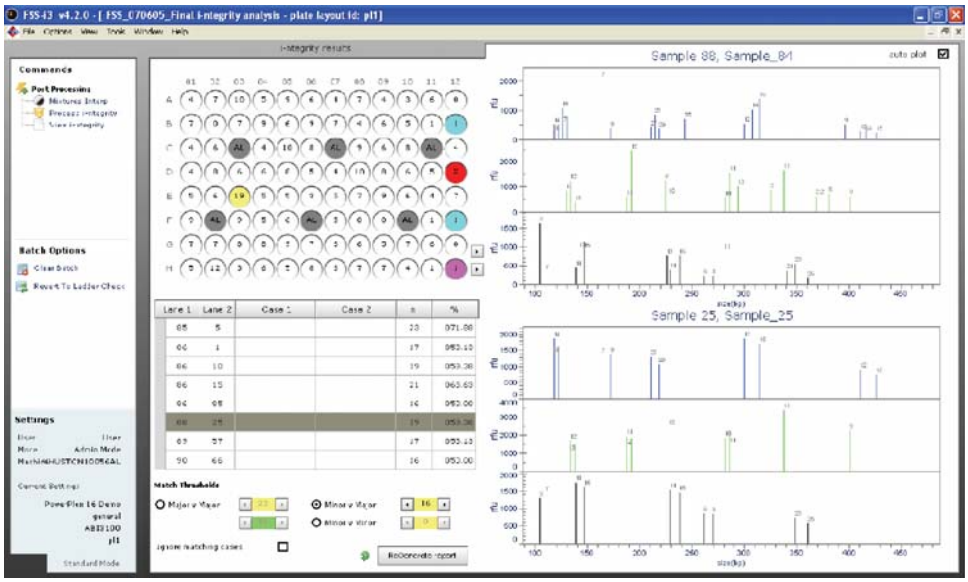

Figure 2. Contamination. The FSS-i3™ Expert Systems Software has a feature called i-ntegrity. i-ntegrity evaluates all samples in a batch for well-to-well contamination. Three different match criteria compare peaks: major-to-major; major-to-minor; and minor-to-minor. The three different match components can be used to evaluate splash over, general contamination from an analyst and duplicate samples.

CONCLUSION

In summary, performing validation is a critical component of a laboratory's quality assurance program. The validation process is an excellent step in evaluating an expert system as a component of the complete set and adopting it into the laboratory's workflow. Application of the different expert systems can revolutionize forensic DNA databanking and casework sample analysis (4). The different expert systems accurately assign allele calls with ease and speed and identify different challenges in the data (5).

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The opinions in this paper are those of the authors and do not necessarily reflect those of the National Institute of Justice.

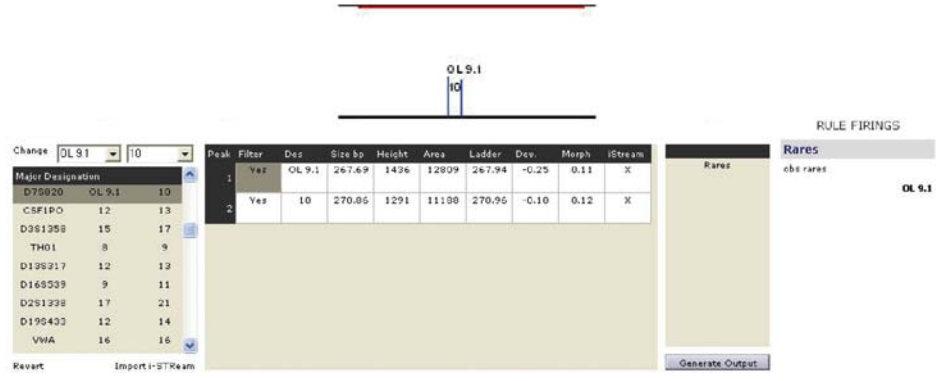

Figure 3. Microvariant. The result presented in this sample is an off-ladder microvariant. The FSS-i3™ Expert Systems Software has accurately identified a "Rares" D7S820 allele, OL 9.1.

REFERENCES

1. DNA Identification Act of 1994, Violent Crime Control and Law enforcement Act of 1994, Title 42, Chapter 136.
2. DNA Advisory Board (2000) Quality assurance standards for forensic DNA testing laboratories and for convicted offender DNA databasing laboratories. This can be viewed online at: **www.fbi.gov/hq/lab/fsc/backissu/july2000/codispre.htm**
3. Butler, J. (2006) Debunking some urban legends surrounding validation within the forensic DNA community. *Profiles in DNA* **9**(2), 3–6.
4. Roby, R.K. and Jones, J.P. "Evaluating expert systems for forensic DNA laboratories," (2005) *Forensic News*, Applied Biosystems.
5. Roby, R.K. *et al.* "The National Institute of Justice's Expert Systems Testbed Project." In: *Proceedings of the Sixteenth Symposium on Human Identification 2005*, Promega Corporation.