

# AMPLITIMES

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As we begin a new year, we are reminded of the strides made in detecting diseases as well as the further research that is needed. Idaho Technology, Inc. (ITI) is pleased to be a part of the ongoing research effort to better detect and diagnose diseases.

In this issue of *Amplitimes*, we look at the progress ITI is making in the real-time detection of infectious disease such as avian influenza. We also examine the advantages of melt profiling in newborn screening using ITI's LightScanner™.

## Real-Time Detection of Avian Influenza

The recent emergence and quick spread of the highly pathogenic H5N1 strain of avian influenza has increased the need for real-time detection. As of December 2006, the World Health Organization has confirmed infection in 258 humans with a mortality rate of 60%. Hundreds of millions of birds have been culled to combat the spread of the disease and to minimize human exposure to infected birds. While these measures help slow the spread of the disease, the possibility still exists that it will mutate into a form that is easily transmissible between humans. Should this happen, it is expected that millions of deaths would occur worldwide, with the majority occurring in developing nations.

Idaho Technology is a leader in real-time pathogen identification. To aid in the identification of this emerging disease, ITI has developed a set of assays designed to identify the H5N1 influenza strain. Two assays were designed against conserved regions within the H5 hemagglutinin subtype: one assay for each of the HA1 and



Avian influenza virus

HA2 regions within the H5 gene segment. These assays are being validated externally and both have been shown to positively identify all H5N1 viral isolates from infected humans (N=12) as well as all H5N1 viral isolates from infected birds (N=14). The validation of these H5 assays is currently in progress and will include specificity testing against non-H5 viral isolates. Both assays are currently available for purchase from ITI.

Idaho Technology has also developed a general influenza surveillance assay that targets a gene segment that is conserved across all influenza A subtypes in both humans and birds. This has been tested internally and was able to positively identify influenza A in all samples (N=12). Internal validation also included specificity testing against a panel of other respiratory viruses which present with similar symptoms (influenza B, parainfluenza, Human metapneumovirus, respiratory syncytial virus [RSV], Human rhinovirus). This assay will also undergo external validation that will include sensitivity and specificity testing. It is anticipated that this general influenza surveillance assay will be available for purchase from ITI this winter.

These assays are available from ITI in multiple formats for the R.A.P.I.D.® System. They can be purchased individually as well as in a kit format (i.e., influenza A and H5). Fluorescence resonance energy transfer (FRET) versions of both assays will also be available for the RAZOR® platform in pouches. Please contact the ITI sales office at (801) 736-6354 for details.

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## LightScanner Melt Profiling in Newborn Screening

Results of biochemical genetic assessments used in prospective newborn metabolic disease screening (tandem mass spectrometry [MS/MS], enzyme assays, quantification of metabolites, etc.) may be confirmed using second tier molecular genetic analysis. Adaptation of means to perform genetic testing in newborn screening (NBS) labs has been technology driven. Initially, particular mutations were analyzed using techniques such as allele specific cleavage, reverse dot blot, and fragment size analysis. Now, melt profiling, provided by ITI's LightScanner and associated reagent kits, can provide a means by which to standardize and simplify gene analysis in NBS laboratories. Melt profiling, using saturating dsDNA binding dye, may be used with an oligonucleotide probe to interrogate genotypes or in a probe-free application using amplicon that tightly flanks the variant of interest.

MS/MS identifies medium chain acyl-CoA dehydrogenase (MCAD) deficiency through elevation of C6, C8, C10, and C10:1 acylcarnitines. Genetic follow-on analysis is often used to confirm the MS/MS result as acylcarnitine profiles among MCAD-affected patients can be highly variable. Figure 1 uses a multiplex short amplicon analysis to assess the common c985A>G mutation and the c199T>C variant that is frequently associated with mild MCAD deficiency identified by MS/MS. Note that all three genotypes are observed at the c985 site (A/A, G/G, A/G) and that a

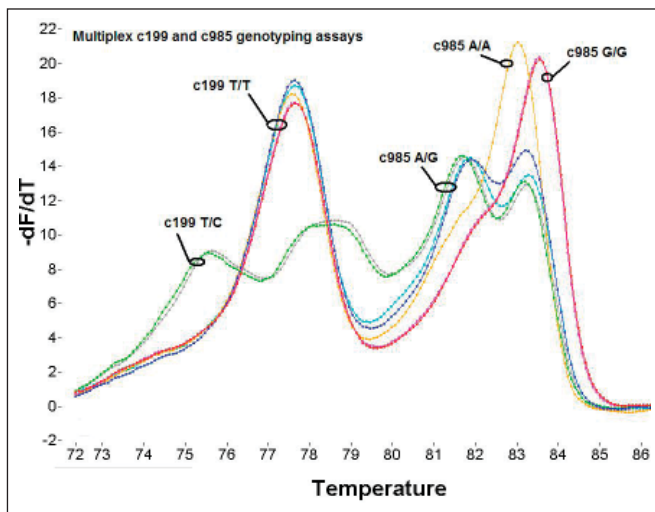


Figure 1. Common c985A>G mutation and c199T>C variant associated with mild MCAD deficiency identified by MS/MS.

compound heterozygote c985A/G c199C/T is also identified. Multiplexing reduces both cost and set-up time while probe-free analysis reduces complexity.

In Figure 2, the sickle cell disease S and C mutations are assessed using an unmodified oligonucleotide probe where fluorescent signal is provided by the saturating dsDNA binding dye. The oligonucleotide matches the S allele and thus has a 1 base pair mismatch to the wild type and a 2 base pair mismatch to the C allele. Note in Figure 2 how the S allele (fully base-paired probe) generates a signal with the highest  $T_m$  while the wild type has an intermediate  $T_m$  and the C allele has the lowest  $T_m$ . Shown in Figure 2 are the following genotypes: S/S, A/S, A/A, A/C, and S/C.

Other assays to support NBS will include sickle cell E, b-thalassemia mutations, long-chain 3 hydroxyacyl CoA dehydrogenase c1528 G>C, isovaleric acidemia A282V, pseudogene derived congenital adrenal hyperplasia mutations, galactosemia mutations, and cystic fibrosis mutations. To further streamline analysis to reduce turn-around time, these assays are being developed in an easy-to-prepare freeze-dried format. Essentially all reagents for NBS (MS/MS standards, IRT kits, DELFIA® kits) are well standardized, and preparing genotyping assays in a standardized format would be beneficial to NBS as well.

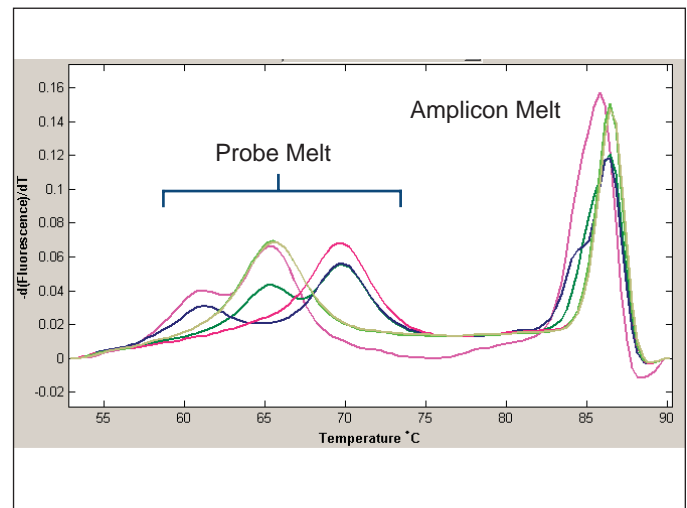


Figure 2. Sickle cell disease S and C mutations using an unmodified oligonucleotide probe.

## Effective OEM Communication

Original equipment manufacturing (OEM) is a lot like having a house built. Just as a homeowner enlists a builder to build a home, so does a customer enlist a manufacturer to manufacture a product. When meeting with the builder, decisions based upon the customer's wants and budget limitations are made. The same is true when a customer meets with a potential manufacturer. Decisions about the following specifications and costs need to be made:

- Labor and overhead
- Reagent raw materials
- Primary and secondary packaging
- Any required capital equipment purchases
- Additional staffing needs
- Shipping
- Licenses and royalties
- Non-inventory items
- Any materials supplied by the customer
- The difficulty and reproducibility of the manufacturing process
- Specific regulatory requirements, e.g., bioburden

Open communication between the customer and manufacturer is essential during this process. Often, after the customer and manufacturer first meet, the need for product secrecy slows the flow of information: customers speak in generalizations and manufacturers are reluctant to disclose intellectual property issues and manufacturing methodologies.

There are a couple of solutions to this dilemma. One is for both parties to sign a confidentiality agreement, and the other is for both to sign nondisclosure agreements.

Confidentiality agreements are customary with many companies, thus requesting and agreeing to an agreement should become standard practice. Nondisclosure agreements are also useful since the customer will eventually have to transfer detailed product specifications, formulations, and manufacturing forecasts to the manufacturer.

No matter what agreement customers and manufacturers make, keeping communication channels open ensures both parties know what to expect from the final product and its associated costs.



## Photo of the Quarter

Skiing Greeley Bowl,  
Alta Ski Resort  
(skier: Rich Abbott  
photographer: David Nielsen)

## Dates to Remember

### January

- 13–17** Plant and Animal Genome XIV  
San Diego, California  
[www.intl-pag.org](http://www.intl-pag.org)
- 28–31** International Forum  
Process Analytical Technology  
Baltimore, Maryland  
[www.ifpac.com/onsite](http://www.ifpac.com/onsite)

### February

- 25–3/2** Society of Armed Forces Medical  
Laboratory Scientists  
Boston, Massachusetts  
[www.safmls.org](http://www.safmls.org)

### March

- 6–8** Food Safety Summit  
Washington, D.C.  
[www.foodsafetysummit.com](http://www.foodsafetysummit.com)

### April

- 14** American Association for Cancer  
Research  
Los Angeles, California  
[www.aacr.org](http://www.aacr.org)
- 16–21** Fire Department Instructor's Confer-  
ence  
Indianapolis, Indiana  
<http://fe.pennnet.com/resource/fdic>

**Editor's Note:** If you have comments or suggestions for articles, please e-mail the editor at [loretta\\_organ@idahotech.com](mailto:loretta_organ@idahotech.com).

**Department of State Note:** The R.A.P.I.D. System and RAZOR Instrument are controlled for export under the International Traffic in Arms Regulations (ITAR), administered by the U.S. Department of State, Directorate of Defense Trade Controls (DDTC) and may not be exported or transferred to any foreign national without prior approval of the DDTC.

## R.A.P.I.D.® and RAZOR® Systems Training

ITI offers training courses for the R.A.P.I.D. and RAZOR systems. Training for two people is included with the purchase of the R.A.P.I.D. or RAZOR instruments, and more can attend for an additional cost. The training courses are three days for the R.A.P.I.D. and one day for the RAZOR. Courses focus on concepts of operation, sample preparation, reagent setup, and software. If you would like to attend or schedule a training course, please contact our training staff at 1-800-735-6544 x. 439.



## LightScanner Webinars on the Internet

The following webinars can be accessed via [www.idahotech.com/LightScanner/webinars-LS.htm](http://www.idahotech.com/LightScanner/webinars-LS.htm):

*Screening Genetic Variants Associated with Dyslipidemia using the LightScanner System*

*High Throughput Genotyping Using Unlabeled Oligonucleotide Detection Probes*

*Somatic Mutation Detection in Tumor Samples by Hi-Res Melting™ Technology*

*The LightScanner System: Achieve High Throughput Mutation Discovery and Gene Scanning*

If you have any questions about the topics of these webinars or would like more information, please contact Rachel Jones at [rachel\\_jones@idahotech.com](mailto:rachel_jones@idahotech.com) or (801) 736-6354 x. 438.



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