Agilent case study: Kevin Legg, Ph.D.

Solving Challenging Problems

Agilent aids research in forensic science

Research scientists tend to relish a challenge. Kevin Legg certainly does.

"Any sort of really nasty problem, like a challenging matrix or unusual sample types—that sounds like fun to me," he says.

Worthy examples are evident in his work at the nonprofit Center for Forensic Science Research and Education (founded by NMS Labs), where Legg is involved in projects with the National Institute of Justice, Department of Defense, the education of forensic professionals, and teaching forensic classes at nearby universities.

Automation plays a key role in two of his major projects—insulin characterization in vitreous humor, and body fluid identification in sexual assault cases. Both projects have challenges that automation helps to overcome.

The need for reproducibility

The first project demanded developing a workflow for characterizing insulin and pharmaceutical analogs, compounds that have been implicated in cases of murder, suicide, and mercy killings. The major challenge is differentiating the various formulations available, an application that is not possible with traditional ligand-binding assays.

"We're the only lab in the Americas who can offer a test for the medical examiners that can differentiate the assorted compounds," Legg says.

"Manual workflows tend to be labor-intensive, and there are problems with reproducibility and repeatability. Challenges that we have solved with automation."

His improved workflow—using Agilent AssayMAP automated sample prep and an Agilent triple quadrupole LC/MS system—has moved beyond the research phase, and is now being used to generate investigational reports for medical examiners.

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"This was a tough project. It took our group several years to get it right," Legg says. "We tried SPE protocols, nano and high-flow 2D-LC/MS, manual immunoprecipitation with magnetic beads, and so on. While these strategies did generally produce acceptable results, we had concerns in terms of the robustness of the workflow. It's one thing to develop a research assay, quite another to create a production-capable solution that can be run by a technician versus PhD-level scientist which stands up to the rigors of validation and ultimately scrutiny from the justice system."

The AssayMAP Bravo automation platform was introduced to achieve the necessary assay robustness for a reproducible workflow. Instead of depending on magnetic beads, AssayMAP Bravo performs affinity purification using powerful micro-chromatography technology that provides consistent binding and low-volume elution. Manual sample prep steps are eliminated, allowing reproducible and easy processing of large numbers of samples.

"I don't think the project ever would have been finished/viable without the automation system," Legg says. "Most of the manual prep workflows are limited to approximately two dozen samples per batch, and if I need to run a calibration curve, controls, and sample blanks, that's 12 samples alone. That's certainly cumbersome—and not at all practical when a typical batch can include a dozen cases as well, or a comprehensive bioanalytical validation study could require upwards of 1,000 injections! With the AssayMAP automation system, we run 96-well plates and shift our focus to other analytical tasks."

The need for speed

The screening of sexual assault cases can involve hundreds of samples, and requires speed.

"What we're trying to do is characterize body fluids that are found on evidentiary material at a crime scene—on cans, bottles, bed sheets, or sexual assault kits. For example—using a panel of protein biomarkers to confirm the presence of vaginal fluid, menstrual fluid, saliva, or seminal fluid," Legg explains. "That's important because it provides additional context, the source or individualization of the DNA sample, that DNA alone cannot provide.

It is possible to identify body fluids using traditional methods such as microscopy to identify cells, but would it be practical? "If you need to analyze several hundred sexual assault swabs via a slide search, that's an enormous investment of time and resources," Legg says.

This is why his approach takes advantage of the Agilent AssayMAP Bravo automated liquid handling platform.

"What we're trying to do is essentially high-throughput proteomics," Legg says. "Instead of performing microscopy on 100 samples, you can take a small aliquot of liquid extract into a 96-well plate, put that into the AssayMAP, which is going to perform a traditional protein digestion and SPE cleanup, and then place the 96-well plate directly into the MS for analysis. The workflow is nearly hands free, significantly faster, more cost-effective, and certainly more accurate. With the automated solution, it's simply a matter of following the SOP, or standard operating procedure."

Legg's research into body fluid identification—a 10-year project—appears to be nearing fruition, and could prove to be a vital supplement to DNA in a court of law.

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