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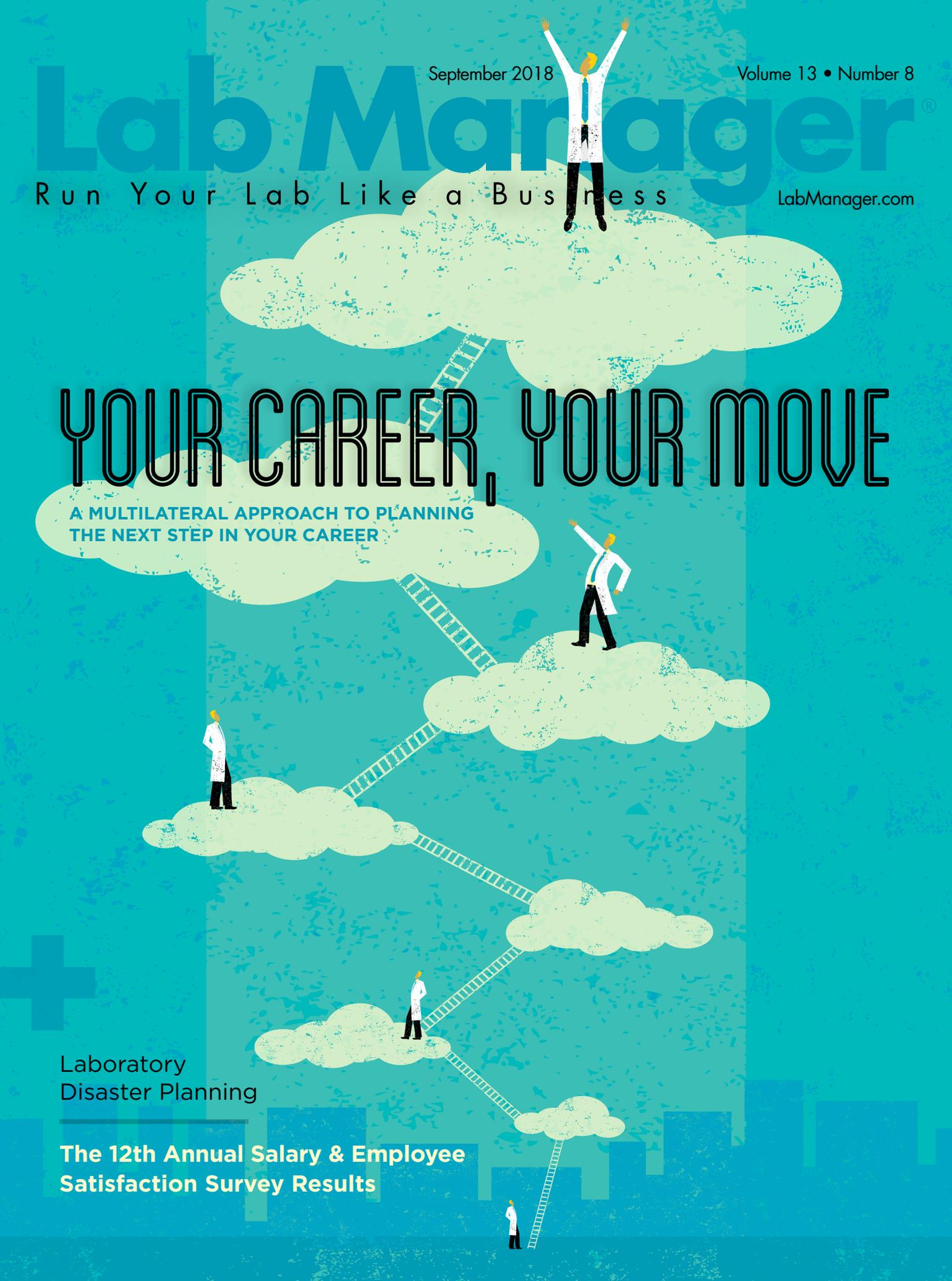
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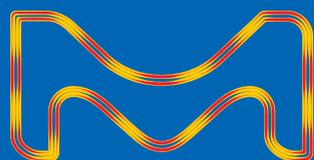
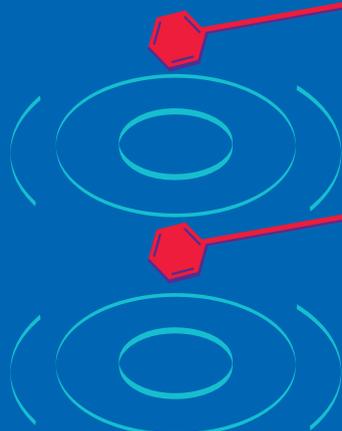
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2019 EDITORIAL VISION

As we approach the final few months of the year, *Lab Manager's* editorial team is finalizing topic ideas for the 2019 editorial calendar—and now is the time to give your input! Is there a specific topic you want to learn more about related to business, staffing, health and safety, or technology? If you have a recommendation for a unique lab to feature in our Labs Less Ordinary series, or want to see a totally new topic covered in one of next year's issues, please email Pam Ahlberg, editor-in-chief at: pama@labmanager.com. We welcome any and all feedback that will help us continue to improve our editorial offerings.

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"An occupation undertaken for a significant period of a person's life and with opportunities for progress."

Whether you're perfectly content in your current career or you're looking for change, this month's issue is brimming with information to help you assess your situation and develop strategies for getting ahead. Beginning with the cover story, in which author Donna Kridelbaugh lays out suggestions for better positioning yourself within your organization and offers a boatload of practical advice for improving your skill set to meet future career goals.

If you are one of those "perfectly content" types, you're not alone. This year's Annual Salary and Employee Satisfaction Survey again paints a happy picture of your scientific cohort. Interestingly, a large percentage of survey participants ascribed their job satisfaction to their organization's culture. "Nearly 60 percent of respondents said corporate culture—defined as an organization's goals, strategies, structure; and approaches to labor, customers, investors, and the greater community—was 'very important' to their overall job satisfaction," says author Lauren Scudato.

For those starting out in their careers, a critical factor in their success is a strong mentoring program. This month's Leadership and Staffing article, "Successful Mentoring," looks at two mentoring programs. One, at Argonne National Laboratory, has had a particularly positive impact on those who participate—both mentors and mentees. What began as a grassroots effort by postdocs has evolved into a formal program for nurturing scientific staff. Among its many benefits, "Individuals are enhanced by feeling

valued and part of an organization that is taking the time to invest in their success," says Dr. Kristene "Tina" Henne. Turn to page 24 to learn more.

This month's analytical expert, Dr. Bryan Tomlin, weighs in on his role at Texas A&M University Center for Chemical Characterization and Analysis and the importance he places on helping students become better prepared for future career success. "I want to teach [students] to be better consumers of analytical chemistry—understand trade-offs, data quality, etc. ... so as they go off and graduate and find jobs, they'll make better use of the analytical tools that they will need in their careers," says Tomlin.

As we head into hurricane season, you would be well served to check out this month's "Laboratory Disaster Planning" (page 36), to learn the hard-earned lessons author Tracy Wieder did from 2001's Tropical Storm Allison, which devastated the lab she managed at The University of Houston Medical School in Houston, Texas.

Involved in cannabis testing, method validation, or clinical genomics? Those topics, and more, are also covered in this month's *Lab Manager*.

Enjoy.

Best,
Pam

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YOUR CAREER, YOUR MOVE

A MULTILATERAL APPROACH TO PLANNING THE NEXT STEP IN YOUR CAREER by Donna Kridelbaugh

When you're a lab manager, it can be challenging to find the time to work on your own career development. From balancing company priorities to effectively mentoring staff, it's easy to overlook your own needs in the process. And while we'd like to think employers have our best interests at heart, they ultimately will do what's best for the company's bottom line. That's why it's so important to always look out for your future career self. One strategy to help empower your career development is to adopt a contractor mind-set. From this perspective, you will be able to continually evaluate career opportunities, identify ways to keep your skills relevant, and take a proactive approach to managing your career.

Adopting a contractor mind-set

Much can be learned from the work traits of an independent contractor. Contractors must take ownership of their own career development and stay abreast of the latest trends and required skills in their field or risk being unemployed. They know their self-worth and what unique services they can offer to clients. Additionally, they are always on the lookout for new opportunities and have the flexibility to quit and move on to a new contract if a project is not challenging or is in an unfavorable work environment.

Thus, there is a lot of career autonomy that comes with shifting to a contractor mind-set. It will help you stay flexible and open to new career opportunities that come along. And during times of outsourcing and downsizing among the science and technology workforce, it allows you to easily identify alternative employment options (e.g., consulting) during any career-transition periods, instead of being left unemployed or taking a job that is less than desirable. And just as important, it will help you stay focused on your long-term career goals, because you are always strategically planning the next step in your career.

Overall, thinking like a contractor will help you build the confidence you need to

make your career interests a priority and advocate for the resources you need to do your job better. In taking this approach in evaluating your contributions as an individual employee, you will be able to focus on what value your skills and expertise add to the company and regularly communicate that to your supervisors.

A couple of activities that will help you develop a contractor mind-set include assessing your career outlook and acquiring the skills needed for the future. Here are some tips on how to do both. In the end, your future

“It pays to gain foresight into how the science and technology job market may change over time.”

Mighty. Small.

career self will thank you for having invested the time now to set yourself up for career success.

Assessing your career outlook

In the long run, it pays to gain foresight into how the science and technology job market may change over time. Hiring trends fluctuate with workforce demands, technological advancements, and funding availability. Thus, lab managers need to stay on top of these trends to make good career decisions.

Fortunately, there are freely available resources to help you regularly evaluate your position for growth potential or the possibility it may become obsolete in the future. In terms of forecasting workforce needs, the U.S. Department of Labor produces a number of useful online resources to track labor market trends. For example, the U.S. Bureau of Labor Statistics maintains the *Occupational Outlook Handbook*, which provides valuable insight on career outlooks (e.g., highest-paying and fastest-growing jobs) across various industry sectors.¹

Another approach is to research where people currently are finding jobs. A number of academic institutions, government agencies, and nonprofit coalitions publish reports related to the career outcomes of scientists (e.g., number of degrees awarded, workforce demographics). An example is the National Science Foundation's Science & Engineering Indicators biennial report.² The National Institutes of Health provides similar reports related to the biomedical research workforce.³ Also, on its website, the nonprofit Future of Research curates a collection of resources related to U.S. institutional career outcomes.⁴

There also are multiple ways to find out who currently is hiring in your field. You can browse through science job boards to see what types of positions are being advertised. Recruiters are another good source of information, as they are in touch with employers' staffing needs and the latest in hiring trends. You can reach out to recruiters directly by contacting recruiting agencies, on networking sites like LinkedIn, or at career fairs and other hiring events. Many recruiting agencies and individual recruiters also publish regular content on hiring topics.

Another item that you may want to add to your research list includes trade publications for your specific industry, which often report on changing labor force trends and survey readers about employment factors (e.g., salary, job satisfaction). For



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example, check out the results from the latest *Lab Manager* Annual Salary and Employee Satisfaction survey in this issue.

Additionally, staying updated on R&D funding news (e.g., grant awards, capital investments) is a proactive way to find out where money is being funneled and thus where potential jobs may be in the future. This information can be found by following relevant companies, foundations, and government agencies on social media to stay apprised of the latest press releases and news related to funding announcements and recent awards.

By taking a multilateral approach to assessing your career outlook, you will be better prepared to make smart career moves and avoid getting stuck on a career route that may be a dead end. This information also will help you determine what you need to do (e.g., acquire skills) to get there.

Acquiring the skills needed for the future

As the science and technology workforce is constantly evolving, so are the qualifications required to fulfill the employment needs of the future. Thus, it's important to continually do a personal skills analysis and identify any additional training you may need to acquire. Overall, the process simply

involves asking yourself where you are now in your career development and what skills are needed to get you where you want to be.

One way to do this is to look at job postings for career paths of interest and review the list of qualifications for key knowledge, skills, and abilities required in that role. You can also conduct informational interviews with professionals who are working in positions of interest. These interviews are informal (e.g., meet for a cup of coffee) and include a casual conversation to find out more about what the person does, how they got there, and any suggestions they may have for making a similar career transition.

Another method for skills identification is to attend trade shows and talk to vendors to stay updated on new technologies that may require additional training. Further, you can take a look internally to see what skills are missing in your own organization and work toward filling those gaps to improve your chances for career advancement and job security.

Collectively, this information can be used to update an individual career-development plan and develop a strategy and timeline for skills acquisition. If you don't have such a plan already or want to create one independent of your employment setting, there are a number of templates available online. One free online application is myIDP from Science Careers.⁵

Once you make a plan, the work begins to find training programs and other options that will fit your needs. First, look to see what employer-sponsored education benefits may be available through your current position. This may include in-house training programs or tuition reimbursement programs. Additionally, there may be the option to rotate through other departments. Other ways to gain needed experience include volunteering for special projects or task forces at work. You likely will need to get manager buy-in to approve any training or time spent on other projects, so be prepared to show how it will benefit the company and tie in to any performance metrics, if possible.



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But if your employer won't pay for training or you are looking to gain skills outside the scope of your current position for a career transition, you have lots of options that you can do on your own time and are affordable. For example, you may consider completing an education program that is designed for working professionals (e.g., competency-based curriculum) or industry-standard certifications offered through trade associations. There also are lots of quality online courses available through platforms like Coursera, and many provide the ability to create individualized programs and certificate options.

Attending workshops at research conferences is another great way to learn new skills in a condensed format. For alternative funding sources to finance your conference attendance, look into options to volunteer (e.g., serving as a poster judge, writing conference abstracts) in exchange for having the registration fee waived. There may be travel grants available through your professional society memberships as well. Additionally, look for any virtual conferences being held, which reduces travel expenses, many of which are free.

And it's never too late to get more hands-on science training. For example, a number of research institutions, foundations, and government agencies offer career transition fellowships and development awards for scientists. As an example of such programs, check out the Harvard School of Public Health's Office for Career Advancement website, which lists programs in the areas of public health and health care.⁶

Donna Kridelbaugh holds an advanced degree in microbiology and is a former lab manager. Connect with her on Twitter (@science_mentor) and visit her website at <http://ScienceMentor.Me>.

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The Twelfth Annual Salary and Employee Satisfaction Survey

CAREER SATISFACTION REMAINS STRONG AMONG LABORATORY PROFESSIONALS
 by **Lauren Scrudato**

For the 12th Annual Salary and Employee Satisfaction Survey, we're taking a fresh look at recurring trends from recent years and discussing new observations this year to provide insight into the careers of laboratory professionals. After reviewing close to 1,000 responses, some of the key points we observed in this year's survey results include slight changes in the types of labs where respondents work, the baby boomer generation still dominates much of the workforce, and employee loyalty remains strong.

Demographics

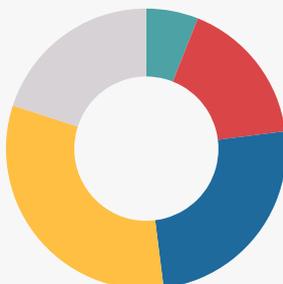
As in years' past, survey respondents continue to represent a diverse variety of industries, from agriculture and food to analytical chemistry, the environment, molecular biology, and others.

But unlike last year's survey, which reported a 6 percent decrease in those working in clinical labs compared to the year prior, this year's clinical professionals show a steady bounce-back—representing 10.5 percent of all respondents. Similarly,

GRAPHS: A snapshot of our survey respondents

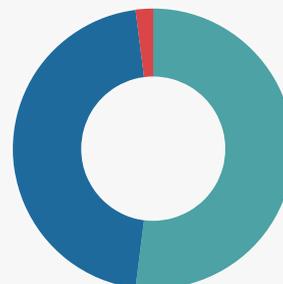
AGE

- Under 30 **6%**
- 30 – 39 **17%**
- 40 – 49 **25%**
- 50 – 59 **32%**
- 60+ **20%**

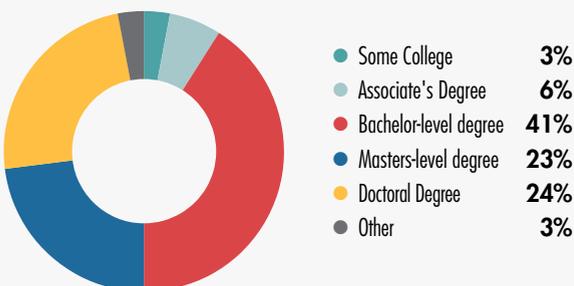


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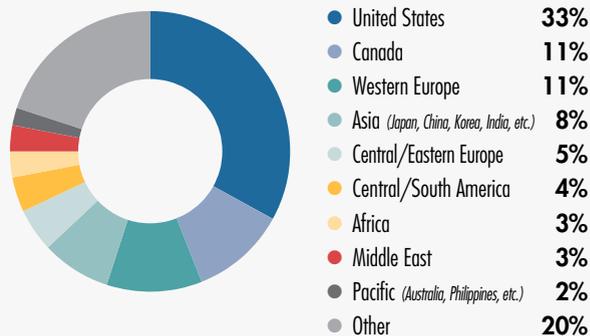
- Female **52%**
- Male **46%**
- Prefer Not to Say **2%**



EDUCATION



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a 2 percent increase was seen this year among those working within a hospital or medical center. Big data and computer science advances, coupled with novel precision medicine therapies and gene editing techniques, have enabled professionals in the medical field to become more informed about once-mysterious diseases in patients, which has also allowed clinical researchers in the lab to become more integrated with medical professionals working directly with patients.

The biotechnology sector continues to steadily grow as well, accounting for 6.4 percent of respondents, while a 2 percent decrease was noted among independent or private research labs.

The ratio of males to females working as lab professionals was split pretty evenly, similar to last year's results. Female survey respondents encompass 46.2 percent of the total audience pool, while 51.9 percent were male.

TABLE 1: Areas of research

Agri/Food	5.3%
Biochemistry	2.1%
Biology	5.3%
Biotechnology	6.4%
Chemistry	7.8%
Chemistry-Analytical	12.9%
Chemistry-Bioanalytical	1.0%
Clinical	10.5%
Drug Discovery	3.3%
Environmental	7.2%
Forensics	1.9%
Genomics	2.2%
Geochemistry	0.8%
Microbiology	5.3%
Molecular Biology	5.6%
Neuroscience	1.9%
Physics	1.0%
Plant Science	0.6%
Proteomics	1.3%
Other	17.8%

The majority of the laboratory workforce still falls within the middle-aged range—about 45 percent of respondents are 45- to 60-years-old. But there was also a minor surge in those aged 60-64, who are still working—14.1 percent this year compared to 11.9 percent last

year. Those who fall into the age range on either side of the spectrum round out the rest of the survey respondents, with 6.5 percent being 65 or older and 6.2 percent being 29 or younger. So while millennials may be making a splash in the job market in other industries, the same cannot necessarily be said among lab professionals.

Education and experience

As expected, about 41 percent of our respondents have a bachelor's degree in science or engineering, which is consistent with last year's report of 43 percent. Nearly 24 percent have a doctorate in science or engineering.

Individuals holding a management position—including corporate, R&D, lab, engineering, core facility, technical/operations, and project management—make up 57.5 percent of the survey audience, which is spot on with last year's demographics. But it is still a decrease from our 10th Annual Survey in 2016 that reported management professionals accounted for 67.7 percent of total respondents.

Nearly half of respondents have worked either full- or part-time as a researcher for more than 20 years, indicating a strong passion for their respective scientific fields—a trend we have seen for years. There was also a noticeable increase in the number of respondents who have been with their current employer for at least 11 years.

TABLE 2: Job Title

Management	57.5%
Academic department head	0.6%
Principal investigator/senior scientist or researcher	5.9%
Research scientist	7.7%
Chemist	4.5%
Clinical researcher	0.3%
Biologist	2.0%
Engineer	0.8%
Professor	3.3%
Post doctorate fellow	1.6%
Graduate/postgraduate/PhD student	1.1%
Technologist/technician/research assistant	6.5%
H&S manager	0.6%
Other	7.7%

Lab size and number of employees within organizations have also remained relatively unchanged. However, the number of organizations with more than 5,000

employees increased slightly, from about 25 percent of respondents last year to 29 percent this year. On the other hand, those with fewer than 25 employees make up just 7.4 percent of this year's respondents, demonstrating that big industry players still significantly outnumber start-ups and smaller corporations.

The number of respondents who manage just one to two employees fell by about 5 percent since last year, from 31 percent to 26 percent, while those managing three to four employees bumped up from 18.5 percent to 19.1 percent. Those managing the largest number of individuals—10–24 employees—went up from 16.3 percent in 2017 to 19.6 percent this year. This increase could suggest that employees are taking on larger managerial roles across different labs and organizations.

Salary and benefits

Respondents represent a wide spectrum of income classes—from those reporting a salary of less than \$25,000 to those earning more than \$150,000. However, the average fell between \$45,000–\$75,000.

TABLE 3: Salaries

Less than \$25,000	6.8%
\$25,000 - \$34,999	4.7%
\$35,000 - \$44,999	7.8%
\$45,000 - \$54,999	11.2%
\$55,000 - \$64,999	12.8%
\$65,000 - \$74,999	12.0%
\$75,000 - \$84,999	9.6%
\$85,000 - \$94,999	7.9%
\$95,000 - \$109,999	9.7%
\$110,000 - \$124,999	5.4%
\$125,000 - \$149,999	5.7%
More than \$150,000	6.5%

Employer-provided benefits remain largely unchanged from previous years. Nearly all respondents (90.4 percent) receive some form of vacation or paid time off, and 86 percent are offered health insurance. Survey results show that half of respondents get a 401(k) employer match as well. Less common benefits provided to respondents by their employers include childcare assistance (5.9 percent), profit sharing (7 percent), and stock options (7.8 percent).

Satisfaction and loyalty

It remains clear that an employee's corporate culture has a direct impact on his or her job satisfaction. Nearly 60 percent of respondents said corporate culture—defined as an organization's goals, strategies, structure; and approaches to labor, customers, investors, and the greater community—was "very important" to their overall job satisfaction, while just 6 percent said it was not important. Thirty-four percent fell in the middle, claiming corporate culture was "somewhat" important.

Most respondents still seem to be content with their current employment situation. The vast majority believe that they will still be working at their current position and in the same organization in the next 12 months, as opposed to looking for new employment. The second most popular answer was that respondents hope to be within the same organization but earn a promotion within that 12-month time frame.

Most respondents also feel that their experience and skill set are adequate for their current position, and a small subset plan to return to school to obtain another degree.

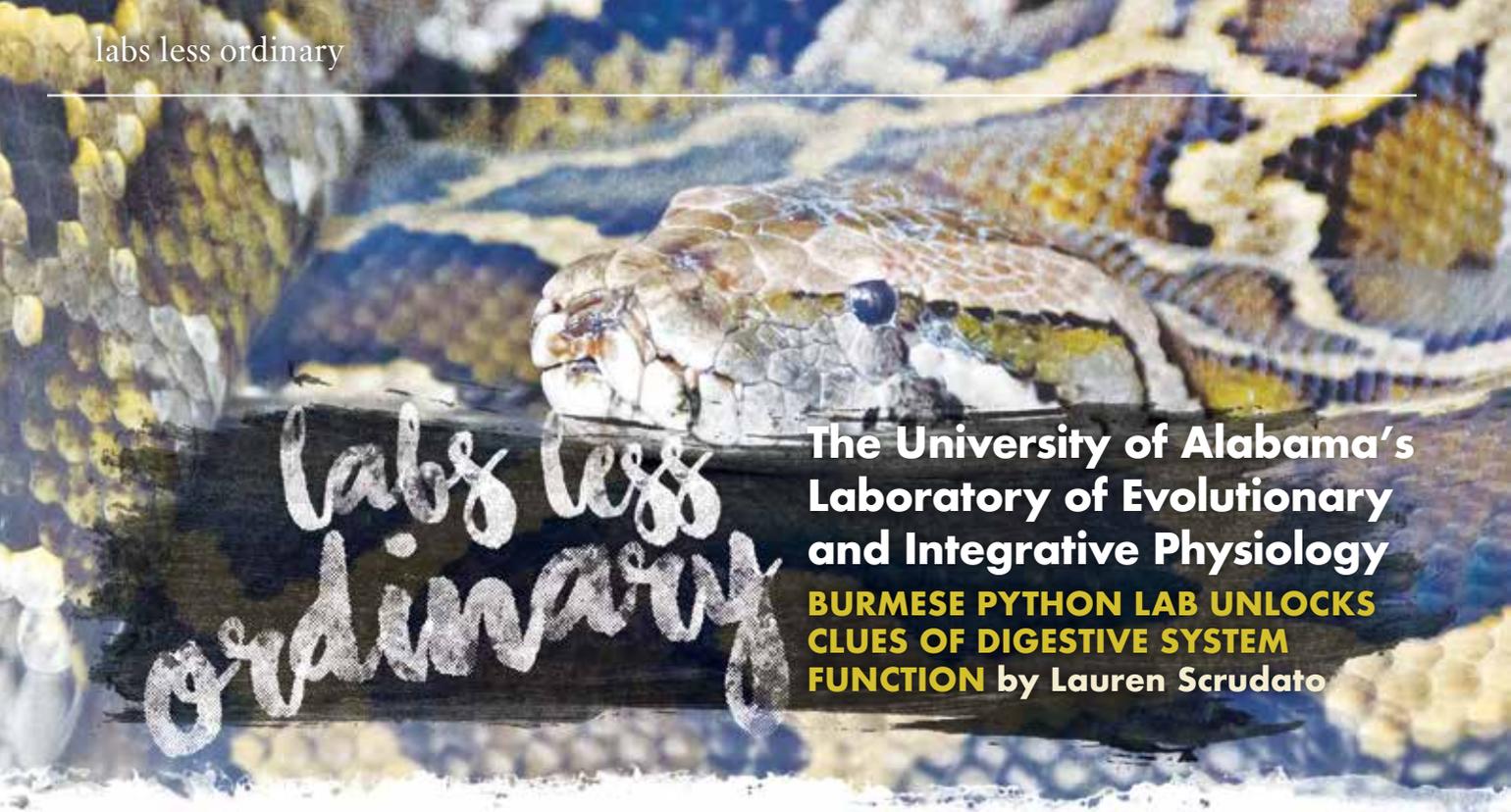
The overall consensus was that employers provide both initial and ongoing training, which helps employees perform their jobs well. Many respondents also felt that they were equipped with all the information, technology, and resources necessary to adequately carry out their duties, but there were some data points that hinted at areas of potential improvement.

Within the section of the survey that asked respondents to select "strongly agree," "agree," "neutral," "disagree," or "strongly disagree" to a list of phrases, the one that received the most "strongly disagree" responses was: "This organization provides training or experiences to help me explore other opportunities within the company," suggesting employees may need to seek out new opportunities on their own without much help from employers. Another phrase that received a high "strongly disagree" rating was: "This organization provides training or education to help me balance work and personal life."

Once again, this year's survey has confirmed trends that have remained consistent throughout the years, while also highlighting potential emerging trends and important changes.

Thank you to those who participated in the 12th Annual Salary and Employee Satisfaction Survey. Your responses contribute to invaluable insight into the field, and we look forward to revisiting this topic next year.

Lauren Scrudato, associate editor for Lab Manager, can be reached at lscrudato@labmanager.com or 973-721-4070.



The University of Alabama's Laboratory of Evolutionary and Integrative Physiology

BURMESE PYTHON LAB UNLOCKS CLUES OF DIGESTIVE SYSTEM FUNCTION by Lauren Scrudato

More than 100 million Americans are currently living with diabetes or prediabetes, according to the CDC. The rate of new diabetes diagnoses continues to climb each year, prompting teams of researchers to investigate potential new treatment options and ways to combat the symptoms of the disease.

The Laboratory of Evolutionary and Integrative Physiology at the University of Alabama, led by Dr. Stephen Secor, is taking a unique approach to learn more about how diabetes, heart disease, and other ailments affect the human body—by using the Burmese python as a model.

Unlike more traditional lab animal models, such as rats or rabbits, the python experiences an unmatched growth of the heart and pancreas within the time frame of just a few days. These extreme changes in the python make it easier to decipher the molecular mechanisms of cellular growth and functional remodeling, which ultimately can help determine the capacity to regulate gastrointestinal performance.

The lab is currently home to about 200 snakes, including about 45 pythons, 25–30 boas, and a variety of water snakes, corn snakes, and other species. The lab also houses a variety of reptiles, including bearded dragons and legless lizards.

Dr. Secor, professor in the Department of Biological Sciences at the University of Alabama, has become known as the “reptile guy” of sorts, having worked with frogs, alligators, turtles, lizards, geckos, and snakes, as well as invertebrates such as tarantulas, scorpions, and centipedes.

▲ A Burmese python

Secor discovered that the Burmese python exhibits an unprecedented increase in metabolic rate after feeding—as high as 40-fold with the digestion of meals. Postfeeding, the python's organs also increase tremendously. Secor has reported a 70–100 percent increase in the python's liver, kidney, pancreas, and small intestine, while its heart mass can inflate up to 30 percent. This all occurs within three days after a meal. These findings, among others from the Laboratory of Evolutionary and Integrative Physiology, are helping contribute to human biology and disease research.

“We can learn the basic mechanisms that underlie cellular remodeling and the capacity to regulate gastrointestinal performance including gastric acid production and intestinal nutrient transport,” said Secor.

Full-circle approach

The lab's extensive work with snakes and other animals explores the “hows” and “whys” of physiological design. The team's projects fall under two long-term interests—the adaptive interplay between feeding habits and digestive physiology and the integrative mechanisms underlying the regulation of physiological performance.

Secor's interest in animals began at an early age while growing up on a horse farm in central New York. He was first introduced to the field of physiological ecology at the University of Oklahoma while studying for his master's degree. Secor earned his PhD at UCLA and

Labconco's ClassMate Fume Hood Gets Straight A's in High Performance

By. **Beth Mankameyer, Sales Engineer**

Newly redesigned and released, the ClassMate full-view fume hood may not look so different, but the new upgrades take this hood from the sidelines to the headlines.

HIGH PERFORMANCE

Labconco's design team added high performance features that allow the new ClassMate to meet the SEFA 1 definition of a high performance fume hood. This means the new design was tested in accordance with the ASHRAE 110 test standard with the stipulations that the sash be fully open and the air exhausting through the fume hood must be traveling no faster than 60 feet per minute (fpm) through the sash opening. There can be no more than 0.05 parts per million (ppm) on average of the tracer gas Sulfur Hexafluoride (SF₆) detected. The new ClassMate test reports show it exceeded the minimum requirements and show an average of 0.00 ppm of detected SF₆. What this means is during the test, less air is exhausted to make the speed of the air travel slowly through the fume hood's sash opening. The slow face velocity paired with the sash being fully open to completely expose the breathing zone challenges the hood, but even with those challenges, 0.00 ppm of SF₆ on average was detected.

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LED lights come standard on the new ClassMate, making it more energy efficient than ever. Requiring half the watts of a typical fluorescent light bulb, and providing three times the rated life hours.

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1. Dr. Stephen Secor holding a green tree python. Credit: Megan Murphy, PhD student **2.** From left: Megan Murphy, PhD student, Dr. Secor, and Anna Reding, undergraduate student, in the lab. Credit: Jarren Key **3.** Mya Montrella, undergraduate student, feeding a python. Credit: Dr. Secor



began work in snake ecology, primarily working with the sidewinder, a small species of rattlesnake. Secor collaborated with Dr. Jared Diamond, professor in the Department of Physiology at the UCLA School of Medicine, to determine whether sidewinders possess adaptive features in their digestive systems that allow them to feed so infrequently in the wild. For six years, the duo worked with sidewinders, then switched to pythons due to their more docile nature and easier maintenance. Secor's work at UCLA was the springboard for developing the Laboratory of Evolutionary and Integrative Physiology at the University of Alabama in 2001.

As Secor explained, the lab has two primary areas of focus. The “Evolutionary” component refers to the approach the team takes in developing questions that have an evolutionary basis. They serve to address the “why” questions. For example, why do certain snakes (those that feed infrequently in the wild) exhibit such dramatic changes to gut structure and function, whereas snakes that feed frequently in the wild do not exhibit such changes? Or, what were the evolutionary pressures that underlie the distinct dichotomy in digestive responses among snakes, and does it translate to other groups of animals?

“To address such questions, one has to take a comparative approach and look at the physiology of multiple species to see to what extent have traits (like wide regulation of the gut) evolved independently or are to some level constrained by phylogeny,” explained Secor.

The “Integrative” component of the lab refers to the approach the team uses to address questions at different levels of organization—from the population level to the molecular level. This falls under answering the “how” questions of physiological response.

“Many labs address the ‘how’ mechanisms, like cellular and tissue responses, and try to explore what molecular signals and step-by-step processes are happening in cells. And then there are those looking from a more evolutionary standpoint and ask, ‘Why is this current?’ ‘What’s been driving these phenotypes and features?’ This lab takes both approaches,” said Secor.

Collaborations

Currently, one of the larger projects taking place in the lab is a National Science Foundation-funded study to examine the underlying molecular mechanisms of intestinal remodeling. The project involves five species

of snakes, an alligator, and a rat. The goal is to dissect the specific molecular pathways that result in the extreme phenotypic changes seen in infrequently eating snakes.

“For this, we are comparing tissue function, histology, and gene and protein expression among infrequently feeding snakes that exhibit the extreme phenotype (Burmese python, boa constrictor, western rattlesnake) with animals that feed frequently and express a very modest postfeeding phenotype (alligator, diamondback water snake, Amazon tree boa, and rat),” said Secor.

RNA sequencing is a key methodology the research team relies on to conduct their work because it allows them to look at gene expression and quantify it. “This methodology allows us to do a full profile on identifiable genes based on their sequence in the snakes and look at how their expression of those genes change[s] the treatment. In our case, comparing a fasting snake with a fed snake or different time points during the digestive process. That is just something we did not have 20 years ago. Now we can do this work in a very short period of time and have it much more complete than ever before,” explained Secor.

In addition to Secor, there are roughly 20 other researchers working in the lab, including postdoc, PhD, and undergraduate students. At any given time, the team could be working on about six different projects.

Throughout his career, Secor has had great success through collaborations with researchers in a variety of other fields of interest. For example, thanks to his work with Todd Castoe, PhD, assistant professor in the Department of Biology at the University of Texas Arlington, the Burmese python was the first snake to have its genome annotated and published in the *Proceedings of the National Academy of Sciences* in 2013. Secor continues to welcome a collaborative approach as the leader of the Laboratory of Evolutionary and Integrative Physiology.

“I have many collaborative projects, and collaboration is certainly necessary given the complexity of the projects and the uniqueness of the study animals. For these projects, it really takes the expertise of different people to advance the work,” said Secor.

Pythons as animal models

Surprisingly, there aren't too many challenges when it comes to working with Burmese pythons in the lab. According to Secor, they are relatively easy to maintain, feed, and grow as they only need to be fed weekly or monthly. They are also fairly docile and can be kept in rack systems. Thinking long term, Secor and his team hope to further their exploration on mechanisms of cellular remodeling by developing and utilizing cell-culturing techniques for python cells.

“I am very curious about pursuing a cell-culturing approach. I'd like to culture snake cells to look at specific signals that are involved in the remodeling process. We hope to transition to a new lab facility that will have cell culture capabilities next year.”

Lauren Scrudato, associate editor for Lab Manager, can be reached at lscrudato@labmanager.com or 973-721-4070.



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ESSENTIAL ANALYSIS INSTRUMENTS MEET SIMPLICITY

Density meters and refractometers continue to evolve to suit researchers' ever-expanding needs

Density meter design and technology have come a long way from primitive origins dating back to the days of the famous Greek mathematician and inventor Archimedes. Their use extends across multiple fields and now includes the production of digital or handheld density meters, among others. But the next phase of their evolution features an intuitive, automated, “all-in-one” solution. Excellence Density Meters from METTLER TOLEDO are based on the oscillating U-tube principle, and guarantee maximum precision with an accuracy of up to 6 digits in density. Users can measure density, specific gravity, and many related values, such as % alcohol and Brix, quickly and reliably. Plenty of benefits have also been infused into the design of Excellence Density Meters. BubbleCheck checks the presence of bubbles in the measuring cell. Temperature control keeps samples at a specific temperature. Automatic viscosity correction corrects the sample viscosity automatically. All of this ensures high accuracy and trustworthy results.

Refractometry has a younger history, dating back to the late 19th century. It continues to be one of the main techniques scientists rely on for chemical analysis. The

refractive index can be used to identify an unknown substance or to assess the purity of a sample. Although the general principle behind how refractometers work has remained relatively unchanged over the years, they have evolved with new features, improved accuracy, and different types to accommodate specific needs.

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“Often density and refractive index are determined for the same sample. Connected instruments let allow researchers to easily synchronize results.”

“Reliable results and automated workflows allow researchers to focus on critical tasks.”

The density or refractive index of a substance is influenced by temperature used to measure it, so proper care must be taken to control or compensate for temperature differences. With Excellence instruments, calibration and verification can be done immediately without needing to manually enter additional texts. The Peltier thermostat maintains the measurement cell at a constant temperature, eliminating worries of any potential temperature variation.

Additionally, the instruments can be upgraded to a dedicated automatic multiparameter system that combines density, refractive index, pH, color, titration, and more to prevent the alteration of samples between individual analyses. Combination with LabX PC software ensures data integrity and compliance, as well as smoother workflows.

Identifying researchers' current needs and predicting the demands of the future lead to the development of innovative instruments worthy of investment. Reliable

results coupled with automated workflows enable researchers to focus on more crucial tasks. METTLER TOLEDO understands the importance of these features and strives to deliver sophisticated solutions to its customers.

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Successful Mentoring

DONE RIGHT, A WIN-WIN FOR INDIVIDUALS AND INSTITUTIONS

by **Bernard Tuls**

Mentorship programs are as varied as the perspectives and opinions about them. In practice, mentoring can be as simple as people meeting occasionally in a breakroom or coffee shop or as complicated as organized in-house initiatives, structured sessions facilitated by external experts, and smart software and apps. And views and attitudes of mentoring run the gamut from seeing them as invaluable to regarding them with quiet acceptance, if not indifference. Yet, hardly anyone disputes the value of mentoring—even when it’s examined through a strict cost-benefit lens.

“Mentoring is incredibly important,” says Dr. Adam J. Schwartz, director, Ames Laboratory, an Iowa-based Department of Defense (DOE) National Lab that specializes in creating materials and energy solutions. “I have seen examples where good mentoring and good mentorship programs have added huge value to individuals and institutions. And I have seen cases where bad mentoring or no mentoring has been a significant negative for an organization or institution.”

Schwartz says that Ames Laboratory has a formal mentoring program that seeks “to provide an additional level of input, guidance, advice, and support outside the supervisor.” He notes that “ideally, supervisors of new staff members will provide appropriate mentoring advice and guidance, but it doesn’t always work out that way.” An independent mentor provides a level of objectivity and can intervene to prevent escalation if there are misunderstandings between supervisors and staff members.

“Our formal mentoring program usually has an established staff member working with a new employee. They meet regularly, with a minimum six-month commitment,

although many of these relationships last for many years,” says Schwartz. The Ames mentorship program has both voluntary and mandatory aspects, and Schwartz says, “We do believe that every new staff member should have a mentor.”

At Argonne National Laboratory, Dr. Kristene “Tina” Henne, postdoctoral program lead, facilitates the postdoctoral mentoring program. Argonne’s formal structured program for postdoctoral researchers was implemented in 2010. “It really started out as a grass-roots effort by the postdoctoral society at the time. They recognized the benefits of mentoring in making career transitions and [in navigating] the pathway to the next phase of their careers,” Henne says.

“If we choose a mentor who doesn’t have enough time and who is not enthusiastic about being a mentor, then we have made a bad choice.”

“Postdoc is a temporary assignment by design. It is meant to be a temporary phase of mentored research where postdocs can build and expand their skills and start to carve out a path toward becoming independent researchers—so mentoring is very important in the transitional phases of their career,” she says.

At Argonne, the postdoc society did a lot of footwork assessing the need, surveying the community, formulating a

proposal, discussing it with leaders, and obtaining approval, according to Henne, who now leads the program. “Actually, I was a postdoc at the time this was proposed, so I was here to help get it off the ground in the early years. Really, it started as something that the postdocs recognized would be of value to their experience here at Argonne.”

Since then, the program has evolved into the formal mainstay for the nurturing of scientific staff it is today. “We recommend that mentors and mentees meet at least quarterly as a bare minimum. We have a set of guidelines that indicate what mentors and mentees may want to work on. One thing that I go over with postdocs coming in the door is to think about where they are in their appointment and what they see as their immediate needs and long-term needs.”

Henne says that a general long-term need of postdocs is to get to the next career phase. “Their immediate needs coming in new to an organization include acclimating, getting to know people, and figuring out how to

navigate the workplace, and those are areas in which a mentor can be of benefit.

“We provide guidance and have developed some tools and checklists for mentees. These include a list of questions that mentors and mentees may want to ask each other. [On our mentoring blogs,] we share regular tips and tools as well as best practices that have worked in other institutions. In general, we try to hold one workshop a year on mentoring skills. We hold regular internal seminars to provide general knowledge on mentoring,” she says.

She says that each postdoc now has a research supervisor or a person he or she works closely with—typically the staff member who hires the postdoc—and that mentoring support is provided by appropriate, more senior staffers outside their line management.

“We have about 15 research divisions that support postdocs. In each division, there is a staff member who acts as a resource, meets with postdocs as they come in, and helps to find a mentor for them. The goal of the

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program is for all postdocs to have mentors; that's our expectation," says Henne.

Schwartz says that the choice of mentor is partially based on the area of activity within the laboratory. "So, if we have someone coming into operations, often but not always we have one of our operational leaders be the mentor," he says, noting that care and attention are expended to ensure good mentor-mentee matches. "We are the smallest of the DOE National Labs, so an advantage is that we . . . know all the people really well. So, we are able to handpick the particular mentor with the right knowledge of the laboratory, the right operational skill set, and the right personality to match with the incoming staff member.

"Having that personality match is often one of the key factors. If we choose a mentor who doesn't have enough time and who is not enthusiastic about being a mentor, then we have made a bad choice. On the other hand, we don't want to overload our outstanding mentors, because we need them to work with up-and-coming mentors to get the entire process working well within our culture," says Schwartz.

Turning to the question of accomplishments, Henne says that the success of a mentoring relationship is based on the goals set up by the mentor and the mentee. "We emphasize that we can put the structure behind the mentorship program, but it is really up to the mentees to drive the relationship and decide what goals they want to set for themselves.

"The keys to success in this relationship are setting expectations up front, spending time early getting to know each other, and understanding what each person brings to the table—because there are benefits to the mentor as well. [Other keys] to success [are] frequent and open communication and honest feedback, which depend on trust as well," says Henne.

One of the key benefits of mentorship programs is that employee improvements extend to the entire organization. "Individuals are enhanced by feeling valued and part of an organization that is taking the time to invest in their success. Increased networking opportunities could lead to new ideas and collaborations.

"This helps to build a culture and a community that is nurturing—as you get more people talking to each other who otherwise wouldn't have, that helps to enhance a culture that is inclusive and values diversity and helps to improve communications along these lines," she says.

Henne says that the benefits reported by staff mentors and echoed throughout the literature include expanding their networks, honing their technical and communication skills, expanding their outlook, and becoming more aware culturally and about new developments in their or related fields. "Sometimes there is the benefit of being viewed as a trusted adviser, as someone who has experience that is valuable and worth sharing, which adds value to the mentor as an individual as well."

Schwartz says, "A good mentoring program develops a new staff member more rapidly, more thoroughly, and with greater job satisfaction than a bad mentor or no mentor." He sees success as the development of new staff members, providing a measure of happiness and quality of life and avoiding personnel issues that could interfere with supervisor and new staff member interaction.

Addressing the uniqueness of lab mentoring, Henne says that mentoring is a broad umbrella term that can take on different forms. She acknowledges that mentoring a researcher to be a successful PI in a lab entails discussing different goals

“The success of a mentoring relationship is based on the goals set up by the mentor and the mentee.”

versus a mentee seeking broad career advice or someone interested in advancing to the next level.

Schwartz says that there are really two main aspects for success in a scientific career. The most important is a combination of technical competence and innovative ability, which are usually measured through scientific publications and other research output. The other important aspect involves the ability to communicate and ultimately drive science and to innovate and lead people, which can be helped considerably by good mentoring programs.

For labs seeking to initiate or better streamline their mentoring programs, Henne advises, "In general, you have to establish a need, because this makes a stronger business case to get buy-in from leadership. I would recommend figuring out the target audience early—is it graduate students, postdocs, or technical staff who you think will be the beneficiaries of the mentoring program? [Then you need to] talk to them, holding some discussions to get to know their needs and interests, which will help you to formulate the goals of the program.

"This leads into making a business case for establishing a mentorship program—and working through the details

for sustaining it—staffing, [naming] responsible staff leaders, and providing some basic tools, [such as] some guidance on what they should be doing as they start their conversations.

“One of the key benefits of mentorship programs is that employee improvements extend to the entire organization.”

“There are different types of mentoring, so it is important to consider the type of format that will work best. For example, when mentees have similar professional development interests and needs, you could have one mentor working with more than one mentee.”

She says there is a lot of available information on how to start and operate a mentoring program, including materials created by the National Postdoctoral Association (APN) (<https://www.nationalpostdoc.org/page/Mentoring-Plans>). The APN provides postdocs and administrators with resources, including information on how to develop mentoring plans and participate proactively in mentoring relationships.

In addition, a range of private and nonprofit companies provides literature, software, apps, and other resources based on a variety of service models, including free access to their websites and paid consultations and expert guidance.

Henne says, “We have come a long way—evolving from a grassroots notion where we believed mentoring will be beneficial to this particular group of employees to addressing questions like what exactly [mentoring is], what a mentor does, [and what a mentee does]. We understand mentoring broadly across the lab; its visibility has been raised, people are finding value in it, and increasingly, people are being identified as successful mentors.”

Bernard Tulsi is a freelance writer based in Newark, Delaware. He may be contacted by email at btulsi@comcast.net or by phone at 302-266-6420.

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Analytical Method Validation

DO IT NOW OR PAY LATER **by Angelo DePalma, PhD**

In his 1996 review of analytical method validation, author Mark Green notes, “Doing a thorough method validation can be tedious, but the consequences of not doing it right are wasted time, money, and resources.”

Over the past few decades, regulatory and standards-setting organizations have revised their validation guidances to reflect current thinking, but the definition of analytical method validation has remained the same: “the process by which it is established, by laboratory studies, that the performance characteristics of the procedure meet the requirements for the intended analytical applications.”

Built into this definition is an implicit component of risk. For an analytical process to “meet the requirements for the intended analytical applications,” analysts must consider the purpose of the assay, inherent analyte and method variability, and the overarching “requirement”—for example, an end product’s use. Within this framework, pharmaceuticals are an excellent model for analytical method validation, given that industry’s regulatory rigidity.

USP (Rockland, MD), the premier pharmaceutical standards-setting organization, lists the goals of validation as quantification of a method’s accuracy, precision, specificity, detection and quantitation limits, linearity, range, and robustness. Industries, and groups within industries, might be more interested in robustness than accuracy or in precision more than robustness. It all depends on meeting “the requirements for the intended ... applications.”

Ed Price, CEO of contract drug manufacturer PCI Synthesis (Newburyport, MA), has cataloged the top 10

questions to ask before undertaking analytical method validation. Based on these considerations, Price recommends that validation experts think ahead to future method testing. “It’s critical... to prepare for further review of all analytical methods developed... The most effective analytical method development assures that lab resources are optimized and that the methods developed can be vali-

dated at each progressive step in the process. If changes to a method are required, it’s best to do so, and document the changes, before moving on to the next validation step.”

Price also stresses the importance of method optimization in order to squeeze all potential benefits

from the selected analytical platform.

Revolution: Risk-based validation

Several standards and government organizations influence pharmaceutical method validation. The U.S. Pharmacopeial Convention sets general specifications for active drug compounds and finished products and describes the types of tests and instrumental methods drug makers must employ to meet those specifications. The pharmaceutical companies work out the specific method, which, per USP guidelines (themselves derived from ICH guidances), are established through a validation process. “Every single method included in the USP must be validated,” says Horacio Pappa, director at USP.

USP’s validation guidelines have served the pharmaceutical industry for nearly three decades. But given the age of USP’s validation guidelines and what has been occurring

“Every single method included in the USP must be validated.”



in the larger industry, USP began to consider whether established validation protocols were still applicable. “Can we continue using 30-year-old concepts in light of technical advances?” Pappa asks. “There is clearly room for discussing a new approach to validation.”

Quality by design (QbD) refers to the practice of monitoring quality during production rather than “testing in” quality through post-production assays. QbD was originally applied to product manufacturing, which makes sense, since “quality” is a concrete attribute.

“We started asking ourselves if the same concepts could apply to analytical methods,” Pappa tells *Lab Manager*. Like quality programs, method validation is not a discrete activity performed once and forgotten. “It exists, and is invoked, on a continuum through the method’s life cycle, which includes method development, qualification, and performance verification.”

The motivation behind rethinking method validation arises from the definition of validation as promulgated by the FDA, ICH, and USP: to demonstrate that a method is suitable for its intended purpose and it measures what you want to measure with appropriate accuracy and precision.

“That idea, although it falls under the official definition of validation, is not reflected in current guidances,” Pappa says. “The current guidances present validation in the form of a checklist. You perform the items on the list, and the method is officially validated. But it’s difficult to conclude after checking all the boxes that the method actually is suitable for intended use. We’re trying to put more emphasis on that demonstration.”

Pappa describes the revamped approach to validation as “a new concept, a journey, a work in progress that is centered on risk.” USP will communicate these recommendations to drug makers through its house organ, the *Pharmacopoeial Forum*, and through workshops where stakeholders will enjoy the opportunity to provide input. If the variability of a method is small compared with the specification, that method will work. “In this scenario, defining what is sufficiently accurate and precise becomes critical. Risk is a function of the method’s criticality.”

Risk-based validation injects understanding and science in what may previously have been blind adherence to protocol.

For example, many drugs under development today have a narrow therapeutic window, meaning the



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effective dose is not very different from the toxic dose. “Because assay results occur over a normal distribution, very likely a fair amount of product, which on average falls within specification, will fall below the lower specification range,” Pappa explains. Method validation should provide evidence that the method detects and quantifies those excursions from specifications.

Utilizing resources

According to Lisa Thomas, senior director, life science mass spectrometry at Thermo Fisher Scientific (San Jose, CA), many labs struggle to plan and execute analytical validation adequately. “Inadequate planning can potentially turn a 10-week validation into a 40-week process, resulting in substantial delays [in] the lab’s ability to process samples.”

Thomas cites expertise, time, and statistical confidence as negative factors affecting validation success.

“Finding scientists who understand quality management can be challenging, as is finding personnel who

understand instrumentation and the science sufficiently to conduct proper method assessment and validation. Small-to-midsize organizations have difficulty dedicating time to method validation, since that activity competes with the daily lab operations.”

Thomas is open to a risk-based approach to validation, calling it a way to ensure the appropriate amount of testing is reflected in the potential implications of not testing. “Over the years, I’ve seen the extremes; too little validation may come at a financial cost in terms of product recalls or tarnishing brand reputation, while too much validation may impede employee effectiveness, innovation, and responsiveness.” For several years, GAMP (good automated manufacturing practices) and guidance bodies have evolved their thinking toward risk-based validation.

A risk-based approach is very much based on the lean principle of keeping only those processes and activities that add value. Pharmaceuticals have some of the best quality practices in place due to the potential effect of their products



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on patient safety, but generally their operations are not lean, according to the management school definition of the term. “For many contract testing labs and clinical reference labs, lean procedures enable them to drive their cost per test down to maintain market competitiveness—often leading the way for lean best practices,” Thomas says.

Only a minimum?

In a recently published application note, scientists from Waters (Milford, MA) demonstrated the validation of a method for detecting metoclopramide, a drug for gastrointestinal reflux, and “related substances,” presumably side products from the drug’s manufacture. The Waters team was looking specifically for assay linearity, detection and quantitation limits, accuracy, repeatability, intermediate precision, specificity, and robustness.

According to Margaret Maziarz, an app note co-author and a principal scientist at the company, those validation objectives reflect regulatory guidelines from ICH, USP, and FDA, “but those are only the minimum validation goals for analyzing this drug by UPLC.” Analysts should be prepared to validate supporting processes that feed directly into the method being validated—think of an inverted pyramid with preparatory processes funnelling into the main act, which is assaying a drug. “Preparing metoclopramide for analysis involves filtration to remove particulates, excipients, and colorants, so validation of filtration should also occur to assure maximum recovery of the drug.”

Long-term stability or storage is a huge quality metric for drugs and other perishable products. For analytical methods, however, analysts also need assurance that their samples, standards, and reagents pass the time test, albeit for a shorter term than for traditional stability assays. “Again, the goal is to ensure that the LC method, including the instrument, provides the expected results reproducibly,” Maziarz explains.

Maziarz was reluctant to discuss risk-based or purpose-based validation, which is understandable given the negative inertia risk-based manufacturing faced when the FDA was pushing this idea a decade ago. To the question of whether validation

might be relaxed in certain less-risky situations, she responded, “To be sure you’re in compliance, simply follow the regulations, whatever they are. If you have questions, you can ask regulators or standards organizations. For pharmaceutical products, there is no such thing as a reduced need for validation. Risk-based validation is a great idea, provided the risk analysis is sound. That may be down the road at some point, but that is for USP and FDA to decide.”

Angelo DePalma is a freelance writer living in Newton, New Jersey. You can reach him at angelo@angelodepalma.com.



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The Eyes Have It

PROTECTING ONE OF OUR MOST ESSENTIAL SENSES **by Vince McLeod**

If there is one thing we are certain about, it is the presence of potential eye hazards in laboratories. In today's modern research and development labs, eye hazards are always present and take many forms. And like our hearing, sight is gone forever if we lose it. Therefore, we should take extra precautions to make sure that never happens.

“It is not a question of when eye protection is needed but rather what type of safety eyewear is correct.”

You really have no excuses, as current safety eyewear is so lightweight and comfortable that most workers don't even notice they are wearing it after a short while. In fact, continued advances in style, fit, and functionality of safety eyewear are great reasons for workers to want to wear modern protective eyewear.

However, if you have a few stubborn employees or disbelievers, you might want to enlighten them about these sobering statistics. Did you know that about 2,000 eye injuries requiring medical attention occur on the job every day?¹ That equates to around half a million incidents per year. And sadly, 90 percent could be avoided, as most eye injuries are due to employees not wearing eye protection at all. And eye injuries account for an estimated \$300 million in medical costs, workers' compensation, and lost time annually.²

The primary eye hazard in labs is chemical splashes, with flying dust, particulates, glass shards, and the like a close

second. Other hazards include ultraviolet light (UVL), lasers, and thermal burns. So it is not a question of when eye protection is needed but rather what type of safety eyewear is correct. What type of eye protection is appropriate for your laboratory work? Keep reading to help answer that question.

Job hazard analyses

As industrial hygienists, we are trained to deal with occupational hazards in a basic three-step process: recognition, evaluation, and control. Prevention—that is, removing the hazard if possible or controlling it with engineering methods, such as putting a safety barrier between the hazard and the worker—always gets priority. Personal protective equipment, such as eye protection, in this case, is considered a last line of defense.

We start the job hazard analysis by identifying the potential eye hazards for the tasks at hand. Determine whether you can eliminate the eye hazards by substituting chemicals, changing the procedure, or utilizing engineering solutions. Evaluate engineering controls such as shielding or guards to prevent particles and splashes from being dispersed or fume hoods or local exhaust ventilation to contain dusts, particles, and vapors.

Eye protection types

Always use safety eyewear that meets American National Standards Institute standard Z87.1-2010.³ Safety eyewear that meets Z87 is tested and must pass stringent requirements for impact, distortion, light transmittance, and lens thickness, among others. Safety eyewear that passes all the tests will carry the Z87+ mark in addition to marks for lens type and use applications.



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HAZARDOUS CHEMICAL
SAFETY

1 **SDS**
KNOW THE HAZARDS

- Employers are required to identify and evaluate the respiratory hazards in their workplaces.
- Retain Safety Data Sheets on incoming hazardous chemicals and make them available to laboratory employees.

2 **SKULL AND CROSSBONES**
ENSURE LABELS ARE CORRECT

- Make sure labels display the identity of the chemical and appropriate hazard.
- The hazard warning must provide users with an immediate understanding of the primary health and/or physical hazards of the hazardous chemical, and the name and address of the manufacturer, importer, or other responsible party.
- Labels on portable containers are not required if the worker who made the transfer uses all of the contents during the work shift; when a secondary container is used for longer than one shift, a label needs to be applied to the secondary container.

3 **PERSON WITH SHIELD**
TRAIN AND PROTECT YOUR EMPLOYEES

- If your laboratory employees use hazardous chemicals, you must develop and implement a written chemical hygiene plan to protect them.
- Provide workers with information and training that ensures their awareness of the chemical hazards used in their work area.
- Periodically measure employee exposures to harmful substances if you suspect that these exposures are routinely above the action.
- Give all employees who work with hazardous chemicals the opportunity to receive medical attention.
- Establish and maintain for each employee an accurate record of any measurements taken to monitor employee exposure and any medical consultation and examination.

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The type of eye protection must match the hazard, and there are definitely types more appropriate for certain hazards. Special hazards such as UVL, welding, or lasers require special safety eyewear.

Safety glasses with polycarbonate lenses are the most widely used type of eye protection. Polycarbonate is a type of plastic that offers extreme resistance to impact due to unique properties of strength and flexibility. Used in “bulletproof” windows in addition to safety glasses and many other applications, it provides excellent protection from flying debris and UVL while being lightweight. However, because its impact resistance comes from being flexible, the material is prone to scratching, so look for safety glasses with hard-coated polycarbonate lenses.

Safety glasses are available in every shape and style imaginable. Today’s well-designed products are lightweight, comfortable, and economical. Keys for a good fit are soft rubber nosepiece and adjustable, rubber-tipped temples that hold well without excessive pressure. Soft, sticky rubber in these areas provides good grip even when the wearer is sweating.

Style, while definitely not a safety feature, it is what motivates many workers to wear their safety glasses. In our experience, facilities that

offer the newer stylish wraparound safety glasses find compliance issues reduced significantly, as workers actually want to wear them. Another advantage of the wraparound safety glasses is that they also provide good protection from airborne debris and meet the OSHA personal protective equipment (PPE) eye and face protection standard for side protection in the presence of flying objects.⁴

Specialty safety glasses

The aging of our nation’s workforce is an undeniable demographic trend. One consequence of this is that many over-40 workers need reading glasses. Manufacturers of safety eyewear have taken notice, and quite a few now offer safety reading glasses. These are offered with polycarbonate lenses that give workers the ability to see things close up as well as read prints and manuals. Safety bifocals are available at a cost much lower than that of prescription safety glasses. In fact, bifocal safety glasses have become a large market, and products now include contemporary sophisticated designs, even the newest wraparounds, in a range of bifocal powers or diopters.

Safety sunglasses are also widely available when the need to work outside is unavoidable. Good-quality safety sunglasses have natural color balance (NCB) gray lenses and provide protection from UVL as well as infrared (IR) and blue light. UV protection is even more important now, as ultraviolet levels have increased recently due to changes in the atmosphere, and UVL also increases at higher altitudes.

Glass or metal buildings and other highly polished, mirrored surfaces reflect light and pose an eye hazard when one is working near water or with intense lights. In this case, polarized safety glasses are needed. Polarized



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lenses selectively eliminate light reflected by surfaces by allowing only certain wavelengths to pass through, which reduces glare considerably.

Alternate light sources, lasers, and radiation pose dangerous risks to the eyes. Hazards depend on the wavelength and power of the light source, duration of exposure, and which structure of the eye absorbs the light. Reflections off surfaces are a serious potential hazard. A competent technical professional should carefully select appropriate safety glasses for lasers or other special applications.

Watch for eye hazards

Sight is one of our most precious senses. Our eyes are very susceptible to injuries that can lead to serious long-term or permanent disability. The low cost, performance, and myriad styles of today's safety eyewear make wearing proper eye protection easier than ever.

Vince McLeod is an American Board of Industrial Hygiene-certified industrial hygienist and the senior industrial hygienist with Ascend Environmental + Health Hygiene, LLC, in Winter Garden, Florida. He has more than 35 years' experience in industrial hygiene and environmental engineering services, including 28 years with the University of Florida's Environmental Health & Safety Division. His consulting experience includes comprehensive industrial hygiene assessments of major power-generation, manufacturing, production, and distribution facilities. Vince can be reached at vmcleodcib@gmail.com.

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Laboratory Disaster Planning

LESSONS LEARNED FROM 2001 TROPICAL STORM ALLISON **by Tracy Wieder**

Tropical Storm Allison hit the Houston metropolitan area on June 5, 2001, proving that storms do not need to be hurricanes to cause tremendous damage to research facilities. The storm dropped 32 trillion gallons of water, causing deadly flooding. Thirty-three thousand people needed emergency shelter after Allison, and there was \$5 billion in damage. Approximately

learned during this event and from subsequent years of experience working in medical research laboratories in both Houston and Miami.

“A little time put into preparation now could save decades of research data and samples down the road.”

\$1.5 billion of the damage was to the Houston Medical Center.¹ The University of Houston Medical School experienced severe flooding. The basement of the Medical School building was completely filled with water, as well as halfway up the first floor. This resulted in the massive loss of research animals, which were housed in the basement. In addition, elevators were no longer functional, resulting in an inability to get liquid nitrogen supply tanks to laboratories located on upper floors. The flooding destroyed backup generators, which were also housed in the basement, leaving liquid nitrogen auto-fill units and -20 and -80 freezers without any power. This led to enormous loss of valuable, irreplaceable research samples, reagents, and cell lines. I was a laboratory manager for a large research lab located in the Medical School building when Allison hit Houston. Many lessons were



▲ Houston experienced massive flooding as a result of Tropical Storm Allison, resulting in flooded streets and highways, preventing staff from getting to work to check on their laboratories.

Preparing a research laboratory for a disaster is essential in allowing research to continue once the disaster has passed. Disasters include severe weather emergencies, but these are not the only disasters to which research laboratories are vulnerable. Fire is also a big risk for research labs, particularly those that store large amounts of flammables, and a risk that many labs often overlook, particularly those located in areas that are not vulnerable to severe weather emergencies. This article aims to address what research laboratories can do before an emergency strikes to better prepare themselves and protect their samples and their data.

Preparing research laboratories for disasters is multifaceted. It does not take long to be well-prepared, and a little time put into preparation now could save decades of research data and samples down the road. As we head once again into hurricane season, preparedness for research institutions in coastal communities could not be timelier. Many of the steps listed below are geared toward disasters that come with some degree of warning, but several of the steps are equally useful for disasters such as earthquakes and fires, which provide little or no advanced warning.

Here are the steps I recommend to all the labs I oversee to prepare for disasters:

1. Back up all electronic data onto a shared computer drive and also to a thumb drive, which you will take with you if you have to evacuate the lab.
2. Keep backup liquid nitrogen on hand so that liquid nitrogen storage tanks can be refilled in the event of an evacuation associated with a natural disaster. Before evacuating, fill your liquid nitrogen storage tanks to the limit allowed by your equipment. Check your operating manuals for info on what that level is. Contrary to common belief, most samples that can tolerate storage in gas phase of liquid nitrogen can also tolerate storage in liquid phase. Make sure you have proper tubes that won't allow liquid to enter the tubes. This applies to auto-fill systems as well; they can be filled up to store your samples in liquid phase instead of gas phase. This could mean the difference between losing all your samples and not losing any samples. After a disaster has passed, electricity could still be out for days, weeks, or even months. Without electricity, elevators do not work. Without elevators, liquid nitrogen tanks cannot be brought up to floors above ground level. Filling up



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storage tanks and having backup supply tanks on hand are critical for getting through the post-disaster period before power returns.

3. Ensure all contact information is current for alarm monitoring systems and make sure all freezers are plugged into emergency power outlets. Move as many samples from -80 freezers into liquid nitrogen freezers as possible based on the tolerance of your samples for storage in liquid nitrogen. Having backup power is a good start, but it is no guarantee against sample loss. Generators are powered by fuel, and if the disaster lasts long enough and people are unable to come refill the gasoline in the generators, then the generators will stop working in generally two to three days. Liquid nitrogen is a safer storage option for any samples that can tolerate liquid nitrogen temperatures.
4. Update laboratory contact lists and ensure all lab members have copies so that staff can check on each other post-disaster.
5. Take photos of all equipment and of the entire lab so that, should the worst happen, pictures will be available to prove to insurance companies that the lab was in good shape before the disaster. Equipment photos may then be used to assist in making lists of costs for insurance claims. This is a very important step for all laboratories to take,

no matter where they are located, as this information is critical for making insurance claims in the event of a fire or a natural disaster, including earthquakes, tornados, hurricanes, mudslides, and flooding.

6. Maintain current chemical and reagent lists so that again, should the worst happen, data will be available for making insurance claims. Keep the lab and equipment pictures along with chemical and reagent inventories on the shared computer drive and back up onto a thumb drive. Again, this is an important step for all laboratories to take. Keep a list of chemicals in the lab outside of the lab itself to be given to firefighters in the event of a fire. If firefighters do not know what chemicals are inside the lab and the risks associated with chemicals exposed to fire (explosions, hazardous gases), they will not be able to enter the labs to fight the fire.

7. Send critical samples for off-site storage. These are samples that are not commercially available and are critical to your research. The best option is to send a box of samples to a collaborator who works in an entirely different region from where your lab is located because this is an inexpensive option, costing you only the price of shipping the samples. If this option is not available to you, you can find companies online that specialize in long-term sample storage and go that route instead.

8. If you work with research animals that are unique and not commercially available, preserve these animal strains through embryo cryo-preservation and/or sperm freezing. Engage a company that specializes in these services to ensure the frozen samples will be properly stored.

9. Before evacuating, unplug all equipment that does not need to stay plugged in, move all equipment away from windows, cover all equipment with plastic tarps, and secure

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the tarps with duct tape to prevent water damage. Do not forget to cover computer equipment as well and to move equipment off the floor if you are located on a lower floor.

10. Check into policies at your institution regarding assigning one lab member as essential personnel to allow them to come check on the lab after the disaster has passed, but before the institution has reopened.

Lessons learned from Tropical Storm Allison:

1. Whenever possible, move animal facilities up. Avoid placing animal rooms in basements or at ground level whenever possible. During Tropical Storm Allison, all loss of animal life was due to animal room locations in the basement.
2. Move emergency generators up from basements and ground floor locations as well. Flooded generators during Tropical Storm Allison resulted in complete loss of power to all freezers in the Medical School building, leading to catastrophic losses of samples.
3. Store critical liquid nitrogen samples in liquid phase, not in gas phase. Liquid nitrogen was the only thing that saved any samples during Tropical Storm Allison. Samples stored in gas phase of liquid nitrogen have very little liquid nitrogen in place to keep samples cold. In situations where liquid nitrogen cannot be secured for weeks at a time, liquid phase storage of samples may be the only thing that saves critical samples.

Taking a few simple steps now can protect your research from devastating loss in the future.

Tracy Wieder, senior manager, University of Miami, Sylvester Comprehensive Cancer Center, can be reached at twieder@med.miami.edu.

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Challenges of Cannabis Contaminant Testing

DIFFICULT MATRIX, COMPLEX PRODUCTS, UNCERTAIN STANDARDS

by **Angelo DePalma, PhD**

Testing cannabis for contaminants could be the easiest thing in the world if it weren't for the many difficulties—some technical, some legal.

Like all herbals, marijuana is a product of its environment, which may contain any number of nasty chemicals, metals, molds, and bacteria. Depending on the intended use, pot's main psychoactive component, tetrahydrocannabinol (THC), may itself be considered undesirable or even a contaminant. Products formulated for sedation or recreation tend to be high in THC and low in cannabidiol (CBD), whereas immunomodulatory or anti-inflammatory preparations stress CBD content.

Concentrated extracts used to formulate tinctures, foods, and recreational products present additional contamination possibilities arising from the extraction process and solvents used.

Cannabis testing labs can rely on established methods for most impurities. The challenge, says Holly Johnson, PhD, chief scientist at the American Herbal Products Association (AHPA; Silver Spring, MD), will be to accurately test unique finished products, like edibles. "Producers and testers face method validation or verification challenges to ensure accurate measurements in new product matrices, to prove that their gummy bear or fudge contains what the label claims." Johnson recalls one producer whose preferred medium was chocolate—relatively simple as analytical matrices go—sending her product to five different testing labs and getting back five widely disparate results.

A further complication: both THC and CBD exist in the plant as inactive carboxylates, THCA and CBDA,

respectively. Heating above 105 degrees Celsius liberates carbon dioxide to generate the active forms of THC and CBD, but ingestion of raw cannabis does not. For oral dosage forms, THCA and CBDA can predominate, so their quantities must be measured individually.



▲ *Sampling in ways that minimize the complexity and heterogeneity of cannabis plants will be critical to establishing analytical standards.*

As noted by cannabis analysis consultant Susan Audino, PhD, the marijuana plant is a difficult matrix on its own, with significant heterogeneity of chemical constituents within the plant. Formulating cannabis into food products complicates analysis even more.

Although 30 US states provide for medical marijuana use, and even though recreational use has been decriminalized in many jurisdictions, possession and transportation of cannabis across state lines remains a federal

crime. Pot's illegality—the “third dimension” in establishing good analytical practices for the growing marijuana product market—introduces danger and intrigue into what would normally be the free exchange of samples, standards, and know-how and the eventual advancement of cannabis analytics.

Most jurisdictions in the US, Canada, and Mexico have settled on testing for an array of potential contaminants, including pesticides, mycotoxins, microbiologic pathogens, gross contaminants, and heavy metals. Testing requirements vary significantly, however, from state to state.

SECONDARY CONSTITUENTS

In addition to CBD and THC, dozens of other cannabinoids have been uncovered, some with medicinal properties. Most exist in the acidic carboxylated form as well. “Some jurisdictions require testing for secondary cannabinoids, some don't,” says Don Land, PhD, chief scientific consultant at Steep Hill Labs (Berkeley, CA),

a leading cannabis-testing laboratory. Some of these chemicals are medically relevant and some aren't, so their future status as “ingredients” or “contaminants” remains unclear.

Other components such as terpenoids and flavonoids, which contribute fragrance and flavor to smoked and edible pot products, might at some point be designated as quality attributes or contaminants, in much the same way as related compounds are now recognized as such in foods.

Since many concentrated cannabis products involve extraction, residual solvents are one type of contaminant for which numerous testing protocols already exist. Supercritical carbon dioxide is often used because it evaporates quickly and completely and is medically innocuous. Butane, another common solvent, also evaporates at standard temperature and pressure. Most jurisdictions impose parts-per-thousand concentration limits on common solvents like hexane, acetone, and ethanol, and parts-per-million or parts-per-billion limits on riskier solvents. “Generic lists of volatile organic compounds



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include 60 or more compounds, but one would hope that a good many of these would never be used to extract products for human consumption,” Land says.

Contaminant concentrations are measured absolutely, based on the weight of plant matter and adjusted for water weight, which is about 10 percent for cured cannabis. A separate microbiological test also quantifies water activity, which is the partial water pressure of the sample divided by the partial pressure of pure water at the same temperature and pressure.

“Bottom line is it may be difficult to analyze for pesticides in a way that does not also negatively affect other analyses.”

PESTICIDES

Over the past several years, food scientists have adopted QuEChERS, the acronym for a pesticide extraction method that is “quick, easy, cheap, effective, rugged, and safe.” As a front end for analysis by liquid chromatography–tandem mass spectrometry (LC-MS-MS), QuEChERS has been applied to pesticide analysis for cannabis. Reporting in *LCGC*, authors from Restek, Shimadzu, Emerald Scientific, VUV Analytics, and Trace Analytics found that QuEChERS performed well for multiresidue pesticide analysis, as shown by acceptable recoveries and relative standard deviations for nearly the entire panel (150+) of pesticides. “Detectability for most compounds was sufficient, and quantitation using matrix-matched calibration was important because of the complexity of the cannabis matrices and the high cannabinoid content that remained in the final extract,” the authors write.

Don Land explains that while off-the-shelf methods have been developed by agricultural and food scientists for VOCs, pesticides, and heavy metals, testing for pesticides introduces complications. “When we test for pesticides, we’re investigating a lot of different compounds at once, each with different polarities and functionalities. QuEChERS tends to deplete samples of certain compounds that may be of interest. Cannabinoids and terpenoids may not be separable from pesticides, making

low detection limits more difficult to achieve for cannabis than, say, spinach, from which pesticides may be more cleanly separated for analysis. Bottom line is it may be difficult to analyze for pesticides in a way that does not also negatively affect other analyses.”

Land’s work, which estimated that more than 90 percent of pot was contaminated with pesticides, was recently cited to illustrate that even approved pot sold legally might not be safe.

STANDARDS AND LEGALITY

A number of organizations are working to develop regulations or recommendations for cannabis testing, an effort in which Steep Hill participates. But as of this writing, no definitive authority exists. “Every state, and in some cases municipality, has its own guidelines,” Land says. Organizations that one would normally turn to—USP and FDA for pharmaceuticals and EPA for environmental toxins, for example—are not much help when the product is cannabis. “If you look closely, it seems that many states have ignored what other jurisdictions have done, so they make the same mistakes over and over. Fixing this patchwork of regulations will take a serious meeting of the minds.”

The prohibition on interstate commerce in cannabis has led Steep Hill to an unusual business model. Instead of establishing a few core facilities in strategic locations, the company sets up autonomous laboratories in every jurisdiction in which cannabis products enjoy legal status. This model may seem like duplication to the extreme, but it is currently the only way to exploit the unusual status of cannabis in the US.

One positive outcome of this approach is that as states confer broader legal status on cannabis products, Steep Hill will be in the position to advise them on what has worked elsewhere and what has failed. And if, as some hope, the federal government eventually creates a friendlier business climate for marijuana products, Land expects his company will participate in formulating guidance and establishing methods for both active ingredient and impurity analysis.

Eventually, producers in need of one-off analyses may be able to turn to analytical service organizations specializing in environmental or food science. However, due to the complexity of the cannabis matrix, its anticipated medical indications, and its numerous dosage forms, Land believes that cannabis specialty labs will be the norm.

“These laboratories will not be the huge money-makers under this scenario. Cannabis testing is not a huge, high-value activity like production. As with other testing markets, customers all want to minimize the negative economic impact of testing. Impurity testing will become commoditized.”



◀ *Don Land of Steep Hill believes cannabis testing eventually will be commoditized.*

The difference under unconditional legalization would be more readily established FDA, USDA, and EPA rules applying to various stages of production and more easily transferred analytical methods. “Once it’s legal, many barriers will fall,” Land says, “but the devil will be in the details in terms of making a marijuana testing service economically viable.”

BY THE BOOK

Holly Johnson of AHPA, which started looking into cannabis in 2010, advises labs interested in the testing market to do things by the book. “Cannabis production facilities should comply with current GMP standards, and testing labs should obtain ISO 17025 accreditation,” she says. GMP refers to “good manufacturing practices” followed by pharmaceutical manufacturers to assure product and laboratory quality. Promulgated in 2005, ISO 17025, which is a requirement for operating a cannabis facility in California, “specifies the general requirements for the competence to carry out tests and/or calibrations, including sampling. It covers testing and calibration performed using standard methods, nonstandard methods, and laboratory-developed methods.”

Johnson believes that ISO 17025 should be the starting point for demonstrating a testing lab’s capabilities, despite the fact that “some states require ISO 17025 accreditation and some do not. State regulations differ on the required analytes, which complicates the establishment of standard analytical methods.”

APHA’s own recommendations are based on GMPs applied specifically to cannabis. Massachusetts, Oregon, Nevada, and Illinois have considered AHPA’s recommendations when formulating their medical marijuana program regulations, as has Americans for Safe Access, a cannabis patient advocacy group.

Johnson also serves on the United States Pharmacopeia (USP) Medical Cannabis Expert Panel, which is nearly finished with a monograph on cannabis flowers and is working on a second monograph on extracts. ASTM, another significant standard-setting organization with hitherto scant involvement with botanicals, has established a committee for cannabis and has recruited experts in manufacturing and laboratory operations.

LEARNING FROM CANADA

With pot poised for unrestricted legalization in Canada by year’s end, and given Canada’s pre-eminence on issues of patient and consumer safety, one would expect that US jurisdictions could benefit from the experience of their neighbor to the north. “Sure we can learn from Canada, but they are still sorting things out,” Johnson says.

Issues still unresolved include marketing of edible products to which children may easily gain access, which Johnson believes could boost prospects for more traditional labs to get involved in cannabis testing, particularly for pesticide and heavy-metal testing that they already do quite well for other industries. Success will depend on arriving at standards, however.

“Reaching a nationally recognized, standard analytical method for pesticide screening that satisfies every state’s requirements will be challenging,” Johnson says. “California requires testing for around 70 specific pesticide residues, but other states often require a more- or less-extensive list. And, depending on the pesticide panel, a lab may need to carry out several extractions and perhaps use more than one method platform to measure all the required compounds.”

Angelo DePalma is a freelance writer living in Newton, New Jersey. You can reach him at angelo@angelodepalma.com.



Dr. Bryan Tomlin

ASK THE EXPERT

CHANGES AND TRENDS IN ELEMENTAL ANALYSIS

by Lauren Scudato

Dr. Bryan Tomlin obtained his doctorate degree in chemistry from Michigan State University, where his dissertation was in the area of nuclear structure studies. After completing his degree, Dr. Tomlin spent five years as a research chemist in the Analytical Chemistry Division at the National Institute for Standards and Technology (NIST), where he focused on the development and application of neutron activation analysis (NAA) for characterizing Standard Reference Materials. He then joined The Dow Chemical Company as a senior R&D chemist in the Analytical Sciences unit, applying NAA in support of varied R&D projects. In 2015, Dr. Tomlin joined the Texas A&M University Center for Chemical Characterization and Analysis as the manager of the Elemental Analysis Laboratory.

Q: What kind of elemental analysis do you most commonly do at the Elemental Analysis Laboratory at Texas A&M University?

A: Oxygen, by far, is our most common analyte. We also frequently do halogen analyses, as well as analyses of various metals.

Q: What key analytical technologies or instrumentation do you use for elemental analysis?

A: We use two kinds of neutron activation analysis (NAA), as well as ICP-MS. We have two resources that are not widely available—one is the research reactor at our university, which we use for thermal NAA, and we have two 14 MeV neutron generators that allow us to do a more specialized type of NAA called fast neutron activation analysis (FNAA). That is what we use for oxygen determinations. So those two pieces of equipment in particular allow us to offer the services we do.

FNAA is a very nice complement to thermal NAA because there are certain elements like fluorine and nitrogen that aren't easy or possible to do with thermal NAA, but they can be done quite well using high-energy neutrons from a generator.

Q: How many researchers are involved in this work at the Elemental Analysis Lab? How many projects are going on at a given time?

A: We have one technician, but we also take advantage of student workers. In a typical semester, we will have one to three students contributing. On average, we have five to 10 projects in the queue per week. Smaller projects can be done within a day, but we do have some larger university-related projects with lots of samples, and those may be completed across a month's time.

Q: What have been the major changes or trends you've observed within the field of elemental analysis in recent years?

A: Speaking specifically to our lab, the big change that's occurred over the last 15 years or so is a push toward increasing

use of ICP-MS as an analytical technique. We purchased our first one around 2001, and since that time, we've seen steady growth of demand for that type of analysis against thermal NAA. That's

“Researchers see that others in their field are publishing with ICP-MS data, so they want to use the same technique.”

been a major change. Part of it is simply due to awareness, because ICP-MS technology is readily available, whereas NAA research reactors aren't so widely available. The broad acceptance of a technique makes people comfortable. Researchers see that others in their field are publishing with ICP-MS data, so they want to use the same technique.

Q: How have those changes affected your lab?

A: It shifted the ratio of the work we do. For decades, we did a lot more thermal NAA than FNAA. Now, as ICP-MS has become more available and can be used to do a lot of the same determinations, the ratio of work we do for fast

neutron, which can't be done with ICP-MS, has grown significantly. It's caused a pretty significant shift in our portfolio of projects in that regard.

Q: What are some of the advantages of using ICP-MS in elemental analysis?

A: Overall, cost is often an advantage for ICP-MS. The most commonly cited drawback to NAA is the high expense, which is largely the result of the regulatory cost of operating a research reactor to provide neutrons. Neutrons as a probe are expensive compared with photons as a probe, for example. A major benefit is the speed of analysis. With NAA, we are often limited by radioactive decay physics. So, if something has a half-life that is very long, you can't speed that up. If you're measuring the radioactive decay of cobalt-60 for example, which has a five-year half-life, the measurement time could be longer. With ICP-MS, if you can prepare a solution of it, you can get a result within minutes. Those are two things that have been pushing broader elemental analysis toward ICP-MS.

But we continue to offer NAA because it's capable of doing things that either cannot be done, or cannot be done well, by ICP-MS—particularly nonmetals. I mentioned oxygen, but we also do a lot of analyses of fluorine, chlorine, and bromine, which NAA does really well. NAA also has the advantage of not requiring sample dissolution. We encounter quite a few things that cannot be destroyed or cannot be readily dissolved, so the two technologies really complement each other even though there's a lot of overlap between the two.

Q: What are some of the main challenges you run into when it comes to elemental analysis?

A: One important lesson I learned during my time in industry is that there tends to be a bias toward selecting the tool over identifying what the problem is. Often, especially with university customers, people come in and say "I need this technique to measure my samples,"

So, understanding what you're doing is important but may not necessarily be the thing that grabs people's attention. One of the things I've learned is that science can be competitive, and there are a lot of other "cooler" techniques that get a lot of attention.

“One of the things I've learned is that science can be competitive, and there are a lot of other 'cooler' techniques that get a lot of attention.”

so the challenge for me is to back up and find out what problem they are trying to solve. Very often, what they think they need is not necessarily the best tool for solving the problem, so the challenge is overcoming the mentality of using analytical chemistry in a "cafeteria" or "à la carte" style, meaning "I choose this technique because it's what I think I should use" versus approaching it from a "I have this problem to solve, let me see what the best tool or technology is for solving this problem." A big part of my job is listening, understanding what it is they are trying to solve, and then perhaps teaching them about different options they have and why I'm recommending one over another.

Q: What advice do you have for lab professionals who are interested in getting into elemental analysis?

A: Bulk elemental analysis tends to be a rather "unsexy" line of analytical work, so working in the field, you often won't be in the "limelight" with exciting research projects. An elemental analysis step may be very early in the discovery process of a project, and will be key, but may not be in the forefront of coverage.

Q: Anything noteworthy for the future of the Elemental Analysis Lab? Goals, new equipment, new research, etc.

A: I have a goal of moving toward ISO 17025 compliance, at least, so that we're meeting all the data quality expectations that industry customers have now or will have in the not-too-distant future. We are moving more toward automation as well—the way things have been done can be labor-intensive and require many manual steps. I see opportunities to increase automation, which would allow us greater throughput.

Finally, our educational role. One of the reasons I came here was to teach students more broadly about metrology. I want to teach them to be better consumers of analytical chemistry—understand trade-offs, data quality, etc. I think there's a space here at the university for that, so as they go off and graduate and find jobs, they'll make better use of the analytical tools that they will need in their careers.

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HPLC

GETTING THE HPLC GRADIENT RIGHT

by Mike May, PhD

To separate solutions, scientists often turn to high-performance liquid chromatography (HPLC). In 1969, Waters Corporation (Milford, MA) produced the first commercial HPLC platform. Since then, HPLC platforms have become increasingly easy to use, even as the technology's capabilities have increased. Nonetheless, producing the right gradient for separation remains a crucial aspect of this analytical technique.

At the Bindley Bioscience Center of Purdue University (West Lafayette, IN), metabolomics research scientist Amber Hopf Jannasch and her colleagues use HPLC to separate complex biological mixtures prior to mass spectrometry (MS) analysis. Jannasch notes that small polar molecules can be a challenge for gradient separation, "but with advances in hydrophilic interaction liquid chromatography, or HILIC, it's becoming less problematic." HILIC columns are made for these very molecules. Still, Jannasch adds, "We have to ensure that the buffers we use for separation are mass-spec-compatible, so we cannot use most ion-pairing types of methods."

"We carefully consider the physical properties of the molecule of interest before starting a column screen."

Other scientists also use HPLC upstream from MS. In the Department of Pharmaceutical Sciences at the University of Tennessee Health Science Center in Memphis, for example, scientists use HPLC-MS to quantify compounds in unknown liquid samples. When asked about the challenges of getting a successful gradient separation, senior research specialist Dejian Ma says, "It varies due to many factors, such as compound properties, column types, mobile phase solvent selection, etc."

Top tips

Despite the advances in HPLC platforms, columns, and reagents, it still takes knowledge and experience to get the best separations. When asked for top tips for getting a successful gradient separation, Jannasch says, "We carefully consider the physical properties of the molecule of interest before starting a column screen." If Jannasch and her colleagues want to pull out a small, polar molecule from a sample, the scientists use HILIC. "If it's a hydrophobic molecule," she says, "C18 will probably work."

For instance, scientists in India used HPLC to study the breakdown of pharmaceuticals made for oral delivery.¹ Specifically, the researchers wanted to study how montelukast sodium—an asthma medication—can be degraded by various oxidative media, including oxygen, in light. Using C-18 columns and a gradient method of HPLC, the scientists separated the degradation products from oxidation for MS analysis.

For most samples, a range of HPLC variables must be adjusted for the best separation. Moreover, optimizing the variables is not always straightforward. To separate four monophosphate nucleotides, for instance, scientists in Japan performed a systematic analysis of HILIC approaches.² They experimented with the buffer concentration, gradient time, and temperature. To separate the four compounds, they used four HPLC columns: ZIC-HILIC, ZIC-CHILIC, NUCLEODUR HILIC, and PC HILIC. The scientists noted that "the HPLC conditions for each column were successfully optimized, [and] optimized HPLC conditions differed from column to column."

In all analytical methods, some of the most complicated challenges arise from unknown samples. In most cases, scientists know what they are analyzing in a sample, but they don't know the concentration. "It's definitely more challenging to analyze a completely unknown sample in order to solve a real-world problem," Ma notes. It gets even more complicated if the range of possible compounds is unknown. Ma shares his top tip for such an analytical challenge: "Before working with unknown samples, make sure that the hardware system performs well by always running a blank or a standard sample first." He adds, "This is probably true for any analytical technique, not just HPLC."

Scouting for a solution

Many approaches to HPLC require some method development. In reverse-phase HPLC, for example, scientists use a scouting gradient when they don't know the best conditions to separate a sample. A scouting gradient helps them determine whether a constant or a gradient method will work best with a sample.

To run a scouting method, scientists increase the percentage of the elution linearly over a run of about a half hour to an hour. The results can be used in various ways to decide how best to separate a sample. If the peaks from the results of running a sample with a scouting gradient take up a small part of the run time—say, one-quarter or less—an isocratic (constant) method can be used. On the other hand, if running a sample through a scouting gradient produces peaks roughly half or more of the run time, then a gradient method will work better. In between, scientists need to make the best guess and see what happens.

In some cases, different gradient methods can be tried on the same sample. A team of scientists from South Africa and France used gradient methods of HPLC to separate forms of amphiphilic hyaluronic acid, which is found in connective and neural tissue.³ The methods included normal- and reverse-phase gradient HPLC. Trying different forms of gradient HPLC can separate samples based on different features. For this work, as an example, the normal-phase gradient separated the compounds based on polar hydroxyl groups in the sample interacting with polar components of the gradient material; the reverse-phase method separated the sample's components based on hydrophobic features of the sample interacting with nonpolar elements on the C8 columns.

Tools to try

Ultimately, a scientist needs a range of ways to approach gradient-based HPLC. Fortunately, various vendors provide some helpful guidelines. For instance, Agilent Technologies (Santa Clara, CA) created its “Good Habits for Successful Gradient Separations,” which notes, “Developing good gradient habits is the key to long-term success.”

As these examples show, no matter how much HPLC instruments and reagents advance, scientists still comprise



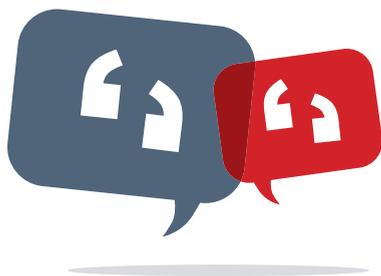
a crucial element of the process. Moreover, getting the best separation depends on some experimenting. The more a scientist knows about what might be in a sample, the more the method can be developed based on knowledge and experience. In an unknown sample, scientists might need to start from scratch—trying different methods to see what does the best job of isolating specific components.

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FOR ADDITIONAL RESOURCES ON HPLC, INCLUDING USEFUL ARTICLES AND A LIST OF MANUFACTURERS, VISIT
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Types of applications survey respondents perform using FTIR:

Quality verification	69%
Contaminant identification	53%
Analysis of thin films and coatings	27%
Failure analysis	25%
Deformation	14%
Monitoring emissions	5%
Other	15%

How many FTIR spectrometers respondents currently have in their lab:

1	64%
2	14%
3	2%
4	1%
5 or more	7%
None	12%

Nearly 41% of respondents are engaged in purchasing a new FTIR spectrophotometer. The reasons for these purchases are as follows:

Replacement of an aging system	63%
Addition to existing systems, increase capacity	19%
Setting up a new lab	17%
First time purchase	10%
Other	21%

WHAT YOU NEED TO KNOW WHEN BUYING AN FTIR SPECTROPHOTOMETER

Fourier transform infrared (FTIR) spectroscopy, a subset of infrared (IR) spectroscopy, uses a mathematical algorithm, Fourier transform, to translate raw infrared data into a spectrum.

TOP 5 QUESTIONS

You Should Ask When Buying an FTIR Spectrophotometer

1. What applications are you using FTIR for? This will determine what type of FTIR spectrophotometer will be the best fit for you. For example, if you will be conducting most work outside the lab, a portable instrument is likely a good fit. FTIR microscopy may also be an option you'll want to consider, depending on what type of research you do.
2. What sort of environment will you be working in? If you are out in the field, in a humid area for example, the instrument should be tough enough to handle the conditions.
3. Who will be using the instrument? If non-experts will be the main users, it makes sense to go for a user-friendly instrument that won't take too much time to learn to use. The complexity of the software is also important to consider.
4. What accessories are available for the instrument and how wide is their range?
5. As with most instruments, you should ask what sort of service and support the company provides for the FTIR spectrophotometer, and its cost in terms of acquisition, running the FTIR, and maintaining the instrument.

TOP PROBLEMS

Experienced When Using an FTIR Spectrophotometer:



➔ For more information on FTIR, including useful articles and a list of manufacturers, visit www.labmanager.com/FTIR

FOLLOW YOUR CELLS IN REAL TIME

Real-time cell based assays are experiencing increased interest in life science laboratories. These assays are usually performed in microplates, allow for continuous monitoring of cellular responses upon stimulation, and offer several advantages to end-point assays. BMG LABTECH's CLARIOstar® multi-mode microplate reader was designed to serve as the ideal platform for the detection of live cell-based assays, in particular in real time. A new BMG LABTECH proprietary technology even encompasses the possibility to study cellular responses in the microplate reader at tissue-specific oxygen conditions and to mimic in vitro disease-like conditions such as ischemia/reperfusion, stroke or tumor hypoxia and growth cycles.

Real-time cell based assays enable researchers to repeatedly perform measurements on the same samples over time, providing a comprehensive analysis of the possible changes occurring in treated cells over the length of an experiment.

For instance, real-time assays allow continuously monitoring and detect changes in cell viability, cytotoxicity, apoptosis, and metabolic parameters such as redox state, pH and oxygen consumption over a period of hours, or even days. In pharmacology, they are used to study the binding kinetics and the activity of agonists/antagonists.

Unlike in endpoint assays, cells are not lysed and can hence be subjected to multiple measurements for prolonged times, directly detecting kinetic changes over time.

Other advantages are the reduced use of reagents a single-plate assay run over time requires compared to multiple separate plates for each time point. This is further enhanced by the capability to detect multiple parameters (multiplexing). Multiplexing decreases sample variability and provides a better internal control.

Measuring cellular reactions in real time requires specific features of the microplate reader. BMG LABTECH's CLARIOstar plate reader was specifically designed for cell based assays. With its revolutionary LVF Monochromator™, this multi-mode reader offers the best sensitivity in its class both for measurements from the top and from the bottom – the latter being the most beneficial when detecting adherent cells. Top or bottom measurement is simply selected by a click in the software, requiring no hardware displacement.

When working with living cells, the possibility to control temperature and atmosphere are absolute requirements. On the CLARIOstar, the built-in incubation system ensures stable temperature at 37 °C, whereas the Atmospheric Control Unit (ACU) actively regulates O₂ and CO₂ between 0.1 to 20%.

A unique feature of the CLARIOstar with ACU is the capability to run gas ramps, depriving the reader of O₂, keeping stable hypoxic conditions and then re-oxygenating back.

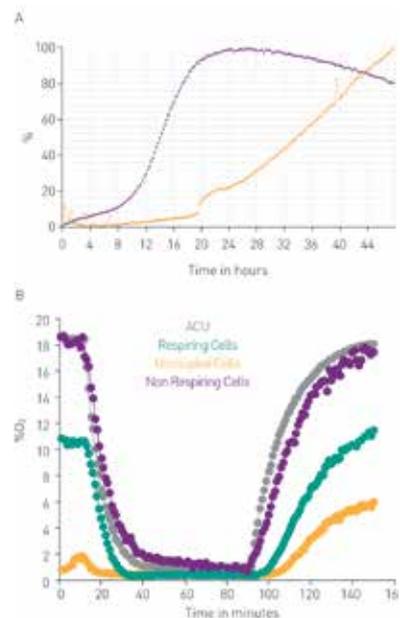
O₂ concentrations can be brought down to 0.1 percent, and ambient O₂ conditions can be recovered within 30 minutes, each. This technology allows researchers to apply hypoxic stress to real-time cell-based assays and can be applied to mimic ischemia/reperfusion, stroke or tumor hypoxia in vitro.

Besides the ability to multiplex different assays, two additional features further enhance the capabilities of the CLARIOstar: the possibility to automatically add reagents to the wells over the whole kinetic, and the ability to detect very fast reactions. In fact, the CLARIOstar can collect up to 100 data points per second for every single well. Thanks to this feature, even very fast cellular responses can be captured with ease.

▼ Fig 1: CLARIOstar



▼ Fig 2:



Two examples of real time cell based assays detected on the CLARIOstar. A: cell apoptosis and necrosis multiplex over 48 hours (BMG LABTECH application note 311). B: gas ramping with cell O₂ consumption measurement (BMG LABTECH application note 309).


The Microplate Reader Company

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George Miles, MD, PhD

ASK THE EXPERT

TRENDS IN CLINICAL GENOMICS

by Tanuja Koppal, PhD

George Miles, MD, PhD, assistant professor, Department of Molecular and Human Genetics, and lab director of the Precision Oncology Laboratory, Lester and Sue Smith Breast Center, Baylor College of Medicine, shares with contributing editor Tanuja Koppal, PhD, some details on the work he is involved with in clinical genomics. He discusses trends in next-generation sequencing, clinical informatics, and molecular diagnostics, all of which are driving personalized medicine.

Q: Can you share some details on the work that you are doing to apply clinical genomics to drive precision medicine?

A: Certainly! I'm delighted to be part of a team that is expanding precision medicine efforts at Baylor College of Medicine and the Texas Medical Center. Specifically, we are building an academic precision oncology laboratory within the Breast Center that will lead and support cutting-edge translational research, including the design and execution of clinical trials. We are utilizing a variety of core pathology

detection, molecular profiling, and monitoring of disease. For the latter, we seek to demonstrate a substantial lead time in adaptive treatment and trial placement relative to current monitoring of disease progression.

Q: Are there new trends and technologies in genomics that you are excited about or you consider to be game-changers?

A: We are now at a point where improvement in patient outcomes will be driven in large part by the integration of complex molecular data into

Add to that the network complexities that are further intertwined with the microbiome, immune response, drug history, and combination drug therapies, and much more! I am excited about the “multi-omic” approach in clinical diagnostics that we are harnessing here at Baylor College of Medicine.

In addition, the development of novel approaches to sequencing nucleic acids is gaining traction now and may help democratize sequencing ability/access, spurring further innovation in research and patient care. I've been fascinated with real-time, native single-molecule sequencing and its potential, once it becomes widely available.

Q: What are some of the changes that have taken place in recent years on the technical and informatics front that have enabled the growing use of genomics technologies?

A: Continuous advances in bioinformatics and big data analytics are helping tackle the problem of efficiently deciphering and interpreting the observed aberrations en masse rather than as individual components. At the very least, this should provide impetus for the design of *in silico* clinical trials followed

“Our approach affords both discovery and surveillance arms that span cancer detection, molecular profiling, and monitoring of disease.”

and advanced molecular technologies, from next-generation sequencing to mass spectrometry, to facilitate individualized patient management plans and better define theragnostic strategies for specific patient populations, such as those who have metastatic breast cancer. Our approach affords both discovery and surveillance arms that span cancer

the clinical space, tailored to a patient's genetic information and corresponding functional phenotype. Cancer (as with any other disease) is a dynamic process and really should be monitored as such. I know that the ability to synthesize and act on panoptic molecular consequences—genomic, proteomic, metabolic—will be a major move forward.

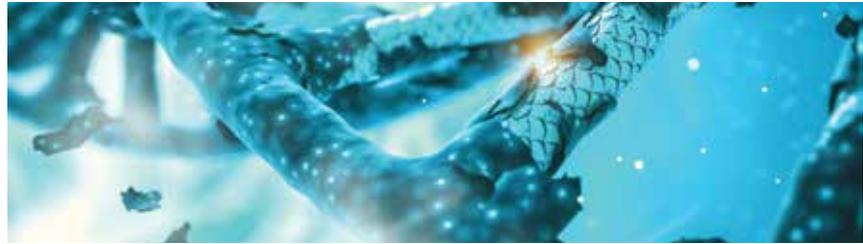
by functional validations. This will help in further demonstrating clinical utility, which of course will feed demand in utilizing genomic technologies as a tool in diagnosis and management.

Q: As laboratory director in the BCM Breast Center, can you share your perspectives on some of the challenges you encounter in clinical diagnosis and therapeutic intervention?

A: There are a number of logistical and operational challenges associated with high-complexity genomic/molecular diagnostics that require close interactions between a number of vested parties at all stages. This requires a very streamlined and efficient workflow. Laboratory/assay development costs remain a concern, particularly for some very complex patient cohorts that we are supporting, such as metastatic breast cancer that requires serial monitoring. Such comprehensive testing at scale (and with much required automation) across individuals and clinical trial populations is particularly challenging and expensive, necessitating a tiered and narrowly focused approach with complementary techniques/molecular platforms supported through various grant mechanisms.

Q: What attempts are being made to tackle these issues in terms of investing in new technologies or expertise?

A: The Breast Center fosters an integrated framework between all shareholders in our precision medicine initiatives, including the following: an in-house biobank staffed with full-time research coordinators who manage patient consent and sample accruals for basic and clinical research, a dedicated



genomics-molecular/core pathology lab, a mouse PDX core facility, focused resources that support biostatistics and informatics in clinical trial management, a high-performance computing center, and so forth. Of course, our

“The recent advances in clinical genomics have really fostered a paradigm shift in genetic testing and personalized medicine.”

collaborations extend beyond the center itself, taking advantage of expertise within Baylor, commercial entities, and surrounding institutions. Additionally, we host a monthly CME-accredited molecular tumor board meeting to discuss challenging cases. This multidisciplinary gathering highlights our expertise and workflow and has proven to be an invaluable resource in patient management and care.

Q: Any advice specifically for our readers who are lab managers in clinical genomics and diagnostics laboratories?

A: The recent advances in clinical genomics have really fostered a

paradigm shift in genetic testing and personalized medicine, and it is an exciting time to be able to bring these next-generation diagnostic tools to fruition in the clinic. Particularly in academic medical centers, the formation of a close working partnership between physicians and labs to best leverage internal expertise and resources is critical.

George Miles, MD, PhD, is an assistant professor in the Department of Molecular and Human Genetics at Baylor College of Medicine, Houston, Texas. He is the laboratory director for the Precision Oncology Laboratory within the Lester and Sue Smith Breast Center/Dan L. Duncan Comprehensive Cancer Center. He completed an MD and PhD at Texas A&M University, College Station, Texas, followed by a pathology residency and fellowship at the National Cancer Institute, National Institutes of Health, Bethesda, Maryland, and a molecular genetic pathology fellowship at Washington University School of Medicine, St. Louis, Missouri. He is board-certified in anatomic and molecular genetic pathology. He is a physician scientist with expertise in cancer genomics and molecular diagnostic assay development that spans academic, government, and private industry settings and is involved in a number of ongoing clinical trials.

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ANTIBODIES AND PROTEIN BIOLOGY

EFFICIENCY, RELIABILITY, AND POSSIBLE DANGER IN PROTEIN QUANTIFICATION

by Brandoch Cook, PhD

Biochemical assays are powerful tools in laboratory biomedical science, and they can often answer questions clearly and digitally in ways other procedures cannot. However, their accuracy is wholly dependent on the fidelity with which one can quantify the relative concentrations of proteins in solution.

To give an idea of the importance of protein quantification, the top three most-cited articles in the history of scientific publication describe techniques for measurement or preparation of protein samples. The all-time champion is the 1951 paper (with over 305,000 citations!) in which its lead author, Oliver Lowry, introduced an eponymous procedure for protein quantification. He and his colleagues adapted the biuret method, a test for the presence of proteins in alkaline solutions revealed by a purple color marking chelation of copper ions by a detection reagent, a chemical reaction first observed in the mid-nineteenth century. They developed and tested a new detection reagent that made the process more straightforward and reliable. This method was a vast improvement in user-friendliness and safety compared with the atavistic Kjeldahl method, which requires the surrogate derivation of protein content via emission of nitrogen liberated in ammonia from a sample exposed to heated sulfuric acid. Today's average lab would need a much bigger fume hood to accommodate this assay.

Ease of use and reliability have been the keywords in subsequent variations on protein assays since the days of Lowry et al. However, most assays rely on similar principles—colorimetric evaluation of a solution via its linear change in absorbance at a given visible-spectrum wavelength when exposed to a protein-binding reagent. One way the Lowry method retains an advantage over contemporary procedures is that it is an “end-point” assay; in other words, the reaction between protein and detection reagent reaches a finite maximum so a single standard curve using a known protein can be used repeatedly to calibrate sample concentrations across multiple experiments.

In other commonly used assays, color continues to deepen indefinitely, and a new standard curve must be generated every time. This is achieved by measuring absorbance across a range of known concentrations in serial dilution of bovine serum albumin or immunoglobulin and calculating the concentrations of experimental samples from the equation generated by the standard curve. If it sounds tedious to repeat this procedure every time you run a protein gel, that's because it is.

Improvements in reliability and throughput

Tedium aside, where Lowry is deficient compared with newer assays is in almost every other aspect relevant to modern biochemical techniques. Therefore, when the BCA assay replaced the Folin-Ciocalteu reagent with bicinchoninic acid, the throughput and reliability of copper-chelate protein quantification improved dramatically. The BCA assay proceeds in one easy step, making it more amenable to simultaneous measurement of many samples, in terms of both time and reagent usage. Moreover, the detection reagent is more stable in an alkaline solution, with less interference from components typical of many protein-containing cell lysates, including detergents, Tris, cations, EDTA, and reducing agents.

Similar in colorimetric principle, although quite different in chemistry, the Bradford assay employs the intrinsic property of Coomassie dye to change from red in acidic solution to blue when bound to protein. This is another straightforward, one-step procedure that allows for high throughput, with the added benefit that it develops more quickly. However, Bradford is also sensitive to the presence of detergents in protein buffers and suffers from high variability. Although the Pierce 660 assay uses a similar procedure while offering more compatibility in buffer constituents, variability is still a problem compared with biuret-based methods. Consequently, BCA has unofficially become the gold standard among biomedical laboratories for protein measurement preparatory to standard procedures such as Western blotting.

Because of differences in chemistry, BCA and Bradford are mutually incompatible in the affinity of one detection reagent over another for particular amino acids. Proteins with excess cysteine, tyrosine, and tryptophan residues will skew BCA assay absorbances because of their affinity for cuprous ions, while the affinity of Coomassie dye for arginine and lysine will do the same, providing inaccurate readings.

“The top three most-cited articles in the history of scientific publication describe techniques for measurement or preparation of protein samples.”

Specialty assays

Finally, for specialists who may be examining small peptides at low concentration or proteins associated with lipid bilayers that can interfere with standard assay reagents, there are

several kits and protocols that allow reliable and reproducible measurement. One example is the CBQCA assay, designed for highly sensitive measurement of proteins in lipid solutions, which uses potassium cyanide (danger!) to stimulate reaction with amine groups, resulting in fluorescent excitation.

In addition to CBQCA, there is a seemingly limitless variety of specialty applications for protein quantification. The table below provides a summary of differences and compatibilities between the more standard assays. Although BCA is perhaps currently the most favored, there are rationales for choosing other assays based on factors such as buffers, time frames, detection limits, and wavelengths particular to spectrophotometer and microplate reader setups. What works best for the end user depends on considerations that are unique to every laboratory's field of interest. This table uses kits provided by Thermo Fisher as a guide, although several are also offered by Sigma-Aldrich and Bio-Rad, and the more adventurous and thrifty among you can always make your own.

Brandoch Cook, Ph.D. is an assistant professor in the Weill Cornell Medicine Department of Surgery in New York, NY. He can be reached at brandoch.cook@gmail.com.

	Lowry	BCA	Bradford	Pierce 660	CBQCA
Chemical reaction	Biuret	Biuret	Protein-binding dye	Protein-binding dye	Amine-reactive dye
Number of reagents	2	2	1	1	3
Number of steps	2	1	1	1	3
Time of incubation	40 minutes	30 minutes	10 minutes	5 minutes	1 hour
Absorbance Wavelength	750 nm	562 nm	595 nm	660 nm	Ex 460 nm /Em 550 nm
Temperature of reaction/storage	Ambient/4° C	37°C/Ambient	Ambient/4° C	Ambient/Ambient	Ambient/-20° C
Detection range	1-1500 µg/ml	20-2000 µg/ml	1-1500 µg/ml	50-2000 µg/ml	10 ng-150 µg/ml
Accuracy	++	++	+	+	+++
Value	++	++	+++	++	+
Incompatibilities	Tris, cations, EDTA, detergents, reducing agents	Reducing agents, thiols, lipids	Low molecular weight samples, detergents	Compatible with more detergents and sample buffers	Tris, glycine, ammonium ions

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ARE YOU IN THE MARKET FOR A FLOW CYTOMETER?

Flow cytometry is a powerful technology that allows researchers and clinicians to perform complex cellular analysis quickly and efficiently by analyzing several parameters simultaneously. Just a few years ago, working with a flow cytometry instrument was generally done by teams of highly trained experts at core facilities, working on massive machines costing more than \$100,000. Today's instruments can be built for benchtop use and are getting ever cheaper. Much of the machine setup is now automated, which will likely continue to democratize the method. Benchtop, micro-capillary flow cytometers, paired with ready-to-use cell analysis kits, enable any researcher to leverage the power of flow cytometry, regardless of expertise or access to a core facility.

Number of flow cytometers used by respondents in their lab

1-2	74%
2-4	18%
5-6	1%
More than 7	7%

Research applications that respondents predominantly use their flow cytometer for.

Immunophenotyping	51%
Cancer	33%
Cell proliferation	33%
Stem cells	32%
Cell cycle analysis	29%
Apoptosis	28%
GFP and RFP detection	20%
Microbiological applications	17%
Marine sample analysis	5%
Other	29%

TOP 5 QUESTIONS

You Should Ask When Purchasing a Flow Cytometer

1. What type of cells do you want to study? Running simple assays involving cell viability or counting often do not require highly sophisticated machines.
2. How many lasers, filters, and detectors do you need? The more lasers and detectors an instrument has, the greater the number of simultaneous colors can be detected.
3. Where will you be in three to five years? How many samples will you be running and what parameters will you be looking at? If your instrument doesn't support your future needs, you may better serve your lab by purchasing a higher-end instrument.
4. How difficult is it to set up and operate? Users should be able to operate a simple two-laser system within a couple of hours, while a more complex system may require considerable training.
5. What are the ongoing costs? Beyond the instrument cost, you'll also be paying for assay reagent kits, filters, and other consumables. Understand the total cost of ownership including repair and maintenance costs before purchasing.

HERE ARE THE TOP PROBLEMS

Experienced by Flow Cytometer Users:

LOW EVENT RATE	40%
NO SIGNAL/WEAK SIGNAL INTENSITY	39%
HIGH BACKGROUND/HIGH PERCENTAGE OF POSITIVE CELLS	32%
TWO OR MORE CELL POPULATIONS OBSERVED WHEN THERE SHOULD BE ONE	22%
HIGH SIDE SCATTER BACKGROUND	16%
HIGH SIGNAL INTENSITY	11%
HIGH EVENT RATE	9%
OTHER	26%

➔ For more information on flow cytometers, including useful articles and a list of manufacturers, visit www.labmanager.com/flow-cytometry



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FOR ADDITIONAL RESOURCES ON HOMOGENIZERS, INCLUDING USEFUL ARTICLES AND A LIST OF MANUFACTURERS, VISIT WWW.LABMANAGER.COM/HOMOGENIZERS

HOMOGENIZERS

GETTING THE MOST OUT OF YOUR HOMOGENIZER

by Erica Tennenhouse, PhD

Homogenizers are used to disperse, emulsify, lyse, or mill samples as diverse as tissue, soil, and cosmetics. Although a simple mortar and pestle can accomplish somewhat similar results, a high level of consistency can be achieved only by a mechanical homogenizer with a decent generator probe. To get the most out of a lab homogenizer, it is crucial to select the right features for given applications and to maintain it properly.

Picking your unit

Homogenizers come in a variety of configurations, including handheld and bench-mounted models with a variety of probes to match the correct sample type. The primary selection criterion for a homogenizer is the sample or material type being processed.

The wattage required will depend on both the sample type being processed and the size of the samples. Processing small volumes requires less power, so a handheld unit is usually the best choice in these cases. But to homogenize a large volume of viscous material, a larger motor may be needed. Certain homogenizer units are capable of processing a wide range of sample volumes, from milliliters to liters.

In many cases, the speed of the process matters as well. For example, faster processing time increases the quality of the yield for nucleic acids and proteins because it gets them into the protective buffer faster. Speed controls are available in some mechanical units to enable the user to adjust the speed in increments of hundreds of RPM.

The friction generated by a homogenizer will cause an increase in temperature during operation. The simplest way to reduce the effect of temperature on the samples is to select the correct generator probe and unit for your application, which will decrease the time spent homogenizing the sample, thus reducing the effect of temperature. Many manufacturers, including those of blender-style homogenizers, have also begun creating built-in cooling systems to combat temperature increases.

Here are some of the most important features to look for when purchasing a homogenizer:

- Easy cleaning of product-contact surfaces
- A low motor noise, since homogenizers are usually located on workbenches in proximity to operators
- Ease of use
- Rapid homogenization
- User control over homogenization parameters through a familiar digital display
- Low heat generation, which is particularly important for labile tissue samples
- A programmable library of methods

Maintenance is key

Once a homogenizer has been selected and purchased, the next challenge is to keep it in good condition. The life of a probe-based homogenizer can be extended with some simple care of the generator probe—the part that actually goes into the sample. Although some homogenizers do not have a generator that can be easily taken apart, purchasing a system that allows for a thorough cleaning once in a while is generally considered a better investment.

The upper and lower bearings need to be replaced on a semiregular basis; the specific replacement time depends on use. While a new generator probe can run more than \$1,000, a pack of bearings costs a mere \$30 to \$40. Thus, small repairs can provide a substantial return on investment in the long run.

Signs that it's time to perform maintenance on your homogenizer include the sample heating up, the generator probe or motor unit becoming hot to the touch, or black residue appearing in your sample. Wearing or discoloration on the internal components, such as PTFE bearings, or your generator probe seizing up are also signs that it's time for service. Additionally, if the generator probe has never been taken apart for a cleaning, then chances are that some maintenance is required.

Erica Tennenhouse, scientific content editor for Lab Manager, can be reached at etenmenhouse@labmanager.com or by phone at 647-500-7039.



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ASK LINDA

ATTRACTING TALENT

QUESTION:

Dear Linda,

I just learned that there is money in my budget to hire two new, badly needed, lab techs. However, I've found that it's much harder these days to find and hold onto qualified candidates. Today's entry-level employees are much different than they were 10 years ago, with most expecting very specific working conditions and opportunities. Given that nobody wants to go through the recruitment, hiring, and training process again and again, are there any secrets to finding and keeping the right people right off the bat?

Thanks,
Meredith



HAVE A QUESTION FOR LINDA?

EMAIL HER AT: LINDA@labmanager.com

ANSWER:

Dear Meredith,

Since the demand for talent always exceeds supply, you have certainly identified what most managers consider their biggest challenge. For most entry-level positions, at this point in time, it's a seller's market. And as that situation continues, so does the weight on lab managers tasked with finding the best.

Instead of a systematic or one-size-fits-all approach, managers need a variety of incentive options when trying to secure talent. Different workers respond to different motivators.

While money is the routine starting point in attracting talent, equally important is management's approach to soft benefits—reward and recognition programs, flextime and work/life balance, career training and development, and other conveniences and services.

Managers should take pains with potential employees to convey the company culture, since it's not obvious from the outside.

Mismatches between employee expectations and the promises and true culture of the workplace—likely the primary reason new hires lose interest and change jobs—can be minimized by a thorough, two-way interview process on the front end.

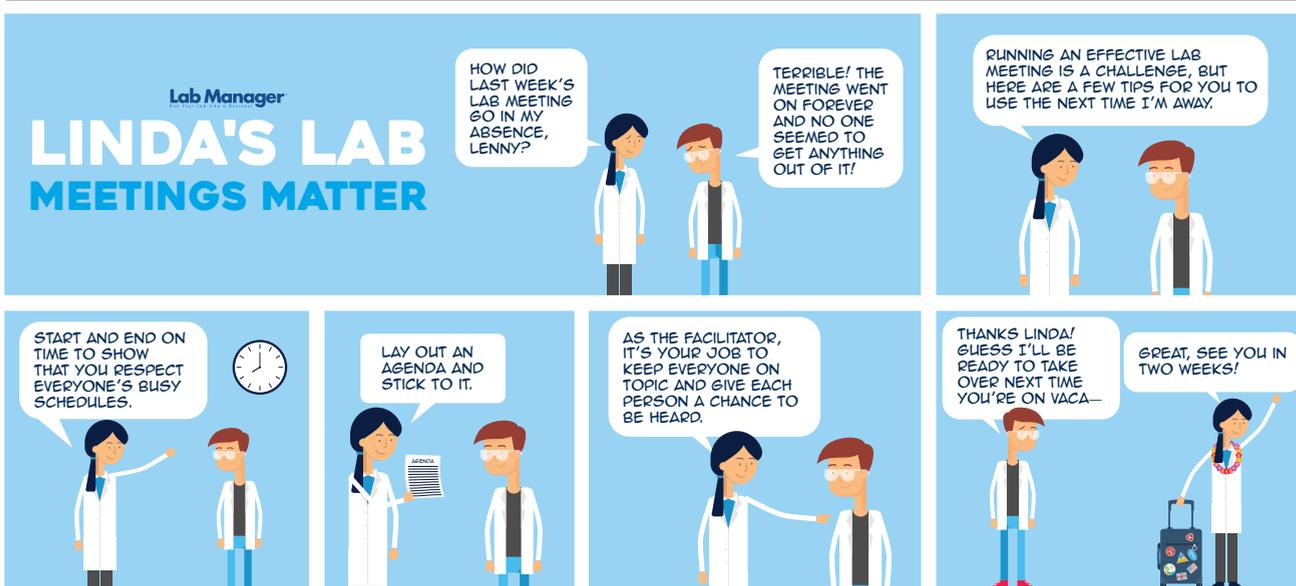
Below are a few more tips that may be helpful in your hiring efforts:

- Showcase your company culture and people
- Add some marketing flare to the job description
- Be realistic about job qualifications
- Implement talent management strategies to fill niche positions
- Develop internal mentoring programs to train new hires in needed skills

While there's no full-proof technique for finding the right person for the job you need filled, hopefully some of these suggestions will prove useful. Good luck.

Best, Linda

FOR MORE INFO: LABMANAGER.COM/TALENT-ACQUISITION



PICKING YOUR MICROPLATES

by Mike May, PhD

In the 1950s, a Hungarian physician, Gyola Takatsy, needed a faster way to test patients for influenza, and that spawned microplates. The first ones consisted of 100 wells that were milled one by one. Eventually, microplates became the new test tube—one of the most commonplace things in life science labs. Still, scientists must consider which microplates to use.

The complexity of present-day microplates is exemplified by the fact that the Thermo Fisher Scientific (Waltham, MA) microplate website lists 922 items. If that number makes it difficult to imagine where someone might start trying to decide what product to purchase, take a look at the company's "Guide to Microplate Formats and Well Designs" to consider the various formats, well shapes, and even colors.

“Sometimes the volume of the wells themselves makes up the key constraint.”

To provide some perspective on microplates, Trevor McFarland, laboratory operations manager at the Oregon Health and Science University Integrated Genomics Laboratory in Portland, made time to answer a few questions.

Plate picking

Most life scientists use some sort of microplate, and probably for more than one application. McFarland, for example, uses microplates for many processes: amplification protocols related to gene expression, genotyping, or methylation microarray projects; Sanger sequencing; fluorescent-based nucleic acid quantification; and qPCR and nucleic acid concentration normalization.

That gives McFarland a good perspective on which plates work for which applications. “Plate selection is specific to the application needed,” he says.

Take fluorescent assays, for example. Here, McFarland explains that “the plates must be black to avoid signal bleed over to an adjacent well.” McFarland and his colleagues often run DNA-quantification assays that use a fluorescent dye, PicoGreen®. For this assay, the scientists include an epMotion liquid-handling robot from Eppendorf (Hamburg, Germany) to automate the process. “The robot pipettes samples, controls, and standards from either a dilution plate—a 96-well microplate—or tubes into a black CoStar Plate—96 wells—that can then be read in a fluorescent microplate reader,” McFarland explains. “This allows us to assay up to 88 samples, including standards curve and control samples, in a single run.”

Other applications need just the opposite. “In the case of a qPCR plate, there is a requirement that the plate be optically clear in order for the signal to be read by the qPCR instrument,” McFarland explains.

In other cases, key features are the volume of the wells in a microplate and the labware's overall size. For microplates that will be used in PCR amplification, for instance, McFarland says the labware must fit the thermocycler being used and have a format compatible with the volumes required. Sometimes the volume of the wells themselves makes up the key constraint. As an example, McFarland says, “In some cases, we use MIDI deep-well plates to accommodate the high volumes needed for precipitation, etc.”

Luckily, today's scientists don't need to go to a milling machine to churn out microplates. Instead, a quick search through online catalogs provides more options than a scientist could create in a career. The bigger challenge is finding the right microplate for the job, but it takes only a little reading to get it right.

Mike May is a freelance writer and editor living in Texas. You may reach him at mike@techtiper.com.

FOR ADDITIONAL RESOURCES ON MICROPLATE TECHNOLOGY, INCLUDING USEFUL ARTICLES AND A LIST OF MANUFACTURERS, VISIT WWW.LABMANAGER.COM/MICROPLATE-TECHNOLOGY

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Particle sizing techniques used by survey respondents:

Laser Diffraction	42%
Dynamic Light Scattering	32%
Sieving	31%
Automated Imaging	14%
Electrozone Sensing	13%
Dynamic Imaging	8%
Electrophoretic Light Scattering	7%
Sedimentation	7%
Other	19%

Nearly 45% of respondents are engaged in purchasing a new particle size analyzer. The reasons for these purchases are as follows:

Replacement of aging system	32%
Addition to existing systems, increase capacity	21%
First time purchase	7%
Setting up a new lab	3%
Other	37%



ARE YOU IN THE MARKET FOR A PARTICLE SIZE ANALYZER?

Particle size, shape, density, and distribution affect the physical properties and chemical behaviors of all products comprised of particles or that use them as ingredients. The size of stationary phase particles affects chromatography retention time, pigment particles dictate hue and finish in paints, and physical dimension imparts mechanical, optical, and electronic properties to nanomaterials. Within critical size domains from nanometers to about ten microns, the physical state can be as important as chemical composition.

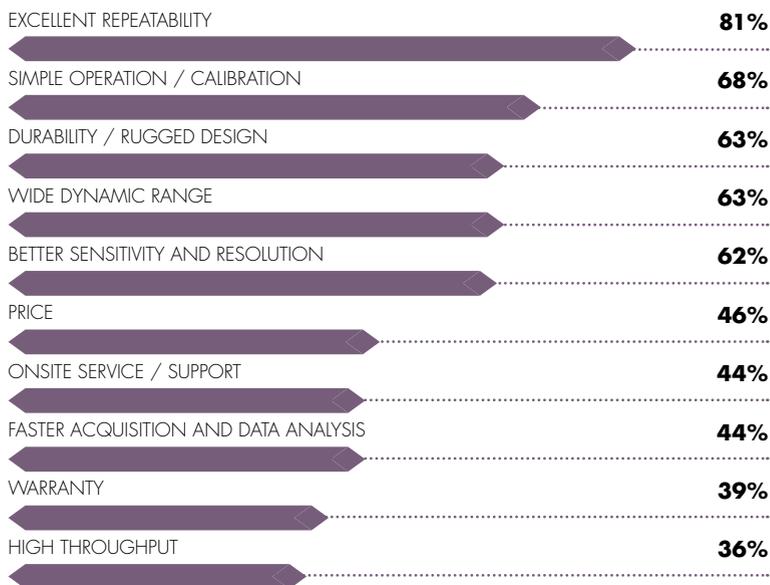
TOP 6 QUESTIONS

You Should Ask When Buying a Particle Size Analyzer

1. What is the size range you need to measure? Unfortunately, no one technique can measure all possible particle sizes, so the range needed will narrow the potential systems that can be used.
2. What exactly do you want to measure and why? Particle analyzers use many different techniques to arrive at measurements. In order to figure out what technique will work best for your application, you need to define what you are trying to measure and why.
3. Are you trying to characterize different particle types in a single sample?
4. In what "state" should the measurements be made? In many cases, measurement of the particles in the "native state" may not be possible.
5. Is measuring the count or concentration (two different measurements!) of the particles along with size/shape important? If knowing an absolute particle count, or a particle concentration is important, then some techniques will be eliminated immediately.
6. How easy is it to generate reliable data? Think about your users and ask what, if any, specific expertise is required for system set-up and routine use. Then, ask to make a measurement to assess this during the selection process.

TOP 10 FEATURES/FACTORS

Respondents Look for When Purchasing a Particle Size Analyzer:



For more information on balances, including useful articles and a list of manufacturers, visit www.labmanager.com/particle-sizing



SEE THE RESULTS OF OUR 2018 VISCOMETER SURVEY

Many industries measure viscosity, though the biggest user is the quality control department utilizing single-point measurement. Research scientists also use viscometers to see how a material reacts to being sheared. The task at hand determines the kind of viscometer to use—different viscometers measure different magnitudes of viscosity and different changes in it. According to one expert, the most important factor to consider when buying a viscometer is robustness, even if users have to give up some sensitivity.

TOP 6 QUESTIONS

You Should Ask When Buying a Viscometer

1. What kind of temperature control and spindle rotational speed control does the instrument offer? Temperature is critical, since viscosity generally rises as a fluid cools. Spindle rotation may also affect viscosity.
2. What range of accessories (ex. sample holders) does the company offer for the instrument?
3. How easy to use is the viscometer? Since most users nowadays aren't experts, an easy-to-use instrument is probably the best fit for most labs.
4. What are the sizes of the samples you'll be working with? This may be an issue when analyzing very expensive materials such as drugs or proteins and cost of ownership is also important for high-volume applications.
5. What is the instrument's measurement range? If you're analyzing petroleum, from crude oil to gasoline, do you want to change out the capillary for each measurement, or use something that works all the way through?
6. What kind of service and support does the company provide?

Viscometer types used by survey respondents:

Rotational viscometer	83%
U-Tube / Ostwald Viscometer	22%
Vibrational Viscometer	7%
Falling Ball Viscometer	4%
Rectangular Slit Viscometer	4%
Other	11%

Frequency of viscometer usage by survey respondents:

Several times each week	40%
Several times daily	30%
Two to three times a month	19%
Less than once a month	11%

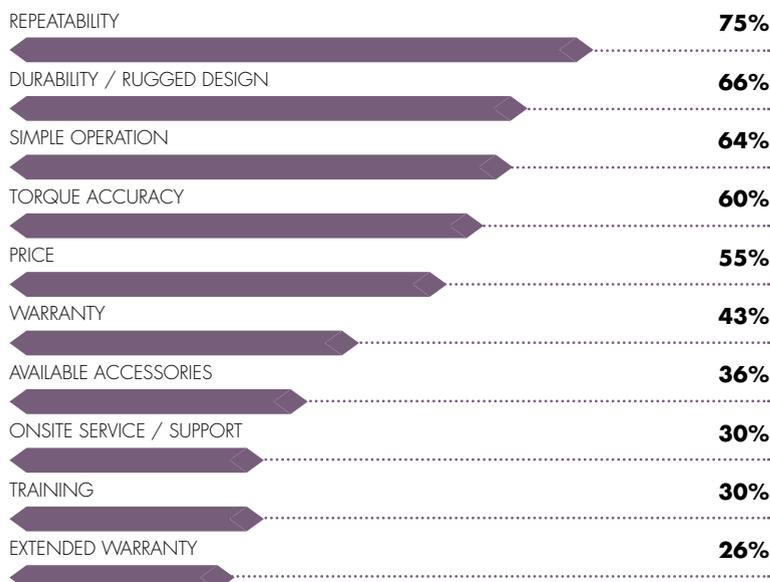
Nearly 58% of respondents are engaged in purchasing a new viscometer. The reasons for these purchases are as follows:

Replacement of aging system	66%
Addition to existing systems, increase capacity	17%
First time purchase	2%
Other	15%



TOP 10 FEATURES/FACTORS

Respondents Look for When Purchasing a Viscometer:



➔ For more information on viscometers, including useful articles and a list of manufacturers, visit www.labmanager.com/viscometers

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PRODUCT SPOTLIGHT

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- Has a working volume of 100-250 mL and features a single Elephant Ear impeller
- Simple to set up and use



Sartorius Stedim Biotech

www.sartorius.com

INFORMATICS

Chromatography Software

Clarity 8.0

- Updated version brings a graphically enhanced user interface
- Provides intuitive approach, excellent performance, and proficient technical support
- Redesigned features include all icons, instrument window, method setup dialog, and more
- Existing users of Clarity Chromatography Software can update to the new version free of charge



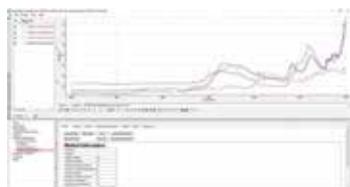
DataApex

www.dataapex.com

Software Suite

Spectral Sage 4

- Emphasizes features that streamline the user experience and enable new capabilities aimed at producing better results when converting near-infrared spectra into actionable results
- Offers the ability to remove stray light from spectra through an automatic operation
- All reports can now be saved as PDF, DOC, XLS, and TXT



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www.galaxy-scientific.com

Drug Discovery Software

StarDrop 6.5

- Features include modeling for drug metabolism prediction and virtual library enumeration
- Enhanced data visualization environment offers more efficient data analysis and project management
- New “dashboard” capability combines multiple interactive plots in a configurable project view



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Spectroscopy Software

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LC-MS Software

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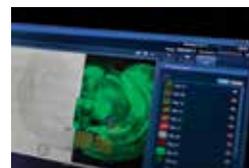
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Software Modules

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NORMALIZING BIOLOGICAL DATA

Problem: Normalization of functional biological data is a key component in the workflow for performing and/or subsequent analysis of raw data to ensure accurate and consistent interpretation of results. As a typical dataset usually contains more than one sample, and as researchers are almost always interested in making statistical comparisons between these samples, some form of normalization is usually required for most experiments performed. Whether comparing different cell types, genetic modifications, or compound treatments, the data must be normalized to a common shared parameter for correct comparison. Normalization of cellular assays can be applied on several levels, including cell number, genomic DNA, and total cellular protein.

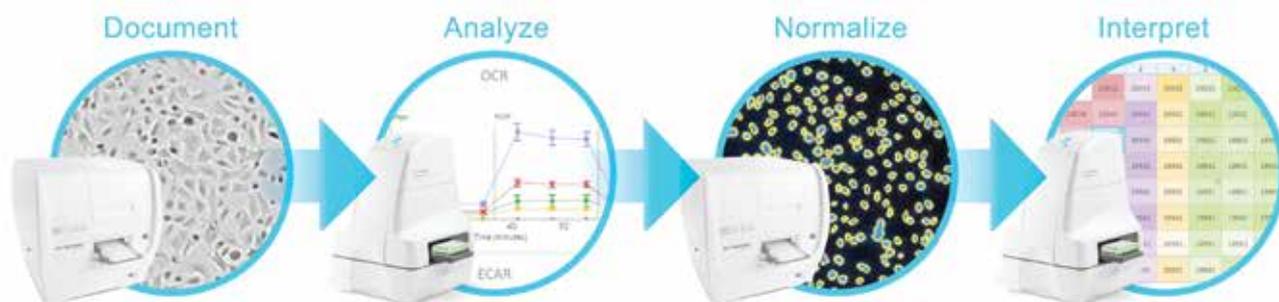
Solution: The solution described combines cellular metabolic analysis technology from Agilent and imaging technology from BioTek Instruments to create a standardized approach for comparing XF data sets, improving assay workflow, and applying normalization values to XF measurements. The novel integration of the Agilent Seahorse XFe Analyzer with the BioTek Cytation 1 Cell Imaging Multi-Mode Reader focuses on a method that uses cell number to normalize. The integrated system is optimized to automate and simplify the acquisition of brightfield (before and after XF assay) and fluorescence (after XF assay) images, via a unified controller that controls both the BioTek and Agilent Seahorse instruments. Specialized software then calculates the cell number in each microplate well, and seamlessly transfers the images and cell counts into the software for XF data normalization. The embedded brightfield images in the software provide visual feedback and quality control of cell seeding conditions, which improves live cell assay reproducibility and XF data quality. An “alert” within the software announces the availability of cell counts and images, and a heatmap display of cell counts enables easy evaluation of cell seeding consistency, making each assay run more robust. The incorporation of the high-quality imagery within the XF software adds another dimension to the data, as researchers can toggle between the XF data, brightfield images, and fluorescence images in a unified software experience. Referencing the images while

analyzing XF data provides evidence and guidance on how to limit variability and improve the reproducibility of the assays. Applying a consistently generated cell count based normalization value ultimately makes interpreting the data and finding relationships amongst the data easier.

The benefits of this solution are:

- Simplified XF analysis with an easy-to-use, reliable, validated, and supported cell count based normalization solution
- Improved XF data interpretation by applying cell count numbers directly to your XF data enabling plate-plate, experiment-experiment, and well-well comparisons
- Documented cell culture condition throughout the XF assay to make better connections between cell seeding conditions and XF data, while providing guidance to detect statistical sources of errors
- Associated normalization values, brightfield and fluorescence images in the XF data analytical software package to provide evidence for making more meaningful biological conclusions
- Enhanced live cell assay reproducibility optimized for XF assays
- Simplified normalization workflow with uncomplicated software and single controller operation to communicate data between both devices

For more information, please visit Agilent.com.



▲ Perform XF analysis with an easy-to-use, reliable, validated, and supported cell count based normalization solution with the Agilent Seahorse XFe Analyzer and BioTek Cytation 1 Cell Imaging Multi-Mode Reader

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STANDARDIZING CELL CULTURE MEDIA

Problem: VERO cells have emerged as a key cell type in the production of many different viruses for both research and vaccine production applications. Fetal bovine serum (FBS) in a basal medium, such as Eagle's Minimum Essential Medium (EMEM) or Dulbecco's Modified Eagle's Medium (DMEM), has traditionally been used to expand VERO cells and propagate viruses. However, the inclusion of FBS can be problematic as it introduces variability, risks for pathogen contamination, and unknown supply reliability. Popular serum-free media for VERO cells, though animal component free, still contain high concentrations of plant hydrolysates. These extremely complex and undefined mixtures, while a major contributor to VERO cell expansion, can complicate manufacturing efforts via the introduction of variable performance, poor reproducibility, and potentially blunted virus production of certain virus types.

In order to successfully progress the numerous vaccines in development today, cell culture media must be designed to consistently deliver optimal performance to propagate the virus of interest. The only way this will be accomplished is through a completely chemically-defined media that will maximize manufacturing yields and avoid any complications that come from unknown components.

Solution: InVitria has leveraged a nonmammalian-based recombinant protein expression system to produce recombinant human serum albumin and transferrin, Cellastim S and Optiferrin respectively, to formulate a virus production media that has complete chemical definition and is free of all blood-derived components. Albumin and transferrin, two of the most prevalent proteins found in serum, play critical roles in many biological processes. Albumin binds and delivers fatty acids, acts as an antioxidant, waste carrier, and free radical scavenger while transferrin regulates iron transport, uptake, and utilization throughout the body. Therefore, it is apparent that, to develop a media formulation without serum, supplementation of these serum proteins becomes the key to success.

Attempts at formulating a serum-free media have been made with the addition of plant-derived di and tri peptides to replace FBS. These hydrolysates are believed to function as a concentrated mixture of balanced nutrients that contribute to the proliferation of cells. However, the unknown compounds present in these mixtures can often interfere with virus productivity, thereby hindering the effectiveness of the cell-based virus production platform.

To circumvent this issue, InVitria's recombinant proteins can be used as a replacement to plant hydrolysates in serum-free media to provide solely positive contributions to the system. Optiferrin has been confirmed to be both structurally and functionally similar to native versions in its reversible iron binding capability as well as its ability to support cell proliferation, differentiation, and productivity. Similarly, Cellastim S has demonstrated structural equivalence to human serum albumin and shown great performance in many different cell culture systems, all while exhibiting high consistency. Combined, supplementation

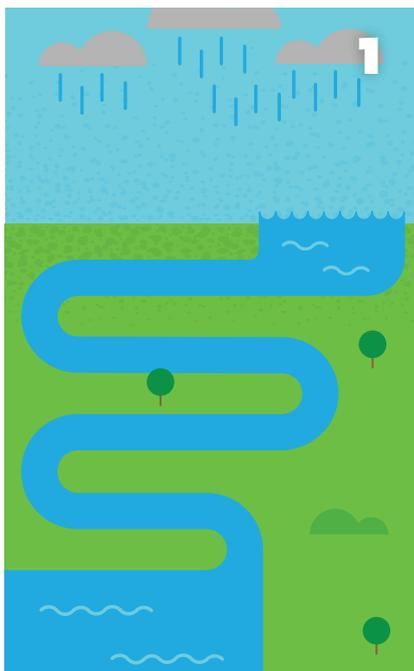
of both Cellastim S and Optiferrin serves as an ideal replacement to undefined plant hydrolysates to create an optimized virus production media for VERO cells.

In a recent poster presented at the World Vaccine Congress, it was shown that OptiVERO can effectively serve as a replacement to FBS and poorly defined plant hydrolysate-containing serum-free media in influenza, flavivirus, Dengue, and Zika vaccine manufacturing. OptiVERO's superior virus production capacity offers many manufacturing benefits, including the removal of batch to batch variability, reduction of safety risks, and increased production capacity, ultimately accelerating vaccine development to provide to patients in need.

For more information, please visit: www.invitria.com.



▲ *OptiVERO Base Media. Credit: InVitria*



LAB MANAGER ONLINE

We look back at our web content since the July issue and look forward to what's in store for the upcoming October issue.

1 From Source to Faucet

Do you know where your water has been? One of our most recent online exclusives is a free downloadable infographic that outlines water's journey from a watershed to your home. Along the way, your water supply can pick up contaminants from a variety of sources. Water treatment plants remove most of those contaminants, but in some cases, they can still end up in the water supply.

Read more at LabManager.com/from-source-to-faucet

2 Trending on Social Media: Mass Spectrometry Can Address Many Shortcomings of Immunoassays

As of August 6, *Lab Manager's* top July issue article posted to social media was our Product Focus on mass spectrometers. This article discussed the growing interest in mass spectrometry to complement or replace immunoassays. As researchers continue to dig deeper into the complexities of biology and the capabilities of current analytical techniques, opportunities arise for new methods to be utilized to overcome traditional limitations.

Read more at LabManager.com/mass-spectrometry-focus

3 Most Popular Webinar

Our most recent top webinar on LabManager.com with 339 registrants was "Top 10 Traps When Presenting Scientific Data—And How to Escape Them." In this Management Matters webinar, Rick Parmely, founder of Polished and Professional LLC, identified 10 common traps that many people fall into when preparing for and making public oral presentations. He also explained how to add life to any oral presentation and better engage the audience. Though it ran on July 10, you can still register to watch on-demand.

Read more at LabManager.com/10-presentation-traps

LabManager.com



NEXT ISSUE ➔

A Lab's Most Valued Resource: People

Lab managers have much on their plates, but what is most critical and sometimes most challenging is managing their staff. From identifying and hiring the best candidates, to getting teams to work effectively together, to maximizing the onboarding process, to choosing the most appropriate supervisory style, lab managers must navigate a sometimes uncertain and uncharted territory.

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