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# Table of Contents

## CANNABIS ANALYSIS

10 Spectroscopy Versus Chromatography for Potency Analysis  
*Brian C. Smith*

An introduction to these techniques and an analysis of their advantages and disadvantages for potency analysis.

## CULTIVATION CLASSROOM

15 It’s Not Too Late: Post-Harvest Solutions to Microbial Contamination Issues  
*Roger Kern and Jacklyn R. Green*

Use the techniques described here to pass the state tests and ensure safe cannabis for consumers.

## NAVIGATING THE Labyrinth: CHALLENGES IN THE CANNABIS LABORATORY

20 Beyond Potency: Fungi, Mold, and Mycotoxins  
*Patricia Atkins*

The world of fungi, mold, and mycotoxins is explored and discussed.

## CANNABIS CROSSROADS

26 Cannabis and Kids: How Two Moms Use Cannabis to Treat Their Children  
*Joshua Crossney*

An interview with two parents that are utilizing medical cannabis in their children’s treatment.

## PEER-REVIEWED ARTICLE

28 Quantitation of Cannabinoids in Dried Ground Hemp by Mid-Infrared Spectroscopy  
*Brian C. Smith*

Applications of a quantitative mid-infrared spectrometer for hemp farmers, hemp extractors, state regulators, and law enforcement are discussed.

## FEATURES

36 Smart HVAC Selection for Successful Cannabis Cultivation  
*Laura Breit*

This article analyzes profitability impacts as they relate to your business by providing a knowledgeable look at the technologies accessible to all sizes of operations and describing the relative economics for various systems.

42 Application of a Simple Genetic Assay to Discriminate Hemp from Drug-Type Cannabis  
*A. Hilyard, S. Lewin, S. Johnson, P. Henry, and C. Orser*

A report on the findings from the initial field evaluation of a molecular DNA-based assay to distinguish the categories of fiber hemp, resin hemp, and THC cannabis based on genetics.

48 GC-TOF Discovery-Based Profiling of CBD Oil Pet Supplements  
*Matthew Curtis, Sue D’Antonio, and Anthony Macherone*

In this study, six different brands of CBD oil pet supplements were obtained and untargeted analyses using gas phase-time of flight mass spectrometry were performed.

54 Environmental Screening of a Cannabis Production and Processing Facility: A Comparison of an Environmental DNA Microarray and Traditional Microbiological Plating Methods  
*Chelsea Adamson and Benjamin A. Katchman*

A case study that demonstrates the utility and necessity of environmental screening in a cannabis production and processing facility.

## OPINION

62 Beyond THC and CBD: Opportunities for Creating Pharmaceutical Targets  
*Frank W. Foss Jr. and Kevin A. Schug*

Commentary on how medicinal cannabis can evolve in a similar manner as the pharmaceutical industry did.

## DEPARTMENTS

09 Cannabis News Focus  
64 Product Spotlights  
66 Application Note  
67 Supplier Profiles
Advanced Weighing Performance
With Legal-for-Trade Certification

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Congressional Committee Passes MORE Act to Legalize Marijuana

November 20, 2019 marked a monumental day in the prohibition of cannabis. The House Judiciary Committee approved a bill that would federally decriminalize cannabis by removing it from the Controlled Substance Act and expunge particular crimes related to marijuana.

First introduced on July 23, 2019, by Chairman Jerrold Nadler (D-NY), the Marijuana Opportunity Reinvestment and Expungement (MORE) Act of 2019, H.R. 3884, aims “to decriminalize and deschedule cannabis, to provide for reinvestment in certain persons adversely impacted by the War on Drugs, to provide for expungement of certain cannabis offenses, and for other purposes.”

“I have long believed that the criminalization of marijuana has been a mistake, and the racially disparate enforcement of marijuana laws has only compounded this mistake,” said Nadler. “While states have led the way in reform, our federal laws have not kept pace with the obvious need for change. With the passage of the MORE Act today, the Judiciary Committee has taken long overdue steps to address the devastating injustices caused by the War on Drugs and to finally decriminalize marijuana at the federal level.”

In a statement from NORML, Executive Director Erik Altieri stated that this was a truly historic moment in our nation’s political history. “For the first time, a Congressional committee has approved far-reaching legislation to not just put an end to federal marijuana prohibition, but to address the countless harms our prohibitionist policies have wrought, notably on communities of color and other already marginalized groups,” he said.

“Opposition to our failed war on marijuana has reached a boiling point with over two-thirds of all Americans, including majorities of all political persuasions, now supporting legalization,” said Altieri. “Congress should respect the will of the people and promptly approve the MORE Act and close this dark chapter of failed public policy.”

Justin Strekal, political director of NORML, also explained in NORML’s statement that in 2018 663,000 Americans were arrested for marijuana-related crimes. “The passage of the MORE Act represents the first time that the Judiciary Committee has ever had a successful vote to end the cruel policy of marijuana criminalization,” he said. “Not only does the bill reverse the failed prohibition of cannabis, but it provides pathways for opportunity and ownership in the emerging industry for those who have suffered most.”

References

Cannabis Science and Technology Receives Folio Ozzie Award

Cannabis Science and Technology magazine is excited to announce that we are the winners of the “New Magazine Design” award in the B2B category at the 2019 Folio: Eddie & Ozzie Awards. For more than 25 years, the Eddie & Ozzie Awards have recognized excellence in uncompromising journalism and gorgeous design across all sectors of the publishing industry. Cannabis Science and Technology submitted the November/December 2018 issue to the Ozzie category of “New Magazine Design.”

“We are thrilled to be recognized for our excellent design work,” said Stephanie Shaffer, publisher of Cannabis Science and Technology and Spectroscopy magazines. “Cannabis Science and Technology was an exciting magazine to launch in 2018 and it has been a truly collaborative effort between the editorial, design, and sales teams. We continue to strive for design excellence while providing our audience with great editorial content.”

Two of our sister publications, CURE® magazine and Medical Economics®, also received Folio awards this year. CURE® magazine won best healthcare/medical/nursing column for a consumer publication for “Will I Reach My Goal? Pondering Life as a Cancer Survivor” by Jane Biehl, PhD, and Medical Economics® won best data visualization for B2B publications for its 2018 Medical Economics EHR Scorecard.

“It is such an honor that several of our publications were recognized for their continued hard work over the past year providing top-quality content to our readers,” said Mike Hennessy Jr., president and CEO of MJH Life Sciences™. “This amazing achievement clearly demonstrates how our team collaborates to produce relevant and practical information that meets the needs of our diverse audience.”
Spectroscopy Versus Chromatography for Potency Analysis

Both spectroscopy and chromatography have been used for decades to measure the concentrations of molecules in samples, and now both techniques have been used to measure cannabinoid profiles in marijuana and hemp based samples. This column briefly introduces these techniques to the novice, and then using the concepts of speed, cost, and accuracy, analyzes the advantages and disadvantages of each technique for cannabis potency analysis.

Brian C. Smith

Both spectroscopy and chromatography have been used for decades to measure the concentrations of molecules in samples, and now both techniques have been used to measure cannabinoids in marijuana and hemp plant material, oils, extracts, and distillates (1–10). I feel the need to write this column because of misconceptions I hear stated by laypeople and experts in the cannabis industry. I have heard PhD scientists say that spectroscopy is “not quantitative.” This is of course nonsense since an equation called Beer’s Law (see “Spectroscopy” section further on) tells us spectroscopy is quantitative, and a shock to me considering I have written a book on quantitative spectroscopy (11). On the other hand, I have heard people berate chromatography by saying it “doesn’t work.” Chromatography does work for cannabinoid analysis as has been proven in the peer-reviewed literature (2,3 and references therein), and in the right hands produces accurate numbers. To try and clear the air, this column briefly introduces these techniques to the novice, and then using the concepts of speed, cost, and accuracy, analyzes the advantages and disadvantages of each technique for cannabis potency analysis.

The Golden Triangle of Chemical Analysis
The goal of any chemical analysis method is to obtain the greatest accuracy, with the fastest speed, at the lowest cost. These three criteria comprise what has been called the golden triangle of chemical analysis, as seen in Figure 1 (for the definition of accuracy please see my previous column [1]).

Note that the criteria are located at the corners of the triangle because they are too often mutually exclusive. For example, many techniques may be accurate but might be slow or expensive to use. On the other hand, techniques that are fast and inexpensive are frequently not as accurate as other available technologies. This is seen when comparing laboratory testing to field testing. Many laboratory instruments may require special utilities like cooling water or nitrogen gas, may be sensitive to temperature, humidity, or vibrations, may be too large to be taken outside the laboratory, or require a trained technician or scientist to be utilized. Thus, these instruments are not necessarily fast or inexpensive to operate, but they will often have the highest accuracy. On the other hand, field testing instruments tend to be fast, inexpensive, and operable by laypeople, but are not as accurate as laboratory testing because of the challenges of performing chemical analyses outside the laboratory. This is reflected in Figure 1 where lab testing is near the accuracy corner, while field testing is listed at the bottom near speed and cost.

Introduction to Chromatography
Chromatography is used to separate a mixture into its individual components. Once the components have been separated, the amount of each component can then be quantified. The word chromatography means “color writing” in Greek because in its original application chromatography was used to separate plant pigments into their separate colors which were viewed during the separation process.

In chromatography the sample to be analyzed is typically in solution. If a liquid needs to be analyzed the sample preparation may be simply a matter of “dilute and shoot” as chromatographers like to say, which means the sample must be diluted enough so that it is in the concentration range where the instrument can quantitate it. So, for example, to prepare a cannabis extract for chromatography, dissolving an appropriate amount in a solvent and diluting it with a further known quantity of solvent may be the only sample preparation needed.
However, the sample preparation for solids, such as cannabis plant material, is more challenging. The sample must be weighed, ground, have solvent added, agitated to promote extraction of the cannabinoids, filtered, and then diluted to the concentration range where the instrument can quantitate it (2). (Note: There have been many cannabis plant material sample preparation methods published, and even more in use. From my experience of monitoring analyses at many cannabis analysis laboratories, the method in reference 2 is best, and I would like to see it become the industry standard.)

Once the sample is in solution it is injected onto a “column” which is essentially a tube filled with a “stationary phase” and a “mobile phase.” The mobile phase can be a gas such as nitrogen or helium, which gives rise to gas chromatography (GC), or a solvent which gives rise to liquid chromatography (LC). The stationary phase is often silica particles that may or may not have a coating on them. The column can be as simple as a glass tube, or as modern as a thin steel pipe capable of withstanding high pressure, hence the technique of high performance liquid chromatography (HPLC). The mobile phase flows through the column carrying the sample molecules with it. For LC gravity can be used to encourage fluid flow, in gas chromatography changes in pressure are used, and in HPLC a pump is used to force fluid through the column.

The separation process for chromatography is illustrated in Figure 2.

In step 1 in Figure 2 the sample is loaded onto the beginning of the column. In LC this may involve simply pouring the sample into the top of the column. In GC the sample is injected into a heated port to vaporize it and it is then swept onto the column. In HPLC the sample is injected and the pumped flow of the liquid mobile phase carries the sample to the column. In the column the molecules in the sample will adhere to the stationary phase as seen in step 2. The pink, orange, and green bands represent three different types of molecules.

The critical element here is that different molecules will adhere to the stationary phase with different strengths or affinities. As mobile phase flows through the column, molecules with a weak affinity for the mobile phase will move through the column faster than molecules with a weak affinity. This is illustrated in step 3 in Figure 2, where the three molecular types have physically separated inside the column. You can almost think of chromatography as a molecular race. The column is the racetrack, the molecules move through it at different speeds, and cross the finish line at different points in time. In this case, the finish line is the end of the column. In step 4 in Figure 2 the green molecules have won the race and leave or “elute” from the column first. It has been separated from the other mixture components and is thus purified. Molecules with stronger affinities for the column will move more slowly and elute later. This is seen in step 5 in Figure 2 as the orange molecules leave the column in 2nd place. It is the difference in affinity for a stationary phase that chromatography uses to separate mixtures into their components.

As different batches of purified molecules leave the column, they can have their molecular structures determined and their concentrations measured. It is this latter measurement that will tell us, for example, the tetrahydrocannabinol (THC) content in an extracted marijuana bud. For quantitation, any number of different techniques can be used such as refractive index or the amount of light absorbed by the sample. For the determination of cannabinoids via
HPLC, an ultraviolet-visible (UV-vis) detector is often used (2) (absolute proof that spectroscopy is quantitative since it is widely used as a chromatographic detector). A disadvantage of GC is that since the sample is heated, the acid form of the cannabinoids decarboxylate, which means GC struggles to quantitate these cannabinoids in samples. This may explain why today many laboratories are using HPLC for cannabinoid analysis.

Spectroscopy

Spectroscopy is the study of the interaction of light with matter (4). Light can be thought of as a wave, and an example of a light wave is seen in Figure 3, which is a plot of light wave amplitude versus time. (Light is properly called electromagnetic radiation [4], but for simplicity it will be called light here.)

To the left in Figure 3 the light wave starts off at zero amplitude. The wave goes up, comes back down, and crosses zero a second time. The wave then goes down, comes back up, and crosses zero a third time. What I have just described is called the cycle of a wave as seen in Figure 3. The distance forward travelled by the wave during one cycle is called its wavelength and is designated by the Greek letter lambda, \( \lambda \). The wavelengths of light can vary from meters to billionths of a meter, and include radio waves, microwaves, infrared light or heat, the visible light that we can see, ultraviolet light that causes sunburns, X-rays, and so on. The fundamental measurement made in spectroscopy is to measure a spectrum. A spectrum can be a plot of the amount of light absorbed by a sample versus some property of light such as wavelength. An instrument that measures a spectrum is called a spectrometer. The infrared spectrum of tetrahydrocannabinolic acid (THCA) is seen in Figure 4.

The y-axis in Figure 4 is in absorbance units, and the height of the peaks is determined by the concentrations of molecules in the sample. The x-axis is plotted in wavenumber (which is related to wavelength). The peak positions are determined by the structures of the molecules present. For the analysis of cannabinoids in samples, infrared (IR) light has typically been used (5–10). It has been used to measure THC, cannabidiol (CBD), and other cannabinoids in marijuana plant material (5,6), hemp (7), cannabis extracts (8,9), and distillates (10).

To use light to measure concentrations in samples we take advantage of Beer’s Law, which is shown in equation 1.

\[
A = \varepsilon lc
\]  

where \( A \) is the amount of light absorbed by a sample, \( \varepsilon \) is the absorptivity, \( l \) is the pathlength, and \( c \) is the concentration.

The absorbance reading is the peak height or area of a peak in a spectrum. The pathlength is the thickness of sample seen by the infrared light beam, and \( c \) is concentration which is of course what we wish to determine. The absorptivity, \( \varepsilon \), is the proportionality constant between absorbance and concentration, and is explained in more detail elsewhere (11). To use Beer’s Law the size of the peaks in a spectrum are correlated to concentration using standard samples of known concentration.

To determine cannabinoids in dried cannabis plant material by IR spectroscopy the only sample preparation typically needed is to grind the sample (5–7). For cannabis extracts, oils, and distillates one can use a sampling method called attenuated total reflection (ATR) (8–10,12) where the liquid is simply spread on a window. It typically takes about 2 min to analyze a sample by IR spectroscopy (12).
Spectroscopy Versus Chromatography: Accuracy, Speed, and Cost

In a previous column, I discussed error, accuracy, and precision (1). Chromatography is an example of a primary method because chromatographs are calibrated using pure standard materials. Spectrometers give secondary analyses because they are typically calibrated using actual cannabis samples and reference cannabinoid data measured on the same samples via chromatography. When combining measurements to determine a concentration, the different sources of error combine via the laws of error propagation to determine the error in the final analysis (13). Long story short, chromatographic methods typically have excellent accuracy because they are primary methods. The accuracy of spectroscopic methods is typically not as good because the chromatographic reference data have error, the spectroscopic analysis adds its own error, and when the two are combined the total error for a spectroscopic analysis is generally greater than that for a chromatographic analysis (13).

However, remember that the “Golden Triangle” of chemical analysis seen in Figure 1 contains three parameters—accuracy, speed, and cost—and the true worth of an analytical method is based not just on accuracy but on all three of these criteria combined. When it comes to speed chromatographic analyses begin to suffer. It can take 5–10 min to prepare cannabis plant material, and another 5–10 min to run the sample through the chromatograph (2,3), which means it can take upwards of 20 min to analyze one sample.

Lastly, let’s talk about cost. The purchase price of an instrument can vary depending upon the make, model, features, and whether it is purchased new or used. An important, but I believe often overlooked, aspect of cost is the cost per analysis—the cost to analyze one sample. Chromatography, particularly for plant material, involves extensive sample preparation. This means consumables such as vials, syringes, filters, and solvent are needed to prepare samples. For HPLC there is the additional cost of environmentally sound solvent disposal. In my experience, the cost of consumables per sample in chromatography can run upwards of $10/sample. Although claims have been made that laypeople can prepare samples for and run a
chromatograph, there is a reason many state regulations require highly trained technicians to run chromatographs (15)—it takes skill to operate them. The need then for highly trained people to perform chromatographic analyses drives up the cost per sample. Assuming it takes 10 min total per sample, and given that skilled technicians earn a good wage, the labor cost per sample with chromatography can be upwards of $15. The total cost per analysis then for chromatography can be $25 or more. Chromatographic instruments also are generally not used in the field because of the need for utilities such as a nitrogen tank for GC, their bulk, and the need for a skilled user.

Spectroscopy on the other hand does not suffer from these problems. For cannabis liquids such as oils, extracts, distillates, and tinctures there is no sample preparation (8–10). For dried cannabis plant material the only sample preparation is grinding, which takes 1 min and can be performed with an inexpensive coffee grinder (6,7). Anyone can do this. There is no need to extract, shake, filter, and then dilute the sample so the consumables cost per analysis for spectroscopy is $0. The labor cost is minimal since anyone can run a sample in about 2 min. In total then the cost per analysis for spectroscopy is about $0. Field portable cannabis analyzers now exist that are fast and inexpensive to use (14).

Spectroscopy and Chromatography
In the big picture chromatography is accurate, but is not fast or inexpensive to use. Spectroscopy is not as accurate as chromatography, but is generally faster and has a lower cost per analysis. What are we to do with this information? Which technique then is better for potency analysis? The answer is neither. As always, one should use the right tool for the right job. In cases where high accuracy is a must, such as for compliance testing or for measuring reference data to be used to calibrate a spectrometer, chromatography should be preferred. For situations such as field testing, or in-house testing, where speed and low cost are important, spectroscopy should be preferred.

Conclusions
We have reviewed how two common cannabis potency methods, chromatography and spectroscopy, work. The two methods were evaluated using the criteria of accuracy, speed, and cost. Chromatography is accurate but can be slow and expensive to use. Spectroscopy is typically not as accurate as chromatography, but is quicker with a lower cost per analysis. Because of its accuracy, chromatography should be preferred for in-laboratory use for applications such as compliance testing. Because of its speed and low cost, spectroscopy should be preferred for in-house or field testing.

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About the Columnist
Brian C. Smith, PhD, is Founder, CEO, and Chief Technical Officer of Big Sur Scientific in Capitola, California. Dr. Smith has more than 40 years of experience as an industrial analytical chemist having worked for such companies as Xeros, IBM, Waters Associates, and Princeton Instruments. For 20 years he ran Spectro Associates, an analytical chemistry training and consulting firm where he improved their chemical analyses. Dr. Smith has written three books on infrared spectroscopy, and earned a PhD in physical chemistry from Dartmouth College.
It’s Not Too Late: Post-Harvest Solutions to Microbial Contamination Issues

Passing state regulations for microbial contamination can be challenging. Excessive levels of mold, yeast, and bacteria can cause health problems for consumers. What happens when your flower doesn’t measure up to your state’s standards? You do have post-harvest treatment options that can save your buds. Microbial contamination can be remediated without damaging your final product.

Roger Kern and Jacklyn R. Green

Now that cannabis cultivation has become accepted as a legal business across most of the United States, growers have come face-to-face with the consequences of failing state tests for microbial contamination. It is a huge disappointment and financial burden to lose your profits and reputation to an unseen enemy—the microscopic menace of the microbial world. During author Roger Kern’s 22 years with The National Aeronautics and Space Administration (NASA), he worked in the area called planetary protection. This lofty-sounding subject ensures that we do not contaminate our planetary neighbors with microbes from Earth when we send exploration spacecraft for science missions. It was his job to ensure that our spacecraft were clean of living and dead microbes. Figuring out how to clean and sterilize sensitive materials was a daily job. Now, it is gratifying to apply that knowledge to help ensure that the cannabis we supply to the consumer is clean and free from microbial contamination.

Microbial Contamination in Cannabis
There are two points in the cannabis product supply chain when failure of the microbial contamination test is most likely:
1. Testing that occurs post-harvest on cured products that have not yet been packaged
2. Testing that occurs on products after they have been packaged. They may have even passed the microbial contamination tests before packaging (1, above).

In general, about 10% of cannabis fails the microbial contamination test at the cultivation facility post-harvest. This 10% can be the difference between profits and business failure. The grower must decide how a failure because of microbial contamination will be addressed. This percentage may be acceptable to a grower who will use the failed plant materials for concentrates to make up some of the lost profits. However, when faced with post-harvest microbial contamination, you may want to implement remediation techniques to ensure that such an event does not occur in the first place and have confidence that your products will pass the state-mandated tests every time products leave the facility.

Let’s take a closer look at the two main threats causing microbial contamination in post-harvest cannabis. The threats are environmental conditions during the harvest, drying, curing, and prepackaging storage; and the microenvironmental conditions of the packaged product across the supply chain from cultivator to consumer.

Microbial growth is enhanced or reduced by environmental conditions. Favorable conditions of temperature and relative humidity can cause microbes to grow and thrive after the plants have been harvested. It is obvious that microbial growth can occur in the large-scale environment of the grow, drying, curing, storage, transport, and retail shelf conditions. However, we must also consider the microenvironment of the cannabis in its final packaging for sale. The cannabis may also experience environmental conditions inside its packaging that promote additional microbial growth. As unusual as it sounds, this microenvironment must also be controlled to ensure that clean and safe cannabis is delivered to the consumer.
Once the cannabis has been packaged in sealed containers and sent to the dispensaries, additional tests for microbial contamination may show an increase in yeast and mold, even to the extent of causing a failure of the state-mandated requirements for microbial contamination. This recently happened in Colorado when state regulators inspected products from 25 dispensaries in Denver (2). There were unanticipated microbiological contamination failures of packaged product taken right from the shelves of these dispensaries: 20 of the 25 dispensaries had failed products. These products were not contaminated at the time of delivery from the supplier, as evidenced by METRC documentation. The origin of this contamination is likely because of conditions within the sealed packages that were favorable to microbial growth. When you consider the large-scale environmental conditions in which the cannabis is grown, harvested, dried, cured, and stored and compare it to the internal microenvironment of the packaging, it makes sense that similar environmental conditions would affect the packaged products in the same way. Just as we have seen that controlled environment agriculture (CEA) is necessary for successful indoor cultivation, we find that a form of CEA is necessary for the microenvironment inside the packaging of the harvested cannabis. You must consider both the large-scale and the microenvironments of the products across the entire supply chain from grower to consumer. Along with your best practices in cultivation, you must work as a team across the supply chain to ensure your products reach the consumer in the pristine state in which they left your grow facility. Plan to work closely with the various people or businesses that handle your product to ensure optimal environmental conditions that support clean cannabis. Mistakes made along the supply chain, by other people, can negatively affect microbial contamination test results, leading to possible questions about the cleanliness practices in your cultivation facility. In the worst case, not considering this source of “down the supply chain” microbial contamination may cause harm to sensitive consumers. Secondary to this is the loss to your profits and reputation that accompanies a failure of this type.

Methods to Reduce Microbial Contamination in Post-Harvest Cannabis
Cannabis cultivators are the beneficiaries of decades of technology developments for consumer safety, including those developed for food safety across its supply chain (3). With cannabis in the mainstream of the consumer products, it is time to take advantage of those developments and use them to create safe cannabis that will always pass the state-mandated tests for microbial contamination. These methods are used to eliminate or greatly reduce the microbial contamination on the post-harvest cannabis and help to create the controlled microenvironment inside the cannabis packaging, necessary for ensuring safe cannabis across the supply chain.

The first step in the process is to kill the microbes in the post-harvest cannabis before it is packaged. The second step is to create the right microenvironment for the packaged cannabis. In sections below, we discuss two microbe-killing methods that will maximize your readiness to pass the state-mandated tests for cannabis post-harvest, before packaging. This is followed by a discussion of a method to ensure that you maintain the right microenvironment in the packaging. By following this two-step process, you will maintain your microbial cleanliness across the supply chain.

Killing Microbes in Post-Harvest Cannabis
There are many methods to reduce total mold and yeast count (TMYC) in post-harvest cannabis, but the two written about below seem to have no impact on the quality of the flower (terpenes, cannabinoids, and appearance). They are effective treatments and are not cost-prohibitive. The decontamination machines may be leased or purchased.

X-Ray Chamber Decontamination
X-ray chamber decontamination is highly effective and has a history of use in medicine and even airport security. X-rays are a form of high-energy electromagnetic radiation, that is, short wavelength light. X-ray wavelengths are shorter than those of ultraviolet (UV) radiation. This means they pack more punch with an intrinsically higher level of energy and therefore are much more effective in killing microbes than UV. The instrumentation for X-ray decontamination is well-tested and uses technology that has been proven for decades. The cannabis is placed in a lead-lined chamber that ensures the safety of the operator during the decontamination process. X-rays are produced from an internal vacuum tube once the lead lined chamber is sealed. The X-rays penetrate the cannabis and kill the microbes. The system is foolproof and does not allow the operator to be exposed to X-rays. It is highly effective in the

Technology developments for consumer safety, including those developed for food safety across its supply chain.
destruction of the full complement of microorganisms to be tested at the state level. After the processing by X-ray chamber decontamination, your cannabis will be ready to pass the state tests.

**Ozone Chamber Decontamination**

Ozone chamber decontamination has a history of use in agriculture to help keep the food supply safe. Ozone is highly effective in reducing the levels of TYMC to meet state standards. Ozone gas (O₃) is a highly reactive oxidizing molecule, meaning it is harmful to cells. It destroys the microbes’ cell wall, which enables the ozone to destroy all of the cell’s components: enzymes, proteins, DNA, and RNA. An ozone chamber generates its ozone gas as needed in a foolproof sealed chamber that cannot be operated until the door is sealed. Once placed in the chamber and sealed in, the cannabis will be exposed to ozone, and the microbes on the surface will be killed. Any ozone not consumed by the disinfection process is deactivated by the system before the door can be opened. This method greatly reduces the number of microbes but does not reduce the number to zero. Ozone chamber decontamination has been demonstrated to be effective against the full range of microorganisms expected to be tested by the states (4).

**Controlling Microbial Growth Inside Packaged Cannabis Products**

In the rare instances when regulators find contaminat ed products, such as flower and pre-rolls, on the shelf from lots that have previously met state standards, it is likely because of post-harvest environmental control of the products. A measurable value, called water activity, must be considered in assessing the probability of on-going microbial growth even while packaged. With an out-of-spec water activity value, plant material that passed the 10,000-count-limit test with a count of 7000 need only double over a period of weeks to render the material noncompliant with a measure of 14,000. The microbial growth that can take place after packaging is the reason that 11 of 29 states now require the testing of water activity, a measure that indicates whether continued microbial growth of the packaged cannabis is likely. We anticipate that additional states will include water activity in their slate of mandated testing.

Our home state of California has established an upper limit for the water activity for flower of 0.65. If flower material is not cured to a water activity measure of at least 0.70, yeast and mold can grow on it. We expect that in the coming years all states will require the same water activity limits for packaged cannabis. If this level of water activity is maintained throughout the cultivation processes and into packaging it prevents incidents such as the recent Denver dispensary test failures (2). Similar to food products, a lower water activity measure should also extend the overall shelf life of the product. If you are interested in a more in-depth understanding of water activity in cannabis, we encourage you to read further about it in a recently published article (5).

How do you achieve the desired water activity of ≈ 0.65 post-harvest if, and when, your state mandates this test? This is not easy; it will require dedication and determination on your part and compliance from all of your team members. This will be a standard operating procedure (SOP) to ensure you are taking all necessary steps to achieve the desired water activity prior to packaging. Ideally, all of your post-harvest processes must be performed in a controlled environment where the relative humidity (RH) is maintained between 55–65%. To achieve the desired water activity in the cannabis of ≈ 0.65, you will need to have a stable humidity across your entire set of post-harvest processes in this range of 55–65% RH. This stable relative humidity is called *equilibrium relative humidity* (ERH). If the relative humidity is not stable and controlled, you will not be assured of the desired water activity when the cannabis is packaged. To accomplish the ERH, you will need to monitor and control the potential effects on relative humidity from the weather, temperature, and people entering and exiting your processing rooms—to name just a few factors. With excellent practices, codified in your SOP, you will be assured that you have done all that can be done to achieve the water activity necessary to pass the state-mandated tests and prevent microbial growth in the packaged products.

Once packaged, the microenvironment in the package can be controlled by including relative humidity control packets, such as Boveda and Integra brands. Both products are designed to maintain relative humidity inside the package at 62%. This will be the ERH in the packaging that translates into the desired water activity measure that will prevent further microbial growth. The packets contain proprietary materials that can both absorb and release water into the packaging based on the relative humidity. The relative humidity will be affected by the temperature, so these packets are protective against some of the negative impact of temperature fluctuations along the supply chain. The packets
are encased in a breathable membrane that permits them to release and absorb pure water with no contaminants. The result is a microbiologically stable cannabis product with longer shelf life and a total yeast and mold count that will pass the state-mandated test.

**Expert Point of View**

We spoke with the Executive Vice-President and a scientist at Radsource Technologies, Inc., George Terry and Justin Czerniawski, PhD, as well as the Engineering Manager of Willow Industries, Adrian Alvarez, to learn about their microbial remediation technologies and gain their perspectives on post-harvest microbial decontamination. Their insights are invaluable as we work to solve this threat that can be costly and lower your profit margin.

**How big is the need for post-harvest decontamination technology?**

**George Terry and Justin Czerniawski:** Huge! Many cannabis regulators require cannabis samples pass total yeast and mold testing before they can be sold in dispensaries. The standards many states use to determine pass or fail criteria for microbial testing, is in the range of less than 10,000 CFU/g on cannabis plant material and 1000 CFU/g on extracts. Although there are many methods to try to reduce the amount of pathogens during the grow (such as approved bacteria sprays, UV light, ozone sprays), mold is a spore, and a living organism that thrives on the moisture in grow operations and proliferates. Post-harvest is the last chance to treat the product prior to it being packaged for consumption. If the packaging process is aseptic, then decontamination at that point is the best option to keep the material clean to the consumer. There are a few articles related to this (6–8).

**Adrian Alvarez:** There is an ever-growing need for post-harvest remediation and decontamination technology in the cannabis industry today. A few years ago, there were less stringent regulations surrounding cannabis contamination, so the market wasn’t really there. But as regulators learned more, new testing laws were created, and the $3B cannabis contamination problem was born. In terms of the numbers, industry estimates state that 10–20% of commercially grown cannabis fails microbial testing, which means there’s a significant need for a decontamination step in post-harvest production.

There are a lot of factors that influence a cultivator’s decision to implement decontamination technology, but in the commercial grows, where a test failure could mean 100+lbs of cannabis is pulled from the supply chain, the need is clear. The only reliable alternative to decontamination technology is turning contaminated flower into concentrates, but these products sell at a lower margin, so businesses usually prefer to have a nonextraction-based decontamination solution on hand.

**How effective is your decontamination approach?**

**Terry and Czerniawski:** While no system can make the legal claim that the product is sterile, we believe our method is the most effective means of reducing the microbial bioburden available. We have used X-rays to decontaminate medical devices and even blood supply for immune compromised patients. Using *Aspergillus niger* as a test strain, one of our clients has shown the eradication of spores from the material to zero.

**Alvarez:** Our technology reduces mold and pathogens on cannabis flower without altering potency, cannabinoids, or terpenes, making it an extremely effective decontamination solution for cultivators. When used for remediation, that is, cleaning flower that has already failed testing once, we have a roughly 89% pass rate, but when our technology is implemented as a kill-step, our pass rate is 94%. There are a lot of factors that explain this difference, but to put it simply, decontamination is most effective when implemented preventative, as this stops mold from growing in the first place. In terms of the numbers, a 6-hour WillowPure treatment provides an average microbial count reduction of over 97% (P < 0.001), and that’s when used on heavily contaminated flower (TY mc greater than 75,000 CFU/g).

**Are there any negative effects, for example, terpene degradation?**

**Terry and Czerniawski:** We have a cold process that does not introduce heat so there is no premature conversion of THCA, no mycotoxin stimulation from pathogen stress, no oxidative aging of the flower, and no accelerated evaporation of volatile organics like terpenes.

**Alvarez:** WillowPure treatment has no negative effects on flower terpene profiles or cannabinoid concentrations, as the plant’s natural, organic resin protects it from the oxidizing effects of ozone and no residue is deposited on the flower during treatment as ozone decomposes into
oxygen. Three years were spent optimizing ozone concentrations and run times, but with hundreds of positive customer experiences behind us, we’re confident in our system’s ability to eliminate mold and pathogens without negatively affecting the product.

Conclusion
Clean cannabis starts with clean cultivation practices (9). Clean practices, combined with the post-harvest decontamination methods described here, should enable every cultivator to pass state-mandated tests for microbial contamination 100% of the time. To maintain state compliant levels of microbial contamination, you should maintain a stable water activity measurement across the full supply chain from grower to consumer. However, with at least 10% of the flower material not passing state-mandated tests for total mold and yeast count, we need safe decontamination steps that do not compromise the quality of the final product. We have highlighted two examples here, X-ray chamber decontamination and ozone chamber decontamination methods. Whether you choose to remediate only the plant material that fails the state test and is returned to you for post-failure processing or introduce a microbial decontamination process for each lot before you send it out is up to you. A conservative approach would be to decontaminate every lot with one of the decontamination methods and use the relative humidity control packet in the packaging materials. This approach will give you confidence that your products will be state compliant and avoid expensive transport and return to your facility for reprocessing should they fail state testing further down the supply chain. Starting clean and staying clean will improve your profitability and maintain your excellent reputation along the supply chain all the way to the consumer.

References

About the Columnist
Dr. Roger Kern
is a scientist and technologist who cares deeply about the cultivation and health of plants in the cannabis industry. With his PhD in microbiology from the University of California, Davis, Plant Growth Laboratory, he solves the most challenging problems in hydroponics, from studying the root microbiome to developing nutrients and lighting systems to ensure plant health and a disease-free lifecycle. He spent 22 years at NASA’s Jet Propulsion Laboratory as a scientist, technologist, and research leader before becoming the President of Agate Biosciences, a consulting firm for project management, systems engineering to cannabis businesses enhancing their financial and technical success in today’s stringent government-regulated environment.

Dr. Jacklyn R. Green
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Beyond Potency: 
Fungi, Mold, and Mycotoxins

Mold and fungi are found everywhere in the world. Due to their size and their spore’s mobility, fungi can spread throughout crops and food stores. When the conditions of humidity and temperature are favorable, the fungi can proliferate into colonies that can either destroy crops by consuming them or poison them with toxic secondary metabolites called mycotoxins. Just like some of the other potential agricultural contaminants (that is, pesticides and heavy metals), fungi, molds, and mycotoxins also must be of concern to the cannabis industry as a factor in product safety and quality. In this column, the world of fungi, mold, and mycotoxins is explored and discussed as they relate to the cannabis industry and the future of analytical testing.

Patricia Atkins

The complexity and range of products produced by the cannabis industry makes it unique. The product range encompasses medicines, recreational agents, and food products. The industry has to worry not only about health effects of an inhalable product but also the effects of a medical preparation and food safety. The exposure risks of contamination from chemicals and microbials must be taken into consideration as the mode of ingestion of inhalation is used. The traditional industries of agriculture, food production, tobacco, and medicinal supplements all battle with contamination issues.

The traditional tobacco industry and now the vaping industry have been the object of scrutiny and testing, which has often showed significant levels of potentially dangerous contamination by chemicals and microbials (1). In the same vein, a large percentage of medical cannabis was found to be contaminated by some type of microbial organism in a study from UC Davis (2). In some cases, these microbial organisms were the cause of a patient’s death.

The food industry also struggles in the battle with contaminants. In the United States, it is estimated by the Centers for Disease Control and Prevention (CDC) that 48 million people get sick from foodborne illnesses and up to 3000 die from foodborne diseases (3). More than 250 agents are known to cause foodborne illness and are introduced through contamination, improper handling practices, and sanitation. These agents can be chemical, physical, or biological.

Biological Contamination

Biological contaminants are by far one of the greatest concerns for illness. Microbes are everywhere and can be beneficial or cause illness and death. The five types of microbes are bacteria, viruses, parasites, protozoa, and fungi. Fungi are a very diverse kingdom of organisms (single and multicellular) which once were considered plants. In the past, the study of fungi was a branch of botany. Now it is known that fungi are more closely related genetically to animals than plants. Fungi are nonphotosynthetic and must obtain nutrients from organic matter. Fungi that derive nutrients from decaying or dead matter are known as saprophytes; while a small percent of fungi derive nutrients from living organisms and are called parasites. There are between 70,000–100,000 known species of fungi and possibly an estimated 3.8 million species in total (4).

The classification of Kingdom Fungi is constantly being debated with the influx of DNA data. Currently the kingdom contains seven phyla (Table I) (5), which span the different forms of fungal organisms from single-celled yeasts to multicellular mushrooms.

Many species of fungi produce biologically active compounds that are used in food production and medicine—where would the world be if we did not have yeast for bread or penicillin. But, there are also many toxic compounds produced by fungi.

“All Fungi Are Edible; Some Only Once” -Unknown

The classical thinking of toxicity from fungus is of the mushroom...
hunter out in the forest foraging for wild mushrooms. The typical mushrooms most of us think about are from the phylum Basidiomycota or club mushrooms. Toxic members of these phyla are infamous such as the Death Cap and Skull Cap. They produce secondary metabolites like amatoxins, phallotoxins, and ergotamines that can easily be avoided by just not picking and eating those mushrooms. The more insidious toxic fungi are from the phylum Ascomycota, which include the molds, yeasts, mildew, and so on. These fast spreading and fast growing fungi are the plague of agriculture. These fungi produce the most common mycotoxins associated with food contamination.

Mycotoxins are organic compounds and secondary metabolites produced by fungus that are capable of causing illness and death. Secondary metabolites are not needed for the normal life cycle of the organism, in many cases the reason for their production is unknown (6). One species of fungi may produce different mycotoxins and some mycotoxins are produced by multiple types of fungi (see Table II). Most of the major mycotoxins of concern in human beings come from a few dozen species from the phylum Ascomycota or the sac fungi.

Cannabis as a crop is particularly prone to the growth of mold and fungi because of cultivation conditions, some of the most common forms of mold are powdery mildew and bud rot. Late harvests can contain significant amounts of water which will increase rot. Cannabis can also be contaminated at many points of harvest and processing, exposing the product to dangerous mycotoxins. Pests can flourish in storage facilities and delays between harvesting and drying can increase mold damage. The amount of drying of the cannabis can affect the potential for mold growth. Plant materials with more than 14% moisture can encourage mold growth. Some mycotoxins, especially aflatoxins and ochratoxins, need oxygen to grow so the reduction of the oxygen in the storage areas can retard growth.

There are dozens of potential contaminants in cannabis including all of the toxins discussed in previous columns (7,8). However, mycotoxin contaminants can be some of the most dangerous contaminants. Toxic and lethal dosages can be quite small for acute poisonings. Aflatoxins: B1, B2, G1 and G2, and ochratoxin A are often of the most concern. Ochratoxin A is has a tolerable daily intake designated by the World Health Organization (WHO) of 5 ng/kg of body weight per day. Ochratoxin A is very toxic with a “Lethal Dose, 50%” (LD₅₀) of 20–25 mg/kg of body weight. WHO recognizes products containing more than 1 mg/kg of aflatoxins as potentially dangerous or life threatening. The U.S. Food and Drug Administration (FDA) has limits for mycotoxins in human and animal feed up to 20 µg/kg for direct human exposure. In the United States, each state with cannabis regulations in place either follows a plan similar to the FDA limit of 20 ppb, but they apply it using various methods. Many of the states recognize and monitor the top five dangerous mycotoxins discussed previously. In states like Maryland and Illinois, each mycotoxin must measure less than 20 ppb. In New Mexico, all five mycotoxins together must be less than 20 ppb. Then, there are states such as Colorado which take an approach in between where the aflatoxins (B1, B2, G1, and G2) must total less than 20 ppb and ochratoxin A must be less than 20 ppb (9).

### Mycotoxin Analysis

The analysis of mycotoxins in products has a lot of

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**Table I: Fungi Phyla**

<table>
<thead>
<tr>
<th>Microsporidia</th>
<th>Chytridiomycota</th>
<th>Blastocladiomycota</th>
<th>Neocallimastigo-mycota</th>
<th>Glomeromycota</th>
<th>Ascomycota</th>
<th>Basidiomycota</th>
</tr>
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<tbody>
<tr>
<td>Unicellular spore forming parasitic fungi</td>
<td>Microscopic fungi with flagellate zoospores found in wet soils and freshwater</td>
<td>Microscopic fungi with flagellate zoospores originally grouped with Chytridiomycota</td>
<td>Anaerobic fungi found in digestive tracts of herbivores</td>
<td>Obligate symbiotic fungi penetrate into the cells of roots of plants and trees</td>
<td>Known as sac fungi which are symbiotic with algae to form lichens and are sometimes parasitic. Contains some yeasts and molds</td>
<td>Large group of club fungi that includes rusts, smuts, puffballs, and mushrooms</td>
</tr>
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challenges. First, molds and fungus are ubiquitous to the environment and agricultural products. The amount of exposure changes with weather, growing conditions, agricultural practices, harvest, and storage conditions. The toxins can be distributed heterogeneously in a batch or harvest. The location and concentration of the toxins can vary greatly. The analytical targets, as we have discussed previously, are very low in the parts per billion ranges. The approach to analysis can be gaged either to screening or targeted analysis.

The simplest types of test are qualitative tests which provide a yes or no answer as to if a toxin is present. Samples are placed in test tubes, well plates, or dipped with testing strips for a cost effective answer if that particular sample tests positive for mycotoxins. For these types of tests, simple colorimetric chemical reactions are employed using a test tube or container with reactant or lateral flow material with reactant that color change in the presence of target analytes.

A second type of test is quantitative. These types of tests can range from quantitative test strips or methods to more complex and sensitive instrumental analysis. In all of these tests, the samples must be extracted and the resulting liquid becomes the testing matrix. Simple quantitative tests like testing machines and strips use readers that are able to calculate chemical changes and equate those changes to a quantitative measurement sometimes as low as parts per billion levels.

In many cases, testing laboratories prefer higher throughput of analysis and use more advanced...
techniques such as fluorometry, chromatography, and mass spectroscopy. Fluorometry is the study of the visible spectrum of fluorescence. The fluorimeter measures the intensity and wavelength distribution of an emission spectrum after excitation by light. Molecules that undergo fluorescence can be measured accurately and to low levels in the parts per trillion range using a fluorimeter. Many mycotoxins (B1, B2, G1, G2) at one time were detected by black light under which they would glow showing their fluorescence. In some mycotoxins they can be treated with a binding agent to become fluorescent and measurable by fluorometry. The first step of testing for mycotoxins with a fluorimeter is to isolate the mycotoxins from the rest of the extracted material using some method of separation like an immunoaffinity or other chromatography columns. Immunoinfinity columns use monoclonal antibodies to isolate target analytes by containing them in the column until they are washed with the proper solvent and concentrated into an extract. Other chromatography columns use substrates that select for size, chemistry, and polarity to retain analytes until the time they are washed into an extract.

Mycotoxin testing is often conducted in conjunction with other types of cannabis testing such as for terpenes, pesticides, and potency. In these multiple target methods, more complex analysis methods are used such as liquid chromatography–mass spectroscopy (LC–MS). These systems can test for multiple targets, but need a high level of expertise to run and are often costly to purchase and maintain unlike simpler test methods.

Conclusion
Cannabis, like every other agricultural product, is plagued by a variety of pests and contaminants. The agricultural practices and processes in the cannabis industry can lead to increased exposure to mycotoxins. There are significant health concerns regarding mycotoxin contamination of products that are not only processed into edible foods, but also used as inhalation products and medicines for children and patients with compromised immune systems. It therefore becomes critical to understand the nature of fungi and mycotoxins and how to reduce them in cannabis products.

References

About the Columnist
Patricia Atkins is a Senior Applications Scientist with SPEX CertiPrep and a member of both the AOAC and ASTM committees for cannabis.
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“The Cannabis Science Conference was an affirming experience for me. It was extremely well organized and attended by a very large group of informed and interested attendees. I found the networking to be very productive and countless people have contacted me subsequently for follow up.”

- Ethan Russo, MD (Director, R&D, ICCI)

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Cannabis and Kids: How Two Moms Use Cannabis to Treat Their Children

An interview with two parents—Moriah Barnhart and Gail Rand—that are utilizing medical cannabis in their children’s treatment.

Joshua Crossney

According to a recent Pew research survey conducted in September 2019 (1), two-thirds of Americans believe that cannabis should be legalized. There still seems to be a unique stigma, however, related to the use of medical cannabis for pediatric patients. For this installment of “Cannabis Crossroads,” I had the pleasure of sitting down with two parents that are utilizing medical cannabis in their children’s treatment—Moriah Barnhart and Gail Rand. These ladies have left no stone unturned when it comes to options for care and have both found cannabis to be a great resource that helps alleviate many of their children’s symptoms.

Can you please introduce yourself and tell our readers which pediatric illness your family is using medical cannabis for?

Moriah Barnhart: My name is Moriah Barnhart. My daughter, Dahlia, was diagnosed with aggressive brain cancer at the age of two. She was actually diagnosed with two types of brain cancer mixed. One of her diagnoses, anaplastic astrocytoma, is not considered curable.

Gail Rand: I’m Gail and I’m a mom to Logan, a happy 10-year-old boy. Logan was diagnosed with epilepsy at age 1, ADHD at age 2, Celiac disease at age 3, and autism at age 4. He uses medical cannabis to help with everything except Celiac disease, but our focus was always controlling his seizures.

Please describe how cannabis plays a role in your child’s treatment.

Barnhart: Dahlia has benefited from multiple different hemp and cannabis products since 2013 for everything from pain, nausea, and vomiting to skin rashes and sleep. Obviously, my great hope in the end is that it also helps cure her.

Rand: Before medical cannabis, Logan had more than 14,000 seizures. Now, Logan has been several years seizure free using tetrahydrocannabinolic acid (THCA), without any pharmaceuticals for seizure treatment. My husband extracts the raw cannabis using dry ice and suspends the kief in olive oil. Logan also takes cannabidiol (CBD) for his development, attention, behavior, and learning. When Logan went seizure free on THCA, he was able to sleep through the night for the first time ever. Although he was initially nonverbal, he has now learned to communicate, which also has a huge benefit of reducing some of his behavioral challenges. Logan’s older siblings, who are typical developing teenagers, now enjoy seeing Logan’s personality flourish, which has dramatically changed the family dynamics for the better!

Can you share details on how you first learned about medical cannabis as a treatment?

Barnhart: I had people straight away bringing me information on cannabis as a potential viable supplement for Dahlia. I specifically remember a cousin, the wife of someone in my church growing up, my sister, and my mom being sold on the idea early on. A few months into looking into cannabis for Dahlia, the CNN documentary came out, but it was talking about hemp for seizures not cannabis for cancer. So, there were really no stories on children using products with THC at the time. There were no doctors to help with dosing and there was really limited information outside of anecdotal adult stories and mice studies.

Rand: In 2012, one of my friends began using CBD to treat her son, Zaki. Her son has the same rare type of epilepsy as Logan, called myoclonic astatic epilepsy (MAE), also known as Doose Syndrome. Zaki went seizure free almost immediately. This made us sit up and take notice as Zaki and Logan always presented similarly with their seizures and reactions to medicine.

In what state is your family located and can you share some of the issues that you have experienced in acquiring quality medical cannabis?

Barnhart: We are located in Florida. First, we went to Tennessee for conventional treatment at St. Jude. Once we saw the benefits of cannabis in an illegal state, we moved to Colorado for better access. But the prices were high and tested products were limited. Even the hemp products touted on CNN’s documentary a few months earlier came with a long wait list. So, we returned home to Florida to remove cannabis from my daughter’s bathroom.
be with her family and I continued to do what I needed to do to help her while working to change laws and relinquish the title of criminal. Now that we finally have a medical program in place, I would say education and cost are the most prohibitive issues. Patient and caregiver education, but also physician education. I wrote the state’s first continuing medical education (CME) program for physicians and have an inside glimpse into how devastating the lack of knowledge among our qualified healthcare providers is. Most medical programs excluded an entire receptor system from their program, and some doctors aren’t interested in learning new information. Others tout themselves as experts while spewing grave falsehoods. For parents and patients who are able to educate themselves, there is still the issue of cost. Since it’s not covered by insurance, everything from doctor’s appointments to product is out of pocket.

Rand: We live in Maryland. With other parent advocates, I was instrumental in getting the first practical medical cannabis bill passed in 2014. I also was the CFO and Patient Advocate for the first licensed grow in Maryland. Originally, we had incredible challenges getting access. CBD was very difficult to get as supply was incredibly minimal. There was a wait list of more than 5000 people for Charlotte’s Web and you had to relocate to Colorado. It continues to shock me how easy it is now to get Charlotte’s Web shipped to our door. It took several years for the program to get up and running to allow access to THCA. Now that the program has been up and running for a few years, consistent quality cannabis is much easier to find.

Can you share a little about how you determined which cannabis strains or cannabinoid profiles worked best for your child and their illness?

Barnhart: As I said previously, Dahlia has benefited from multiple different hemp and cannabis products over the years. We were able to make our own product in her name from one of the organic plants that helped her in her better times before there were so many hemp products on the market. In worse times, when she needed more THC to see relief, we went through multiple products at different doses. There’s really no one-size-fits-all in botanical medicine. A product that works for one person may not work for another. A product that works for one person at one time may not work for them as well for other symptoms or at a different time. Our bodies gain a fast tolerance to cannabis and one may need to change doses or products. It’s very rare even with conventional medicine that two children with the same diagnosis are going to see the same results from one medicine protocol, and in my experience, this is especially true with cannabis.

Rand: We tried CBD at first for Logan’s seizures but it did not have any impact on his seizures. However, we continued with the CBD as we believed it helped with his learning. Without knowing that we were trying CBD because this was several years before CBD was more widely understood, Logan’s school commented about how they believe “the lights went on in his brain.” He began to communicate and we got to see his personality for the first time. For CBD, Logan has had a lot of benefit from Charlotte’s Web. Eventually, we tried THCA at a 5 mg/day dose, which helped reduce his seizures. At 10 mg/day, he went completely seizure free. Because of the variety in the market, we have tried many different THCA strains with various terpenes and have not seen a change in the success of the treatment. Since then, he has continued to blossom in ways that we never thought possible.

This interview has been edited for length and clarity. To read the full interview, please visit: www.cannabissciencetech.com/cannabis-crossroads/cannabis-and-kids-how-two-moms-use-cannabis-treat-their-children.

Reference

About the Columnist
Joshua Crossney is the columnist and editor of “Cannabis Crossroads” and a contributing editor to Cannabis Science and Technology magazine. Crossney is also the president and CEO of CSC Events. Direct correspondence to: josh@jcanna.com
Quantitation of Cannabinoids in Dried Ground Hemp by Mid-Infrared Spectroscopy

A novel, small, portable, general purpose quantitative mid-infrared (IR) spectrometer has been invented and applied to the analysis of dried, ground hemp. The unit was calibrated using cannabinoid concentrations determined by high performance liquid chromatography (HPLC) at a state licensed laboratory, and mid-IR spectra measured on the same samples. The analyzer was validated using the leave-one-out cross validation method. Mid-IR calibration models for delta-9-tetrahydrocannabinol (Δ⁹-THC), tetrahydrocannabinolic acid (THCA), total THC, cannabidiolic acid (CBDA), cannabidiol (CBD), total CBD, cannabigerolic acid (CBGA), and cannabichromene (CBC) were constructed. The accuracy for the determination of total THC, which is important in determining the legality of a hemp crop, is ±0.04 weight percent, more than sufficient for compliance testing. The analyzer requires little sample preparation, features push button operation, produces results in 2 min, and at a cost of $0/sample. Hemp is a naturally variable material, so obtaining representative data on a grow requires averaging results from many samples. The speed and ease-of-use of mid-IR spectroscopy makes this feasible, as opposed to chromatography where typically only one or a few samples from a grow are analyzed so representative data are not obtained. Applications of this analyzer for hemp farmers, hemp extractors, state regulators, and law enforcement are discussed.

Brian C. Smith

Because of the 2018 Farm Bill, growing industrial hemp in the United States is legal if the sample contains not more than 0.3 dry weight percent (wt.%) total tetrahydrocannabinol (THC) (1,2). There is a need then to test hemp samples to insure they comply with the new law. In the past, cannabis and hemp have been analyzed for cannabinoid content via high performance liquid chromatography (HPLC) (3,4) or gas chromatography (GC) (5). However, chromatography suffers from several problems. Samples must be weighed, ground, extracted, vortexed, diluted, and filtered before injection (3–5). To perform these many manual sample preparation steps properly takes a skilled analyst several minutes and involves the use of expensive consumables. Also, chromatography runs can take at least 5 min or more (3–5), which when combined with the sample preparation time means it takes at least 10 min to analyze one sample. Between the consumables and labor, the cost per sample can be $20 or more. It is hard to imagine laypeople having the time or skill to perform these steps properly, which is why cannabis regulations require highly trained scientists to operate chromatographs (2).

Another concern with chromatography is the lack of representative sampling. Cuttings from adjacent plants in a grow, and even buds from the same plant, can vary by several weight percent in their cannabinoid content. This is illustrated in Table I, which shows the results for five different cannabis strains. The numbers represent weight percent tetrahydrocannabinolic acid (THCA) as measured by HPLC, and the low, medium, and high values represent samples take from different positions on the same plant (3).

Plants from five different strains in Table I are represented. Note that the potency variation across an individual plant can vary by almost 3 wt.%. This means cannabis is a heterogeneous, naturally varying material. The scientifically correct way then to sample a cannabis grow is to collect composite samples from many places in a grow, analyze them, and average the results (6,7). This may mean dozens or even hundreds of samples need to be analyzed, and these analyses must be done over time to insure a hemp grow does not go above the 0.3% total THC limit. Analyzing this many samples by chromatography would be prohibitively expensive. Because of the time, expense, and trouble of chromatographic analyses, typically only one sample from a grow is analyzed. This is by definition not representative (6,7). The danger here is that by using chromatography hemp
farmers may be obtaining nonrepresentative data on their grow, misleading them as to its legality and economic value.

Another problem with chromatographic techniques is the lack of standard reference materials and methods for hemp analysis, leading to the problem of inter-laboratory variation—different laboratories obtaining markedly different cannabinoid values on the same samples (8–10). A large part of this problem is variation in chromatographic sample preparation techniques across cannabis laboratories resulting in varying extraction efficiencies and significant offsets between potency values on the same samples (8). An analysis technique that involves little sample preparation would go a long way towards solving this problem. There is then a need for a faster, easier, and less expensive test with minimal sample preparation to measure cannabinoids in hemp, and that will allow more representative data to be obtained on hemp grows.

Mid-infrared (IR) spectroscopy has been used for decades for qualitative and quantitative analysis of samples (11–13). More recently it has been used to accurately measure cannabinoid profiles in marijuana plant material (14), to study the rate and mechanism of potency degradation in cannabis oils (15), to measure cannabinoid and terpene profiles in cannabis oils (16,17), and to measure potency in cannabis distillates (8). The latter study found that for measurements of THC in cannabis distillates mid-IR was four times more precise than HPLC. Mid-IR analyses feature little sample preparation, no expensive consumables, and a 2-min sample analysis time.

Given the success of mid-IR in measuring cannabinoids in marijuana, marijuana extracts, and distillates, the application of mid-IR to the analysis of dried, ground hemp was investigated. Such analyses would allow hemp farmers to monitor their grows and to harvest at the right time thereby lessening the risk of losing their crop (18). The molecules in hemp of economic value include cannabidiolic acid (CBDA) and cannabidiol (CBD). An analyzer that quantitates these molecules would allow hemp farmers to conduct rational experiments varying soil, water, light, fertilizer, and other inputs to maximize the amounts of CBDA and CBD generated by a grow while minimizing the cost of production. Such an analyzer could also be used for on-the-spot testing to set the price when hemp biomass is bought and sold.

State governments are currently tasked with determining the legality of hemp crops (19). Many states collect samples from a grow and then transport them to a laboratory for analysis (19). This is expensive and time consuming. Additionally, it can take state laboratories weeks to complete their analyses. An accurate, portable field hemp analyzer would allow state governments to perform hemp compliance testing on the spot saving taxpayer dollars and giving more representative data.

There has been controversy around the legality of shipping hemp across state lines in the United States. For example, in early 2019 hemp shipments in two states were seized by state law enforcement agencies (20), who argued that hemp that contained any THC violated state law. While the courts sort this out, truck drivers sit in jail and valuable hemp is sequestered.

### Table 1: Weight percent THCA values as measured by HPLC at low, medium, and high positions on the same plant. Five strains are represented.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Low</th>
<th>Medium</th>
<th>High</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.1</td>
<td>4.9</td>
<td>4.5</td>
<td>0.8</td>
</tr>
<tr>
<td>2</td>
<td>17</td>
<td>16.9</td>
<td>15.4</td>
<td>1.6</td>
</tr>
<tr>
<td>3</td>
<td>16.2</td>
<td>17.2</td>
<td>18.1</td>
<td>1.9</td>
</tr>
<tr>
<td>4</td>
<td>20.4</td>
<td>21.8</td>
<td>18.9</td>
<td>2.9</td>
</tr>
<tr>
<td>5</td>
<td>18.7</td>
<td>18.1</td>
<td>18</td>
<td>0.7</td>
</tr>
</tbody>
</table>

![Figure 1: A picture of a dried, ground hemp sample being applied to the mid-IR spectrometer’s sampling window.](image)
in government warehouses (20). If law enforcement agencies had a field portable hemp analyzer, they could test hemp on the spot for compliance, perhaps avoiding false arrests and needlessly seizing legal plant material.

**Experimental**

Hemp samples were prepared by taking approximately 5 g of dried plant material and grinding it for 1 min in a standard coffee grinder. These samples were scanned using a BSS 3000 Hemp Analyzer (Big Sur Scientific). The BSS 3000 is about 6-in. x 5-in x 4-in., weighs about 3 lbs, and is portable. The BSS 3000 is a general purpose quantitative mid-IR spectrometer, and was equipped with a triple bounce zinc selenide (ZnSe) attenuated total reflectance (ATR) crystal (8–10). Ground plant material was placed on the BSS 3000’s sampling window, as seen in Figure 1.

The powdered sample is then secured with a clamp, which can be seen in the background in Figure 1. The clamp features a mechanism that slips at a given torque to insure reproducible pressure is applied to all samples. Mid-IR spectra were measured from 1250 to 952 cm\(^{-1}\) at 12 cm\(^{-1}\) instrumental resolution. This spectral region was chosen because THC and other cannabinoids absorb strongly here, enhancing sensitivity.

Big Sur Scientific Cannabis Analyzer software was used to control and scan the analyzer. The software runs on a standard personal computer equipped with the Windows 10 Pro operating system. Data is transferred from the analyzer to the computer via a USB cable. The software allows the user to choose different sample types, such as dried ground hemp, extracts, or distillates. A background spectrum is run, and then the user is prompted to scan three separate aliquots of each sample. The calibration models are applied to each spectrum in turn, the cannabinoid weight percents are predicted, then these are averaged to ensure representative data, and the results are presented to the user on the computer screen. The user then has the option to print the results, copy and paste the results into a different application, or generate a certificate of analysis in Adobe Acrobat PDF format. A video on how the BSS 3000 is used to analyze hemp is available (21). Mid-IR spectra of 12 standard hemp samples, spanning a range of cannabinoid concentrations, were measured in triplicate for a total of 36 reference spectra. The same samples were analyzed via HPLC to yield cannabinoid wt.% values. HPLC analyses were performed by ProVerde Labs, a state licensed, ISO certified, third party laboratory.

To build mid-IR calibration models, HPLC cannabinoid weight percents and mid-IR spectra were input into the Big Sur Scientific Model Builder software. Spectra were pre-processed by calculating their first derivative using the Savitsky-Golay algorithm (13) using a second order polynomial and filter width of three points. The purpose of the derivative is to remove any baseline offsets that may be present in the spectral data. Both THCA and THC exhibit a strong absorbance around 1160 cm\(^{-1}\). The size of this absorbance is thus proportional to the total THC concentration in a hemp sample. This is illustrated in Figure 2.

First derivative spectra are shown as these are the spectra that were used in building the calibration model. The size of derivative features follow Beer’s Law and are proportional to concentration (13). The top trace in Figure 2 is from a hemp sample containing 0.77 wt.% total THC, the bottom trace is from a hemp sample that contains 0.27% total THC. The features are clearly of different sizes, and it is the variation in the size of these features that gives rise to the correlation between total THC values measured by HPLC and mid-IR as discussed below.

Pure cannabinoid standards were not used to calibrate the spectrometer because it would have been inappropriate (13). In use, the spectrometer analyzes dried, ground hemp not solutions of pure cannabinoids. For the spectroscopic
models to be applicable to actual hemp samples, spectra and cannabinoid weight percents for dried, ground hemp samples must be used to build calibrations. A partial least squares algorithm (PLS1) was used to build the calibration models (13). The advantage of this algorithm compared to traditional single peak Beer’s Law analyses is that it works well even if spectral peaks contain overlaps from multiple analytes (13). Thus, there is no need for each analyte to have a spectrally resolved peak for quantitation to be achieved (13). Separate mid-IR calibration models for delta-9-tetrahydrocannabinol (Δ9-THC), tetrahydrocannabinolic acid (THCA), total THC, CBDA, CBD, total CBD, cannabigerolic acid (CBGA), and cannabichromene (CBC) were developed.

Models were validated using the leave-one-out cross validation method (13). In this technique, one standard sample’s spectra and reference concentrations are removed from the data set, and a calibration is generated using the remaining standard spectra and cannabinoid weight percents. This model is then applied to the spectra of the standard sample left out to give mid-IR predicted concentrations. This process is completed in turn for each standard sample until each one’s data has been left out, yielding a set of predicted concentrations as measured by mid-IR, which are compared to the HPLC reference cannabinoid values for the sample set to calculate accuracy. This is a validation because the sample’s data whose concentrations are being predicted are not included in the calibration model applied.

Accuracies were calculated as average cross validation standard errors of prediction (ACVSEP). The need for this metric arises because for each calibration sample there was one HPLC cannabinoid reference value and three mid-IR predicted values. This arose because spectra of each sample were measured in triplicate, and thus three mid-IR predicted values for each standard were generated. The first step in determining the ACVSEP is to calculate the average predicted concentration by mid-IR for a standard sample and given analyte using equation 1.

\[ C_{apc} = \frac{\sum C_p}{n} \]  

[1]

where \( C_{apc} \) is the average predicted concentration by mid-IR for a particular sample and analyte; \( C_p \) is a predicted concentration for that sample and analyte; and \( n \) is the number of predicted concentrations for a standard. In the present case \( n = 3 \).

In the present case, there were 12 HPLC and 36 mid-IR concentration values for each analyte. Equation 1 is used to calculate the average predicted mid-IR value for each standard. We now have 12 HPLC and 12 average mid-IR predicted values for comparison.

The ACVSEP for a particular analyte is calculated using this data set and equation 2.

\[ ACVSEP = \sqrt{\frac{\sum (C_{ref} - C_{apc})^2}{N-1}} \]  

[2]

where \( C_{ref} \) is the reference sample weight percent value by HPLC; \( C_{apc} \) is the average predicted value as calculated using equation 1; and \( N \) is the number of reference samples. The ACVSEP is the standard deviation of the HPLC reference values and the \( C_{apc} \) values as determined by mid-IR. The ACVSEP is an excellent measure of accuracy since it is determined by how well the model predicts concentrations for samples not included in the calibration (13), which is precisely how calibrations are used in real life. Correlation coefficients were determined for the mid-IR calibration models by plotting predicted wt.% values for the standards as determined by mid-IR versus the values for the same samples as measured by HPLC.

### Results and Discussion

The accuracies, range, and correlation coefficients (\( R^2 \)) for the determination of cannabinoids in dried, ground hemp by mid-IR are seen in Table II.

The total THC calibration has an accuracy of ±0.04 wt.%, more than accurate enough to determine

<table>
<thead>
<tr>
<th>Cannabinoid</th>
<th>Range, Wt.%</th>
<th>( R^2 )</th>
<th>Accuracy*, Wt.%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total THC</td>
<td>0.2–0.8</td>
<td>0.95</td>
<td>±0.04</td>
</tr>
<tr>
<td>THCA</td>
<td>0.2–0.8</td>
<td>0.96</td>
<td>±0.04</td>
</tr>
<tr>
<td>THC</td>
<td>0.0–0.5</td>
<td>0.94</td>
<td>±0.03</td>
</tr>
<tr>
<td>Total CBD</td>
<td>6.0–20.0</td>
<td>0.99</td>
<td>±0.9</td>
</tr>
<tr>
<td>CBDA</td>
<td>4.0–20.0</td>
<td>0.97</td>
<td>±0.95</td>
</tr>
<tr>
<td>CBD</td>
<td>0.2–0.9</td>
<td>0.97</td>
<td>±0.11</td>
</tr>
<tr>
<td>CBGA</td>
<td>0.1–0.4</td>
<td>0.81</td>
<td>±0.05</td>
</tr>
<tr>
<td>CBC</td>
<td>0.03–0.12</td>
<td>0.94</td>
<td>±0.02</td>
</tr>
</tbody>
</table>

*Average cross validated standard error of prediction
whether a hemp crop is above or below the 0.3 wt.% limit as stated in federal law (1,2). The accuracies for the other cannabinoids are surprisingly good for a 2-min, no sample preparation required analysis of a naturally variable material. Table II indicates mid-IR can accurately quantitate total THC, THC, THCA, total CBD, CBD, CBDA, CBGA, and CBC in dried, ground hemp plant material. Given the half-dozen cannabinoids quantitated, this method gives not only potency measurements but also a cannabinoid profile.

To establish the correlation between two analytical methods, a plot of the results on the same samples obtained using both techniques can be made and is called a correlation chart (13). In the present case, correlation charts are constructed by plotting the reference cannabinoid wt.% value for standard samples as measured by HPLC versus the C_spc value for the same samples as measured by mid-IR. The correlation chart for total THC is seen in Figure 3.

A measure of the quality of the correlation between two methods can be derived from this chart by calculating the "correlation coefficient" or R², value (13). An R² of 1.0 means there is perfect agreement between the two methods, which doesn’t happen because of measurement error (13). A correlation coefficient of 0 means there is no correlation between the two methods. The correlation coefficients for all the cannabinoids determined in hemp by mid-IR are seen in Table II. The correlation chart for total THC in hemp, relating values as measured by HPLC versus those obtained on the same samples by mid-IR, is shown in Figure 3.

A measure of the quality of the correlation between two methods can be derived from this chart by calculating the "correlation coefficient" or R², value (13). An R² of 1.0 means there is perfect agreement between the two methods, which doesn’t happen because of measurement error (13). A correlation coefficient of 0 means there is no correlation between the two methods. The correlation coefficients for all the cannabinoids determined in hemp by mid-IR are seen in Table II. The correlation chart for total THC in hemp, relating values as measured by HPLC versus those obtained on the same samples by mid-IR, is shown in Figure 3.

Table II also lists the concentration range of the standards used for each calibration, which is the range over which each model can be expected to be quantitatively accurate.

The mid-IR spectrometer used in this study requires little sample preparation for hemp apart from drying and grinding. There is no weighing, dissolving, or filtering to prepare samples as in chromatography (3–5). Mid-IR also requires no consumables, so the cost per analysis is $0, unlike chromatography where the consumables and solvent used cost several dollars per sample, not to mention the at least 10 min of labor required to run one sample (3–5). The mid-IR analyzer used in this study comes pre-calibrated and features push-button operation so anyone can use it. Lastly, analyzing a sample takes about 2 min, significantly faster than chromatography (3–5).

The ability of mid-IR spectroscopy to determine cannabinoids in dried, ground hemp opens up some unique applications. Hemp farmers can take numerous cuttings of a plot, dry them, grind them, and then easily analyze them by mid-IR spectroscopy. By measuring more samples than is typical with chromatography, a representative picture of a grow is obtained, which is more valuable to the farmer. In essence, HPLC provides a snapshot of a grow, mid-IR provides a motion picture. Since the latter provides more information, it should be preferred. The ability of mid-IR spectroscopy to measure CBDA, CBD, and total CBD—molecules of value in hemp—means hemp farmers can conduct rational growing experiments to maximize the CBD and minimize the THC content of their crops.

State regulators currently only sample a fraction of the plants from a particular grow for testing (19). This is because samples are collected and taken to an offsite location for slow and expensive chromatographic testing. Whether the handful of samples measured is representative of a grow needs to be questioned (6,7). Instead, a portable mid-IR unit would allow state regulators to analyze many samples on the spot, giving a more representative picture of a grow, saving time and money.

Hemp extractors can also make use of a portable, accurate, mid-IR hemp analyzer to assess the
potency of hemp biomass prior to purchase. For example, when purchasing plant material a hemp extractor can use mid-IR to analyze the material on the spot to ascertain what they are truly buying and set the proper purchase price. The spectrometer described above can also analyze cannabinoids in extracts and distillates (8,15,16).

A portable hemp analyzer could also be of utility to law enforcement. There has been great controversy since the passage of the Farm Bill about the legality of transporting hemp across state lines (18,20). A portable, easy-to-use, and accurate mid-IR spectrometer could be used by law enforcement to determine the THC level of hemp shipments roadside. Shipments that are legal could be sent on their way, reducing false arrests, lessening the sample load for forensic laboratories, and thus saving time and taxpayer money. Samples of shipments that test above the legal limit could be forwarded for confirmatory testing. Of course, analytical instrumentation produces data, is it up to district attorneys and law enforcement officials to determine who to arrest, how to prosecute, and what evidence to introduce in court. A quantitative, field portable hemp analyzer will give them more evidence to work with when making these decisions.

Conclusions
It has been shown that mid-IR spectroscopy can be used to accurately measure Δ²-THC, THCA, total THC, total CBD, CBDA, CBD, CBGA, and CBC in dried, ground hemp samples with good correlation to HPLC values measured by a state licensed, ISO certified laboratory. Unlike chromatographic methods, mid-IR analyses are fast, easy, inexpensive, and there is little sample preparation. This allows multiple samples to be scanned and averaged, giving hemp farmers a more accurate and representative picture of a grow, proving of greater value to hemp farmers. State regulators could use mid-IR for field compliance testing, hemp extractors are using mid-IR to assess biomass potency at the point of sale, and law enforcement could use such a device for roadside testing of questioned material.

Acknowledgement
I would like to thank Onchiotta Ador-netto and the folks at Hudson Hemp for providing hemp samples.

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21) https://www.youtube.com/watch?v=DhQlp-cwQh0&t=3s.

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Creating a Safe Supply Chain

Learn how to better navigate compliance to ensure profit and product safety throughout the supply chain.

Successfully managing risk in the cannabis industry through compliance, traceability, standard operating procedures (SOPs), and more is key to safeguarding your growing business. Cannabis Science and Technology (CST) sat down with Allison Kopf, founder and CEO of Artemis, to discuss the daunting pressures of risk management and how cultivators can better navigate compliance to ensure both profit and product safety throughout their supply chain.

CST: Can you tell us about Artemis—what problem do you solve, and how do you solve it?

Kopf: Artemis is software designed for enterprise growing operations. We work with specialty crops such as lettuce, tomatoes, and cannabis, and some commodities like hemp. We manage all of the risk involved in the operation, with a focus on automating back-office functions that run the farm day to day—production, calendaring and scheduling, crop planning, labor planning, compliance, and risk management.

CST: Is there a right or wrong way to approach risk management?

Kopf: The easy answer is yes; the hard answer is, what does that look like? You have to break down the supply chain and where the risk is occurring: There’s the consumer side, and what is risky for them, and the business side, and what’s risky for the business and the operation itself—and these risks can be managed differently.

One of the first things I notice when I visit a customer’s site is whether there is or isn’t thought and intention from leadership around risk management: Does the operation have a product safety plan? Are SOPs adhered to in the day-to-day processes? Is there a way to document everything happening on the farm? Who’s touching each plant and at what point?

The first question to ask is does your company have a built-in strategic vision with a clear stance on its compliance goals? And second, is there an operational mechanism for carrying that vision out? Do you have the right SOPs, the right task ownership and data information systems, etc.? Ultimately, these details will reduce risk throughout the supply chain—for the business and the consumer, but there are varying degrees of it. You don’t have to have it perfect, but you have to know how to ask the right questions and how to dig into the right things to ensure you’re paying attention and have the resources to carry those things out.

CST: Why is compliance so intimidating to growers, and does it have to be?

Kopf: It’s two-fold: There is a voluntary desire to be compliant and mandated regulatory compliance, which I think is where the nervousness comes in. No company wants their consumers to get sick from their product, but what happens is an operator is trying to keep their business...
afloat and create everything from scratch, and at the same time, government regulations are changing daily, which makes it really stressful.

Compliance is scary because you don’t want people to get sick, but you also don’t want fines or to be shut down. Being nervous is OK, but it doesn’t have to be this terrifying experience. It can be well-regulated and well-managed. This is the heart of what we are building at Artemis—how do you use technology as a tool to make the compliance experience less stressful, and how do you get in front of all these issues, so the risk is minimal?

**Kopf:** Whether or not a company has good compliance practices is decided by company culture—I genuinely believe that. It’s easy to operate a business legally without having a culture around compliance. That isn’t a bad thing, but you want to make sure you’re legal and compliant.

The flip side is an attitude of above and beyond what’s mandated by state law—spending extra time training employees, creating SOPs for traceability, or investing in technology to ensure compliance. It’s about creating a culture that traces all steps forward and all steps back.

**CST:** We are currently in the midst of the most exciting time in this industry history. What has the Artemis journey taught you about the challenges your customers now face in the market, and what are the next big challenges?

**Kopf:** We are seeing a lot of macro trends around the industry: mergers and acquisitions, roll-ups of license applications, a changing regulatory landscape, and the pressure to compete in pricing consolidation. I don’t see these trends changing over the next few years, but growers will get better at managing them. For example, who are the licensees who know how to operate multi-state operations and can open up across the United States at scale or in a pricing squeeze?

The next challenge is where will we end up on a federal level if regulations maintain on a state-by-state basis? As growers start operating across state lines, they have to deal with different regulations in different states. We’re going to see a lot of technology, which will cut costs and increase accessibility, but the more technology you have, the less standardized it becomes across state lines, which is a challenge. We’re also going to see a wave of data concerns in the next few years, i.e., making sure companies are properly handling data with good policies and security mechanisms in place. In the past, a security breach didn’t kill a company, but soon, it will hinder a company’s growth and turn growers away.

There is also an opportunity to focus on training programs and training growers to operate large enterprises. One of the big challenges is how do you hire good talent, and how do you retain that talent? How do you make sure you have enough people to get the work done? This is standard across any agricultural field right now, but talent retention, training, and needing labor will be a huge issue over the next few years.

**CST:** What sort of industry-unique opportunities do cannabis cultivators have right now, and how are they dependent on risk?

**Kopf:** The biggest opportunity is scale—how do you scale a business that is federally illegal in legal states where each state requires different compliance mechanisms and has different customer bases? It’s hard enough to scale a business as it is, if it were federally legal; when you introduce the compliance structure to this, it’s exponentially more difficult.

On top of that, how do you set up the right technology systems that accurately track data and sales state to state? How do you hire people and scale up teams in different states? All of these things are risky in their own right. Having a good management team that understands the risks and can implement the right technology with the right SOP is so critical.

Artemis scales easily and carries the compliance mechanisms across states. We have data tracking, so you can visualize everything on a farm-by-farm basis. Having the right technology and investing in the right people and the right training all have to happen at the same time. You have to try to reduce risk as much as you can, which is fun and tremendously exciting, and it’s a big opportunity. But the key is, do you understand the risk, and can you manage it correctly?

We’re going to see a lot of technology, which will cut costs and increase accessibility, but the more technology you have, the less standardized it becomes across state lines, which is a challenge.
Smart HVAC Selection for Successful Cannabis Cultivation

By providing a knowledgeable look at the technologies accessible to all sizes of operations, and describing the relative economics for various systems, this article analyzes profitability impacts as they relate to your business. Other topics discussed include: the impact of short-term cost savings compared to long-term profits when it comes to heating, ventilation, and air conditioning (HVAC) selection; the ways in which HVAC economics play a large role in profitability; real-world case studies to illustrate concepts in action; and codes and regulations associated with grow room design that should have an impact on HVAC decision making.

Laura Breit

In cannabis cultivation, heating, ventilation, and air conditioning (HVAC) systems will be one of the largest expenses your business comes across when you consider the upfront purchase and installation costs alongside long-term operating and maintenance costs. HVAC system design can affect almost every aspect of your business and business goals, from scalability for future growth to meeting sustainability goals. Because of the significant impact on your budget and your day-to-day operations, it is vital to select an HVAC system that will help optimize your operation from the start.

All other considerations aside, all of the systems you select and design for your facility must make economic sense or they won’t make sense at all. Taking the bottom line of your business into consideration is perhaps the most important factor when it comes to system selection, for HVAC and beyond. To optimize your cultivation operation, you must find a balance between cost and efficiency. Considering both the short and long term impacts of your HVAC decisions will lead to success for your business.

Successful Cannabis Operations Depend on HVAC Performance

Because of the important environmental factors that accompany cannabis cultivation (such as temperature, humidity, and so forth), HVAC performance can truly make or break the success of your operation. Your HVAC system directly controls temperature and humidity and therefore the ability of your HVAC system to control these factors can directly impact your cultivation process. There are many elements to consider to select an HVAC system that will help optimize your operation, but ultimately it will depend on the unique needs and goals of your business. Unfortunately, there is no “one size fits all” approach.

Budget is something to keep in mind throughout the entire system selection process. When considering budget, think about both the upfront costs and long-term cost effectiveness. If a system costs more upfront but is more efficient and will save money in the long term, it may be worth the spend. On the other hand, be cautious of buying a more expensive system because of popularity or branding traps. The equipment you select and systems designed as a result must make sense for your unique operation.

Cultivation Economics

Including engineers in the design of your cultivation facility offers the opportunity to design systems specifically for your business that consider your budget, facility size, geography, and other important factors. Energy models are also an important step in analyzing an operation, which can investigate different technical and economic conditions at play. An energy model is a simulation of a building that attempts to estimate the energy consumption of the facility, including the impact of various energy related items such as building fenestration, insulation, air conditioning, lights, and hot water. These can be very simple or more detailed, depending on the goals of the operation.

As engineers, we generally consider three factors when making system selection recommendations: first cost, annual costs, and payback. First cost includes the cost of the equipment as well as the cost of labor for contractors to install the system. Annual costs include energy costs (derived from energy modeling) and maintenance costs to keep the system functioning as designed. When we look at these two costs together, we are able to determine the payback period, which gives us information to help optimize your cultivation operation and your budget.

The time it takes to recover the additional cost through savings is called the payback period. As an example, imagine you are comparing two systems, System A and System B. System A is the least expensive and least energy efficient system. Often times this is
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a system that meets the energy code minimum, and no more, or perhaps it’s the system that is currently installed at an existing facility that is looking at upgrades. We call this the “Base System.” System B is more expensive, but also more energy efficient. Most people will understand that System B will cost less to run, but it’s hard to know if it’s really worth it to spend the extra money to purchase System B. With the help of a simple energy model, we can estimate how long it will take you to recoup the money you spent on System B, as compared to System A. The result is what we call the payback period. As seen in Figure 1, it will take 0.5 years to payback the initial investment of the more expensive, more energy efficient system.

**System Case Studies**

We have put together four system case studies to highlight the impact system selection can have on your bottom line. Between all four systems, we are looking to compare first cost, annual energy costs, maintenance costs, and payback. Specifically, we are comparing them all to System 1, which we call our baseline system.

Please note that the payback dollars were calculated utilizing a number of assumptions, including a geographical location in Sacramento, California. These results are tied completely to the specifics of this example or model, so we caution you in using these results for your facility. The purpose is to highlight that working with your engineer to understand the long-term costs of your system can significantly impact your bottom line.

**System 1**

System 1 is an HVAC system with the lowest first cost of any of the systems included in this case study (Figure 2). This is called the baseline system as it’s the system to which all other systems will be compared.
compared. It includes residential air handlers with direct expansion air conditioning units (DX condensing units) and standalone dehumidifiers. This is a residential-style system, where condensing units reject heat from the space using refrigerant pumped between an indoor fan unit and condensing unit. The dehumidifier’s purpose is to give the system extra capacity to remove moisture from the air.

With a relatively low first cost of $220,000, but an annual energy cost of $265,000, System 1 results in an estimated five year cost of $1.545 million (Figure 3). Other variables that could impact the first cost and operational costs of System 1 include code considerations, which may require additional equipment and impact the first cost, such as the potential requirement for air side economizers, as well as the varying levels of efficiency available in this type of equipment.

System 2

System 2 is a popular system for cultivation facilities that uses basic fan coils, an air-cooled chilled water system, and standalone dehumidifiers (Figure 4). The chiller produces chilled water, which is then pumped to fan coils that absorb the heat from the space. The standalone dehumidifier removes excess moisture.

Though System 2 has a higher first cost of $536,000, the annual energy cost comes out comparable to System 1 at $258,000, resulting overall in a higher five year cost of $1.826 million (Figure 5). Other variables that could impact the five year cost of System 2 include code considerations, which may require additional equipment and impact the first cost and payback. These can include things such as the addition of variable speed pumps or a drycooler for free-cooling mode, which would add first cost but decrease energy costs. Another potential drawback when comparing this system to Systems 3 and 4, which we introduce below, is that tight temperature and humidity setpoints can be difficult or more costly to achieve. In addition, modifications need to be made to this system if you are in a cold climate, which could add to first cost and increase the energy cost, thereby increasing the five year cost of this system.

System 3

System 3 is a highly efficient packaged unit with modulating compressors and hot gas reheat for humidity control (Figure 6). This system uses hot gas reheat as the dehumidification process, which is more efficient than the standalone dehumidifiers seen in the last two systems. In a typical system, either dehumidifiers will be utilized to handle the dehumidification load (not very efficient), or the
HVAC system will continue to cool the air to remove moisture in the room, resulting in subcooling the air which means heat will need to be added back into the room. Normally, the compressor heat goes to the condenser and is rejected out of the room. In this example system, the cooling coil subcools the air to remove the moisture from the air, then the hot gas reheat function utilizes the heat rejected from the compressor to reheat the air to a desired supply air setpoint as dictated by the cooling requirements of the space, adding to its efficiency and allowing for tighter temperature control.

System 3 also uses inverter driven compressors, which are more efficient and offer better temperature control. This type of compressor uses a drive to control the compressor motor speed, regulating cooling capacity.

System 3 has a first cost of $377,000 and an annual energy cost of $202,000—lower than both System 1 and System 2. The estimated five year cost of this system is $1.387 million (Figure 7). Other variables that could impact the five year cost of System 3 include physical considerations regarding where units need to be located, as well as utility incentives which could lower the first cost significantly. Additionally, this assumes no redundancy which could impact the first cost of this system, as additional units or controls may need to be considered if redundancy is a requirement of the facility.

**Figure 8:** System 4: High-end heat pumps with cooling tower and hot gas reheat for humidity control.

**Figure 9:** System 4 performance results.

<table>
<thead>
<tr>
<th>System #1</th>
<th>System #2</th>
<th>System #3</th>
<th>System #4</th>
</tr>
</thead>
<tbody>
<tr>
<td>First Cost</td>
<td>$400,000.00</td>
<td>$600,000.00</td>
<td>$800,000.00</td>
</tr>
<tr>
<td>5 Year Energy Cost</td>
<td>$(200,000.00)</td>
<td>$(400,000.00)</td>
<td>$(600,000.00)</td>
</tr>
</tbody>
</table>

**Figure 10:** Payback periods. The graph below is a payback graph. The point where the line crosses the x-axis marks the payback point in years. The interception of the y-axis is the initial cost in dollars.
where the units need to be located, simplified controls with tight setpoint control, as well as utility incentives which could lower the first cost significantly.

Payback Analysis
Remember that when we introduced the idea of payback at the beginning of this article, we defined the payback period as the time it takes to recover the additional cost of a piece of equipment as compared to a baseline system through savings. When you look again at Figure 9, you can conclude a few things:
- System 1 has the lowest first cost system (blue bars).
- System 4 has the highest first cost system.
- System 3 has the lowest overall five year cost.
- System 2 has the highest overall five year cost.

A few other conclusions, as depicted in Figure 10 include:
- System 2 never pays back when compared to System 1 (red line). It’s likely not a good choice for this facility, unless there are other reasons driving this system choice.
- System 3 (green line) pays back the fastest, with a payback period of just three years. Anything less than five years is generally considered a quick payback period, and with utility incentives, this system may come in at an even lower cost, so it deserves consideration.
- System 4 (yellow line) does payback, but the payback period is 12 years. This is generally considered a long payback period so there would need to be other reasons to choose this system (that is, tight control setpoints that would impact plant health and therefore your bottom line).

Final Thoughts
As outlined above, there are many factors to keep in mind when selecting an HVAC system for your cannabis facility. First costs, annual costs, and payback all need to be considered to find the balance between cost effectiveness and efficiency that is right for your unique operation. It is also important to think about your set point precision requirements, code requirements, and redundancy requirements, which are often different from business to business.

We advise our clients to be skeptical of any “one size fits all” system type, as the right decision for your facility is highly dependent on your unique operation and business goals.

Whether you have an existing facility or are looking at building a new facility, making informed decisions about your HVAC system can have a significant impact on your bottom line. Your HVAC selection can directly impact the profitability and growth of your business.

Laura Breit, PE, is a professional engineer with Root Engineers in Bend, Oregon. Direct correspondence to: laura@rootengineers.com
Application of a Simple Genetic Assay to Discriminate Hemp from Drug-Type Cannabis

The explosion in the hemp industry within the United States has created confusion for state regulators and hemp farmers. The passage of the 2018 Farm Bill provides for the widespread planting of hemp containing less than 0.3% tetrahydrocannabinol (THC). While some hemp is being grown for fiber and seed, most of the 2019 hemp harvest is intended for extraction of cannabidiol (CBD). Therein lies the anxiety, since resinous hemp is often above that 0.3% THC threshold. An alternative to relying on THC content alone is to distinguish the categories of fiber hemp, resin hemp, and THC cannabis based on genetics. Here we report on the findings from the initial field evaluation of one such molecular DNA-based assay. The assay ID markers correctly identified 420 out of 420 individual seed, leaf, and flower cannabis samples. Correlation with a subset of available cannabinoid data shows that while low in THC content, 72% of these resin hemp samples fall outside of the 2018 Farm Bill’s definition of hemp of less than 0.3% THC.

A. Hilyard, S. Lewin, S. Johnson, P. Henry, and C. Orser

The Agriculture Improvement Act of 2018, otherwise known as the 2018 Farm Bill, was signed into law on December 20, 2018. The 2018 Farm Bill legalized the cultivation and sale of hemp at the federal level effective January 1, 2019 (1). The change in federal status had broad ramifications for the hemp and cannabidiol (CBD) industries in the United States. Until December 20, 2018, hemp was categorized as a Schedule I substance under the Controlled Substances Act. It was illegal at the federal level to cultivate, possess, or distribute the hemp plant or CBD derived from the hemp plant. Almost all CBD products were illegal at the federal level. Notwithstanding, the CBD industry has flourished across the United States operating in the gray zone created under the federal policy in state jurisdictions where cannabis business is legal and regulated to some degree.

Concomitant acreage of hemp has quickly grown to more than 500,000 acres in the U.S. alone with much more under licensure. There have been many challenges associated with this ramp up in hemp cultivation starting with the definition of hemp as Cannabis sativa L. with a \( \Delta^9 \)-tetrahydrocannabinol (\( \Delta^9 \)-THC) concentration of not more than 0.3%. Some might strictly interpret that 0.3% to mean the \( \Delta^9 \)-THC content only, others might take additional steps to account for tetrahydrocannabinolic acid (THCA) content. This is hardly the only point of confusion. Is moisture content taken into account? What is the analytical method? How are extracts and infused products regulated? Who performs...
this analysis? Each of these questions add layers of confusion and slow the growth of the industry.

Let’s consider the specification of Δ9-THC, for example. Most of the THC in the plant is not Δ9-THC but THCA. Therefore, if only Δ9-THC is considered, 17% of THC cannabis from the tightly regulated medical and adult-use cannabis industry in Nevada could also be categorized as hemp no matter how much THCA is present. If THCA is accounted for, a significant percentage of the resin hemp being grown under the current hemp industry expansion may be in a gray zone, since the high end of total THC content variation in these plants seems to be around 1% or higher.

The 0.3% THC distinction is artificial and creates confusion, but there actually are three distinct phenotypes of *Cannabis sativa* L. (see Figure 1) with an unmet need for easy discrimination. The molecular assay described in this article was developed as a DNA-based alternative to differentiate fiber hemp, resin hemp, and THC cannabis. This simple evidence based genetic test provides a clear picture of what hemp farmers are growing and thereby provides an alternative to the 0.3% THC cutoff as proposed in the 2018 Farm Bill.

The assay uses two single nucleotide polymorphisms (SNPs) that were previously identified by Henry in 2018 (2). Based on data from 192 cannabis DNA samples, we proposed the use of a set of two SNPs found in two disparate regions of the cannabis genome; the mitochondria and the THCA synthase gene (Table I). These two molecular markers provide a rapid and cost-effective means to differentiate fiber hemp, resin hemp, and THC cannabis.

### Materials and Methods

**Cannabis sativa** L. Plant Samples

Hemp samples including seed, leaf, and flower were collected in Vermont, North Carolina, Colorado, and Nevada for DNA extraction and testing on the Tru-Hemp ID assay (GroSciences). Additional analysis on Tru-Hemp ID markers was performed on samples accessed from retention held at Digipath Labs in Las Vegas, Nevada under a state approved method development R&D project. Additional analysis on Tru-Hemp ID markers was also performed at VSSL in Kelowna, British Columbia. The Tru-Hemp ID data output is available in real time, and the Solas 8 instrument (MatMaCorp) can be linked to a remote computer, or cellphone. Eight samples can be tested in parallel, and the reaction takes approximately 2 h. The associated DNA prep method adds about 1 h. Representative graphic displays of the amplification of the two observed outcomes for each of the two SNPs is shown in Figure 3.

**Chemotyping of Large-Scale Population Samples**

Cannabinoid and terpenoid analyses followed the approach described by Orser and colleagues (3,4) and was extended to a total of 8859 individual cured cannabis flower samples. Briefly, cannabinoid certified reference standards for THCA, cannabidiolic acid (CBD), Δ9-THC, CBD, tetrahydrocannabinolic acid (THCV), cannabidivarin (CBDV), cannabigerol (CBG), cannabigerolic acid (CBGA), cannabichromene (CBC), Δ8-THC, and cannabinol (CBN) were obtained from Cayman Chemical as 1.0 mg/mL solutions in methanol. Cannabinoid analysis was undertaken using high performance liquid chromatography with diode array detection (HPLC-DAD) using an Agilent Technologies 1260 UPLC system equipped with a G4212A DAD, G1316C temperature-controlled column compartment, G4226A autosampler, and G4204A quaternary pump. All cannabinoid data is reported on an as is basis per Nevada regulations.

**DNA Isolation and SNP Detection**

DNA isolation of field samples was completed using the MagicTip Plant DNA Isolation kit (MatMaCorp) as per manufacturer instructions. Briefly, a lysis buffer is applied to about

---

**Table I: Distinction by single nucleotide polymorphisms**

<table>
<thead>
<tr>
<th>Category</th>
<th>Mito_318’683</th>
<th>THCAS_8’374</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fiber hemp</td>
<td>A/A</td>
<td>A/A</td>
</tr>
<tr>
<td>Fiber, seed, and by-product production</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resin hemp</td>
<td>C/C</td>
<td>A/A</td>
</tr>
<tr>
<td>Oil production - CBD most abundant</td>
<td></td>
<td></td>
</tr>
<tr>
<td>THC cannabis</td>
<td>C/C</td>
<td>G/G or G/A</td>
</tr>
<tr>
<td>Oil production - THC most abundant</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

---

**Figure 1:** Phenotypes of *Cannabis sativa* L.
5 mg of sample which is crushed in the buffer using a supplied silica-based substrate (MagicTip). Following a heating step, a binding buffer is added, thus allowing DNA from solution to bind to the MagicTip. Removal of the MagicTip into a new tube containing an elution buffer leaves the sample debris behind and facilitates elution of the bound DNA. Per manufacturer instructions, 1 µL of isolated DNA was used as template for genetic analysis in a Combined Sequence Amplification and Detection (C-SAND) assay (MatMaCorp). The C-SAND assay is a customized detection assay that is conducted using a four-channel fluorescence detection platform, the Solas 8 (MatMaCorp). The C-SAND assay was designed to detect two SNPs, one mitochondrial and one genomic in cannabis sativa. Briefly, the assay is a two-step padlock probe-based method that begins with hybridizing the custom-designed probes with their desired nucleic acid target found in the sample. Once hybridized, the probes are then ligated to form a circle and are subsequently amplified. Detection takes place during the amplification step using fluorescently labeled primers that hybridize with the previously formed circles. Each target has been assigned a channel on the Solas 8 and is detected independently.

**Calling Algorithm**

The calling algorithm uses first-order kinetics reaction properties (inflexion point detection) in combination with a measure of the closeness of the two signals associated with a specific SNP. Various indicators are tracked during the reactions to perform on-the-fly analysis. The analysis is then consolidated by a measure of the similarity between the two fluorescence signals at the end of the run. Aggregating values from the similarity measure, the end gain, and the inflexion point detection allow the Solas 8 to make the call at the end of the run without having to compare a results library of known sample genotypes.

**Results**

To illustrate the confusion created by defining hemp based on Δ⁹-THC content under 0.3%, we applied that guidance to the cannabinoid analytical data for a set of 8859 individual THC cannabis flower samples. Approximately 17% of these THC cannabis plants could be categorized as hemp and therefore would be legal under the 2018 Farm Bill even though they contain up to 31% THCA (Figure 2). The red line in Figure 2 represents the 0.3% Δ⁹-THC content cut off, and samples below that line could potentially be considered hemp even though they are high in THCA. This is clearly not the intention of the rule.

The intention of the rule is to distinguish THC cannabis from hemp. We
tested 420 samples on the Tru-Hemp ID markers to attempt to define the relevant categories genetically. The observations for all 420 individual samples analyzed by Tru-Hemp ID were found to be accurate based on phenotype (Table II) and the chemical analyses when those data were available (Table III).

While only 10 fiber hemp samples were available for testing, they all resulted in the expected Mito marker A/A and the SW6 THCA synthase marker A/A. As well, results for 100 resin hemp samples all reported the Mito marker as C/C (differentiating them from fiber hemp) and the SW6 as A/A in common with the industrial hemp, as expected. The most abundant category was THC cannabis and these samples showed the Mito marker A/A, THCA synthase marker A/A, and the Mito marker C/C. The SW6 marker was variable, but the data were consistent with the expected banding patterns.

### Table II: Tru-Hemp ID results

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Fiber and seed hemp</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Resin hemp</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>THC cannabis</td>
<td>0</td>
<td>0</td>
<td>234</td>
<td>76</td>
</tr>
</tbody>
</table>

### Table III: Analytical cannabinoid data for Tru-Hemp ID categorized samples

<table>
<thead>
<tr>
<th>Category</th>
<th>Samples</th>
<th>Samples with Analytical Data</th>
<th>Avg. Total THC%</th>
<th>Avg. Total CBD%</th>
<th>Samples Above 0.3% THC</th>
<th>Sample % Above 0.3% THC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fiber hemp</td>
<td>10</td>
<td>0</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>Resin hemp</td>
<td>100</td>
<td>47</td>
<td>0.40</td>
<td>9.16</td>
<td>34</td>
<td>72</td>
</tr>
<tr>
<td>THC cannabis</td>
<td>310</td>
<td>110</td>
<td>20.92</td>
<td>0.14</td>
<td>110</td>
<td>n/a</td>
</tr>
</tbody>
</table>
marker as C/C (in common with resin hemp) and either heterozygous A/G or homozygous G/G at SW6.

In Table III, the available correlative analytical cannabinoid data is reported by category for the samples evaluated. THCA and CBDA are accounted for in this data, in line with the majority of current regulatory strategies. Notably, 72% of resin hemp samples, 35 out of 48 samples tested, had a THC content greater than or equal to 0.3%. In Figure 4, a logit graphical representation of the distribution of THC content observed across all samples tested to date with the Tru-Hemp ID clearly demonstrates the separation of hemp samples shown in green dots with the drug-type THC cannabis shown in red dots where a cut-off representing 0.3% THC would eliminate 72% of the hemp samples. Note: The logit is the log of the odds ratio = log[percent/(1-percent)] used to transform percentage and proportion data. The logit for 0.3% (decimal 0.003) is equal to the log base 10 of (0.003/(1-0.003)) = -2.52157. Moving the THC cutoff to 1% THC would be inclusive of all but one outlier.

Discussion
The current legal distinction for hemp is not evidence-based and introduces confusion that impacts the pace of economic development. The arbitrary 0.3% Δ9-THC comes from a 1976 study describing cannabis taxonomy and was not intended as a legal distinction (5). Legal confusion leads to economic consequences for hemp businesses. This confusing guidance criterion has become a burden on law enforcement where testing procedures vary widely from state to state and CBD seed stocks are often not stable enough to guarantee a consistent phenotype.

Hemp industry regulators, farmers, processors, distributors, and retailers all require an evidence-based compliance framework. Results from Tru-Hemp ID markers demonstrate that evidence-based compliance strategies are available now. More comprehensive genetic identification and distinction strategies will undoubtedly make their way into the cannabis industry, and this simple example represents just one of many ways these technologies will be useful for both farmers and regulators.

In addition, improved learning algorithms for near infrared (NIR) screening technology for cannabinoid content is coming online that complements the use of a genetic screening in the field or laboratory (6). Economic growth will be best supported when all parties can come together to form a regulatory strategy that coordinates the intentions of law makers with evidence-based realities. Considering their similarity and the overlap of related regulatory issues, regulation of the legal cannabis industry (THC cannabis) along with the hemp industry should be considered in a single comprehensive framework.

Acknowledgements
The authors acknowledge the participation of those hemp farmers from North Carolina, Vermont, and Nevada that participated in this beta-testing of Tru-Hemp ID. Britni Gonzales, Kevin Hong, Alexis Middleton, Benoit Sommervogel, and Stephen Webb assisted with technical aspects of DNA preparation and the Tru-Hemp ID assay.

References

A. Hilyard, S. Johnson, and C. Orser are with Digipath Inc., in Las Vegas, Nevada. S. Lewin is with MatMaCorp Inc., in Lincoln, Nebraska. P. Henry is with VSSL, in Kelowna, British Columbia, Canada. Direct correspondence to: cindy@digipath.com.
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Cannabidiol (CBD) oils manufactured specifically for pets is a rapidly growing market. Many pet owners are supplementing their pets’ diet with products derived legally from hemp. In this study, we obtained five lots of six different brands of CBD oil pet supplements and performed untargeted analyses using gas phase-time of flight mass spectrometry. Chemical features of the raw data were extracted and annotated, and differential statistical analysis using commercially available bioinformatics software was used to examine each product.

Matthew Curtis, Sue D’Antonio, and Anthony Macherone

Materials and Methods

Chemicals and Standards

Five bottles of six commercially available CBD oil pet supplements (n = 30) were purchased online. High purity dichloromethane (DCM) was used to solubilize and dilute the oil samples. A C₅–C₃₀ alkane mixture was used to define retention indices, and an isotopically labeled polyaromatic hydrocarbon (PAH) mixture containing naphthalene-d₈,acenaphthene-d₁₀, chrysene-d₁₂, and perylene-d₁₂ was used as internal standards.

Instrument Hardware and Software

An Agilent 7890/7250 gas chromatography-quadrupole time-of-flight (GC-QTOF) system was used for all analyses. Mass Hunter software was used for acquisition and qualitative analysis. Mass Hunter Unknowns Analysis software was used for feature finding. Mass Profiler Professional software was used for
for differential statistical analysis. See Tables I and II for GC and QTOF parameters, respectively.

Sample Preparation
A 10 µL aliquot of each CBD oil sample ($n = 30$) was transferred to 5 mL volumetric flasks and 5 mL DCM was added (500-fold dilution). The flasks were capped and briefly vortexed. A 50 µL aliquot of each solubilized sample was added to 950 µL DCM (10,000-fold final dilution factor) in 2-mL autosampler vials and the isotopically labeled PAH mixture was added to each vial at 400 pg/µL. All 30 CBD oils were mixed equally to create a pooled quality control (QC) which was prepared as defined above. The alkane mixture was used as is. Pure DCM was used as solvent blanks.

Data Acquisition
A randomized batch sequence was created in MS-Excel. Five replicates of each CBD sample were collected, $n = 180$. Throughout the data collection sequence, DCM blanks were run 21 times, the alkane mixture was run four times, and the pooled QC was run four times. Data was collected in traditional 70 eV mode and in low energy mode at 14 eV.

Data Analysis

Feature Finding
To identify real spectral features in the raw data, Mass Hunter Unknowns Analysis software used the SureMass signal processing algorithm and Library Search to identify and annotate chemical entities in the raw data. Commercially available spectral libraries included a proprietary high-resolution accurate mass (HRAM) pesticides personal compound database-library (PCDL), and the unit mass Fiehn (1) and NIST14 (2) spectral libraries.

Differential Statistical Analysis
Mass Profiler Professional software (MPP) was used to determine relationships among the sample groups and variables. In this study, the sample group was named “Product” and the variables were Sample 1, Sample 2, Sample 3, Sample 4, Sample 5, Sample 6, Alkanes, Pooled, and DCM Blank. The statistical model was ANOVA.

<table>
<thead>
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<th>Table I: GC parameters</th>
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<tbody>
<tr>
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</tr>
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</tr>
<tr>
<td>Run time</td>
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<tr>
<td>He quench gas</td>
</tr>
<tr>
<td>N2 collision gas</td>
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<td>Injection volume</td>
</tr>
<tr>
<td>Injection type</td>
</tr>
<tr>
<td>Split/split-less inlet</td>
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<td>Septum purge flow</td>
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<td>Gas saver</td>
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</tr>
<tr>
<td>MSD transfer line</td>
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</table>

Results and Discussion
Quality Control
The chemical composition of CBD oils extracted from hemp was quite complex. In the 70 eV experiments, 840 entities were annotated, and 74 entities were identified in the low energy...
(14 eV) experiments. We identified fatty acids, fatty acid esters, di- and triglycerides, terpenoids, cannabinoids, tocopherols, steroids and sterols, essential oils, fragrances, alkanes, alkenes, and alcohols in the CBD oil samples. In Figure 1 chromatograms of the DCM blank, the alkane mixture, the pooled reference QC, and an unknown CBD product are shown.

Figure 2 is a principle component analysis (PCA) that was performed as a quality control measure on all identified chemical entities, and demonstrated that each CBD oil sample was differentiated from one another and were unique compared to the pooled reference standard, DCM, and the alkane mixture. Figure 3 is a Box-Whisker plot illustrating the variance of the internal standards in each sample over the entire dataset.

### Statistical Analysis

In the MPP environment, the chemical entities were filtered by those present or marginally present, then by the frequency that they appear across the entire dataset. This filtering removes chemical entities that have very low abundance and do not appear often in the data. This was followed by ANOVA with $p < 0.05$ (5% occurrence by chance) and a fold-change $>2$ (two-times more abundant than the median ion abundance over the entire data set). The result of these processes trimmed the number of chemical entities to 152 out of 843.

The purpose of PCA is to reduce a complex dataset to a

<table>
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smaller number of variables (principle components). It often provides visualization of the variation and interrelationships in a dataset. After performing the statistical analysis defined above, the three-dimensional (3D) PCA plot in Figure 4 demonstrated the uniqueness and similarities amongst the CBD oil products collected at 70 eV. Table III is a select list of the annotated compounds after statistical analysis. Interestingly, psychoactive Δ9-tetrahydrocannabinol (THC) was identified in all the samples. This is perhaps not unusual since hemp and products derived from hemp may legally contain up to 0.3% THC by weight. There is evidence of acid catalyzed conversion of CBD to THC (3), and in situ synthesis of THC from highly concentration CBD oil diluted in dichloromethane (unpublished data). However, in soon to be published work using liquid chromatography–mass spectrometry (LC–MS), these two products contain >0.3% THC by weight. Figure 5 is a normalized Box-Whisker plot of the THC ion abundance and a plot of the raw THC intensities.

**Low Energy Mode**

A series of low energy experiments were performed on a subset of the samples. In these experiments, all GC-QTOF parameters remained the same except for the electron voltage, emission current, and the source temperature which were lowered to 14 eV, 0.8 µA, and 200 °C, respectively. Low energy mode often increases the molecular ion response and improves elemental composition calculations. Figure 6 is the 70 eV and 14 eV chromatograms and spectra for an unknown compound. The 14 eV spectra better retained the molecular ion and therefore improved elemental analysis and compound identification from a known mass spectral library. The correct identification was hentriacontane (C₃₁H₆₄) with a mass accuracy of -0.27 ppm. Figure 7 is the HRAM extracted ion chromatograms (EIC) and mass spectra for CBD and cannabichromene (CBC). It should be noted that in the previously mentioned unpublished work, this sample contains 22% by weight total CBD and about 1% by weight CBC. In this qualitative study, the relative abundance of CBC is an order of magnitude

![Figure 5](image1.png)

**Figure 5:** (a) Box-Whisker plot of the normalized THC abundance. (b) Raw THC intensity values. Black horizontal line estimates the 0.3% cut off.

![Figure 6](image2.png)

**Figure 6:** Low energy 14 eV provided molecular ion information for the correct identification of a hydrocarbon. The 70 eV library match was heptacosane (C₂₇H₅₅) but the compound is hentriacontane.
lower than CBD which agrees with the quantitative work using LC–MS.

Conclusion
In this study, six commercially available CBD oil pet supplements were analyzed using GC-QTOF in discovery (untargeted) mode at both 70 eV and in low energy mode at 14 eV. The data was analyzed in a qualitative manner using chemical feature finding and differential statistical tools. The CBD oil products contained a multitude of chemotypes synthesized by the hemp plants. All products contained CBD in variable relative abundances. Compared to traditional analysis at 70 eV, low energy mode (14 eV) offers a second level of information from the samples. This workflow can be applied for multistrain analysis, cannabinomics, terpenomics, or plant metabolomics.

Disclaimer
Agilent products and solutions are intended to be used for cannabis quality control and safety testing in laboratories where such use is permitted under state or country law.

References
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Table III: Select list of annotated compounds

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<td>Vitamin E</td>
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<tr>
<td>Dicyclopentadiene diepoxide</td>
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</table>

Matthew Curtis and Sue D’Antonio are with Agilent Technologies in Santa Clara, California. Anthony Macherone is with Agilent Technologies and The Johns Hopkins University School of Medicine in Baltimore, Maryland. Direct correspondence to: amacher1@jhmi.edu
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- Extended Release Coating
- Taste Enhancement
- Appearance / Identification

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Environmental Screening of a Cannabis Production and Processing Facility: A Comparison of an Environmental DNA Microarray and Traditional Microbiological Plating Methods

As the cannabis industry continues to expand and become more heavily regulated, the need for screening tools that detect microbial contamination increases. While screening has primarily focused on the raw product, there has been little emphasis on the actual facilities in which that product is processed, which has the potential to be a contaminating source for the cannabis product. The following case study was performed to demonstrate the utility and necessity of environmental screening in a cannabis production and processing facility. Samples were collected for assessment of microbial contamination across 11 locations throughout the facility. Each sample was assessed by traditional microbiological plating and an environmental DNA microarray to compare the effectiveness of both methods.

Chelsea Adamson and Benjamin A. Katchman

Environmental surveillance is increasingly appreciated for its utility in public health efforts, including those related to agriculture and quality control (1). The presence of pathogenic organisms in production facilities is concerning because it indicates that there may be a reservoir of the contaminating organisms within the facility (2). Importantly, these pathogenic organisms may be capable of posing a significant risk to both human health and agricultural products. More specifically, the presence of agricultural pathogens in horticultural operations may
increase the risk of agricultural disease, thereby increasing the likelihood of agricultural losses and rejected batches of product. These concerns persist not only in agricultural operations, but also in food and drug production.

Provided the potential ramifications of microbial contamination in such a facility, it is important that practices in risk reduction are diligently followed and maintained. An important component of risk reduction is the continuous screening and monitoring of a facility for microbial contamination. Importantly, while an initial investigation may be able to detect and identify contaminating organisms present within a facility, subsequent screening and surveillance is necessary to confirm the effectiveness of decontamination methods, verify sources of contamination, and validate the cleanliness of a facility, as well as identifying any new instances of contamination that may occur.

Currently, the golden standard for the screening, detection, and assessment of microbial contamination has been through culturing on agar plates (3). While traditional plating methods offer a visual confirmation of microbial presence and viability, and do not require sophisticated equipment, this method does pose several disadvantages. For instance, plating is incredibly time-consuming, can be laborious, and requires the expertise of a skilled microbiologist. Additionally, a comprehensive microbiological analysis, which encompasses the detection of multiple types of organisms, often requires the use of multiple plates and media types, increasing material and labor costs; even so, species-level identifications require secondary methods of confirmatory testing.

Though their use has not yet become standard in the industry, the use of microarrays for broad-spectrum microbial detection and identification has been well-studied for its many applications in clinical and agricultural settings (4). Microarray technology is able to identify microorganisms at the family, genus, and species-level of classification. Multiplexed in design, microarrays are capable of simultaneously detecting a multitude of microbial isolates within a single sample in a matter of a few hours, making the array more cost-effective and less labor-intensive than traditional plating methods. While microarrays do require specialized equipment and training in molecular techniques, the mechanism of recognition of specific DNA sequences imparts a high degree of both specificity and sensitivity in microbial detection.

This study was conducted in an effort to highlight the utility and necessity of environmental screening of an agricultural production and processing facility. The study also compares the proficiency of traditional microbiological plating and the environmental DNA microarray used in this screening.

Methods

Sample Collection

First, 56 swabs (each suspended in 4 mL of buffered peptone water) were provided to the collaborating facility for this study. Sampling locations and intervals of sample collection were determined at the discretion of the facility contact. Immediately following sample collection (performed according to the procedure outlined in PathogenDx Product Insert: Enviro® Environmental Swab), swabs were homogenized by vortexing and aliquoted into 1 mL samples for analysis via traditional microbiological plating and PathogenDx Enviro®.

Traditional Microbiological Plating

Each sample was plated on a general medium for bacteria (Tryptic Soy Agar, TSA) and fungi (Sabouraud Dextrose Agar with chloramphenicol, SDA) to capture as many microbial contaminants as possible.

In addition to a neat sample, a 1:10 and 1:100 dilution was plated for each swab collection to ensure that individual isolates could be visualized in the event that heavy concentrations of microbial contaminants within the samples resulted in overgrowth of the plates. For each concentration, 100 µL of sample was plated.

Sample collections spanned across 11 distinct rooms within the facility:

- Veg 1
- Veg 2
- Propagation
- Post-Harvest
- Dry Room
- Mother Room
- Clone Room
- 3rd Party Lab Sampling Room
- Packaging
- Packaging 2nd Room
- Inventory

Documentation was provided for each sample collection, including date of collection, site of collection, and whether the swab site was swabbed prior to or after decontamination procedures were performed (“dirty” and “clean” designations, respectively). Schematics were also provided for seven of the above locations (Veg 1, Veg 2, Propagation, Post-Harvest, Dry Room, Mother Room, and the Clone Room).

Sample Analysis

Upon arrival, swab collections were homogenized by vortexing and aliquoted into 1 mL samples for analysis via traditional microbiological plating and PathogenDx Enviro®.

Sample collections spanned across 11 distinct rooms within the facility:
PathogenDx EnviroX Microarray

Microarray analysis was performed on 1 mL of the original sample according to the procedure outlined in the PathogenDx Product Insert: EnviroX Environmental Swab.

Results

For each sample, the results of the traditional plating and EnviroX methods were compared directly; a representative example of the comparative analysis is demonstrated in Figure 1, which displays the results for swab number one (located in Veg 2 within the facility). Microbial contamination is apparent, and robust, on both TSA and SDA plates, representing bacterial and fungal growth, respectively. While a dilution effect can be observed in the growth on the SDA plates, the growth observed on the TSA plates remained too concentrated to discern individual colonies. Interestingly, swab number one was collected after cleaning and disinfectant procedures had been conducted for this particular swab site (“clean” designation). While unable to definitively identify the contaminants from the plates alone, several categorical and species-level identifications were ascertained by the EnviroX microarray, including total aerobic bacteria (TAB), total enterobacteriaceae (TE), bile-tolerant gram-negative (BTGN), and total yeast and mold (TYM); species-level identifications included Aeromonas spp., Pseudomonas spp., and Cladosporium spp. Similar trends were observed throughout the remaining study samples.

Of the 56 swab collections analyzed, all samples tested positive for microbial contamination through both traditional microbiological plating and EnviroX analysis methods (Table I). In comparing the number of swabs with confirmed growth on TSA (bacterial) to the number of confirmed detections on TAB (bacterial) on the EnviroX array, EnviroX displayed equal or greater sensitivity in detecting contaminating microorganisms as compared to the microbiological plating in all cases (Table II). The same trend was observed, to a greater extent, in comparing the number of swabs with confirmed growth on SDA (fungal) as compared to the number of swabs with confirmed detection of TYM (fungal). Notably, TE and BTGN are more defined subcategories of TAB and consequently, were detected at a lower frequency than TAB.

The composition of microbial species detected was variable depending on the location analyzed (Table III). Veg 1 presented with the highest degree of variability with the detection of seven species-level identifications (Aeromonas spp., Pseudomonas spp., Pseudomonas aeruginosa, Fusarium oxysporum, Candida spp., Penicillium spp., and Mucor spp.). In contrast, the Clone Room presented with the lowest degree of variability with the detection of only one species-level identification (Pseudomonas spp.). Given the high degree of consistency in organisms detected across different locations within the facility, the frequency of locations that tested positive for each organism was calculated (Table IV).

### Figure 1: Bacterial-specific plates are displayed in the top row and the fungal-specific plates are displayed in the bottom row (concentration of sample decreases from left to right [neat, 1:10, and 1:100 of the original sample]). This sample has been designated as “clean,” indicating that the sampling was performed after decontamination procedures had been conducted. The corresponding EnviroX microarray data is displayed on the right. Above analysis represents swab sample number one, from the Veg 2 room.
<table>
<thead>
<tr>
<th>Swab Number</th>
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<th>Micro Plating</th>
<th>EnviroX Technology</th>
<th>Species-Level Identifications</th>
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</table>
(6 out of 11 locations). Many microbial species observed were isolated to singular locations within the tested locations (Pseudomonas aeruginosa, Mucor spp., Aspergillus terreus, and Aspergillus fumigatus).

Provided the species-specific identifications from the EnviroX analysis, temporal and spatial relationships in the distribution of microbial contaminants were evaluated. An observed temporal relationship is observed in Figure 2, which displays the contamination present at two sites within Veg 1, before (“dirty”) and after (“clean”) decontamination procedures were utilized. Looking at the two sets of agar plates, there is evident reduction of microbial burden after decontamination procedures were utilized, but they were not sufficient to remove all microbial contamination at these two sites. Interestingly, the microarray data indicated that four species of microbial contaminants were present before decontamination (Aeromonas spp., Pseudomonas spp., Fusarium oxysporum, and Candida spp.). After decontamination, only Candida had been removed from the sites, and the other three species still remained.

Among others, spatial relationships were observed in the distribution of Golovinomyces and Cladosporium spp. throughout the facility. As demonstrated in Table IV, Pseudomonas spp. was detected in 82% of the locations swabbed, and was the most

(continued): Microbial characterization of swabbing collections by traditional microbiological plating methods and EnviroX microarray technology

<table>
<thead>
<tr>
<th>Swab Number</th>
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<th>Micro Plating</th>
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<td>Clone Room</td>
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<td>✓</td>
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<tr>
<td>40</td>
<td>Veg 2</td>
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<td>✓</td>
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</tr>
<tr>
<td>41</td>
<td>Veg 2</td>
<td>✓</td>
<td>✓</td>
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<tr>
<td>42</td>
<td>Veg 2</td>
<td>✓</td>
<td>✓</td>
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</tr>
<tr>
<td>43</td>
<td>Veg 2</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
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<tr>
<td>44</td>
<td>Post-Harvest</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>45</td>
<td>Post-Harvest</td>
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<td>46</td>
<td>Post-Harvest</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
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<tr>
<td>47</td>
<td>3rd Party Lab Sampling Room</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>48</td>
<td>3rd Party Lab Sampling Room</td>
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<td>✓</td>
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<tr>
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<td>3rd Party Lab Sampling Room</td>
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<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>50</td>
<td>Packaging</td>
<td>✓</td>
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<td>✓</td>
</tr>
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<td>51</td>
<td>Packaging</td>
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<td>✓</td>
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<tr>
<td>52</td>
<td>Packaging</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>53</td>
<td>Packaging 2nd Room</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>54</td>
<td>Packaging 2nd Room</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>55</td>
<td>Inventory</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>56</td>
<td>Inventory</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
</tbody>
</table>

TSA = tryptic soy agar, SDA = sabouraud dextrose agar (with chloramphenicol); TAB = total aerobic bacteria; TE = total enterobacteriaceae; BTGN = bile-tolerant gram negative; TYM = total yeast and mold
widely distributed organism within the facility. Golovinomyces spp. was the second most prevalent, appearing in 6 of the 11 locations swabbed. When broken down by location, it was observed that Golovinomyces spp. was detected in 86% of the swab sites within the Post-Harvest room (7 of 8 swab sites). In addition to the Post-Harvest room, Golovinomyces spp. was identified in the Dry room, 3rd Party Lab Sampling room, Packaging, Packaging 2nd room, and Inventory. Followed closely behind, Cladosporium spp. was present in 5 of the 11 locations swabbed, and was primarily concentrated in Veg 2, where all 9 of the swab sites within the room tested positive (Figure 3).

### Discussion

This study highlights the utility of environmental screening as a tool to evaluate potential microbial contamination within an agricultural production and

### Table II: Summary of microbial characterization of swabbing collections by traditional microbiological plating and EnviroX microarray methods

<table>
<thead>
<tr>
<th>Location</th>
<th>Number of Swab Sites at Location</th>
<th>Traditional Plating (Number of Swabs with Confirmed Growth)</th>
<th>EnviroX (Number of Swabs with Confirmed Detection)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>TSA SDA TAB TE BTGN TYM</td>
<td></td>
</tr>
<tr>
<td>Veg 1</td>
<td>10</td>
<td>10 10 8 10 7 10 10</td>
<td></td>
</tr>
<tr>
<td>Veg 2</td>
<td>9</td>
<td>9 9 9 9 5 9 9</td>
<td></td>
</tr>
<tr>
<td>Propagation</td>
<td>3</td>
<td>3 3 2 3 2 3 3</td>
<td></td>
</tr>
<tr>
<td>Post-harvest</td>
<td>8</td>
<td>8 8 5 8 2 3 7</td>
<td></td>
</tr>
<tr>
<td>Dry room</td>
<td>8</td>
<td>8 8 2 8 0 1 4</td>
<td></td>
</tr>
<tr>
<td>Mother room</td>
<td>5</td>
<td>5 5 4 5 2 5 5</td>
<td></td>
</tr>
<tr>
<td>Clone room</td>
<td>3</td>
<td>3 3 3 3 1 3 3</td>
<td></td>
</tr>
<tr>
<td>3rd party lab sampling</td>
<td>3</td>
<td>3 2 1 3 0 0 3</td>
<td></td>
</tr>
<tr>
<td>Packaging</td>
<td>3</td>
<td>3 2 1 3 0 1 2</td>
<td></td>
</tr>
<tr>
<td>Packaging 2nd room</td>
<td>2</td>
<td>2 0 1 2 0 0 2</td>
<td></td>
</tr>
<tr>
<td>Inventory</td>
<td>2</td>
<td>2 2 2 2 0 2 2</td>
<td></td>
</tr>
</tbody>
</table>

TSA = tryptic soy agar; SDA = sabouraud dextrose agar (with chloramphenicol); TAB = total aerobic bacteria; TE = total enterobacteriaceae; BTGN = bile-tolerant gram negative; TYM = Total yeast and mold

### Table III: Microbial species identified at each location

<table>
<thead>
<tr>
<th>Location</th>
<th>Species-Level Identifications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Veg 1</td>
<td>Aeromonas, Pseudomonas, Pseudomonas aeruginosa, Fusarium oxysporum, Candida, Penicillium, Mucor</td>
</tr>
<tr>
<td>Veg 2</td>
<td>Aeromonas, Pseudomonas, Cladosporium, Fusarium oxysporum</td>
</tr>
<tr>
<td>Propagation</td>
<td>Aeromonas, Pseudomonas, Fusarium oxysporum, Candida</td>
</tr>
<tr>
<td>Post-harvest</td>
<td>Aeromonas, Pseudomonas, Golovinomyces, Penicillium</td>
</tr>
<tr>
<td>Dry room</td>
<td>Pseudomonas, Golovinomyces</td>
</tr>
<tr>
<td>Mother room</td>
<td>Aeromonas, Pseudomonas, Candida, Aspergillus terreus, Aspergillus fumigatus</td>
</tr>
<tr>
<td>Clone room</td>
<td>Pseudomonas</td>
</tr>
<tr>
<td>3rd party lab sampling room</td>
<td>Cladosporium, Golovinomyces</td>
</tr>
<tr>
<td>Packaging</td>
<td>Pseudomonas, Golovinomyces, Botrytis, Cladosporium, Candida</td>
</tr>
<tr>
<td>Packaging 2nd room</td>
<td>Cladosporium, Golovinomyces, Botrytis</td>
</tr>
<tr>
<td>Inventory</td>
<td>Pseudomonas, Cladosporium, Golovinomyces</td>
</tr>
</tbody>
</table>

### Table IV: Prevalence of each microbial species identified during study (11 locations total)

<table>
<thead>
<tr>
<th>Microbial Species</th>
<th>Number of Locations that Tested Positive for Specified Microbial Species (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aeromonas</td>
<td>5 (45)</td>
</tr>
<tr>
<td>Pseudomonas</td>
<td>9 (82)</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>1 (9)</td>
</tr>
<tr>
<td>Fusarium oxysporum</td>
<td>3 (27)</td>
</tr>
<tr>
<td>Candida</td>
<td>4 (36)</td>
</tr>
<tr>
<td>Penicillium</td>
<td>2 (18)</td>
</tr>
<tr>
<td>Mucor</td>
<td>1 (9)</td>
</tr>
<tr>
<td>Cladosporium</td>
<td>5 (45)</td>
</tr>
<tr>
<td>Golovinomyces</td>
<td>6 (55)</td>
</tr>
<tr>
<td>Aspergillus terreus</td>
<td>1 (9)</td>
</tr>
<tr>
<td>Aspergillus fumigatus</td>
<td>1 (9)</td>
</tr>
<tr>
<td>Botrytis</td>
<td>2 (18)</td>
</tr>
</tbody>
</table>
processing facility. While largely harmless, many of the microorganisms detected in this surveillance carry potential risks to both human health and agricultural products and yields.

Highly ubiquitous in the environment and agricultural samples, it is unsurprising that *Pseudomonas* spp. was the most prevalent isolate in this study; while some species of *Pseudomonas* can be harmful, many serve as plant commensals and are not especially concerning (5). Conversely, the detection of organisms such as *Golovinomyces* spp. and *Cladosporium* spp. are more concerning. The presence of *Golovinomyces* spp. is particularly problematic from an agricultural perspective because these species are a major cause of powdery mildew in plants, which is capable of reducing or destroying agricultural yields (6). By comparison, *Cladosporium* spp. is relatively ubiquitous in the air, but can be a significant allergen and can pose health concerns in susceptible individuals (7). Without environmental screening, microbial contamination such as this may go undetected, imposing risks to agricultural yields and human health. Provided such screenings, steps can be taken to reduce contamination, modify decontamination procedures as necessary, and monitor facilities to ensure rapid detection of any recurrence of contamination.

Further, this study emphasizes the advantages of utilizing the PathogenDx Enviro<sup>®</sup> microarray technology in microbial detection as compared to traditional microbial plating. In addition to producing results in a more cost-effective and rapid manner as compared to traditional plating, the Enviro<sup>®</sup> microarray displayed equal or greater sensitivity in detecting microbial contamination in all sample cases.
In fact, there were many cases in which the agar plates displayed no growth but Enviro\textsuperscript{X} detected contamination, for both bacterial and fungal isolates. In addition, Enviro\textsuperscript{X} provided speciation of many of the contaminants present, a distinguishing characteristic that could not be determined by the agar plating alone. Notably, without these species-level identifications, the observed temporal and spatial relationships could not have been ascertained. While some patterns can be observed from the agar plates, the species-level identifications could not be made without further experimental analysis. Furthermore, without species-level identifications, the degree of risk associated with the specific contaminants present cannot be fully appreciated. Taken together, these data support the usefulness and need for environmental screening in agricultural processing facilities, and highlights the critical advantages in utilizing microarray technology for microbial detection, as opposed to traditional microbiological methods.

References
4) T. Kostic and A. Sessitsch,

Chelsea Adamson is a microbiologist and Benjamin A. Katchman is a principal scientist at PathogenDX in Tucson, Arizona. Direct correspondence to: bkatchman@pathogendx.com
Beyond THC and CBD: Opportunities for Creating Pharmaceutical Targets

Taking a look back, we can see that the modern pharmaceutical industry evolved from more crude preparations and administration of natural product extracts. With consideration also given to what makes an effective therapeutic compound, it is interesting to think how the medicinal cannabis business will evolve through a similar lens.

Frank W. Foss Jr. and Kevin A. Schug

The cannabis and hemp industries, a longtime taboo area at the never ending frontier of science, are now expanding at alarming rates. As we see individual states passing favorable legislation for medical and recreational cannabis, the Federal Farm Bill has opened the door for industrial hemp, and with it, a massive influx of cannabidiol (CBD) and CBD-infused products. While a large majority of these burgeoning business entities are pursuing extensive cultivation efforts to produce potent flower material and isolate products at a more expeditious and economical rate, perhaps there are other opportunities on the periphery that are worth investigating. This becomes more apparent if we look at the approach that the pharmaceutical industry took with natural products during its nascent stages; an approach that continues to produce blockbuster medicines today.

Prior to the evolution of the modern pharmaceutical industry, early biopharmacology involved the prehistoric use of plants, insects, animals, molds, and raw chemicals. Early healers understood that preparations and extracts of these materials had beneficial effects for people suffering from disease, or had psychological effects that were considered desirable. In the preindustrial age, these preparations contained a vast number of molecular components, which could cause unwanted effects including lethal toxicity. Many modern medicines are linked to these millennia-old remedies.

If we use the history of opium poppy (Papaver somniferum) as an example, we see that many elixirs were prepared by extraction with ethyl alcohol and water, and the cultivation of opium plants may be traced to Mesopotamia ca. 3400 B.C. Physicians in the 18th century recognized that use of the Papaver elixir had tonic effects on heart failure, asthma, and gastrointestinal illnesses. They also learned that attaining therapeutic doses of the underlying molecule required chronic consumption of morphine, which is the largest constituent of opium. Even then, this was considered deleterious.

In 1848, Georg Merck first separated and characterized Papaverine from opium. Papaverine is a benzylisoquinoline alkaloid and occurs naturally in opium at a concentration of 0.5%. Papaverine was rapidly recognized as beneficial for cardiac and pulmonary conditions. Demand for Papaverine rapidly outpaced the supply that could be obtained from biological sources. This led to a quest to synthesize Papaverine from de novo sources. This synthesis was first successfully accomplished in 1879 and has had numerous improvements since then. The isolated molecule nonselectively relaxes smooth muscle in the body—smooth muscle being the only form of muscle in cardiac walls, blood vessels, pulmonary airways, and the wall of the intestines. Papaverine has a half-life of ca. 1 h. The use of oral preparations required that dosing be performed 6–10 times a day.

Subsequently, Verapamil was first synthesized in 1962, and while not a direct analogue of Papaverine, thermal degradation and methylation of the pyrrole ring of Papaverine gives the structure of Verapamil. Verapamil is also a calcium channel blocker. This is particularly significant given its tonic effects on smooth muscle and its primary use as an anti-arrhythmic. The take home point is that Verapamil was approved as an ethical drug and has produced more than $100 billion dollars in revenue since its approval.

Another example of drugs used for many centuries as a folk remedy is Digitoxin and Digoxin. Both are isolated from the flower of the Foxglove plant (Digitalis sp.). Digitoxin was used for heart failure and Digoxin continues to be used today, both for heart failure and as an anti-arrhythmic. Both are cardiac glycosides, which have extremely long half-lives: 3–5 days for Digoxin and 7–9 days for Digitoxin. Both molecules have very narrow therapeutic windows. The therapeutic dose and fatal dose of Digitoxin are almost identical. This has greatly limited the usefulness of either as a medicine.

Both Papaverine and Digitoxin illustrate the two ends of the spectrum of biopharmacologic efficacies using botanicals as medicine. One is too short, the other too long. To be a useful medication, a therapeutic agent should have a half-life in the body that allows for once or twice a day dosing, a therapeutic
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window that is wide, and minimal undesirable side effects relative to the primary action sought. However, knowing their structures and activities has led to a long-term and fruitful medicinal development of biologically inspired structures that improve human health.

Reviewing this history of bringing botanicals from anecdotal folk medicine through to science-based pharmaceuticals, we can expect the following as we take cannabis and hemp through the same journey. Initially, we should expect that any useful molecules may not be grown in sufficient quantities to fill the demand for an international market. Almost all of the molecules of interest that are present in cannabis and hemp (such as cannabinoids, terpenes, flavonoids), are expressed in exceedingly small quantities. Mendelian genetic manipulation is already being applied to amplify the concentrations of these constituents; however, laboratory genetic modification and chemical synthesis are also underway. The caveat with genetic modification is that this modality of increased production may also introduce or amplify compounds that interfere, inhibit, or produce side effects in a full-spectrum product, or they may delete compounds that are necessary for the full useful effect. Similarly, the production of individual cannabis and hemp-based compounds through chemical synthesis may produce products of limited therapeutic value.

This is in fact a major point of contention in the cannabis and hemp sectors. Anecdotal evidence already suggests that isolated use of either tetrahydrocannabinol (THC) or CBD does not match the beneficial effects of total extracts from cannabis and hemp. While the use of full-spectrum cannabis and hemp products is widely accepted, we can expect upon possible federal legalization that the pharmaceutical industry will apply regulatory and industry pressure to pursue only isolated molecules or their derivatives. In any eventuality, it is highly likely that newly isolated molecules will require chemical modification. The botanical molecule may have issues with biologic half life, target selectivity, or toxicity that limits or eliminates its use as a medication. Chemically modified analogues of cannabis derived molecules can produce a new species that overcomes these limitations. Regardless, investigation of purified molecules found naturally in cannabis and hemp are likely to indicate potential receptors that could be targeted through rational drug design, in which modifications to botanical progenitors lead to new avenues of clinical care.

The cannabis and hemp industries are in the midst of massive changes allowing for new frontiers in science to be more fully explored. Two things will limit the opportunity for medical advancement and discovery using cannabinoids, terpenes, and flavonoids: capitalization of that research and regulatory push back from U.S. federal authorities. As of this writing, the U.S. Food and Drug Administration (FDA) has issued three cease and desist letters for making false and unsubstantiated medical claims regarding the use of cannabinoids and it appears that there will be a fourth coming. Regulatory limits and possibly outright bans on the use of CBD as an additive and adulterant in multiple products are possible. Given the history of the FDA, we should expect that there will be an upcoming battle on the use of hemp and cannabis as nutraceuticals. The FDA may begin pushing for research into cannabis and hemp for use as ethical medications, while limiting or banning their use as nutraceuticals. Only those who have started down the path of doing basic research into the safety and efficacy of cannabis and hemp, from the testing of isolated molecules and their modes of action through structured patient clinical trials, will survive.

Frank W. Foss Jr. and Kevin A. Schug are with the Department of Chemistry and Biochemistry at The University of Texas at Arlington, in Arlington, Texas. Direct correspondence to: ffoss@uta.edu or kschug@uta.edu

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Avoiding Contamination in Cannabis Quality Control Sample Preparation

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Patient and consumer safety is a critical factor during manufacturing of your products. In addition, false test results in product quality testing can lead to unnecessary scrapping of batches and product recalls. Good pipetting practices using filtered and pure pipette tips help ensure contamination-free pipetting to prevent contamination during sample preparation, ensuring true test results and patient and consumer safety.

Preventing contamination is paramount to ensuring patient and consumer safety, the quality control of your products, and accurate results of your analytical testing. To prevent contamination requires identification of potential contaminant mechanisms so they can be addressed.

This paper discusses the three contamination types that originate from pipetting: pipette-to-sample, sample-to-pipette, and sample-to-sample contamination.

Pipette-to-Sample Contamination

This type of contamination occurs when a contaminated pipette or pipette tip contacts a sample.

In daily laboratory work, pipette-to-sample contamination can be avoided by following these simple guidelines:

- Clean and properly maintain your pipettes.
- Select a tip with the relevant purity class for your application.
- Use (sterilized) SafetySpace filter tips or tip-cone filters.

Pipette tips are available in multiple purity grades and are generally divided into three categories:

- no purity certification
- certified free of contaminants, like DNase, RNase, and endotoxins
- sterilized to be free of microbial life

Contaminants such as endotoxins are difficult to remove, so it is very important to prevent contamination during manufacturing. To certify the absence of these contaminants, a third-party laboratory performs quality testing. Sterilization after manufacturing ensures that the tips are free of microbial life (bacteria, fungi, molds, and so on) when delivered to customers.

Pipette tips can also be a potential source of leachables—trace amounts of chemicals originating from materials or process equipment that can contaminate the samples. Examples of leachables include heavy metals, antioxidants, pigments, biocides, and surfactants. High-quality tips manufactured from 100% virgin polypropylene in a high-quality manufacturing facility are free of leachables.

Sample-to-Pipette Contamination

This type of contamination occurs when the pipetted liquid or aerosol particles from it enter the pipette body. To minimize the risk of sample-to-pipette contamination, the following precautions are recommended:

- Always release the pipette’s push button slowly to prevent aerosol formation and uncontrolled liquid splashing within the pipette tip.
- Hold the pipette in a vertical position during pipetting and store the pipette in an upright position. This prevents liquids entering the pipette body.
- Use SafetySpace filter tips to prevent aerosol transfer from the sample into the pipette body.

Sample-to-Sample Contamination

Sample-to-sample contamination occurs when aerosol or liquid residue from one sample is carried over to the next sample. This may happen, for example, when the same pipette tips are used multiple times. To avoid carry-over contamination:

- Use SafetySpace filter tips to prevent aerosol transfer from the sample into the pipette body.
- Always change the pipette tip after each sample.
- If you suspect pipette contamination, autoclave or disinfect the pipette according to the manufacturer’s instructions.

Conclusions

Proper pipetting techniques and use of sterilized filter tips are key to avoiding the three major contamination types during sample preparation. Contamination-free pipetting ensures reliable results during microbiological, heavy metal, and chemical analysis.
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Business Development-Cannabis Testing
Dr. Anthony Macherone,
Senior Applications Scientist
Cameron George,
West Coast Sales Director

www.agilent.com/en/promotions/cannabis
Anton Paar USA

COMPANY DESCRIPTION
Anton Paar develops, produces, and provides analytical instrumentation used in research, development, and quality control. Anton Paar’s cannabis instrumentation portfolio includes microwave digestion systems for safe and efficient sample preparation prior to heavy metal and trace element analysis. Additionally, Anton Paar offers particle analysis instruments that measure particle size as an indicator of bioavailability and measure zeta potential as an indicator of product stability over time.

CHIEF SERVICES SUPPORTED
Anton Paar Services include pharmaceutical instrument qualification to support your compliance and traceability needs, Anton Paar Certified Service directly from the manufacturer, and Anton Paar’s ISO 17025 calibration, guaranteeing the traceability of your equipment to the International System of Units (SI).

MAJOR PRODUCTS
Multiwave 7000 Microwave Digestion System
With Multiwave 7000 you can digest different samples—cannabis plant, oils, edibles, or creams—in the same run, using the same method.

Multiwave GO Microwave Digestion System
Processing 12 samples in one run, Multiwave GO is the most economic tool for routine digestion of a wide range of samples, including biological materials, edibles, cosmetics, and pharmaceutical samples. It has the smallest footprint, is easy to handle, and comes at low investment and running costs.

Litesizer™ 500
Litesizer™ 500 is ideal for measuring the particle size and zeta potential of beverages infused with CBD emulsions.

STATES SERVED
Nationwide

www.anton-paar.com /cannabis
CEM Corporation

COMPANY DESCRIPTION
CEM Corporation is a leading global company specializing in scientific solutions for critical laboratory applications. We design and manufacture systems for analytical laboratories, life sciences, and processing plants worldwide. Our product portfolio includes innovative instrumentation for sample preparation for elemental and chromatographic analysis.

We have been deeply involved in the cannabis industry for many years and are familiar with testing procedures and regulations. We are founding members of many cannabis committees that are working on standardization of testing and reporting of data. We are passionate about transcribing this information into systems to help your business grow and have the knowledge to support you along the way.

CHIEF SERVICES SUPPORTED
World-class application and service support are what set CEM apart from other instrument manufacturers. Our mission is to simplify your testing procedures with easy-to-use systems that do the work for you. With built in methods, onboard support videos, and live applications and service support ready to help when you need it most, CEM is your partner in sample prep.

MAJOR PRODUCTS
• EDGE: Automated extraction system for potency, pesticides, terpenes, and mycotoxins. The EDGE automatically performs solvent addition, sample extraction, filtration, and cooling in as little as 5 minutes.
• MARS 6: Microwave digestion system for trace metals sample prep of all cannabis and cannabis containing products.

STATES SERVED
Nationwide

CEM Corporation
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USA

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LOCATIONS OF OTHER OFFICES AND FACILITIES
France, Germany, Ireland, Italy, Japan, United Kingdom, Local agency representatives in all world regions

www.cem.com
Cornerstone Scientific

COMPANY DESCRIPTION
Distributor of scientific supplies used in cannabis testing laboratories including chromatography, biosciences, general laboratory, and safety products. Modern, easy to use website with products not offered by large distributors.

MAJOR PRODUCTS
• HPLC columns
• Low adsorbing and labelled autosampler vials
• Filtration media including paper for cannabis testing
• Vial centrifuge
• Syringe filters
• Weigh boats
• Pipette cleaners
• Color-coded centrifuge tubes

STATES SERVED
Nationwide for the USA.
Chem Service, Inc.

COMPANY DESCRIPTION
Established in 1962, Chem Service is accredited for ISO 17034 and ISO 17025 and registered and certified to the ISO 9001 Quality Management System for the design, development, production, distribution, and servicing of organic neat and synthetic reference materials.

CHIEF SERVICES SUPPORTED
Chem Service, Inc., produces high purity chemicals for use as reference materials and for other laboratory purposes. More than 95% of their standards grade materials have a certified purity of 98.0% or greater. Standards-grade chemicals are clearly labeled with an expiration date that is based on years of experience in handling and testing. Products are packaged in small quantities to minimize storage, waste, and disposal requirements. Organic and inorganic chemicals, solutions, and mixtures are available to meet a wide range of specialized laboratory needs.

MAJOR PRODUCTS
• Pesticide residue standards
• Metabolite standards
• Cannabis pesticide standards
• Residue solvent standards
• Terpene standards
• Flame retardants/PBDE
• EPA standards
• USP standards
• ISO standards
• DIN standards
• Explosive residues
• PCB congeners
• Petroleum hydrocarbons
• Fatty acid methyl esters
• High purity inorganics
• Surfactants
• Anti-oxidants
• Ultraviolet inhibitors
• Plasticizers
• Biological stains
• Phthalate standards
• Polynuclear aromatics
• Vitamins
• Carbohydrates standards
• Forensic standards
• Personal care product standards

STATES SERVED
US and international
Milestone Inc.

COMPANY DESCRIPTION
With over 50 patents and more than 25,000 instruments installed in laboratories around the world, Milestone has been widely recognized as the global leader in metals prep technology for the past 30 years. Committed to providing safe, reliable, and flexible platforms to enhance productivity for cannabis testing labs, growers, and processors, Milestone is the acknowledged industry leader in microwave technology for metals digestion and solvent-free terpene extraction.

CHIEF SERVICES SUPPORTED
Within the cannabis industry, Milestone offers innovative solutions that help chemists accurately test for metals and extract full-profile terpenes.

- **Metals testing:** With increased state regulations, cannabis growers are required to conduct trace metals testing to ensure a safe and high-quality product. Obtaining the analytical data required to produce quality products starts with the crucial step of sample preparation. Milestone offers a complete suite of microwave sample prep and clean chemistry productivity tools that allow testing labs to meet their unique throughput needs.

- **Terpene extraction:** Milestone’s solvent-free microwave terpene extraction solutions provide efficient and fast production of terpenes, providing a complete terpene profile with superior quality over all other extraction techniques for growers and processors alike.

MAJOR PRODUCTS
For Cannabis Testing Labs:
- **ETHOS UP:** A flexible and high-performing rotor-based platform that aligns to the needs of cannabis testing labs for determining trace elements in wide array of cannabis samples
- **ultraWAVE:** Single reaction chamber microwave digestion technology, capable of processing mixed batch cannabis samples with disposable glass vials for ICP/ICP-MS analysis

For Processors and Growers:
- **ETHOS X:** A game-changer in the production of high-quality terpenes, uses microwave technology to produce full-profile terpenes

STATES SERVED
Nationwide

Milestone Inc.

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LOCATIONS OF OTHER OFFICES AND FACILITIES
Boldone Soriole (BG), Italy
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www.milestonesci.com/cannabis
Neta Scientific, Inc.

COMPANY DESCRIPTION
For 20 years, Neta Scientific, Inc., has been a leading distributor of laboratory supplies and solutions. Our mission is to offer our customers access to a robust catalog of world-leading brands to more effectively conduct cutting-edge research and development. We are an MBE and WBE certified business, headquartered in Hainesport, NJ with representation throughout the United States.

CHIEF SERVICES SUPPORTED
We specialize in aligning with industry leaders to offer an extensive catalog of leading brands to researchers in the pharmaceutical, biotechnology, and academic industries. As a diverse aggregator and neutral provider, our products are trusted, preferred, and always offered at a fair market price. Our Laboratory Equipment Asset Procurement Services (LEAPS) division provides new and existing customers within the life science industry with convenient access to end-to-end lab services and insights.

MAJOR PRODUCTS
Our key product categories include chromatography and spectroscopy, life sciences, labware, chemicals and reagents, small and large bench instruments, and cold storage. Additionally, as a diverse and neutral provider, we offer third-party support for major brand suppliers and more in-depth catalog aggregation.

STATES SERVED
Nationwide

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KEY PERSONNEL
Winfred Sanders,
President

Dragan Karajovic,
VP of Business Development

Alicia Thomas,
Marketing Operations

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Proton OnSite

COMPANY DESCRIPTION
Proton OnSite, a wholly owned subsidiary of Nel ASA, Oslo, Norway (OSE:NEL), manufactures hydrogen, nitrogen, and zero air generators that you can rely on. Our advanced proton exchange membrane (PEM) electrolysis systems, coupled with uncompromising attention to excellence and quality, enable us to deliver, install, and support gas generation on every continent. No other company has as large of a commercial fleet or as long of a history with reliability as Proton OnSite. Exclusively focused on customer solutions, we have the experience and products to ensure success.

CHIEF SERVICES SUPPORTED
• Cannabis sciences
• Environmental sciences
• Food and beverage industries
• Forensic sciences
• Life sciences
• Petrochemical industry
• Pharmaceutical industry

MAJOR PRODUCTS
Proton OnSite offers hydrogen, nitrogen, and zero air gas generators to fit the needs of any laboratory. These units are suited to provide ultra-high purity gas with consistent and predictable results. Also, Proton OnSite is the only provider of “Lab Server” solutions, allowing large labs to supply numerous mass spec instruments from a single, central generator. Our line of on-site gas generators is a simpler, less expensive, and less complex approach to supplying superior gas for laboratory and scientific applications. These generators are compact in size and available in stackable configurations to maximize your available lab space.

Proton OnSite

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KEY PERSONNEL
Kim Georgiades, Inside Sales

www.ProtonOnSite.com
Restek Corporation

COMPANY DESCRIPTION
Chromatography is what Restek does, and chromatography is who we are. We are an independent, international, and diverse team of employee-owners not bound to a specific brand of instrument or geographic region. We live and breathe phase chemistry, peak separations, resolution, and inertness because while chromatography may be a necessary tool in your business, it is our business. And it is a business that we directly serve across 100+ countries and six continents with unrivaled Plus 1 service, applications, and expertise.

From LC and GC columns to sample preparation, reference standards to accessories, Restek is your first and best choice for chromatography.

CHIEF SERVICES SUPPORTED
Restek provides the industry with accurate, fast, and reliable analytical testing workflows. We support this in Restek blogs, application notes, presentations at conferences and seminars, customer visits, and webinars.

MAJOR PRODUCTS
Restek offers cannabis workflow testing solutions and analytical consumables for potency, terpenes, pesticides, residual solvents, and mycotoxins. These products include CRMs, sample prep, columns, and application notes for various testing methodologies.

STATES SERVED
International

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KEY PERSONNEL
Ashlee Girardi,
Business Development Manager - Cannabis

www.restek.com
Sartorius Lab Instruments GmbH & Co. KG

COMPANY DESCRIPTION
The Sartorius Group—A trusted partner for the biopharmaceutical industry and laboratories
Sartorius supplies high-quality laboratory instruments, high-grade consumables, and excellent services. Customers are from research and quality assurance laboratories of the pharmaceutical, chemical, and food industries as well as from the academic sector. The product portfolio focuses on high-value laboratory instruments such as lab balances, pipettes, and laboratory water purification systems. Moreover, a wide range of consumables, such as laboratory filters and pipette tips are offered. In laboratory weighing technology, Sartorius ranks as the world’s second largest equipment supplier, and enjoys a strong position among the leading global suppliers for consumables, pipettes, and laboratory water purification systems.

CHIEF SERVICES SUPPORTED
As your value-added partner, we offer a complete range of services from conceptual design, contract testing and research, and detailed engineering to regular preventative maintenance to ensure ongoing productivity and efficiency.

MAJOR PRODUCTS
Sartorius enables fast, precise, and reliable results in your cannabis analysis.
States that have passed laws permitting usage of medicinal and recreational cannabis products are also calling for rigorous testing of cannabis derivatives to ensure consumer safety.
Sartorius offers a variety of high-quality products that ensure the most accurate test results and allow your testing lab to run smoothly at all times. Our products not only support you in performing super-fast determination of your samples’ moisture and weight, but also in the process of safely preparing of your samples for analytical or microbiological analysis.

STATES SERVED
About 60 sites in more than 30 countries, thereof 22 production facilities, including all sales subsidiaries and commercial agencies present in more than 110 countries.

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KEY PERSONNEL
Wallace Harvey, Regional Business Manager, USA

www.sartorius.com
Schuler Scientific

COMPANY DESCRIPTION
Schuler Scientific is an ISO 9001:2015 accredited organization, located in Englewood, Colorado. We provide a broad range of innovative instruments for precision weighing, moisture determination, and electrochemical analysis.

We are a company of experienced managers who have worked for years, in every aspect of instrumentation, engineering, sales and marketing, and manufacturing. We brought our considerable experience together in Englewood, where every aspect of quality control, and final inspection are conducted on every product.

Customer service and technical support may be our strongest suit, and it’s always been local.

CHIEF SERVICES SUPPORTED
We offer a broad range of balances and scales, calibration weights, and electrochemistry products. With a focus on our weighing portfolio, we offer analytical weighing solutions which range from ultra-microgram readability balances (0.0000001 g) to high capacity scales (60,000 g). Our ISO 17025:2017 calibration lab offers certificates of calibration for new balances and recalibration of balances.

MAJOR PRODUCTS
Schuler Scientific provides a wide range of professional weighing instruments, moisture analyzers, OIML class weights, ISO 17025:2017 metrology services, and electrochemistry products. All analytical and precision balances come with an industry leading five year, no-hassle warranty. Our balance portfolio is broad, ranging from ultra-microbalances to high capacity scales. We have four different lines of NTEP approved balances, totaling 21 individual NTEP (legal-for-trade) approved balances. Any of our balances can also be purchase with a calibration certificate, thanks to our ISO 17025:2017 registered laboratory.

STATES SERVED
North America; including the U.S., Canada, and Mexico

Please visit us at www.schulersci.com
Shimadzu Scientific Instruments

COMPANY DESCRIPTION
Shimadzu is one of the world’s leading providers of analytical instruments for applications in a broad range of industries. In the United States, Shimadzu has been at the forefront of working with cannabis testing laboratories and has a comprehensive understanding of the requirements of this evolving industry. From seed to sale, from accurate potency testing and terpene profiling to contaminate testing for pesticides, residual solvents, heavy metals, and mycotoxins/aflatoxins, Shimadzu offers scalable solutions to meet your testing needs for today and tomorrow.

CHIEF SERVICES SUPPORTED
As medicinal and recreational cannabis markets continue to grow, analytical testing will ensure that consumers are receiving accurately labeled products that are free from contamination. Shimadzu is ready to assist you as you grow your laboratory. We offer a full suite of testing instruments, research platforms, scientifically validated methods, and a variety of leasing programs to meet evolving requirements. In addition, our expert team of scientists is readily available to help your cannabis testing laboratory succeed, assisting with method development, instrument training, and maintenance to ensure your systems operate at an exceptional level.

MAJOR PRODUCTS
Shimadzu offers a full suite of analytical instrumentation to help grow your lab. These instruments include:
- HPLC
- GC
- GC–MS/MS
- LC–MS/MS
- Q-TOF LC–MS
- ICP-MS
- Moisture balances
- Online SFE-SFC-LC–MS/MS
- MALDI-TOF MS
- Consumables

STATES SERVED
Nationwide

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www.GrowYourLab.com
UCH, Inc.

COMPANY DESCRIPTION
UCH, LLC is a vertically integrated manufacturer and supplier of analytical products for the cannabis testing industry. We offer the largest selection of QuEChERS, solid-phase extraction products, UHPLC and HPLC columns, and the best application support team available. Featured products for the cannabis industry, unique to UCH, include Chlorofiltr®, for the selective removal of chlorophyll from cannabis extracts leaving planar pesticides intact, SpinFiltr®, which combines dSPE clean-up and 0.2 µm filtration in a single step, and new this year, LipiFiltr®, our targeted cleanup cartridge for the analysis of pesticides in oil-based cannabis products.

CHIEF SERVICES SUPPORTED
UCH has 30+ years of experience as the premier supplier for chromatography consumables. We combine this with world class technical support and application development.

MAJOR PRODUCTS
• ENVIRO-CLEAN® SPE cartridges
• Chlorofiltr® Sorbents
• QuEChERS
• Quick QuEChERS
• Syringe Push-Thru cartridge formats
• LipiFiltr®
• SpinFiltr®
• SELECTRA® HPLC columns

STATES SERVED
Nationwide and international through distribution.

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KEY PERSONNEL
Jody Hallett,
Director of Sales & Marketing
Brian Kozlowski, Marketing Specialist & Product Manager
Chris Embert,
Graphic Specialist

unitedchem.com
Ace Glass Inc.

COMPANY DESCRIPTION
Ace Glass Incorporated has been a leader and innovator of scientific glassware and lab equipment for more than 80 years. Ace provides a wide variety of U.S. manufactured standard and custom scientific glassware including glassware specifically designed for the cannabis processing industry. This includes distillation equipment, extraction equipment, and custom glass.

We work directly with businesses, including distributors. If you have a glass idea and want to realize it, speak to one of our staff today because . . . Quality Cannabis beings with Quality Glassware

CHIEF SERVICES SUPPORTED
We offer scientific glass manufacturing services including standard and custom options specifically designed for the cannabis industry.

MAJOR PRODUCTS
- Short path distillation systems
- Large scale reactors (up to 200 L) for ethanol extraction or winterization
- Large scale filter reactors (up to 150 L) for recrystallization, ethanol extraction, or winterization
- Rotary evaporators
- Heating mantles
- Heating tops (fabric for top of boiling flask)
- Filtration apparatus
- Custom creations
- Modular support stands
- Instatherm® (a closer more efficient heat)

STATES SERVED
International


Ace Glass Inc.

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KEY PERSONNEL
Jeff Kramme,
President
Liz Dejesus,
General Sales Manager

DigiVac

COMPANY DESCRIPTION
DIGIVAC is a family-owned business that has been operating for more than 30 years. It is the home of Vacuum Engineering Excellence. We build American made vacuum instrumentation and scientific controls. Our products are designed to meet the practical needs of our customers accuracy and reliability. Our gauges are calibrated under actual vacuum against a NIST standard with unmatched accuracy. DIGIVAC serves OEM, reseller, and provides direct customer support and sales. We strive to solve tough R&D, process, and manufacturing challenges with scientific controllers and vacuum instrumentation. We back our designs with smart and responsive technical support.

CHIEF SERVICES SUPPORTED
What makes DIGIVAC different: We have a team of highly experienced engineers that know vacuum and distillation applications. They thrive on innovation and can customize gauges and controllers to meet your needs. We have a deep understanding of short path distillation (SPD) and how vacuum improves processes and efficiency. Our relationships in the industry supports in-laboratory product testing that speeds innovation and product development to meet the growing and evolving needs of the cannabis and plant oil processing industries.

MAJOR PRODUCTS
• VPC: provides innovative yet simple vacuum control for rotary evaporator distillation and isolation of plant oils.

The VPC delivers precise control of target vapor pressure and is designed to tolerate harsh chemicals.
• Bluetooth Bullseye Precision Gauge has patented vacuum graphing and has become the standard for measuring SPD processing. Combine this with the Vacuum Gauge app to enable remote monitoring. Set low and high alarms plus email data and graphs for documentation.
• STRATAVAC 2018 with bleed: Often installed in distillation systems including SPD and wiped film to show multiple vacuum levels across a system with simple and precise vacuum control.

STATES SERVED
USA Nationwide and Canada

DigiVac
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KEY PERSONNEL
Tim Collins, EE, MBA, CEO, President
Tom Bassi, R&D Manager
Jennifer Collins, MBA, Director Sales and Marketing

www.digivac.com

www.CannabisScienceTech.com | CANNABIS SCIENCE AND TECHNOLOGY
Drummond Scientific Company

COMPANY DESCRIPTION
Drummond Scientific is a manufacturer of a wide range of microdispensers, micrinjectors, micropipettes, pipet controllers, and innovative liquid handling tools for the laboratory.

CHIEF SERVICES SUPPORTED
Full technical service at our headquarters that will handle any problems you might have with the units we offer.

MAJOR PRODUCTS
- Pipet-Aid pipette controllers
- Microinjection
- Microdispensers
- Capillary micropipettes
- Hemato-Clad glass
- Manifolds

STATES SERVED
Worldwide both domestic and international

Drummond Scientific Company

MANUFACTURING & PROCESSING

ANALYTICAL INSTRUMENTS

For over 70 years, Drummond Scientific has been a leading manufacturer of a wide range of microdispensers, micrinjectors, micropipettes, pipette controllers and innovative liquid handling tools for the laboratory. Demand the best... Demand Drummond.
FRITSCH Milling & Sizing, Inc.

COMPANY DESCRIPTION
Since 1920, Fritsch has engineered and manufactured a wide range of milling and grinding systems for agricultural, food, and pharmaceutical applications. The PULVERISETTE 19 (P-19) Precision Cutting Mill system sets a new standard for the cannabis industry, allowing controllable particle size output, reproducible performance regardless of end user, throughput of > 1 lb. per min average, heat mitigation, and a dust-free, easy cleaning design. Recent scientific studies have demonstrated that there is no loss of valuable cannabinoids or terpenes using this precision milling system. Oil extractions and preparation of pre-rolls or other ingredients can be optimized like never before. For the analytical testing laboratory, Fritsch offers several cryogenic milling options for individual flower or edible samples. For high-throughput sample preparation...

CHIEF SERVICES SUPPORTED
Manufacturer of precision milling, grinding, and particle characterization systems

STATES SERVED
Nationwide

MAJOR PRODUCTS
• P19 Cutting Mill
• P14 Rotor Mill
• P11 Knife Mill
• A3 Sieve Shaker
• A22 & A28 Particle Analyzers
Pall Laboratory

COMPANY DESCRIPTION
Pall Laboratory provides dependable, high-performance products that expedite and simplify cannabis processes. Pall is the largest and most diverse filtration and separation company in the world, and the global leader in the development of new filtration, separation, and purification technologies. Pall Laboratory is committed to providing many sample preparation and detection tools used in cannabis, basic research, genomics, proteomics, and combinatorial chemistry applications including scalable chromatography, tangential flow filtration, and sterile filtration products.

CHIEF SERVICES SUPPORTED
• Cannabis analytical testing products for pesticide analysis, potency testing, microbial testing/PCR, and nucleic acid purification
• CBD and THC oil production
• R&D process validation to scale to production
• Sample preparation, filtration, and separation
• Protein separation and recovery
• Sterile filtration
• Scale up solutions to food grade production

MAJOR PRODUCTS
• Syringe filters
• Vacuum filtration
• Multiwell filter plates
• Microbial growth and filtration
• Positive pressure filtration
• Filtration capsules
• Membrane filters, disc, sheet, rolls

STATES SERVED
Worldwide

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KEY PERSONNEL
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Sales Manager

Joseph Baaklini
SLS Director

pall.com/lab
A PASSION FOR CHEMISTRY & CANNABIS

At Extract Consultants we help our partners build successful products that deliver repeatable and consistent results. Our terpenes, flavors and effects blends are specifically designed to work in minimal amounts with low add-back rates that maintain uniformity, taste and effect. We source our ingredients from only the highest quality suppliers. Our team of internationally renowned chemists and formulators, along with our advanced processing equipment and in-house lab testing, confirm purity and consistency to ensure that our products deliver every time.

From formula fulfillment to custom creations, we strive to provide virtually any terpene, terpene blend, essential oil blend and flavor desired backed by our logistical and regulatory support.

ExtractConsultants.com
BOOST YOUR CANNABIS SAMPLE PREPARATION WITH MULTIWAVE GO AND MULTIWAVE 7000

- Complete digestion of different samples - even in the same run with Multiwave 7000
- Multiwave GO offers the most economically-efficient microwave digestion with the smallest footprint
- Easy handling with disposable glass vials in Multiwave 7000 and tool-free vessels in Multiwave GO
- Compact benchtop devices with integrated touchscreens and highly-efficient cooling designs

Get in touch: www.anton-paar.com/cannabis