







ith the growth of the cannabis market, laboratories are under increased pressure to provide robust, reliable, and accurate analytical testing to help ensure product safety and quality. Two critical areas of investigation are heavy metals and microbial testing.

Here. the three-part *Top Tips for Success* in cannabis analysis series (sponsored by Agilent) continues with look at best practices for testing for heavy metals and microbials in cannabis. The full series includes:

- Potency and Pesticide Testing, Part 1
- Heavy Metals and Microbial Testing, Part 2
- Residual Solvents and Terpenes Testing, Part 3

Each workflow offers tips about samples preparation techniques, methods, quality control, reporting and analysis, instrument maintenance, and more.

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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/country law.

INTRODUCTION TO HEAVY METALS TESTING

eavy metals analysis in cannabis is an area that has made it onto consumers' radars across the recreational and medicinal markets. For instance, the dangers of mercury and arsenic are widely known. So to promote consumer confidence in product safety, brands must clearly communicate which elements are present and at what levels they are present.

What may be lesser known is that the cannabis plant's system is adept at the uptake and accumulation of heavy metals. Specific processes can occur at the root/soil interface in certain soil media that can draw in heavy metals. For this reason, cannabis is being explored as a phytoremediation tool to remove contaminated elements from soil. In certain growth applications, however, the uptake can happen unintentionally. This makes heavy metals analysis all the more relevant for cannabis products. Importantly, specific concerns must be managed throughout the analytical workflow to achieve sufficient data integrity and compliance.

SAMPLE PREPARATION FOR HEAVY METALS TESTING

ust as a chef must prepare food correctly before cooking, analysts must prepare cannabis samples properly before their characterization. Regardless of the quality of the cookware, if the chef fails to follow the recipe, the dish will fall short of expectations. Similarly, the chemical technician must follow guidelines and use best practices for success. The path to a favorable outcome starts with using high-purity reagents. Although using less expensive solvents is an easy way to reduce costs, it is also a quick means of producing unacceptable data or higher detection limits. Using ultra-pure water is critical for this reason. In addition, different kinds of acid are used during different digestion and sample preparation protocols, and the grade of those acids can have a large impact on data quality. This is especially important when analyzing for less common metals such as zinc or nickel. Consequently, trace metal or higher-grade acids must be used. Ensuring that the appropriate acid is employed for the type of analysis is just as vital.

One of the most common techniques for cannabis metals sample preparation is microwave digestion. Compared to a beaker digest, analysts have far better control of the conditions with microwave digestion. In addition, microwave digestion excludes the risk of losing volatile analytes such as mercury.

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It should be noted that in the wide range from industrial hemp to medicinal products, the type of sample plays a role in the sample preparation process. It determines which reagents are used, how representative samples are collected, and the digestion parameters. For example, certain industrial hemp applications allow the inclusion of more stalk material so that more of the plant is utilized and less waste is generated. This introduces more organic content, which affects the different approaches for sample digestion and analysis. For the sample digestion, the more stalk of the cannabis plant that is incorporated, the more silica is typically present. Trying to account for that to ensure a complete digest changes aspects of the digestion. Thus, it is important to understand the matrix. New cannabis laboratories could face numerous complications by accepting a broad array of sample types before all factors of sample preparation have been thoroughly developed and validated.

The choice of containers used for cannabis sample preparation can also have a significant impact on data quality. The wrong vessel can introduce interferences that can lead to unusable data and can be very difficult to trace. For this reason, glass containers should be generally avoided. Acid-cleaned plastic containers are recommended for best results.

Note that even ready-to-use containers should be rinsed before use to remove any

dust or other contaminants. Nitric acid or hydrochloric acid may be used, depending on the sample type, and 10% HCl is common. The process for cleaning plasticware should be well defined and strictly followed.

Moreover, minimizing the number of labware items to which a sample is exposed minimizes the possibility of contamination. A best practice is to weigh reagent and make a standard solution directly in the tube that will be used for the analyses.

Although it may seem more expensive, recognizing and managing the various issues of sample preparation will be cost effective in the long run by eliminating common sources of error and protracted troubleshooting.

METHODS FOR HEAVY METALS TESTING

ccurate testing of cannabis products is necessary to ensure consumer safety and meet regulations. The lists of metals and their acceptable limits vary by state and will likely incorporate more elements as the industry evolves.

A well-established technique, inductively coupled plasma-mass spectrometry (ICP-MS), has emerged as the ideal technology for trace metals analysis, making it quite apposite for cannabis plants and products. Along with outstanding sensitivity, the technique provides rapid multi-element analysis in a single run. Although there are no standardized methods or federal guidance for metals analysis of cannabis, some states do specify the use of ICP-MS for the measurements. State methods for related matrices can be adapted, or EPA Method 6020 provides labs with a foundation of an approved, validated ICP-MS method on which to base some of their parameters.

Method parameters change depending on the type of cannabis samples and the analytes being measured. For example, the acceptable levels of metals in products for use in vape cartridges are very different than those for an edible. Analysts must understand what levels of detection they need to achieve with the different analyses. Fortunately, ICP-MS can accommodate all the requirements.

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Heavy Metals & Nutrients

Testing for Cannabis

For reliable measurements, several aspects of the ICP-MS workflow should be evaluated. A crucial piece of the metals analysis is the use of high-purity reagent gases in the ICP and collision cell. This is particularly important for the quantification of trace quantities. Ultrapure helium is used in the collision cell of the Agilent system for optimal results. Note that other manufacturers may recommend a different gas for their systems.

addition, method setup should be accomplished by well-trained personnel, as it is easy to generate data of marginal quality, and unskilled employees may not recognize a problematic issue. Parameters such as rinse times, radio frequency (RF) powers, and torch distance require some knowledge of the ICP-MS process and its intricacies. For streamlined setup, laboratories can take advantage of the Agilent ICP-MS Cannabis Analyzer with ICP Go. This comprehensive system includes installation, methods, workflows, and extensive training so that the lab can be ready to perform their validation in a matter of days. This system is enormously beneficial for analysts who are unfamiliar with ICP-MS; ICP Go software sets up the methods with the appropriate aerosol, dilution, half mass correction, and other parameters for user-friendly operation. Agilent's experts train the users on instrument operation and helpful tips, as well as understand their state's requirements.

Thus, the ICP-MS Cannabis Analyzer gives new labs a significant head start on their setup and workflows. Nonetheless, they should be prepared for either additional training opportunities or for their processes to evolve along with their sample matrix variety. As they are validating different sample matrices, they must evaluate and optimize the experimental parameters every time.

Several components of the metals method are critical for dependable results. To avoid carryover, rinse composition and time must be evaluated for each sample type. As the sample is drawn into the instrument, it travels through peristaltic tubing, which can suffer from residual elements left behind. Therefore, it is important to do an adequate rinsing with acid to remove the residue. Dilute nitric acid or hydrochloric acid is recommended, depending on the samples, with a sufficient rinse time to ensure cleanliness. Using HCl for the matrix and rinse will assist in washing out mercury.

The correct interface cones must be used as well. As an integral part of the ion optic stream, the cones focus the analytes of interest. In general, nickel-plated cones are preferred for most applications. Further inside the instrument, relatively high RF

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power (1500–1600) is necessary and common for cannabis analysis, compared with other matrices. This is typically complemented by aerosol dilution, which helps reduce some of the matrix interferences.

The use of Agilent's half mass correction is encouraged for the measurement of samples containing rare earth elements (REEs). As REEs have relatively low second ionization potentials, they readily form doubly charged ions, which can interfere with the analytes of interest. By engaging the Agilent software's half mass correction, the interferences are resolved and corrected automatically. Note that this results in a calculated value rather than a direct measurement. The best REE doubly charged element correction is obtained by using an ICP triple quadrupole mass spectrometer, which entails a significant cost investment. For current cannabis analysis activities, however, a quadrupole ICP-MS is sufficient. While triplequadrupole instruments are available from multiple manufacturers, they are not required technology at this time.

Laboratories could benefit from monitoring changes to applicable laws as well as the cannabis market. Along with being flexible to manage changes, this would help future-proof the lab. In addition, it would empower them to handle regulations from additional jurisdictions in case the industry eventually permits samples to cross state lines.



REPORTING FOR HEAVY METALS TESTING

eporting and analysis are usually driven by jurisdictional requirements and which facet of the industry is involved. Different states have different lists of required metals for testing, and different product types can also have different metals of concern. As such, reports may vary from state to state and lab to lab. For example, there is no requirement for the means of reporting quantifications of metals that are below the state-defined limit, which leaves it up to the labs to decide how to report them. For probity, they may choose to provide all the measured quantities. If a lab reports the measured level of a toxic heavy metal that is well below the threshold, however, buyers will nonetheless see that the metal is present in the product. As a result, they may decide not to purchase the product based on that report, disregarding the noted safe level.

Alternatively, labs may choose to simply provide a list of metals that were found below a particular threshold. This practice is common for preventing customer confusion and driving its purchase. This example illustrates the effect of reporting on the cannabis market and the need for judicious design of Certificates of Analysis (CoAs).

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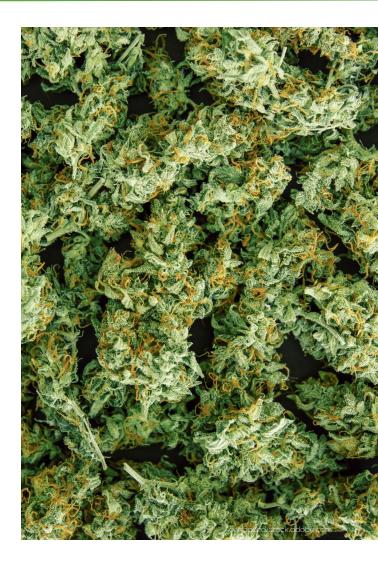
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As there is no such thing as a matrix blank for cannabis metals analysis, laboratories must understand that the calculation of the Limit of Quantitation (LOQ) takes into account not just the matrix, but also the efficiency of digestion, limitations of reagents, and other factors in the analytical process. The LOQs for each analyte are not required items in CoAs but should be included so that customers can make informed decisions. They also serve to highlight the capabilities of the lab. Nonetheless, some laboratories feel that LOQs are confusing to customers and opt to omit them. Still others abstain from including them in order to prevent the comparison of their analytical performance to that of other laboratories. As thresholds are typically set by the type of product, labs must validate that their method can meet the required limits for the various products they test. Accordingly, reporting requirements may also vary for the diverse sample matrices and sources. Clarity and consistency in reporting is the key to customer confidence.



QUALITY CONTROL FOR HEAVY METALS TESTING

uality control (QC) is essential for regulatory compliance as well as consumer safety and confidence. Laboratories in jurisdictions where QC parameters are not well-defined can consult EPA Method 6020. This method can help with determining the usage of QC samples, matrix spikes, duplicates, and other best practices that should be incorporated into a quality program. As the QC parameters help validate the data set, their prudent establishment is vital.

Inter-batch variations are common in cannabis, which is typical for all plant-based materials. However, the industry is still working to manage this issue. Ensuring that the sample is as homogenous as possible during sample preparation may help reduce the variation, albeit slightly.

Good-quality reference materials are important for QC in any industry. Although third-party standards are available for cannabis analysis, their veracity has come into question. To address this void, NIST is developing plant-based reference materials for cannabis laboratories. This will enable analysts to use credible reference materials to validate recoveries across their entire methodology.

MAINTENANCE FOR HEAVY METALS TESTING

Ithough most modern ICP-MS systems are extremely robust, components will eventually wear out or fail. Manufacturers offer service agreements, which can be enormously helpful by providing efficient, reliable repairs. Aside from major instrument repairs, routine maintenance issues can be performed by laboratory personnel. For example, the vacuum system will need regular oil changes. In addition, the cones are considered consumable items that must either be cleaned or replaced on a fairly routine basis. That schedule is based on the number of samples being run and how dirty the matrix is.

The peristaltic pump tubing also requires frequent attention to promote precision and stability of the analysis. In addition to ensuring that the tubing is clean and in good condition, it must be released during the shutdown procedure. Failing to release it can have detrimental effects on its long-term utility.

Chiller maintenance is commonly overlooked, which can quickly lead to expensive repairs. The use of antimicrobial agents is important, and the fluid should be changed on a relatively routine basis over a defined schedule. Based on the chiller's manufacturer, the appropriate fluid should only be

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employed. Moreover, as running ICP-MS can generate a thermal load, the laboratory should be designed to handle the heat. This is advantageous for consistency of the analysis, as it will ensure stability of the operational temperature on long runs.

Agilent's ICP-MS Cannabis Analyzer and other ICP-MS instruments in their portfolio have a proven track record of being exceptionally robust, stable, and user friendly. Their experts will install and set up the instrumentation in addition to providing user training. They are also available for consultation and advice when a question or concern arises. Routine maintenance is straightforward, and well-trained service engineers are ready to help with any issues.





eavy metals analysis in cannabis entails the use of ICP-MS for rapid, sensitive, multi-element detection. Given the wide variety of product types and regulations, method development is decidedly complicated. Not only are there different matrices to consider, but the lists of analytes and their acceptable limits also change among jurisdictions and continue to be revised. There are no standard methods, reference materials, or matrix blanks. Thus, experienced analysts are needed to develop and validate appropriate methods. Sample preparation is critical, as it significantly impacts the quality of the results. Instrument parameters must be optimized, and



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the components require regular monitoring and maintenance. Quality control programs, combined with consistent workflows and reporting, are crucial for compliance, as well as consumer safety and confidence.

Choosing a single vendor to supply the technology, software, consumables, and support helps streamline the analytical process and provide stability. Agilent offers a suite of dependable, robust ICP-MS instruments, including the ICP-MS Cannabis Analyzer, which makes metals analysis easier than ever. The Cannabis Analyzer dramatically shortens the time it takes to develop and optimize a new method, verify its performance, and obtain regulatory

approval. It combines the powerful Agilent 7800 ICP-MS system with its standard High Matrix Introduction (HMI) technology, plus an optional Agilent SPS 4 autosampler. The system comes with an optimized analytical method, ICP Go software interface, ICP-MS MassHunter software, and a consumables starter kit. The analyzer package includes expert assistance to help with setup, method transfer, and operator training. This allows laboratories to be set up and running as quickly as possible, with a knowledgeable Agilent team ready to assist as the lab evolves.

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Agilent ICP-MS
Cannabis Analyzer

INTRODUCTION TO MICROBIAL TESTING

icrobial testing is one of the most controversial aspects of cannabis testing. There is a large divergence of opinions regarding acceptable strategies for analysis. Traditionally, the majority of methods utilized were brought over from the food industry simply because they were easier to implement and incorporated standard technologies. The relative novelty of cannabis analysis and the uniqueness and diversity of the cannabis matrix, however, requires innovation. As such, many laboratories and states have pushed new approaches to employ different technologies and platforms. This has led to some conflicting, passionate conversations in the cannabis world.

SAMPLE PREPARATION FOR MICROBIAL TESTING

efore the sample preparation for microbial testing can begin, the analytical laboratory must choose the testing platform (or platforms) that is appropriate for their state requirements. The sample preparation will depend upon the desired outcome of the measurements.

Two predominant technologies are currently used for cannabis microbial testing: culture-based testing and molecular-based testing. The first entails traditional plate culturing, which is well established and commonly used in the food industry. As that industry's current gold standard, many scientists have familiarity with this straightforward technique, however the cannabis matrix has unique properties (i.e., terpenes) that may impact plating results. For labs working toward microbial testing for total yeast and mold, different coliforms, *Salmonella*, *Aspergillus*, and so forth, the methods are well known.

The second technology uses quantitative polymerase chain reaction (qPCR), which is considered by some a new technology platform, but in fact has been around since the 1980s. It is used extensively in the research and clinical diagnostics fields (including flu and COVID-19 testing), and has become very popular in the food and environmental testing space as the pitfalls of

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culture-based testing are exposed. As culture and molecular methodologies are drastically different, it is important to understand the chosen test before preparing a sample.

As one would expect, the laboratory set up for each of the two tests varies widely. Traditional plate culture techniques need different forms of media and typically call for an autoclave, incubator, and other microbiology equipment. The instrumentation and consumables differ with gPCR. Labs must ensure that the equipment needs for their chosen analytical platform are deliberated and accommodated. With both technology platforms, the sample requires some level of sterility. Safeguarding against the introduction of additional microbes to the sample during preparation and analysis is vital for success. Accidental contamination can occur easily, such that certain cleanroom practices are necessary that are often not needed in other types of testing.

It takes an experienced microbiologist or mycologist to correctly interpret the live culture plates because complexities may cause errors with novice experimentalists. For example, the definition of total yeast and mold may change, or some fungi may be moved to different phyla. With molecular methods, if laboratory technicians can perform a reasonable extraction, the technology offers advantages over plate culturing. In general, it is easier to detect for the specific DNA of an organism, removing the ambiguity of identifying and counting colonies on plates. Ensuring that a lab's technology platform is supported by appropriately trained personnel is critical.

METHODS FOR MICROBIAL TESTING

What methods should a lab use for microbial analysis of cannabis?

When no guidance is provided by the state, the laboratory must decide which analytical strategy to follow for microbial testing. It is important to understand that plate culture and qPCR actually measure different constituents in the cannabis sample. The culture-based technique measures species that grow in a given medium and time frame. The types of microorganisms that grow depend on the food provided by the growth media. In addition, the media may contain antibiotics or other components that will only allow specific organisms to grow. Instances in literature show that there are different media on the market that can have negative impacts on microbial growth. As such, the lab must select the correct plate technology for the target microbe. Then the resulting live, culturable organisms are measured.

The mechanism for qPCR measurements, however, is fundamentally different in that it is detecting the DNA of organisms. Molecular methods are, by definition, species specific. The science behind this methodology is more quantitative and easier to define, as it has numerical values associated with it. Consequently, many new labs have opted for this approach. Importantly,

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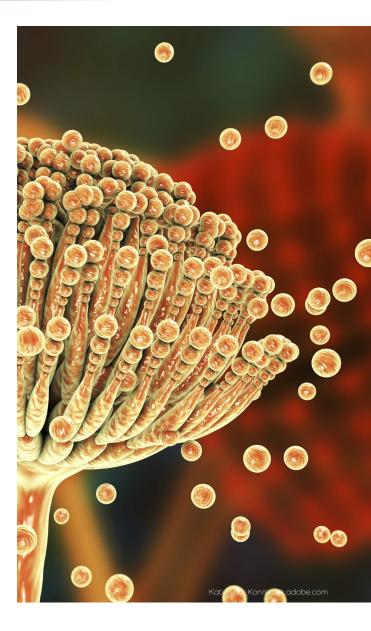
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it does not matter if the organism is dead or alive; a positive test will result if its DNA is detected. This can lead to different test results when compared to plate culturing, which only measures live organisms growing on the media. There are solutions available to solve the live dead issue that can be applied in sample prep, and remove dead DNA from the qPCR reaction. Unfortunately, the differences between plating and DNA-based techniques have not yet been well rationalized across the two platforms.

Endophytic and epiphytic organisms are present on cannabis plants. The fungus endophyte, and Aspergillus is an documented to have caused illness, and a few deaths, in immunocompromised cannabis users. In some cases, these organisms provide positive growth environments for plants, but quantifying them and maintaining viability is extremely difficult using plate culturing. This is an instance in which gPCR-based technology would be advantageous because DNA is simply being extracted and the analyst does not need to manage the growth process and quantification.

Although it offers benefits, qPCR is sensitive to pipetting errors. It requires the repetitious pipetting of small volumes reproducibly so it is important that pipettes be calibrated. Without this, broad errors in testing will occur. One way to address this is with laboratory automation platforms, such as the Agilent Bravo Liquid



Handler. A robotic liquid handling system delivers time savings with extreme levels of precision and method robustness. While automated platforms come at a cost, the significant time savings, enhanced accuracy, increased throughput, and maximized reproducibility may render them worthwhile.

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The two testing methodologies consume dramatically different amounts of time, from sample arrival to data reporting. The speed of qPCR makes it appealing, as data can be reported within one to two days of sample receipt. Plate culturing takes significantly longer, as analysts must wait for the microbes to grow. In many cases, that can take nearly a week. The time differentials should be considered due to the impact on sample throughput.

Many laboratories choose to continue with plate culturing because it is so well-established. However, any labs that are not currently interested in doing qPCR should be prepared for it, because over the long term, it appears that the scientific community will move toward qPCR-based technologies for environmental and food safety testing (cannabis included). In addition, states will likely gravitate to qPCR because it provides a quantifiable number and removes the ambiguity related to someone discerning

which type of microorganism, and how many colonies worth, was grown in a medium.

AOAC and ASTM are important because they are two of the few organizations that are approaching standardization across broad consensus strategies. They promote sound science and help laboratories produce data that has scientific reproducibility across different jurisdictions. They also provide a level of confidence in the data because standardized methods customarily incorporate quality control criteria.

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Cambium Analytica relies on instruments and expertise from Agilent

REPORTING FOR MICROBIAL TESTING

ifferent regulatory bodies stipulate different microbial reporting limits. The thresholds vary across product types as well as states. Thus, laboratories need to realize that one detection limit does not work for all products. Moreover, the thresholds for cannabis are often very different than other industries. Most states have adopted extremely strict requirements promoting high product safety margins, which earns good public relations for the state and a high level of safety for consumers. It is critical that labs fully understand the state-specific requirements on the product types being tested, then measure and report accordingly.

In most cases, laboratories report the culture-based estimated concentration of microorganisms using the colony forming unit (CFU). This value is more of a statistical calculation; it is a probability number as opposed to a hard count. More quantifiable data is acquired and reported using qPCR technology. The strategy for quantification should be well-defined by the laboratory, followed by consistent reporting, particularly as the microbial testing of cannabis evolves.

QUALITY CONTROL FOR MICROBIAL TESTING

uality control programs with all workflows are crucial, and microbiology is no exception. It is imperative for laboratories to ensure that their analysts are adequately trained and do not cause contamination of the samples. Culture-based quality assurance calls for comparison of the cultured sample to a known cultured organism for identification. While there are standardized reference materials for that, much of the interpretation is based on the analyst's discretion. This can lead to variability in results due to plating type and what is considered a colony (e.g., size and shape). Different plating mediums can provide a different result on the same sample and even



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the same sample on the same plate can have significant standard deviations

For qPCR, positive controls are required. From a DNA standpoint, that is relatively easy to achieve. Most qPCR assays have accompanying DNA controls built into their workflows to provide assurance that the assay itself worked properly. These can include internal cannabis controls and users can also add certified reference material samples to verify the effectiveness of the assay.

Finally, there are different microbial thresholds for different pathogens and between different cannabis products (e.g., flower, oils, extracts, and chocolates). For some target organisms, there is a pass/fail measurement, in which their mere presence can cause the failure of a product batch. This is an area where the two testing methods diverge. Because qPCR measures cellular DNA, it could result in

the failure of a batch that may have passed a culture-based test since studies suggest that a significant number of microbes do not effectively grow on culture plates. Labs need to be aware of this issue. Labs should comprehend the differences of the two analytical techniques in order to understand contradictory pass/fail data.

MAINTENANCE FOR MICROBIAL TESTING

egardless of the microbial testing technique, some form of regular maintenance will be necessary. As noted earlier, culture-based measurements employ incubators and perhaps an autoclave. These pieces of equipment require a routine maintenance and calibration to ensure that appropriate temperatures are being maintained. Furthermore, when generating potentially biohazardous waste or culturing organisms, the lab must be prepared with designated decontamination procedures for both the immediate and surrounding work areas. It is absolutely imperative to have procedures in place that will avoid taking cultured organisms outside of the lab or bringing in new ones unintentionally.

qPCR systems, are robust instruments, and some, such as the Agilent AriaMx Real-Time PCR System, do even not require annual adjustments, such as dye or plate location calibrations. Agilent does offer kits (Sybr Qualification plate) that users can run routinely to ensure that the instrument is functioning properly. Service and preventative maintenance agreements are also available in which staff will visit the lab to help maintain the technologies, clean the instrument, and

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check the platform's performance. In addition, most manufacturers offer a type of rapid service agreement. This option does not necessarily involve a service engineer's visit to work on an instrument; in some cases, the manufacturer will quickly send a new qPCR in exchange for the defective one. This can happen in as little as one day, depending on the terms of the agreement. The rapid service agreement can be an enormous benefit for laboratories that do not have repetitive technology inhouse. As it minimizes downtime, the fast response can become an essential tool for providing redundancy without actually having redundant equipment.



SUMMARY AGILENT CANNABIS MICROBIAL TESTING SOLUTIONS

he controversy of cannabis microbial testing is currently unresolved, as divergent analytical techniques and evolving regulations have not yet led to a single, universally adopted methodology. Although both culture-based testing and qPCR analysis provide effective results, it appears that the future of microbial testing will lie with qPCR. This is due to its more quantitative nature and unambiguity of the measurement. While both techniques require sterility controls, the laboratory set up and analyst training vary greatly. Testing labs must take that into account, along with throughput and reproducibility of the data. Regardless of the testing strategy, the ultimate benefit is product safety for consumers.

Agilent has partnered with Medicinal Genomics to provide a complete workflow with highly selective and sensitive qPCR assays for the unequivocal identification and quantitation of microbes, yeasts, and mold as mandated by various regulatory agencies. This workflow shortens the time to achieve results and reduces false positives and negatives compared to culture-based plating methods by using qPCR to detect the unique DNA sequence of

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Aspergillus (AOAC certified test), Salmonella, E. coli, and all other state-required microbial targets.

The Agilent AriaMx real-time qPCR system, the Bravo automated liquid handling platform, and the Medicinal Genomics PathoSeek Microbial Safety Testing assays are part of a validated (by Medicinal Genomics) and efficient workflow that comprises an internal cannabis control and the ability to detect single or multiple targets per sample. The workflow can be processed manually or automated with the Bravo platform. In California, 46 samples can be tested for Salmonella, STEC (Shiga toxin-producing E. coli), and four Aspergillus species in one 96-well plate. In contrast, the same measurements following AOAC plating guidelines would require 1,104 plates, a more than 10-fold increase compared with the Agilent AriaMx solution.



Microbial Testing for Cannabis & Hemp



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/country law.

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