Cannabis Science and Compliance

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Learning Objectives for Introduction to Cannabis Safety and Science

- Role of science in cannabis
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  - Scientific Method
  - Responsibility
  - Current Landscape of cannabis science
  - Future of cannabis science
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Science

Cannabis science can mean different things to different people. There is the body of knowledge that is all knowledge of cannabis and then there is the scientific method as it is applied to cannabis applications. Cannabis has been a companion species to humans for longer than we have written records. The first evidence of humans using cannabis comes from 10,000 years ago in pottery found in Asia with cannabis fiber used as a rope. Cannabis science has all but missed the last 100 years of scientific advancements. We are now just catching up.

It is important to clarify what science is. The scientific method is the basis of science. The scientific method begins with observation of phenomena. These observations are gathered and combined to form a hypothesis. Experiments are designed to verify that predictions made by the hypothesis support the hypothesis. The results are used to support, refine, or discredit the hypothesis. This process is repeated until enough experiments and observations have refined and verified the hypothesis to the point it becomes a theory. A theory is a much stronger version of a hypothesis.

In terms of our current western medicine model this process unfolds through clinical trials. The development of a medicine begins with the discovery of a molecule that has potential of therapy. This molecule is then purified to the point of being a single molecular identity. This molecule often has a single target. Then the molecule is evaluated for whether it can be dosed and whether it has toxicology concerns. A formulation screen is performed determine if the therapeutic molecule can be delivered to a person in a way that it will work. If this is achieved it is then dosed in escalating amounts to mice, rats, or some other animal to see when toxicity occurs. If the molecule proves to be toxic it may be abandoned at this point. If the molecule appears to be deliverable and has acceptable toxicity limits it is evaluated for efficacy. This evaluation takes into account dosing regimens, health, gender, genetic, age, medical conditions, and a host of other variables that could affect its safety or efficacy.

This approach is very laborious, expensive, and complicated. This is assuming a single molecule with a single target that responds to dose escalation. Cannabis has hundreds of potential active and co-active molecules capable of working together in countless combinations. Its action often involves multiple receptors and cascading biochemical reactions. These factors combine to make cannabis evaluation via our current method for developing and understanding medicines extremely difficult. It is difficult to imagine the data complexity arising from the many potential formulations of cannabis preparations. Our current approach for evaluating medicine may not be suitable to unlocking the full potential of cannabis as a therapeutic. Significant advancement in our understanding of pharmacology will need to be realized before the science of cannabis as a medicine can be fully utilized in a pharmaceutical model. It may be that cannabis helps usher in a new age of medicine. An age of highly personalized medicine that balances systems and provides a buffer between human health and disease without the drastic, invasive, aggressive approaches medicine has increasingly had to take in order to treat disease which has advance beyond the stage where prevention and early intervention are still possible. Cannabis with its intrinsic ability to modulate systems optimizing balance and wellbeing may very well make it common for it to be used in the maintenance of optimal health and as a preventative of disease more like a nutritional supplement. Cannabis in its various forms will likely continue to be an important tool in treating disease, probably increasing in its scope and application towards various conditions but its greatest value may be in understanding how it can be applied in maximizing health and preventing illness in the first place.

Scientific study will continue to advance our understanding of cannabis. Whether we are creating solutions to our environmental issues, energy needs, nutritional requirements, or human health science will support these efforts.

Cannabinoids

Cannabinoids of interest

Cannabigerolic Acid	CBGA
Cannabigerol	CBG
Tetrahydrocannabinolic Acid	THCA
D9–tetrahydrocannabinol	THC
Cannabidiolic Acid	CBDA
Cannabidiol	CBD
Cannabinol	CBN
Cannabichromenic Acid	CBCA
Cannabichromene	CBC
Tetrahydrocannabivarinic Acid	THCVA
Tetrahyrdrocannabivarin	THCV
Cannbivarinic Acid	CDVA
Cannabivarin	CBDV

Cannabinoids are the active ingredients mainly responsible for its pharmacological effects on humans. Cannabinoids are produced by animals for a variety of reasons. They have been implicated in most metabolic functions and are understood to promote homeostasis. It is possible that they play a role in encouraging infants to thrive by increasing appetite and promoting a sense of wellbeing. Dr. Raphael Mechoulum, in addition to being the first scientist to elucidate the structure of THC, discovered the endocannabinoid system. This system contains multiple cannabinoid receptors have been identified and more are expected to be discovered. These receptors are found throughout the body and play a key role in its functioning. Anandamide which is similar in activity to THC is one of several cannabinoids produced by humans. It is named for Ananda the Sanskrit word for Bliss. These receptors are glycoprotein receptors with retrograde signaling capabilities. These receptors can be activated by plant and synthesized cannabinoids as well endocannabinoids. There is increasing evidence that suggests some activity associated with terpenes as well.

Phytocannabinoids are produced by the cannabis plant. Over 100 phytocannabinoids have been identified in the cannabis. It is thought that cannabinoids are produced by the plant plays a protective role similar to terpenes which are concentrated in the same resinous glands on the surface of the plant. There are no phytocannabinoids that are known to be harmful to humans. There are a number that are associated with beneficial activity. Many therapeutic goals are met through phytocannabinoid action and some people just like the way it makes them feel. The cannabimimetic effects or phytocannabinoids are understood to be modulated by each other and by terpenes in what has been called the entourage effect.

Terpenes

Alpha Pinene
Beta Caryophyllene
Eucalyptol
Limonene
Linalool
Myrcene

Hundreds of terpenes have been identified in the cannabis plant. Terpenes are responsible for the taste and aroma of cannabis and are thought to have cannabimimetic activity. An intensive area of cannabis research includes terpenes and how they contribute to human health. Terpenes are found everywhere in nature as well as everyday life. They are produced by plants primarily as a protective function. Their smell, taste, and effect on humans has been known and utilized for so long that most people have never thought of them. People seldom describe the terpene profile they experience while remarking on various experiences although the majority of the experience is likely coded by the terpenes that were present. The chemical communication of aroma is only a part of the role terpenes play in our biology. Individual odors can be described using fairly simple terms but when someone describes the fresh scent of clean laundry or the overpowering inspiration realized in a pine glade the terms of description become much more complex much

like the difference of whole plant based cannabis forms compared with the effects of isolated forms of cannabinoids.

Decarboxylation

Decarboxylation is the chemical conversion of THCA into THC or any acid form cannabinoid into its neutral form through the loss of carbon dioxide.

Why it is important

Each cannabis molecule has its own distinct properties in how it behaves and how it impacts the biology of humans. THCA is non-psychoactive while THC is psychoactive. There are many other meaningful differences that can be accomplished by changing one molecule into another.

We are just beginning to unlock all of the applications cannabinoids have in human health and decarboxylation doubles the number of potential molecular identities to study and use and informs cannabis preparations based on which form is desired. Each acid form cannabinoid will decarboxylate at specific temperature rates.

Processors of cannabis who which to create specific formulations of acid/neutral cannabinoids must understand which cannabinoids they are decarboxlating and how their process affects the rate of conversion as a function of time. Careful consideration must be given to heating environment, cannabis amount, matrix, and desired final composition.

Heat can drive a number of chemical changes in cannabis constituents. Applying heat to cannabis will decarboxylate the acid forms into the neutral forms but using the wrong temperature can result in loss of yield by either not converting enough or by converting the desired compound further into degradation products. There are a number of resources to guide appropriate times and temperatures for decarboxylation.

These resources should be considered starting points as different processing conditions will have to be optimized to the specific times and temperatures to maximize yield.

Solubility

Solubility is a measure of a substances ability to be in dissolved into a solution with a given solvent.

Why it is important

Understanding solubility is important in guiding decisions for materials to extract cannabinoids as well as finished product composition. Ultimately extract artists must choose an extraction method that balances yield, quality, safety, and cost with their choice of extraction solvent being heavily influenced by the solubility of cannabinoids and terpenes solubility in that solvent. Understanding solubility is helpful to extract artists choosing their solvents and is also useful to analytical chemists in developing their test methods. Accurate analysis of cannabis products absolutely requires an understanding of solubility.

Terms

Solvent – a material that dissolves another material

Solute – a material that is dissolved into another material

Solution – a mixture of materials in liquid form

Polar – a substance that has an uneven distribution of charges at the molecular level

Solvation – the dissolving of a solute into solution

There are a number of approaches to determining a suitable solvent. Trial and error has been the mainstay of many cannabis extractors. A more systematic approach to solubility experiments typically involves dispensing a compound to a series of neat and blended solvent systems and then mixing for a period of time and then analyzing the amount of the compound in the solution. The higher the concentration of the desired

compounds in the solution the better the solvent is for extracting the compound(s) of interest. By evaluating the structure of a compound chemists can often determine the approximate polarity of a material and make informed decisions regarding solvent choices prior to or instead of formal designed experiments.

The easy way to predict a compounds solubility is to remember that like dissolves like. Meaning that things with similar chemical properties are likely to be better at forming solutions than things with differing chemical properties. One of these chemical properties is polarity. Polarity is a measure of a compounds electronic charge distribution at the molecular level. Things that are said to be polar will dissolve nicely into polar solvents but not as well or at all in non-polar solvents. The best examples are water and oil. Water and oil do not mix well and tend to separate. Water is polar and oil is non-polar. Cannabinoids are very non-polar and dissolve readily in non-polar solvents such as hydrocarbons, organic solvents, and fats/oils. Cannabinoids do not readily dissolve in polar solvents such as water.

Water based solvents are a poor choice for extracting and formulating cannabis products. Due to the insolubility of cannabinoids in water based media products like cola, tea, and coffee require co-solvents or other additives to get sufficient amounts of active ingredients to be available in these products.

While many hydrocarbons are well suited for dissolving cannabinoids and terpenes some are toxic and many are considered undesirable by consumers and regulatory bodies.

Butane is a good example of a hydrocarbon that is very good at extracting a desirable cannabinoid and terpene profile but is considered undesirable in the finished product. It should be noted that while butane is considered undesirable there is currently insufficient evidence that it is harmful in small amounts. OSHA guidelines allow workers to breath air with up to 800 ppm butane for as much as 8 hours a day five days a week as a recommended exposure limit and most states that regulate butane levels in cannabis concentrates limit those levels to well below that.

Solubility guides the selection of an extract solvent as well as a finished product formulation. Often times non-polar hydrocarbons, oils, and fats are used to extract and or concentrate cannabinoids from the cannabis plant material. You also see many edible cannabis products containing oils and fats as bases because of the solubility of cannabinoids in these materials. Areas of development include finding techniques and additives to make cannabinoids more soluble in aqueous based finished products and finding safer and more desirable ways to extract cannabinoids into concentrated forms both in extraction method and in subsequent solvent removal from the concentrate.

Safety

Industrial Safety

Cannabis workers need to be protected in their work environment just as any industrial manufacturer does. There are a host of safety considerations to take into account and the key to a safe work environment is a culture of safety. A culture of safety includes all levels and members of an organization taking personal responsibility for the safety of their work. Planning, design, training, and consistent safety consciousness need to be the reality of a manufacturing facilities very core. A lack of commitment to safety at any level, at any time puts the health and well-being of workers and visitors at risk.

Safety training should be deployed prior to allowing an individual being allowed to work in a given area. All employees should receive general safety training prior to beginning work for an organization. Safety training deployed quarterly, or at a minimum annually. The goal of a safety program is to reduce the likelihood of injury or death associated with the facilities operations. Formal training and a visible safety conciseness is recommended because if the safety program is very successful in preventing injuries or close calls people may lose vigilance towards potential risks. By routinely deploying formal training and having a visual safety presence in a facility it will remind employees and visitors to be focused on safety and further demonstrates the commitment of safety on the part of the organization and its leadership.

Each work environment should have personal protective equipment, training, and other tools to keep individuals working in those areas safe. Each environment will have its own unique needs based on the potential threats in that environment. As an example cultivation safety tips are given below.

Cultivation rooms

1. Dedicated garments that protect the plants from external contamination and the growers from exposure to concentrated chemicals and harmful UV radiation
2. Eyewear to protect growers from UV damage to their eyes, chemicals, and other threats to the eyes.
3. Growers should limit the time spent exposed to the lights and use sunblock when appropriate to further protect against skin damage. – Care must be taken not to allow sunblock to contaminate the cannabis plants.
4. Dust masks or other breathing apparatus should be used anytime physical or chemical air quality poses a risk to workers in a grow room
5. CO2 alarms should be inside and outside each grow room to notify workers if the levels are unsafe.
6. Footwear that is slip resistant.
7. Routine audits and checklists are a good way to focus attention on areas of concern and make awareness a routine part of an employee's daily work.

Emergency response training should include procedures to be followed, training, and practice.

Medical

There should be a trained first aid responder who can be summoned in the event of a medical emergency and all employees should be trained in how to act in the event of a medical emergency. Calling for help,

calling emergency medical services, and supporting the victim until help arrives are the minimum things that should be included in each employee's medical safety training.

Fire

Employees should be trained on what to do in the case of a fire. They should be familiar with emergency exit options and routes, the location and appropriate use of fire extinguishers, where the fire alarm pulls are located, and where they should meet outside the building to be accounted for in the event of an evacuation. Having designated fire wardens is a good way to organize and facilitate orderly and effective fire evacuations.

Industrial Accident

Employees should be trained in how to safely respond to an industrial accident. How to identify an issue, how to protect yourself to becoming a victim of an accident, how to mitigate the situation in the event of an accident, and how to elevate a response if an accident is beyond their means to handle alone safely.

MSDS Sheets

MSDS sheets are material safety data sheets. They contain a large amount of information about a material that is in the facility. They have important information on how to safely use and store a material as well as information on what to do in the event of an accidental exposure. Key information to be familiar with that can be found on an MSDS include;

1. Emergency contact information
2. Emergency first aid
3. What to do in the event of a spill
4. How to properly store the material
5. Chemical reactivity, incompatibilities, and special precautions

Having each employee trained on how to use an MSDS sheet can prevent injury or damage and will go a long way in mitigating and damage or injury that occurs in the event of an accidental exposure or release.

Cannabis has a very good safety profile for healthy adult humans. There is no record of a lethal dose of cannabis in human history. There are a number of concerns to human health with regard to consuming any drugs. Drugs can interfere with a number of important biological process, sometimes stopping them all together. Drugs can suppress the central nervous system to the point where the body can go into cardiac arrest. Drugs can cause the metabolism of other drugs to slow down to the point that the other drugs build up in the body to toxic levels. Drugs can also be toxic to any number of cells, tissues, or organs in the body.

Cannabis does not shut down the central nervous system.

Cannabis does not interfere with the metabolism of other drugs.

Cannabis is not toxic to the cells, tissues, or organs of the human body.

Cannabis does not shut down the central nervous system because the portion of the brain which governs heart rate and respiration are not compromised by cannabis use. Both breathing and heart rate can be affected though. Cannabis use can cause tachycardia (rapid heart rate) with onset approximately 8 minutes after inhalation and lasting up to twenty minutes thereafter. This effect is usually relatively mild, similar to jogging up a flight of stairs, and there is no evidence that it leads to medical emergencies. People with heart conditions should consult with their healthcare team and be cautious in titrating their dosage.

Cannabis has not shown to interfere with metabolism of other drugs to the point where there is a toxic buildup of the other drug which would inform physicians to advise against combining cannabis therapeutics with other medicines however supervised medicinal cannabis usage is very new and as we learn more this could change. It is important to note that the cannabis would not cause the toxicity in the case of it interfering

with the metabolism of other drugs, it would be the other drug building up in your system that would lead to toxicity. In some cases it might make sense to consider not using the other drug in other cases cannabis may be counter indicated.

Staying with the theme of drug-drug interactions cannabis may have a synergistic effect with a number of other drugs. Cannabis may increase the effects of opioid based medicines allowing a patient to consume less opioids to achieve the same level of pain relief. Cannabis may increase the intoxicating effects of alcohol which would increase the dangers associated with driving, operating equipment, or doing anything else where judgement and coordination are important to safety.

Cannabis can also lower blood pressure. Lower blood pressure can cause dizziness and or light-headedness lasting for several hours after consumption. Most people do not experience noticeable symptoms of reduced blood pressure however people should be aware of this side-effect and take appropriate precautions. Particularly people with a pre-disposition for low blood pressure, or who are taking medication for low blood pressure should consult their primary and referring physician prior to consuming cannabis and take precaution is becoming familiar with how cannabis will affect them.

Other health considerations for cannabis include the effect of inhaled cannabis on the lungs, the effect of cannabis on the brain, and the potential for psychological disorders from consuming cannabis. The scientific literature to date does not show a causal link between cannabis use and any negative outcomes associated with these areas of concern. Continued study and evaluation of cannabis use and any potential deleterious effects from it will be required to determine what impact cannabis use has on these areas of concern. Right now there is insufficient or conflicting evidence to suggest that cannabis use causes problems in these areas.

Inhaled cannabis by smoking or vaporizing is not statistically linked to increased instances of lung cancer or loss of lung performance. One of the active ingredients or cannabis THC is a bronchodilator and may prove an effect therapeutic for COPD and asthma. Inhaling cannabis can irritate the lungs causing coughing with

onset immediately upon inhalation and lasting for varying amounts of time with most coughing subsiding within five minutes.

Although most recent scientific evidence suggests that cannabis does not cause psychological disorders in healthy adults, people who are risk for substance abuse and or psychological conditions should take extra caution and consult with their appropriate medical care provider(s) prior to consuming cannabis.

Patients, caregivers, and other cannabis professionals should take every measure possible to ensure that medicinal cannabis does not find its way into the possession of non-patients. Preventing access by children and pets is especially important as they can have severe adverse reactions to cannabis use.

A child who has been appropriately qualified as a cannabis patient should only use cannabis under the careful supervision of a legal guardian or caregiver.

A pet who has been appropriately qualified as a patient by a veterinarian should only consume cannabis under the close supervision of a caregiver or guardian.

Patients should store the medicine in such a way as to prevent its unauthorized use and in such a way that it is clear to anyone that it is medicine containing active cannabis ingredients.

As the properly cultivated cannabis plant material itself does not pose a significant (or really any) threat to a healthy adult who consumes it much of the safety concern with cannabis comes from intentional adulteration or unintentional / intentional contamination.

Examples of intentional adulteration include adding things to cannabis to make it appear more desirable. There have been reports of people adding pulverized glass to cannabis to make it appear more resinous and therefore more potent. There have been reports of people adding lead and other weight adding components to cannabis to artificially increase its weight. There have been cases reported where people have added cleaning products that enhance the smell of cannabis.

All of these instances are cases of intentional adulteration to mislead the consumer into believing that the product is either of better quality or greater quantity than it actually is. Legal access to regulated cannabis will make these intentional adulteration practices less common and with the accompanying laboratory services available to patients and consumers eventually there should be no instances where consumers are being exposed to this type of product.

Inappropriate cultivation practices are the main source of intentional contamination. This happens when cultivators use plant growth regulators, supplements, or pesticides in an effort to obtain the highest yields they can. There are supplements and pesticides that can be used in a way that does not result in harmful residues on the finished product.

Unintentional contamination issues result when cultivators, processors, and other handlers of the product introduce heavy metals, microbiological contaminants, residual solvents, common debris, or any other unwanted material to the finished product. Cultivators should educate themselves on the best practices and techniques for minimizing contamination of their grow operation. This includes being knowledgeable about the soil, water, nutrients, and supplements they are using. Being knowledgeable about when and how much of what type of materials can safely be used in their cultivation practice. Knowing the proper environmental conditions for their plants. Keeping sanitary conditions in their grow operation and protecting from contamination of their grow operation.

Cultivators should be well versed in remediation techniques that do not result in contamination of the finished product. Cultivators should appropriately flush their plants prior to harvesting for drying and curing. The healthier the plant the more resistant it will be to pests, microbiological infestations, and disease.

Processors should ensure the appropriate technique and environment for drying and curing plants. Sanitary conditions, tight control of light, temperature, and heat are essential. Measures to protect against contamination from the outside should be taken.

Extractors should be knowledgeable about their technique and material. The highest quality solvents should be used. Safety measures should be considered for flammable solvents. Common consideration is minimizing the concentration of solvents outside of a closed loop system so as to avoid explosion or asphyxiation. Careful consideration should be given to ventilation and ignition sources when working with solvents. Proper maintenance of extraction equipment is necessary to prevent equipment failure which could lead to injury or death of workers in the extraction work area and to protect the quality of the finished product. Where necessary purging processes must ensure that residual solvents are below levels that can result in harm to the consumer.

Trimming, weighing, and packaging should all be done in a conscientious manner with attention being payed to precision and prevention of contamination.

Packaging should protect the product from damage, contamination, and deterioration. Packaging should avoid appearing to mimic branded products. Packaging should avoid making the contents appear desirable to children. Packaging should inform the patient as well as protect the medicine. Careful consideration should be given to labeling. Labeling should be compliant with state laws and regulations. Labeling should be easy to read and understand. Packaging and labeling should make it obvious that the contents contain active medical ingredients.

Labeling should (at a minimum) inform the consumer as to the following; ingredients active and otherwise, allergens, calories if applicable, side-effects, dosage information, serving size, drug interactions, poison control phone number, patient name, packaging date, storage conditions, expiration date.

Patients should be carefully advised in the selection of medicine, routes of administration, side-effects, and what to expect overall. Patients should be advised on properly titrating their dosage. Becoming familiar with the effects of a specific medicinal product, route of administration, and dose prior while in a safe and supported environment and prior to being in a position where being under the influence may affect the

performance or decision making ability. Examples of situations to be cautious of include but are not limited to operating a motor vehicle, operating machinery, handling sharp objects, cooking and so forth.

There are a wide variety of experiences that people can have while under the influence of cannabis that can impact their motor skills and cognitive function. Factors that can affect how cannabis use impacts the individual includes age, gender, genetics, fed vs. fasted state, medical condition, and experience.

Common side effects to cannabis use include a change in motor and mental functioning, perception of time, dry mouth, red eyes, tachycardia, lowered blood pressure, interference with short term memory, increased appetite, anxiety, paranoia, drowsiness, improved sense of well-being, euphoria, giddiness, talkativeness, introversion, extraversion, and hyperactivity.

Properly produced cannabis products are very safe for healthy adults to consume. The vast array of active chemical constituents in cannabis make it an unmatched source of potential therapeutic compounds. The main safety concern with cannabis comes from contamination and irresponsibility. Cannabis professionals and consumers are well positioned to protect against both contamination and irresponsible practices. With access to legal regulated cannabis becoming more widespread any harms associated with cannabis should be reduced or eliminated.

Cannabis Testing

A huge change in cannabis safety and quality has resulted from regulated medicinal cannabis programs. When Massachusetts released its regulations for medicinal cannabis it was the first state to have required testing of the cannabis product with specific limits and controls on some of the more concerning contaminants commonly associated with cannabis. For the first time ever cannabis quality assurance and quality control was mandated to protect consumers. Cannabis testing laboratories are springing up across the country to meet the requirements of regulated medicinal cannabis.

Cannabis testing laboratories existed prior to mandatory testing and some of these laboratories are excellent at what they do. The real change that regulation has brought is the focus on consumer safety and credentialing requirements. The laboratories that were operating prior to this have to adjust their operations to meet these changes and the ones with the right resources in place will be very well positioned to meet the regulated requirements and provide consumer protection services to cannabis users.

Cannabis testing requirements vary from state to state and the requirements are continuing to evolve as experience dictates. It is likely that over time all states with regulated cannabis markets will have similar, if not the same testing and credentialing requirements. For the time being this is an exciting time as cannabis, science and technology, regulation, and consumer preferences come together and coalesce into best practices and what will be the future of cannabis quality.

In Massachusetts laboratories providing testing services for RMDs are required to be ISO 17025 accredited or approved by the Massachusetts department of health. ISO stands for International Organization for Standardization. The 17025 stands for the area of focus that the standard will be applied toward – in this case testing services. The purpose of this requirement is to ensure that a laboratory is capable of performing the tests that it says it can perform, in the way that it says it can perform them, and that it can demonstrate that it has systems in place to ensure that it does consistently perform those tests the way that the say they can.

Laboratories that are ISO 17025 accredited have demonstrated to the greater scientific community that they are capable of performing the tests that they claim and that they have a system in place to ensure that each test result the provide is accurate. This is important to ensure that consumers can be assured that the material they are consuming has been screened by a laboratory that knows how to perform the analysis and is routinely scrutinized to ensure they consistently provide valid results. Consumers can trust the labeled product is free of contaminants and knows the identity and potency of the active ingredients.

Steps to accreditation

- Review policies procedures
- Review validation data
- Review proficiency test results

Audit laboratory records and personnel to verify objective evidence that policies and procedures are consistently followed

Surveillance audits are performed annually to ensure each functional area is consistently compliant.

ISO accreditation requires a laboratory to have their laboratory governed by normative documents which describe the policies, procedures, and practices that will be followed in performing tests and reporting test results. These normative documents are collectively referred to as a quality system. A quality manual usually serves as the overall governing document that states how the laboratory will ensure the quality and integrity of the test results and references the many normative documents that detail the various procedures and policies that personal shall follow to accomplish their work.

Some of the important documents a laboratory will need are listed below.

Standard operating procedures – These documents are used in training and as reference material for analysts. They often describe how to operate an instrument or perform a general task.

Test Methods – These documents are the specific details an analyst must perform in order to execute a given test.

Forms – These are documents used to record standalone information.

Logs – These documents are used to record ongoing information.

Policies – These documents describe the organizations rules of behavior for employees.

Method validation is an important to the accreditation process. Laboratories must demonstrate through validation that the test methods that they use result in consistent, reproducible, and accurate results. Method validation requires a series of experiments that determine what factors affect the validity of the test result. Every aspect of the test method is evaluated at its extremes to determine where the test will become invalid. This information is compared to the procedure the laboratory uses to perform the test and the test procedure must have controls to prevent the test reaching the limits that would invalidate the result. Failure to do so will result in a lab failing to get its accreditation status.

Validation requirements vary from test to test but some of the more common requirements are listed below with a brief description;

Specificity	**Separation of target analyte from other components of the test sample**
Accuracy	**How close the measured value is to the nominal value**
Linearity	**Correlation of concentration of an analyte to the response of the measuring devise**
Precision	**How close a series of measurements are for the same sample**
Range	**Concentration range of an analyte over which the analyte is accurate, linear, and precise**
Detection Limit	**The lowest amount of analyte that can be detected**
Quantitation Limit	**The lowest amount of analyte that can be accurately measured**
Robustness	**The reproducibility of the results under various conditions**

A proficiency test is often a part of or in addition to a validation. To perform a proficiency test a laboratory must have a different, unaffiliated laboratory, that is also ISO accredited, provide them a 'blind sample'. This blind sample is a sample prepared with the analytes the laboratory claims they can measure but is provided to the laboratory with unknown concentrations of analytes.

The testing laboratory must analyze this sample and report the results to the provider of the PT. The provider of the PT has prepared the sample to known composition of concentration(s) and identities. The PT provider then compares the results of a laboratories test results to the known or nominal concentration of the PT sample.

During the initial accreditation process and during subsequent surveillance audits the ISO accreditor will review validation and PT results. Validated methods and PT samples are required for each test that is a part of an accreditation scope. PT samples must be performed prior to a laboratory receiving accreditation and must be performed routinely for a laboratory to keep its accreditation. Method validations are performed prior to receiving scope accreditation and after any significant changes are made to the testing method.

Scientific soundness and integrity of character are of utmost importance to a testing laboratory. A testing laboratory must be competent in understanding how to perform tests and must be disciplined in its day to day operation. Patients are depending on laboratories to provide accurate information so that they can appropriately dose their medicine and feel confident that their medicine is free of harmful contaminants. The accreditation process does a good job of ensuring laboratories are doing what they are supposed to but it is of utmost importance that a laboratory self-regulate to the highest standard of accuracy and integrity of test data. Constant vigilance is key.

The state of Massachusetts requires all cannabis medicine dispensed by RMDs to be tested for and meet specific requirements for safety and potency. It is important to note that the requirements listed below pertain to the regulations at the time of this writing November 2015, these requirements will likely undergo changes as regulatory bodies fine tune them to best fit the needs of patients.

The cannabinoids THC and CBD must be quantified and labeled on all cannabis products. Additional cannabinoids will likely be added to the active ingredient profile as cannabis knowledge increases. Most

laboratories provide cannabinoid profiles that include THCA, CBDA, CBGA, THC, CBD, CBG, and CBN. There are laboratories with profiles including many more cannabinoids, however the availability of certified reference material is usually lagging behind demand. Cannabinoid profiles with 25 or more different cannabinoids will likely be standard in the near future.

Terpenes are not currently required to be profiled by regulations. Terpenes are of great interest to a number of patients and researchers due to their activity. They have a noticeable impact on the qualitative experience of the cannabis user and may have therapeutic application beyond that. Market forces will continue to drive terpene profile analysis until clinical data demonstrates a medically based need for these compounds to be included in the active ingredient label of cannabis products.

The safety tests include screening for microbiological contamination, toxins, pesticides, heavy metals, and residual solvents. These screens were chosen based on their likelihood of being present in cannabis and how likely they are to cause harm to the consumer. Regulators needed to consider how much cannabis might be consumed by a patient as well as what the health condition of the patient might be. Many of the conditions that are listed as qualifying a patient for cannabis might make the patient more susceptible to harm from contaminants. The 5 ounce allowable monthly limit of cannabis means that patients can be consumer considerable amounts of medicine. The guidelines listed below are intended to prevent harm to patients resulting from cannabis consumption.

Unprocessed cannabis material, processed cannabis material, and cannabis extracts all need to be analyzed for microbiological contamination. The types of contamination and the limits are given in colony forming units in the table below.

Material	Total Viable Aerobic Bacteria	Total Yeast and Mold	Total Coliforms	Bile Tolerant Gram Negative Bacteria
Unprocessed Material	100,000	10,000	1,000	1,000
Processed Material	100,000	10,000	1,000	1,000
Extracts	10,000	1,000	100	100

Pathogenic strains such as E Coli and Salmonella spp must not be detected at all in a one gram sample. Environmental conditions for growing and finishing cannabis plant material can contribute to microbiological infestations. When cultivators and processors allow the environmental conditions to become too warm and humid infestation can get out of control compromising the quality and safety of the finished product.

It is important to strike a balance between what the plant needs to grow and thrive and what would discourage microbial infestation. Once the appropriate conditions are determined careful environmental controls must be in place to keep conditions favorable for the plants growth and process and unfavorable for microbes.

As with most things prevention is key. Efforts should be made to reduce potential sources of introduction of harmful microbiological entities to the growing, processing, and packaging rooms. Some things to consider in reducing contamination risk.

Growing and processing rooms buffered from other areas with change rooms.
Dedicated uniforms for workers in the growing and processing rooms.
- Uniforms should include overalls, gloves, and hairnets
- Uniforms should be worn only in the change rooms and work areas
- Uniforms should be cleaned on a schedule that maintains their cleanliness

Limiting access to sensitive areas.
Rigorous and routine cleaning of sensitive areas.
Using only sterile material inputs and processing equipment in sensitive areas.
Rigorous and strictly enforced hygiene practices for employees in sensitive areas.

Sneeze guards for trimmers.

Unprocessed cannabis material, and cannabis concentrates need to be analyzed for mycotoxin contamination. Mycotoxins including aflatoxin B1, B2, G1, and G2 as well as ochratoxin A must be below 20 micrograms per kilogram of material (20 parts per billion). Mycotoxins are produced by certain species of fungi. The best protection against mycotoxins is preventing fungi infestation of the plant material and processed plant material.

Massachusetts does not allow the use of non-organic pesticides in the cultivation of cannabis. Finished plant material needs to be screened for pesticides. The table below lists the pesticides screened for. Pesticides on this list must not be detected at levels above 10 micrograms per kilogram (10 parts per billion).

Abemectin	**Daminozide**	**Paclobutrazol**
Acequinocyl	**Etoxazole**	**Pyrethrins**
Bifenazate	**Fenoxycarb**	**Spinosad**
Bifenthrin	**Imazalil**	**Spiromesifen**
Chlormequat Chloride	**Imidacloprid**	**Spirotetramat**
Cyfluthrin	**Myclobutanil**	**Trifloxystrobin**

An alternative approach to specifying the pesticide screen as above would be to allow some safe pesticides at specified limits and have testing laboratories screen for those pesticides in addition to an unpublished list of unapproved pesticides. Producers using only the approved pesticides at appropriate levels would pass the screen. This would allow producers to take advantage of less harmful pesticides and protect their crops from unwanted infestations. Having a list of pesticides that laboratories test for may incentivize a producer to choose a pesticide that is not on the list and may be more harmful than an approved pesticide – if such a list of approved pesticides was available.

The best way to avoid the need for pesticides is to prevent infestation by minimizing contamination of the growing room with pests. Once an infestation has been identified using biological pest control measures

may prevent the need for chemical treatment. If approved pesticides are used careful attention to application protocols and remediation techniques should by employed to ensure that the finished product has levels of residue that meet the regulated standard.

Finished plant material, cannabis, and cannabis concentrates all need to be analyzed for heavy metals. The types of metals and the limits are given in ug/kg in the table below.

Metal	Finished Plant Material	Cannabis Resin and Cannabis Concentrates
Arsenic	353	2352
Cadmium	145	964
Lead	212	1411
Mercury	701	470

These contaminants are most likely to be introduced through soil, water, nutrients, or other plant treatments (pest control, supplements, etc.). Typically these contaminants are found in extremely low concentration in the soil or other source but bio-accumulate in the plant. As the plant grows water, soil, nutrients, and other inputs are continuously added and the trace contaminants build up over time.

The best way to reduce heavy metal contamination of finished plant material is to use water, soil, and nutrients that are of the highest quality and contain the lowest amounts of these trace contaminants. Extensive flushing of plants prior to harvest is a good way of removing salts and other undesirable residues from the plant and may also help reduce heavy metal contaminants. Ultimately the products need to be analyzed by testing labs to ensure they have levels below the regulated limits.

Cannabis concentrates using the solvents listed in the below table must be screened for solvent residues in the finished concentrate material. The solvents have individual limits of residue.

Solvent	Upper Limit	Solvent	Upper Limit
Acetic Acid	276.5	Heptane	276.5
Acetone	276.5	Hexane	16.0
Acetonitrile	22.7	Isobutyl Acetate	276.5

Anisole	276.5	Isopropyl Acetate	276.5
N-Butane	1.0	Methanol	165.9
I-Butane	1.0	2-Methoxyethanol	2.8
1-Butanol	276.5	Methyl Acetate	276.5
2-Butanol	276.5	3-Methyl-1-Butanol	276.5
Butyl Acetate	276.5	Methylbutylketone	2.8
Tert-Butylmethyl Ether	276.5	Methylcyclohexane	65.3
Chlorobenzene	19.9	Methylethyl Ketone	276.5
Chloroform	3.3	Methylisobutyl Ketone	276.5
Cumene	3.9	2-Methyl-1-Propanol	276.5
Cyclohexane	214.6	N-Methylpyrrolidone	29.3
1,2-Dichloroethene	103.4	Nitromethane	2.8
Dichloromethane	33.2	Pentane	276.5
1,2-Dimethoxyethane	5.5	1-Pentanol	276.5
N,N-Dimethylacetamide	60.3	1-Propanol	276.5
N,N-Dimethylformamide	48.7	2-Propanol	276.5
Dimethyl Sulfoxide	276.5	Propane	1.0
1,4-Dioxane	21.0	Propyl Acetate	276.5
Ethanol	276.5	Pyridine	11.1
Ethyl Acetate	276.5	Sulfolane	8.8
Ethylene Glycol	34.3	Tetrahydrofuran	39.8
Ethyl Ether	276.5	Tetralin	5.5
Ethyl Formate	276.5	Toluene	49.2
Formamide	12.2	1,1,2-Trichloroethylene	4.4
Formic Acid	276.5	Xylene	120.0

Solvent-less extraction methods such as bubble hash, rosin-tec, and dry sift are all good ways to create concentrated cannabis forms without contaminating the product with solvents. Supercritical extraction with nitrogen, oxygen, carbon dioxide, and other solvents deemed safe without limits is another alternative to hydrocarbon and organic solvent based extractions. Extraction with hydrocarbons and organic solvents is very popular among both producers and consumers due to the high yield efficiency and favorable taste and aroma profile. Careful technique and deliberate purging processes can lower the residual solvent levels in these products to safe levels. Heat and vacuum are both employed in removing these solvents from the finished materials.

To screen for all of these potential contaminants scientists employ a number of analytical techniques an instruments capable of accurately quantitating each analyte being evaluated. A significant amount of training and experience is required to develop these analytical techniques. Often times a degree in the physical sciences is a pre-requisite for a position in a laboratory. The instrumentation is very advanced technology and usually very expensive to obtain. Having capable scientists and analyst is key to success in the laboratory.

Each group of tests requires its own unique set of instruments and techniques. While it is often the case that a given test can be performed by a variety of techniques using different instrumentation it is often common to find a consensus of the most suitable technique for the purpose of the test. Listed below are the most suitable methods to achieve the desired results but by no means the only approach and as regulatory and market forces change so too will the most suitable methods for testing. The most common instrumentation used in the analysis of cannabis are the liquid chromatograph coupled with a ultra-violet detector (LCUV), the gas chromatograph with a headspace auto-sampler and flame ionization detector (HSGCFID), the triple quadrupole mass spectrometer couple with a liquid chromatograph (LC/MS/MS), various systems for automated culturing and measuring of microbiological entities, and inductively coupled mass spectrometry (ICP/MS).

Different techniques of analysis require different processing techniques. A generic view of sample processing is given below.

- Sample is weighed
- Sample is extracted
- Sample is cleaned
- Sample is diluted
- Sample is introduced to the instrument
- Sample is separated into analytes of interest
- Analytes of interest are measured
- Measurement data is processed to obtain a measured value for the analytes of interest
- Weighing and dilution factors are used to back calculate the original composition of the test sample
- Results are reviewed for accuracy

- Results are reported

LCUV Sample Analysis

The LCUV is the most suitable way to measure cannabinoids, both the acid and decarboxlated or neutral species can be separated and detected, quickly, accurately, and at levels meaningful to the consumer and regulatory bodies. LCUV is an analytical method that uses chemical affinity competition to separate analytes of interest from each other and other sample components and then measuring the analytes by absorption of light as a function of analyte concentration.

A solvent system with a variable affinity for the sample components is used to carry sample components to a solid bed of materials with a fixed affinity for the components. The solvent composition is modified at controlled rates to change the affinity of the constituents while the solid material remains constant. As the solvent affinity changes the different components with their differing affinities for the solvent overcome their affinity for the solid material and eventually are carried from the solid material to the detector. Due to the different affinities of the components they separate from each other as the solvent changes and they form bands and reach the detector at different times. This separation allows the analyst to determine which analyte they are measuring based on the time it takes to reach the detector.

The detector uses a beam of light to determine the amount of analyte in a solvent. As the analytes pass in front of the beam of light the detector registers less light. The amount of light loss can be correlated to a concentration of analyte.

An instrument with the liquid chromatography attached to a mass filter and detector can be used to measure analytes giving increased separating power and significantly lowering detection limits while extending dynamic range which increases both capability and performance. This becomes important when a large number of analytes are being analyzed at once or when the analytes are being measured at extremely low

concentrations or both. An eighteen analyte pesticide screen is a good example. The LC/MS/MS instrument uses that same separating power of the LCUV system but then further separates analytes by mass to charge ratio. Instead of the separated compounds being introduced to a UV detector they are introduced as ions into a mass filter. The mass filter separates the analytes based on their ionized mass. These are further separated by fragmenting the ions and separating them by fragment ionized mass. The result of these multiple separation methods and the ion detecting selectivity and sensitivity complex analyte mixtures can be analyzed at very low concentrations very effectively.

A gas chromatograph equipped with a flame ionization detector and headspace autosampler is the next workhorse of the cannabis lab. This instrument is suitable for measuring both terpenes and residual solvents. Due to the detection method it is less suitable for measuring cannabinoids however techniques can be used to overcome the challenges posed by the decarboxylation of the sample in the detection process making HSGCFID a viable alternative to the preferred LCUV method of analysis of cannabinoids. The HSGCFID uses an oven to agitate and heat sample components into a gas phase before introducing them to a solid material. Like the LCUV method it uses competition between the affinities of the analytes for the solid phase to separate the components. A combination of gas and temperature variation is employed to exploit this competition and accomplish the separation. Once separated and freed from the solid phase the analytes are consumed by a flame furnace. The ions of the combusted material are counted and the count is correlated to a known concentration of analytes.

There are number of approaches to screening cannabis for microbiological contamination. The original plating technique is still used by some laboratories while some labs have chosen more advanced and automated systems using the same basic principles as the plating technique. The most advanced methods employ DNA amplification and measurement – quantitative polymerization chain reaction monitoring (QPCR).

Plating techniques involve inoculating a petri dish containing culturing media with cannabis samples. After the samples are incubated in this media for a suitable amount of time the plates are evaluated under a microscope and the microbial colonies are counted. Different media types culture different microbial entities so the identity of the species is determined by which media is used.

Recent advances in technology employ extraction of microbes from cannabis samples, introducing this extract to cards that contain the culturing media and other ingredients. These cards are incubated and then read by an instrument which is able to calculate the colony forming units from the original sample.

QPCR is very sensitive, accurate, and capable of determining a vast multitude of different species from one another. The technique is very sensitive to analyst technique and has not been found to be suitable for quantifying analytes in all of the required matrices (edible, concentrate, etc.) and so is not currently widely used. As these obstacles are overcome laboratories may shift from other methods to QPCR.

DNA technology is becoming increasingly popular in determining the gene sequences of cannabis plants. This information can inform the identity of the plant, its lineage, and its potential active ingredient profile. Some people are using the gene sequence of desirable strains of cannabis as a component of intellectual property filings.

By patenting a specific strain a breeder can create strains that can be the legal property of the holder of the patent. This is a departure from the ownership of a cannabis strain being based on possession of the physical plant or seed and changing it to owning the rights to the very essence of that plant – its genetic fingerprint. Issues regarding the federal status of cannabis need to be sorted out before these patents can be defended legally.

The most suitable method for measuring heavy metals in cannabis samples is inductively coupled mass spectrometry. Samples are digested by dissolving in concentrated acid and exposing them to microwave radiation. The digested samples are then introduced into the source of the instrument by a nebulizer. This

nebulized sample is further plasmarized into ions and introduced into a mass filter to further separate prior to measuring with an ion detector. Atomic absorption instruments are also employed in the analysis of metals however detection limits and processing time make the ICPMS the choice most laboratories are employing.

Thought Provoking Questions

- How can science contribute to the cannabis movement?
- What will cannabis science look like in 5 years? Ten years?
- What are the major cannabis technology challenges science can help overcome?
- How can the safety of cannabis be improved?
- How can you use science in your cannabis efforts?
- What does the legitimization of cannabis as medicine mean for healthcare going forward?
- How can knowledge of the entourage effect improve our ability to use cannabis as a therapeutic?
- With all of the many uses of cannabis already what cannabis applications are yet to be employed?

Glossary

- Accreditation – Recognition that an institution maintains a set of standards
- Adulteration – To make impure by the addition of undesirable additives
- Asphyxiation – Being deprived of oxygen
- Bronchodilator – A substance that when ingested opens the lung passageways
- Cannabimimetic – Compounds which have activity on cannabinoid receptors
- Chromatography – A process of separating compounds from each other
- Decarboxylation – The removal of a carboxylic acid group from a molecule – when THCA is heated it is decarboxylated loosing carbon dioxide and becoming THC
- Drug – A substance which has a physiological effect when ingested or otherwise introduced into the body
- E Coli – A bacterium found in the digestive track of animals (including humans) which under certain circumstances can cause disease
- Fungus – Spore producing organisms that feed on organic material
- Hydrocarbon – Compound consisting of carbon and hydrogen bonds – typically referencing petroleum products
- ISO – International Organization for Standardization
- Marijuana – A term people use while attempting to reference cannabis
- Mildew – Thin film of fungus
- Mold – Multicellular fungus
- Mycotoxin – Toxic substance produced by fungus
- PT – Proficiency Test – A test performed by a laboratory which measures their reported result against the nominal or known value
- Residual Solvent – Solvent that remains in a finished product after processing
- RMD – Registered Marijuana Dispensary
- Salmonella - A bacterium found in the digestive track of animals (including humans) which under certain circumstances can cause disease – a different species from E Coli
- Solubility – The ability of one substance to dissolve in another substance
- Tachycardia – Abnormally rapid heart rate
- Terpene – Volatile unsaturated hydrocarbons found in the essential oil of plants
- Titration – Slowly increasing cannabis dose to achieve therapeutic effect
- Validation – A body of work that demonstrates that an analytical method performs as stated