Microbiology and the Cannabis Industry, is your Cannabis Testing Method Truly Validated?

Patrick M. Bird

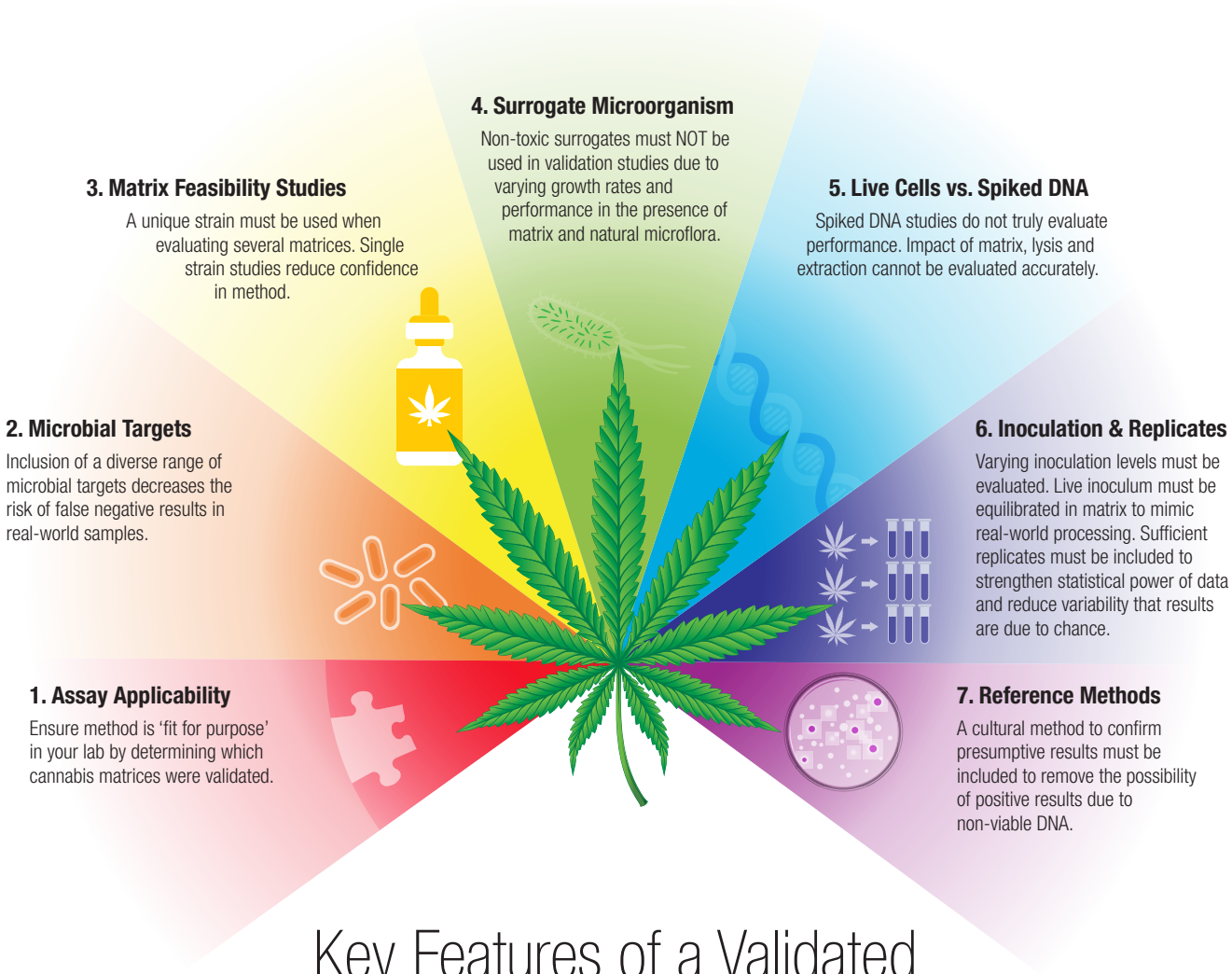
Microbial Testing

Introduction

One of the fastest growing markets in the world, the production and sale of cannabis and cannabis infused products (CIP) is becoming a lucrative, legal industry. By the end of 2025, it is estimated that the global legal marijuana market will reach sales of ~$150 billion dollars[1]. In 2018, the legal marijuana industry grew by $10 billion in the US alone[2]. The rapid expansion can be attributed to the increase in legalization (and/or decriminalization) at the state level in the US, and at the national level globally (Canada, Uruguay, Netherlands, Spain, South Africa), but other factors, including an increase in the use of medical marijuana to treat more clinical conditions, and a demand from consumers for new products for adult use has also accelerated the market's growth[3]. The expansion into new products, specifically foods, has resulted in an increased scrutiny of manufacturers. Similar to food producers, cultivators and distributors of cannabis and CIP must comply with regulatory requirements, establishing safety plans that certify the quality of their manufacturing processes and conduct routine laboratory screening of their products for specific microbial and chemical contaminates[4,5]. However, unlike the food industry, there remains high uncertainty with some of the methods being used in the cannabis industry as the validation procedures used to certify them have been less then rigorous. In order to be utilized by food producers, method developers must validate their assays according to globally recognized standards. For the cannabis industry, the validation of methods for microbial contaminates has not been established, and methods currently being used may not be "fit for purpose" as the designs of these validations do not conform to globally accepted validation practices. The objective of this report will be to discuss the flaws in these validation designs (selection and use of live microorganisms, number of test replicates evaluated, use of surrogate strains, etc.) and provide guidance on improving study designs for future validations.

1 Assay Applicability

When developing a new assay, the first steps a method developer will take is to determine the applicability of the assay. By identifying an assay's target markets, a method developer can then select the food items that will be included in the validation study. The study can be designed to be broad enough to cover key commodities ensuring that the method can be rapidly adopted. This process has often been overlooked in the cannabis industry, as the scope of several validation studies is much less than promoted applicability of the assay. Under current validation guidelines, methods are only validated for matrices (or food categories) evaluated during the validation study and that additional matrices need to be verified by the end users. When adopting these technologies, laboratories in the cannabis industry must determine which items were validated by the method developer and then determine how to verify the method is appropriate for matrices they will be testing in their facilities. Both components (validation and verification) are necessary to ensure a method is "fit for purpose" in that laboratory.



Key Features of a Validated Cannabis Microbial Test Method

2

Microbial Targets

A second crucial aspect to the applicability of a method is determining the range of targets the assay can detect, which includes the assay's ability to discriminate target analytes from other closely related species. For the cannabis industry, laboratory testing has focused on three main microbial contaminants: *Salmonella*, Shiga-like toxin producing *Escherichia coli* (STEC) and select *Aspergillus* species. The first two strains are bacterial pathogens with a zero-tolerance policy in food products. There are greater than >2700 serotypes of *Salmonella*, and hundreds of different STEC strains. It would be impracticable (if not impossible) to validate a method for all strains of an organism. Internationally recognized validation guidelines have therefore agreed that a set number of strains should be evaluated prior to approval. For most microbial targets, a minimum of 50 different isolates are required (the exception being for *Salmonella*, where 100 isolates are required). Testing a wide range of strains demonstrates the robustness of a method and indicates it should be able to detect a majority of strains that could be encountered by end users. Many cannabis validations have greatly reduced this number (<10 strains evaluated). This raise concerns that these methods may not have the ability to detect a diverse range of strains, and because of this, contaminated product may reach consumers. Expanding these validations to include more isolates is essential to demonstrating they will detect these zero-tolerance targets.

3 Matrix Feasibility

After determining the applicability of the validation, a method developer will then outline the framework for the matrix study testing. This framework will include details surrounding the processes for matrix inoculation, evaluation of test replicates and final data analysis. When conducting matrix studies, a single inoculation strain should be used per matrix, but when evaluating several matrices, a unique strain should be used for each matrix. Results generated using a single inoculation strain for multiple matrices significantly reduces the robustness of the data. It demonstrates the assay can detect that specific strain but gives little confidence you can extrapolate the performance of the assay to other strains. In an ideal situation, the strains selected for inoculation in each matrix will have been isolated from that matrix, or one with similar physiochemical characteristics.

4 Surrogate Microorganisms

Another misconception that exists in the validation studies of some cannabis methods is the use of surrogate organisms. While this may be a common practice for process validations in manufacturing facilities to ensure that pathogenic microorganisms are not brought into the facility or for "proof of concept" studies used to generate some initial data on an assay, the use of surrogate organisms is inappropriate for matrix study validations. For example, the use of a non-toxic *E. coli* in place of an STEC strain may be preferred from a safety standpoint and can steer method developers on the right path for enrichment incubation time. However, this approach can't be used to validate a method for several reasons. First, the growth rates of surrogates may differ from those of the target strains. This could result in data that may indicate a shorter enrichment time is possible, when in fact the use of a true target strain could prove otherwise. Secondly, surrogate strains may perform differently in the presence of matrix and its naturally occurring microflora. This may also result in the generation of inaccurate data. Using surrogates can be beneficial in the early development of an assay, but it does not provide evidence that the assay will perform accurately when used on real-world test samples and is not an acceptable practice for validation studies.

5 Live Cells Versus Spiked DNA

As previously mentioned, "proof of concept" evaluations are early developmental studies designed to evaluate the feasibility of an assay and do not conform to the rules of method validation. They can be very useful for method developers but should not be mistaken for validations. One example of the difference between the two is the use of bacterial DNA in lieu of live microorganisms. Several method developers have used this practice to "validate" their methods for the cannabis industry. For certain components of a validations, such as linearity studies, stability and lot-to-lot evaluations, using bacterial DNA is a commonly accepted practice to evaluate a method. This can allow for rapid data generation and determination of the repeatability and precision of an assay however it does not truly evaluate the performance of a method. An assay's ability to detect pure DNA in a matrix cannot be directly correlated to its ability to detect DNA from live organisms. DNA studies are not able to evaluate an entire method from start to finish. They exclude the impact that matrix (and background microflora) can have on the growth of the target organism, the efficiency of the lysis process, and the ability to isolate the organism after detection. Each of these parameters is key to understanding how a method will perform with real world test samples.

6 Inoculation and Matrix Equilibration

After determining which microorganism (live strains) to use with each matrix, the next step is to perform the inoculation of the target strain into the matrix and ensuring that the organism has been equilibrated into the matrix. Traditional validations will incorporate three separate levels of contamination: non-inoculated level, high inoculum level and low inoculum level. The low level, the most important level tested during a validation study, ensures that the method is able to detect organisms at its limit of detection (LOD). Validations are designed this way to provide confidence that a method will work in situations where the organism has been diluted in the matrix to very low levels. Several cannabis methods have only produced data at a high inoculum level where the method should detect the target analyte but have not demonstrated the technology can work at low levels where the target analytes can still cause illness if consumed. Equilibration of the organism in a matrix may cause sub-lethal injury (mimicking what may occur during processing) resulting in slower growth of the organism. If no equilibration period is observed, a method may overestimate its ability to detect an organism in a shorter time frame. Several known cannabis studies have been conducted by inoculating a matrix and testing the product using the assay after a very short holding period (1–2 hrs). This practice falls outside recommended guidance (48–72 hrs. for perishable matrices, 2 weeks for non-perishable matrices or frozen matrices) and may result in inaccurate performance of the method.

7 Replicates

Just as crucial to the inoculation process, is designing your validation to include a sufficient number of test replicates for each contamination level. The study guidelines for both qualitative and quantitative validations allow for more accurate interpretation of data, strengthening the statistical power of the data generated and reducing the variability that the results are due to chance (which can occur if fewer than the recommended number of replicates is used). One final key to ensuring the validity of the data generated by testing multiple replicates at several levels of contamination is to blind code the samples prior to analysis. If analysts know that a sample should be positive (think high level inoculum) it can impact the way they conduct the test and influence the final results. By having the analysts unaware of the contamination level, this testing bias can be removed.

8 Refrence Methods

For a majority of validation studies, the alternative method is compared to a cultural reference standard, traditionally the method that regulators use to evaluate that commodity. For the cannabis industry, no reference cultural method exists. The lack of a reference method may lead to issues in how to interpret results. It is important to verify that a validation with no reference method includes some confirmatory steps to ensure that that presumptive results are accurate. All test portions, regardless of presumptive results, should be confirmed, and in the case of *Salmonella* validations, a secondary enrichment, utilized in all reference standards, should be included. If all samples are not confirmed, it can lead to false claims about the accuracy of a method. It could be possible that some presumptive positive detection is the result of cross reactivity of a closely related strain or detection of non-viable DNA. These "positive" results appear to make a method look like it is working, when it may be producing inaccurate results. It can also lead to non-detection of a target analyte that truly is present in the sample. Ensuring the accuracy of the preliminary result is key in a validation study, and while no reference method exists, method developers must ensure that some cultural confirmation is occurring.

9 Setting The Standard

During my review of these "validations" it stood out to me that me that not all method developers are taking the bare minimum approach to their cannabis validations. One organization that continues to maintain the same high level of standards for validations regardless of industry is Bio-Rad Laboratories. Bio-Rad (Hercules, CA), a global leader in the clinical diagnostic and food safety industries, uses their knowledge and understanding of the requirements for method validation to design validations that go above and beyond the "status quo" of the current cannabis validation market. In addition to ensuring that their methods meet the the highest levels of validation, Bio-Rad works with industry leaders to ensure that their validated methods are also "fit for purpose". They work with manufacturers and testing laboratories to conduct extensive method verification studies on newly created products. These studies continue to demonstrate Bio-Rad's commitment to the highest level of consumer safety.

CONCLUSIONS

Method validation is a complex process and globally accepted validation standards exist to provide guidance to method developers. While variations exist between the guidelines, there is a common thread throughout these standards that provide the backbone for acceptance of validly produced data. As method developers move into the cannabis industry, it will be critical for them to use these resources to safeguard that their methods are accurate, reliable and properly evaluated to ensure that safe products are reaching consumers.

1. Grand View Research (2018) *Legal Marijuana Market worth $146.4 Billion by 2025* https://www.grandviewresearch.com/press-release/global-legal-marijuana-market
2. Reisinger, Donald (2018) *The Legal Marijuana Industry is Soaring, And 2019 Could Be Its Best Year* http://fortune.com/2018/12/27/legal-marijuana-industry-sales/
3. BDS Analytics (2018) *BDS Analytics' Top 10 Cannabis Market Trends for 2018* https://bdsanalytics.com/bds-analytics-top-10-cannabis-market-trends-2018/
4. California Cannabis Portal (2019) *Cannabis Regulations (Currently in Effect – January 2019)* https://cannabis.ca.gov/cannabis-regulations/
5. Colorado Department of Revenue, Enforcement Division (2019) *MED Laws and Regulations* https://www.colorado.gov/pacific/enforcement/med-laws-and-regulations

Patrick M. Bird

Pat has been an active member of the food safety community for the last 15 years serving in numerous roles including manufacturing and laboratory management. He is a member of the AOAC Research Institute team as a technical consultant, where he works on the development of validation outlines for method developers. Pat has authored and/or co-authored over 40 peer reviewed publications and was part of the validation team that has won three awards for AOAC Multi-Laboratory Study of the Year, most recently in 2018.