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MEDICINAL GENOMICS

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Independent Regulatory Review Commission

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Pennsylvania Medical Marijuana Program Director

Dear Director,

As industry leaders in cannabis and pathogen genomics, we have spent decades working with quantitative polymerase chain reaction (qPCR) and culture-based methods for the detection of microorganisms. We are experts in the field with over 40 patents related to qPCR and DNA sequencing based methods for detecting microorganisms. Kevin McKernan, Chief Scientific Officer at Medicinal Genomics Corporation (MGC) managed the Research & Development team for the Human Genome Project at the Whitehead Institute of Massachusetts Institute of Technology. He has over 56,272 citations related to his work in this field. Our scientists recommend microbial testing specifications that will ensure that cannabis is safe for patients. Due to our concerns for public health and safety, we feel that the Pennsylvania Department of Health Office of Medical Marijuana's microbial standards for testing established under § 1171 a.30 should be modified to reflect ongoing efforts at the AOAC, USP, FDA, and CDC, which are consistent with our findings at MGC.

The presence of microorganisms is common in natural products, such as cannabis flowers. One must be able to differentiate between harmless and/or beneficial microbes and human pathogens that have contaminated the cannabis plant and/or manufactured products. Examples of human pathogens that have been detected in cannabis are Shiga toxin producing *E. coli* (STEC), *Salmonella* spp. (all species are pathogenic), *Aspergillus flavus, A. fumigatus, A. niger, A. terreus* [1-26].

Current testing requirements for microorganisms in states with medical cannabis programs are diverse. Some states require a subset of the following tests: total aerobic bacterial microbial count (TAMC), total yeast and mold (TYM), total bile-tolerant Gram-negative bacteria (BTGN), and total *E. coli*. Action levels for enumeration tests for each product type are dependent on the state's rules. All microbial tests have action levels as colony forming units (cfu/g or cfu/ml), which is the number of colonies that grow on the surface of an agar plate. On the other hand, California, Alaska, Oregon, Montana, and Vermont have or have drafted rules that require testing for the six human pathogens listed above with an action level of none detected per gram of product.

In the above Guidance, subsection "Microbiological testing: Appropriate methods for performing include plating & culture. See reference in U.S. Food and Drug Administration Laboratory Methods"

Our first concern is stating that the appropriate method for microbiological testing is a plating system per the US FDA Bacteriological Analytical Manual (BAM). A universal aspect of a test validation study is to use the sample type that will be ultimately used; in this case cannabis. The **only** cannabis AOAC certified plating method using cannabis as the sample type is for total yeast and mold. There are **no** BAM methods that have been validated using cannabis as the sample type.

Therefore, MGC recommends that the statement quoted above should be modified to read "Microbiological testing:
Appropriate methods are a certified Performance Test Method approved by the AOAC International or an alternative method approved by the Office of Medical Marijuana, which may include molecular methods, such as a qPCR method".

When one compares qPCR *vs.* plating methods, the primary advantage of using qPCR detection assays are that they are designed to identify unique short DNA sequences either shared by a "group" of bacteria, such as all *Salmonella* species and STEC subtypes or a specific genus and specie, such as the 4 different pathogenic *Aspergillus* species. If the unique sequences are present, then the qPCR test will detect it. Therefore, a qPCR test is very specific, very sensitive, and possesses a rapid turnaround time (6 hours) *vs.* plating methods that are less specific, less sensitive, and has a very slow turnaround time of days for colonies to form on a plating system. Moreover, MGC has developed a method to remove the DNA that originated from dead cells by using a DNA nuclease enzyme, incubation, & nuclease inactivation step before amplification to detect any DNA originating from live pathogens [27].

MGC's qPCR methods are going through additional validation according to AOAC's Standard Method Performance Requirements (SMPRs). AOAC has 3 SMPRs for testing the human pathogens listed above (see #1-3 below).

1. Detection of *Aspergillus* in Cannabis and Cannabis Products
   https://www.aoac.org/wp-content/uploads/2019/10/SMPR-2019_001.pdf
2. Detection of *Salmonella* species in Cannabis and Cannabis Products
   https://www.aoac.org/wp-content/uploads/2020/07/SMPR-2020_002.pdf
3. Detection of Shiga toxin-producing *Escherihia coli* in Cannabis and Cannabis Products
   https://www.aoac.org/wp-content/uploads/2021/02/SMPR-2020_012.pdf

Medicinal Genomics is a member of AOAC's Cannabis Analytical Science Program (CASP) Microbial Contaminants Working Group. This working group's objectives are to:

- Develop SMPRs that use cannabis as the sample type
- Extend a call for methods for each SMPRs
- Form expert review panels to review candidate methods
- Deliver consensus-based validated Performance Test Methods (PTMs) & Final Action Official Methods for the cannabis industry

NOTE: MGC has an AOAC validated qPCR PTM for the detection of the 4 *Aspergillus* species and has an AOAC validated qPCR PTM for the detection of *Salmonella* spp. & STEC. The sample types for both tests are for cannabis flower, infused products, oils, and concentrates.

In contrast, plating methods have major disadvantages when being used to either enumerate a group of microorganisms and/or detect specific bacterial and fungal pathogens.

- Cannabinoids, which can represent 10-20% of a cannabis flower's weight, have been shown to have antibiotic activity. Antibiotics inhibit the growth of bacteria [28-29]. *Salmonella* & STEC bacteria are very sensitive to antibiotics, which may lead to either a false negative result or lower total counts on plates *vs.* qPCR methods.
- Plating methods cannot detect bacterial and fungal endophytes [30-31] that live a part or all of their life cycle **inside** a plant. Examples of endophytes are the *Aspergillus* pathogens and

Fusarium. Methods to break open the plant cells to access these endophytes for plating methods also lyses these bacterial and mold cells (killing these cells in the process). Therefore, these endophytes will never form colonies, which will lead to either a false negative result or lower total counts on plates *vs.* qPCR methods.

- Selective media for mold plating methods, such as Dichloran Rose-Bengal Chloramphenicol (DRBC) reduces mold growth; especially *Aspergillus* by 5-fold. This may lead to a false negative result for this human pathogen. In other words, although DRBC medium is typically used to reduce bacteria; it comes at the cost of missing 5 fold more yeast and molds than Potato Dextrose Agar (PDA) + Chloramphenicol or molecular methods. These observations were derived from study results of the AOAC emergency response validation [32].
- MGC has recently identified four bacterial species associated with cannabis that **do not** grow at plating system incubation temperature and therefore. They are *Aeromonas hydrophila, Pantoea agglomerans, Yersinia enterocolitica,* and *Rahnella aquatilis.* This will lead to lower total counts on plates *vs.* qPCR methods.

In the Medical Marijuana Harvest and Final Product Acceptance Criteria table, the microbial analytes to be tested are:

Microorganism	Action Level
Salmonella	Absence in 1g
E. Coli	Absence in 1g
Mold and Yeast	• 10,000 CFU/g Harvest Lot
	• 1000 CFU/g Process Lot
	• 10,000 CFU/g Final processed flower at product testing
Total Viable ABMC	• 10,000 CFU/g Harvest Lot
	• 10,000 CFU/g Process Lot (including flower at product testing)
BTGN	• 1000 CFU/g Harvest Lot
	• 100 CFU/g Process Lot
	• 1000 CFU/g Final flower at product testing

Our second concern is that total count tests, such as Mold and Yeast, Total Viable Aerobic Bacterial Microbial Count, and BTGN bacteria **do not** test directly for the presence of any human pathogens. The American Herbal Pharmacopoeia's *Cannabis* Inflorescence *Cannabis* spp. monograph [33] states that total microbial counts **must never** be used to pass or fail a cannabis sample. In other words, total count results **do not** provide any information about the presence of any pathogenic microorganisms in the cannabis sample, which may cause harm to patients. Moreover, approximately 25 pest control agents that contain either non-pathogenic beneficial bacterial or fungal strains are available to prevent infection that could lead to reduction of cannabinoid yield or total crop loss. Required total count tests may cause cultivators to use toxic chemical pesticides instead of harmless biological agents.

Therefore, MGC recommends to the PA DOH OMM to modify the list of microbial analytes to be tested for the product types by adding five pathogen specific tests, which are:

1. Shiga-toxin producing *Escherichia coli* (STEC)
2. *Aspergillus flavus*
3. *Aspergillus fumigatus*

4. *Aspergillus niger*
5. *Aspergillus terreus*

NOTE: *E. coli* was substituted with STEC, because CDC says that "Most *E. coli* are harmless and actually are an important part of a healthy human intestinal tract." Shiga toxin producing *E. coli* is the correct test to require, because it is the most dangerous of the 6 *E. coli* pathotypes to human health.

The United States Pharmacopeia stated that "Many states with legalized cannabis markets now require that all cannabis goods intended for consumption by inhalation be tested for the four pathogenic *Aspergillus* species (*A. flavus, A. fumigatus, A. niger,* and *A. terreus*). When inhaled, all four of these species are known to cause a variety of immune lung disorders, ranging from asthma, allergic bronchopulmonary aspergillosis, and hypersensitivity pneumonitis to invasive and life-threatening systemic fungal infections in immunocompromised hosts."

The number of states and territories that require microbial testing rules for inhaled cannabis products (flower, pre-rolls, *etc*) was 26 in 2019 [30] and 39 in 2022 [31]. A comparative analysis of the required microbial testing rules for all jurisdictions with legal cannabis programs in 2019 and in 2022 showed that the percentage of states and territory that require the detection of the pathogens listed above has increased during this 3 year period (see the following table).

Microorganism ('19)	# (%)	Microorganism ('22)	# (%)	% Increase
Salmonella species	22 (85%)	*Salmonella* species	37 (95%)	10%
STEC	4 (15%)	STEC	17 (44%)	29%
4 *Aspergillus* species	8 (31%)	4 *Aspergillus* species	21 (54%)	23%

NOTE #1: States & territory that require STEC testing are AK, CA, CO, CT, FL, IA, MI, MS, MT, NM, NY, OK, OR, SD, VT, WA, and Guam

NOTE #2: States & territory that require pathogenic *Aspergillus* species testing are AK, AL, AZ, CA, CO, CT, DE, FL, HI, IA, MI, MO, MS, MT, NM, NV, NY, OK, OR, SD, VT, and Guam

Since other states and territories with legal cannabis programs are in the process of modifying or drafting their microbial testing rules and new states & territories will legalize medical cannabis in the future, we predict that the percentage of jurisdictions requiring the detection of microbial pathogens for cannabis products will continue to increase.

I thank you for your time and consideration. If you have any questions, please feel free to contact me.

Respectfully,

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