PathogenDx
Setting the standard in DNA testing

January 2, 2020

Andrea Sundberg
NM Department of Health Medical Cannabis Program P.O. Box 26110
Santa Fe, NM 87502-6110

Dear Ms. Sundberg;

Please find PathogenDx Inc's response to New Mexico Regulations pertaining to the proposed rule revisions under 7.34.4 NMAC specifically related to:

- Section 7.34.4.10C(1) under Microbial testing

As of Dec. 27, 2019, 2,561 hospitalized vaping-related cases have been reported to the CDC; 55 people have died from the Vitamin-E Acetate crisis. Each of these cases resulted through the process of inhalation. What we put into our bodies and more importantly our lungs has a critical bearing on our health and safety, and we have painfully seen the consequences of this with national catastrophe impacting the well-being of consumers and patients in every state. Cannabis will continue to be inhaled whether medically or via adult-use.

As Cannabis is introduced for Medical purposes, potential microbial contamination becomes a major safety and health concern, in that many of the patients taking the drug may be immune compromised due to chemotherapy or age, in that the median age of therapeutic cannabis users is higher than that of the recreational market.

An understanding of the range of microbial contaminants in Cannabis has evolved rapidly in the past several years, due to seminal research papers by Thompson and colleagues (1,2), by McKernan and colleagues (3,4) and via a white paper review from the Cannabis Safety Institute (5), Bear-McGinnis (11) and clinical papers by by Kagen et al (9) dating back to 1983, and (10) Cescon et al

The pilot studies from Thompson and McKernan are particularly informative, because in both instances assumptions were not made as to the role of any pathogen. Instead relatively bias free Next Generation sequencing was deployed.

McKernan concluded from their pilot study that,
"the toxigenic *Penicillium* species: *P. paxilli, P. citrinum*, *P. commune, P. chrysogenum, P. corylophilum, Aspergillus* species: *A. terreus, A. niger, A. flavus*, *A. versicolor* and *Eurotium repens*. In addition, a pathogenic species *Cryptococcus liquefaciens* was

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detected. The fungal microbiomes of the different samples differed significantly in the number and diversity of species present".

Thompson et.al found, in their NGS pilot *the presence of,*
"E. coli, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *P. fluorescens* and *P. putida*, *Acinetobacter baumannii*, and *Stenotrophomonas maltophilia*. Although limited in coverage, WMGS reads from the samples analyzed mapped to the fungal genomes targeted: Sample 138-011 read (n=534) mapping supports the presence of *Alternaria alternata* and *Cladosporium sphaerospermum*. reads from sample MJ150-008 (n=658) indicated the presence of *A. fumigatus*, *C. laurentii* and *M. circinelloides*, all well-known causes of invasive fungal infections in immunocompromised hosts. We found numerous Gram-negative bacilli and fungal pathogens contaminating medical marijuana". Which are also known to be dangerous to immunocompromised patients.

As the authors of both the Thompson and McKernan pilots have both argued, these preliminary findings from next generation sequencing (NGS), the 'gold standard' in molecular typing. Both pilot studies were obtained via analysis of less than 2-dozen cannabis isolates from a number of growers.

What the pilot data do suggest is that, even among the sampling that was done, there is substantial variation among the small numbers of samples studied in terms of the relative abundance of the several pathogens detected and even the identity of the pathogens seen in each sample.

Based upon those findings, we summarize 3 important general principles of cannabis contamination, as deduced from the recent wide-ranging NGS data published within the past 6 months (1-4) and from the conclusions drawn by The Cannabis Safety Institute in 2015 (5):

1). Especially for the fungal contaminants, a much larger range of contamination was detected than previously generalized (in 2015 by the Cannabis Safety Institute). The systematic incidence of *P. paxilli, P. citrinum,* as seen by McKernan (3.4) was especially worrisome, since it was previously un-suspected and could be highly toxic to immunocompromised users. Kagen(10) concluded as far back in 1983 through a peer reviewed clinical study in the Journal of Clinical Immunology that:

The possible role of marijuana (MJ) in inducing sensitization to Aspergillus organisms was studied in 28 MJ smokers by evaluating their clinical status and immune responses to microorganisms isolated ,from MJ. The spectrum of illnesses included one patient with systemic aspergillosis and seven patients with a history of bronchospasm after smoking of MJ. Twenty-one smokers were asymptomatic. **Fungi were identified in 13 of 14 MJ samples and included *Aspergillus fumigatus*, *A. flavus*, *A.***

***niger*, *Mucor*, *Penicillium*, and *thermophilic actinomycetes*.** Precipitins to Aspergillus antigens were found in 13 of 23 smokers and in one of 10 controls, while significant blastogenesis to Aspergillus was demonstrated in only three of23 MJ smokers. When samples were smoked into an Andersen air sampler, A. fumigatus passed easily through contaminated MJ cigarettes. **Thus the use of MJ assumes the risks of both ,fungal exposure and infection, as well as the possible induction of a variety of immunologic lung disorders.**

2). The substantial variation in the number and nature of pathogen contamination among individual isolates, as seen in both pilots, in cannabis samples obtained from a number of growers, suggests that the range of pathogenic bacteria and pathogenic fungal contamination may be much larger than previously suspected. As the geographic range of cannabis cultivation is extended to many states, it may be necessary to continually update the list of important cannabis contaminants.

3). As both Thompson (1.2) and McKernan (3.4) have suggested, and as had been suggested by The Cannabis Safety Institute before them (5) the measurement of "Total Yeast and Mold" and "Total Bacterial Load" may be viewed as relatively useless analytical tests: the reason being that in both the bacterial and fungal complement of cannabis, the incidence of a toxic bacterial or fungal sub-fraction, may be unrelated to the very large excess of non-toxic bacteria or fungi in any sample.

Testing Recommendations to New Mexico, based on the scientific literature and observations made above.

1). **Bacteria**. Testing should be performed to explicitly detect toxic bacteria, especially the **E. coli** and **Salmonella** strains. Given that P. botulinum has been implicated in the early NGS testing, it should also be considered for addition to the New Mexico bacterial test panel so that its true incidence may be understood. **Bacterial subtyping for Enterobacter** should be considered for retention. Total bacterial load should be abandoned as a test, given that it produces a meaninglessly high false positive rate.

2). **Fungi**. Testing should be performed to explicitly detect toxic yeast and mold, especially the toxic **Aspergilli (flavus, Niger, terreus, Fumigatus**) and **Penicillium (citrinum, paxilli)** which have been implicated as present in pilot studies. Total Yeast and Mold load should be abandoned as a test, given that it produces a meaninglessly high false positive rate in many instances.

3). **Nucleic Acid Tests Should be Deployed in a way that Bypasses Cell Culture**. The references Cited (1-5) all suggest that great care be taken in the interpretation of plate based culture methods, in that pathogen viability may be lost

during cannabis processing (especially drying) and during ambient temperature transit from the grower/processer to the testing lab (e.g. see ref 6).

Such Pre-analytical variables are likely to affect cell viability but not DNA yield, thus methods should be found to obtain a DNA-based estimate of bacterial and fungal contamination in ways that are not based on culture enrichment, given that culture based enrichment skew the pathogen profile.

4). **Nucleic Acid and Culture Based Methods Should both be Used Mindfully**: **Understanding meaning of Culture vs Culture independent methods Microbial Contamination**. The traditional argument for the superiority of plate-based microbial culture analysis or culture independent analysis after a preliminary fluid based or plate based culture enrichment is that both such culture based approaches reveal the identity of the "culturable" sub fraction of a microbial contaminated sample, where as a nucleic acid test, done without enrichment gives the "Total" microbial load: both "culturable" and "nonculturable".

Given the rapidly growing diversity of both the bacteria and fungi of interest in cannabis testing, the references cited (1-5) all argue that culture conditions must be fine-tuned and validated to accommodate the diverse growth needs of the different microbial antigens. The finding of culturable material is nearly always a solid finding. However, there is now substantial data to suggest that potentially viable microbial contamination may reside in a sample, but especially when many different pathogens must be detected at the same time, the diversity of multiple culture conditions needed may yield a distribution of "culturable" pathogens that is greatly skewed relative to the true distribution of "potentially culturable" pathogen in the sample, or in the extreme case (as often seen for fungi) the production of overt false negatives: i.e. potentially-culturable pathogens which simply did not amplify under the culture conditions chose.

5) It has been clearly demonstrated that enrichment culturing of microbes can introduce bias, both positive and negative, which can yield inaccurate representation of the original microbial population in the sample.

Scientific evidence has been presented in a number of peer-reviewed scientific articles which include: 1) Kerr, J.R. (1999) *Bacterial inhibition of fungal growth and pathogenicity*. Microbial Ecology in Health and Disease, 11:3, 129-142; and 2) Dunbar, J., White, S., and Forney, L. (1997) *Genetic Diversity through the Looking Glass: Effect of Enrichment Bias*. Applied and Environmental Microbiology, 63(4), 1326-1331.

Additional scientific evidence has been provided that shows how enrichment yields inaccurate results specifically as it relates to cannabis include McKernan, Spangler, et

al,(2016) Metagenomic analysis of medicinal Cannabis samples; pathogenic bacteria, toxigenic fungi, and beneficial microbes grow in culture-based yeast and mold tests.

6) In addition, common enrichment protocols permit aerobic and facultative anaerobic bacteria to grow but does not permit obligate anaerobes, such as Clostridium botulinum, to grow. This renders many laboratories from adequately testing for obligate anaerobes which can pose a very significant health hazard to consumers of products inhabited by them. Molecular methods can be used to negate the bias effect of enrichment culturing, as well as permit screening for presence of obligate anaerobes.

7) **Recommend adding Clostridium botulinum, producer of the life threatening botulinum toxin** (Peck, M. W., Stringer, S. C. & Carter, A. T. Clostridium botulinum in the post-genomic era. Food Microbiol. 28, 183–191 (2011)) as a required organism as it is a relatively common bacterium found in soil samples and it has been associated with outbreaks involving food oil products (Centers for Disease Control and Prevention (CDC). Type B botulism associated with roasted eggplant in oil--Italy, 1993. MMWR Morb. Mortal. Wkly. Rep. 44, 33–36 (1995), and Morse, D. L., Pickard, L. K., Guzewich, J. J., Devine, B. D. & Shayegani, M. Garlic-in-oil associated botulism: episode leads to product modification. Am J Public Health 80, 1372–1373, (1990)) due to the microenvironment of a hydrophobic and anaerobic conducive towards C. botulinum cell and spore growth, This is particularly analogous to the oils and other hydrophobic extracts using materials from Cannabis, and ingestion of C. botulinum cells/spores can find niches within the digestive tract to permit growth and production of the botulinum toxin.

In the context of those arguments, the cannabis testing industry needs to host a discussion to assign the proper use of both nucleic acid and culture based methods. In the area of food safety testing, Nucleic Acid testing is now considered the most conservative type of test, when a large panel of pathogens must be measured in parallel: especially when the nucleic acid testing can be done without a potentially-skewed pre-culture step which precedes the nucleic acid test: see for instance the USDA White paper ref 6)

If you have any questions please contact Dr. Michael Hogan, mhogan@pathogendx.com or Dr. Carl Yamashiro, cyamashiro@pathogendx.com, and Dr. Ben Katchman, bkatchman@pathogendx.com

Regards,

Milan Patel

PathogenDx
Setting the standard in DNA testing

CEO
PathogenDx

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1). Thompson GR,, Tuscano JM. **Adverse health effects of marijuana use.** N Engl J Med 2014; 371(9):878-9.

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(9) Kagen et al. **Marijuana Smoking and Fungal Sensitization**. J Allergy Clin Immunol. 1983.

(10) Cescon et al. **Invasive Pulmonary Aspergillosis Associated with Marijuana Use in a Man with Colorectal Cancer**. Journal of Clinical Oncology. 2008.

(11) Bear-McGuinness. **Thousands of Types of Fungi, Bacteria Found in Californian Cannabis**. Analytical Cannabis. 2018.

From: Gonzales, Martinik, DOH
To: Woodward, Chris, DOH; Jimenez, Billy, DOH
Cc: Sundberg, Andrea, DOH
Subject: FW: [EXT] Error in New version of rules
Date: Thursday, January 2, 2020 10:20:30 AM

See below from Kathleen O'Dea. Andrea, I think this should be considered as public comment?

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-----Original Message-----
From: Kathleen ODea <kkodea@scepterlabs.com>
Sent: Thursday, January 2, 2020 10:19 AM
To: Peralta, Matthew, DOH <Matthew.Peralta2@state.nm.us>; Gonzales, Martinik, DOH <Martinik.Gonzales@state.nm.us>; Kunkel, Kathy, DOH <Kathy.Kunkel@state.nm.us>
Subject: [EXT] Error in New version of rules

Matthew,
There is glaring error in the new version of the proposed rules. Please see proposed table for testing solvents. Ethylbenzene is not the same as meta xylene. Meta xylene is a separate chemical. Meta xylene and para xylene cannot be separated so the footnote is incorrect. Otho xylene can be separated from meta and para but with great difficulty and there would be no reason to do so.
Your rules state that ethylbenzene is the same as meta xylene. This is incorrect. The footnote states that ortho and pera cannot be separated. This is incorrect. Also, since meta xylene has been improperly identified as ethylbenzene the action level is incorrect

Please correct this. It is embarrassing for New Mexico to memorialize into law such an obvious error In addition the proposed rules require a calibration curve that contains the highest action level for certain solvents(2000 ppm) This is not possible. CRM is available at a maximum of 1000 ppm.
Thank you.
Kathleen ODea

Sent from my iPhone