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DH, MMRegulations

From: kevin mckernan <kevin.mckernan@medicinalgenomics.com>
Sent: Monday, April 5, 2021 3:44 PM
To: DH, MMRegulations
Subject: [External] Open Comment period
Attachments: MCR_Rebutal_BLMv9.doc

Hello,

We are submitting these comments to the open comment period.
http://www.irrc.state.pa.us/regulations/RegSrchRslts.cfm?ID=3301

Office of Medical Marijuana, Department of Health

Room 628, Health and Welfare Building

625 Forster Street, Harrisburg, PA 17120

Thank you,

Kevin McKernan
CSO Medicinal Genomics.

Send via email RA-DHMMregulations@pa.gov

Office of Medical Marijuana, Department of Health

Room 628, Health and Welfare Building

625 Forster Street, Harrisburg, PA 17120

Dear Mr. Collins,

Medicinal Genomics would like to comment on a letter submitted by MCR Labs regarding Total Yeast and Mold (TYM) testing and the various errors presented in the letter.

Ms. Naccarato claims:

"Furthermore, independent assessment has found qPCR is not fit for purpose for quantitation of organisms for three main reasons" yet provides no citation for this independent assessment

While the industry is waiting for a final ruling from AOAC, we thought it would be helpful to provide perspective on why we believe MCR's claims are misguided and invalid.

1. TYM qPCR does target ITS DNA and these ITS sites can have copy number changes in genomes however these copy number changes do not result in zero copies or the organism isn't viable. The additional copies that exist in some organisms makes ITS qPCR even more sensitive in genomes where this exists and can provide sub-CFU sensitivity. All fungal organisms have ITS DNA, yet only 1% can be cultured. So, while qPCR may over-estimate the number of organisms present due to copy number changes in genomes, culture vastly under-estimates risks by a factor of a 100 or more. This is mostly due to the fact that organisms only culture under very special circumstances. In addition, yeast and molds, require more time to culture than bacteria. These competing bacteria obscure the capacity to read the slower growing fungi on agar plates. Often antibiotics are required to enable slower growing yeast and molds to grow while killing the bacteria. These same antibiotics (Chloramphenicol), are known to inhibit the growth of many fungi like Aspergillus while still allowing chloramphenicol resistant bacteria to thrive. Aspergillus is the most dangerous pathogen found on cannabis and any culture-based method that uses antibiotics that inhibit the growth of this organism should be banned from use. Ms. Naccarato uses such a platform known as Tempo which does not have Aspergillus terreus covered in their inclusion studies. Because of this limitation with culture-based approaches, some states have moved to species specific testing with Aspergillus using qPCR. We have published on the shortcoming and liability this platform presents in cannabis testing here: https://osf.io/vpxe5/

2. DNA extraction efficiency can vary in some DNA extraction methods. This problem has been addressed with Inclusion and Exclusion testing of control organisms purchased from reputable biobanks like ATCC. We have demonstrated DNA extraction from over 105 organisms with these

protocols (https://help.medicinalgenomics.com/yeast-and-mold-assay-validation-data). This is an argument that works against plating based technology, as 99% of organisms do not culture unlike molecular techniques which can address all living things. In our survey of 52 Yeast and Molds purchased from ATCC, 96% of them were isolated and PCR amplified successfully. This is simply not an issue for qPCR but is a substantial issue for culture-based organisms.

3. Standard curves are an argument for qPCR not against it. qPCR enables internal controls to be present to address the concern of PCR efficiency. There is no such internal control for culture or plating. Absence of any growth in the plating experiment is indiscernible from a failed experiment. This is a major limitation to plating and should be banned for this reason alone. The variability of PCR organism to organism needs to be compared to the same variability seen in culturing these organisms. If only 1% of the organisms can be cultured but 96% can be qPCR'd, qPCR is in the best interest in consumer health.

4. The letter suggests there is no standard for TYM in cannabis. While this has been true during rapid cannabis legalization for the last few years, AOAC has since addressed this by issuing a number of SMPRs to properly validate testing methods for cannabis. Our firm is a member of AOAC's Cannabis Analytical Science Program responsible for developing cannabis standards. The link below contains more information on the various SMPR's that have been released. Our firm is participating in all of these studies to validate our qPCR methods to the highest standards that exist in the world. AOAC also have a draft SMPR for TYM which they plan to release at the end of the current study which we would be glad to provide.

   https://www.aoac.org/scientific-solutions/casp/

To put these comments into context, Figure 1 on the following page is an inclusion and exclusion testing validation for a commonly used TYM test. This is public information included in the product literature.

Figure 1. 3M Total Yeast and Mold Inclusion and Exclusion testing.

Results **Comparison to Customer Method, 25 °C**

Inclusivity

	3M Petrifilm Yeast and Mold Count Plates	aPDA Agar
3 days	5/19 strains (79%) had growth	15/19 strains (79%) had growth
	Strains with no visible growth: *Alternaria alternaria* ATCC 6663 *Chaetomium globosum* ATCC 6205 *Cladosporium cladosporiodes* ATCC 16022 *Scopulariopsis acremonium* ATCC 58636	**Strains with no visible growth:** *Alternaria alternaria* ATCC 6663 *Chaetomium globosum* ATCC 6205 *Cladosporium cladosporiodes* ATCC 16022 *Scopulariopsis acremonium* ATCC 58636
5 days	20/20 strains (100%) had growth	18/19 strains (95%) had growth
		Strains with no visible growth: *Scopulariopsis acremonium* ATCC 58636

Exclusivity

	3M Petrifilm Yeast and Mold Count Plates	aPDA Agar
5 days	4/19 strains (21%) had growth	1/19 strains (5%) had growth
	Strains with visible growth: *Aeromonas hydrophila* ATCC 7965 *Bacillus atrophaeus* ATCC 51189 (1 colony) *Bacillus cereus* ATCC 11774 (1 colony) *Salmonella enteridiidis* ATCC 13311 (1 colony)	**Strains with visible growth:** *Aeromonas hydrophila* ATCC 7965

You will note only 79% of the Yeast and Mold grew in 3 days despite most people running these plates for 48 hours (Table 1). 21% of the bacteria plated grew on the TYM plates. Compared to qPCR, where 96% of the Yeast and molds were detected and zero bacteria were detected. There is far better sensitivity and specificity with qPCR. The qPCR validation also used 52 Yeast and Molds compared to just 20 for the 3M TYM plate. No such cannabis matrix data is publicly available for the Tempo platform that MCR currently uses.

Table 1. Comparison of plating and qPCR.

	Yeast and Mold Detected	Off Target Bacteria	Organisms in Study
3M TYM	**79%**	**21%**	**20**
PathoSEEK qPCR	**96%**	**0%**	**52**

The 3M YM plates also produce different results when compared to a different growth media (aPDA). This is something we also noticed in our AOAC validation study. This study was performed by an independent lab in Michigan which noted **100-fold** differences in CFUs based on the plating medium one used (Figure 2). The conditions that produce less CFUs (and thus more passing cannabis samples) rely on antibiotics like Chloramphenicol which are known to limit the growth of the most pathogenic microbe found on cannabis (Aspergillus).

The following papers describe this effect.

- https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2606932/
- https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4054225/
- https://pubmed.ncbi.nlm.nih.gov/25497707/
- https://link.springer.com/article/10.1007/BF00413493
- https://www.medicinalgenomics.com/benchmarking-qpcr-to-culture-pda-vs-drbc/

Figure 2. Plating cannabis samples on DRBC (Dichloran Rose Bengal with Chloramphenicol) compared to Potato Dextrose Agar (PDA) with Chloramphenicol and PDA alone. One can see 100X variance in the counts based on the selected media used. This data was generated in the AOAC study by our organization and then replicated by a third-party lab working with AOAC.

Sample	DRBC						
	10^{-2} CFU/g	10^{-2} CFU/g	10^{-2} CFU/g	10^{-3} CFU/g	10^{-3} CFU/g	10^{-3} CFU/g	Final Result CFU/g
Low A	0	4	1	1	0	0	170
Low B	0	3	1	1	1	0	130
Low C	2	1	0	0	0	0	100
Low D	2	5	2	0	2	0	300
Low E	2	0	2	0	0	0	130
	Average CFU/g						170
	PDA with Chloramphenicol						
	10^{-2} CFU/g	10^{-2} CFU/g	10^{-2} CFU/g	10^{-3} CFU/g	10^{-3} CFU/g	10^{-3} CFU/g	Final Result CFU/g
Low A	8	16	12	1	3	2	1200
Low B	8	12	8	1	0	3	930
Low C	13	19	13	1	2	1	1500
Low D	3	12	21	2	0	1	1200
Low E	9	7	4	0	3	3	670
	Average CFU/g						1100
	PDA						
	10^{-2} CFU/g	10^{-2} CFU/g	10^{-2} CFU/g	10^{-3} CFU/g	10^{-3} CFU/g	10^{-3} CFU/g	Final Result CFU/g
Low A	127	133	124	32	32	21	14000
Low B	151	157	101	26	20	28	15000
Low C	TNTC	TNTC	TNTC	41	45	37	41000
Low D	147	141	123	32	26	26	15000
Low E	138	102	119	23	15	24	13000
	Average CFU/g						20000

5. The failure to discern bacteria from Yeast and Molds on the Tempo is documented in the literature (Figure 3).

Figure 3. Reprinted with permission from page 6 of McKernan *et al*. *Microbiological examination of nonsterile Cannabis products: Molecular Microbial Enumeration Tests and the limitation of Colony Forming Units*.

produces ACC and Tanzawaic acids[47]. It is unclear to what extent this expression affects pH in culture and how specific pH is as a proxy for CFU/g in a mixed microbiome growth environment.

To test this theory we sent a chloramphenicol resistant, salicyclic acid producing *Pseudomonas aeruginosa* culture to two testing laboratories using the TEMPO® system and received failing TYM (9x10^4) counts and cloudy cartridge images with passing BTGN results. This implies chloramphenicol resistant bacteria that can alter the pH of the broth can create false positive Yeast and Mold tests with this platform. Pseudomonas should trigger a BTGN according to the USP.

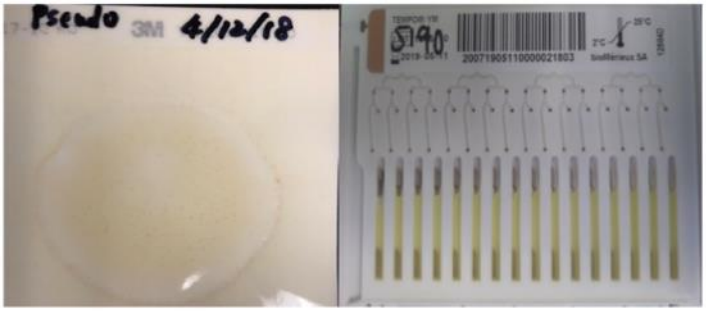

Figure 3B- Bacterial growth in common TYM assays. *Pseudomonas aeruginosa* ATCC#10145 plated on 3M TYM Petri-film and in Biomerieux TEMPO YM Cartridge. *Pseudomonas aeruginosa* is chloramphenicol resistant and produces salicylic acid. TEMPO detection is based on a pH sensitive fluorescent dye.

Pseudomonas aeruginosa is responsible for 11-14% of hospital acquired infections and is particularly problematic with Cystic Fibrosis patients[48]. CF patients are experimenting with cannabinoid use for the reduction of fibrosis. Corbus Pharmaceuticals is pursuing modified cannabinoids for CF patients in FDA trials. 100,000 CFU/g of TAC are allowable but only 1,000 CFU/g are allowable for BTGN. Any lab using only Biomerieux will be vulnerable to passing samples with 1001- 99,999 CFU/g of Pseudomonas.

Unlike the methods used by MCR, we have published over 131 cannabis flower microbiomes in NCBI to validate the specificity of our tests (Table2). There is no test on the market that has more validation data substantiating its performance than our PathoSEEK qPCR platform. Well over 2 million samples have been run through the platform at over 300 laboratories within the United States. We look forward to having AOAC accreditation in 2021.

- https://f1000research.com/articles/4-1422
- https://f1000research.com/articles/5-2471
- https://osf.io/vpxe5/

Table 2 https://www.coindesk.com/sec-lbry-unregistered-securities. NCBI accession numbers of the Sequencing verification for the MGC TYM PathoSEEK assay. No sequence verification exists for the culture-based platforms being used on Cannabis.

SRR4255463	SRR4255501	SRR4255532	SRR4255568	SRR4255589
SRR4255466	SRR4255502	SRR4255533	SRR4255569	SRR4255590
SRR4255467	SRR4255507	SRR4255540	SRR4255570	
SRR4255468	SRR4255508	SRR4255541	SRR4255571	
SRR4255469	SRR4255509	SRR4255542	SRR4255572	
SRR4255470	SRR4255516	SRR4255543	SRR4255573	
SRR4255477	SRR4255517	SRR4255544	SRR4255577	
SRR4255478	SRR4255518	SRR4255545	SRR4255578	
SRR4255479	SRR4255519	SRR4255546	SRR4255579	
SRR4255487	SRR4255520	SRR4255553	SRR4255580	
SRR4255488	SRR4255527	SRR4255554	SRR4255581	
SRR4255494	SRR4255528	SRR4255555	SRR4255582	
SRR4255495	SRR4255529	SRR4255562	SRR4255586	
SRR4255497	SRR4255530	SRR4255563	SRR4255587	
SRR4255500	SRR4255531	SRR4255564	SRR4255588	

Conclusions

- qPCR can detect a wider array of fungal pathogens as most pathogen do not culture. Use of antibiotics in culture-based systems needs intensive scrutiny as these have been shown to inhibit the growth of the most pathogenic fungi.
- qPCR can be multiplexed to have internal controls which help to safeguard against negative results being mis-interpreted from failed results.
- Standards are in the process of being agreed upon in the cannabis industry and it would be premature to make technology choices or mandates in 2021 before these standards have reached consensus.
- qPCR assays used in the cannabis industry have more public and open access data for evaluation than any test on the marketplace.