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DATA VALIDATION REPORT

Laboratory Result Comparison

Prepared for:

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Project Narrative

Summary

This report summarizes the results of the data validation performed on samples collected and submitted by AMCO to two testing laboratories, CannTest and Steep Hill, both located in Anchorage, AK.

On December 1, 2017, the Alaska Marijuana Control Office (AMCO) submitted CannaBanana muffin samples to both laboratories. Each of two muffins in three different retail packages were cut in half and submitted as separate samples. One half of each muffin went to CannTest and the other half of each muffin went to Steep Hill for testing and reporting.

On December 22, 2017, AMCO submitted additional samples of three matrices: cookie crumbs, capsules, and flowers. CannTest and Steep Hill each received approximately half of each sample.

	Potency (THC)				
	CannTest		Steep Hill		RPD
Sample name	mg/serving (unless otherwise noted)	mg/g	mg/serving (unless otherwise noted)	mg/g	
01-0992	5.26	0.34	11.9	0.58	52
02-0992	6.02	0.36	8.33	0.57	44
01-5332	5.66	0.29	8.00	0.41	34
02-5332	4.96	0.26	7.26	0.41	45
01-5922	6.28	0.28	8.86	0.45	47
02-5922	4.47	0.28	7.31	0.38	30
Cookie crumbs	13.8	NR	19.8	1.31	36
Capsules	4.40	NR	8.18	12.8	60
Dried Flower	24.70%	NR	16.2%	184	42
Dried Flower	12.8%	NR	12.1%	137	5.6

RPD = relative percent difference
NR = not reported

This report documents the review of the potency and microbial data. The samples were analyzed by CannTest and Steep Hill utilizing different extraction protocols for potency, but similar analytical techniques. The two labs used different microbial test methods.

TEST METHODS

Test	Method
Cannabinoid Potency Extraction	Agitation/QuEChERS Salt (CannTest)
	Sonication/Agitation(Steep Hill)
Cannabinoid Potency Analysis	HPLC – UV
Microbial	Plating (Steep Hill)
	qPCR (CannTest)

A summary of the results is provided below.

Laboratory Result Comparison

	Microbial					
	CannTest			Steep Hill		
Sample name	*Salmonella*	*E.coli*	*Aspergillus niger*	*Salmonella*	*E.coli*	*Aspergillus niger*
01-0992	ND	ND	ND	ND	ND	Detected
01-5332	ND	ND	ND	ND	ND	Detected
01-5922	ND	ND	ND	ND	ND	Detected
Cookie crumbs	ND	ND	ND	ND	ND	ND
Capsules	ND	ND	ND	ND	ND	ND
Dried flower	ND	ND	ND	ND	ND	ND
Dried flower	ND	ND	Detected	ND	ND	Detected
Dried flower	ND	ND	ND	ND	ND	ND

ND = Not detected

AMCO requested the Environmental Health Laboratory (EHL) perform data validation to investigate the differences in the results.

Briefly, the data review, with assumptions made, reproduced the reported results; however, several pieces of documentation needed to support the data were missing and there is a general lack of accuracy assessment occurring at the labs. The recommendation is for, 1) a complete assessment of lab operations as a follow-up to that of A2LA. Essential items to cover in this assessment are verifying implementation of critical parameters (e.g. incubation temperatures and times, calibrations of support equipment), procedures (e.g. laboratory homogenization and sub-sampling protocols), and general adherence to the laboratory SOPs) 2) accuracy controls (including all applicable positive controls for microbial testing and frequency) must be incorporated into lab activities, and 3) a regulation update that incorporates framework for regular oversight of the laboratories. Details of observations are provided in the following report.

I. DELIVERABLES/DOCUMENTATION

The laboratories provided data and documentation necessary to reproduce the reported results. However, sufficient documentation was not provided to demonstrate complete adherence to laboratory standard operating procedures (SOPs). Additionally, review of the SOPs found select procedural descriptions missing.

II. TECHNICAL ASSESSMENT

Reviewers

The data were reviewed utilizing laboratory SOPs as a point of reference and also experience auditing and reviewing data from municipal and commercial laboratories for SOP conformance, QA/QC activities and conformance to State of Alaska (SOA) , EPA, and industry published methods. The Alaska State Environmental Health Lab (EHL) performed the reviews. The reviewers currently serve as Laboratory Certification officers for the SOA Drinking Water Program and Laboratory Evaluation Officers for the SOA Dairy Program.

Potency

Methodology. Both laboratories utilize high performance liquid chromatography-ultraviolet detection (HPLC-UV) analysis for potency testing. Each laboratory monitors a different wavelength (CannTest 230 nm; Steep Hill 272 nm) to determine presence/absence of and to quantitate the THC and CBD constituents of interest. The data review and an online literature search did not reveal one wavelength as better than the other from quantitative and qualitative perspectives.

Instrument Calibration. Both laboratories submitted instrument calibration raw data, for which the EHL was able to reproduce the linear regression analyses the laboratories generated for determining calibration acceptance. The linear regression analyses were both compliant to a correlation coefficient criterion of 0.995. CannTest used calibration data acquired over a year prior to the analysis of the samples, and Steep Hill used calibration data acquired about two months prior to sample analysis. The difference in lifespan of a calibration curve is statistically acceptable given performance of accuracy assessments and calibration checks within SOP prescribed intervals and QC criteria. Both labs performed calibration checks, but neither lab performed accuracy assessments.

Analytical chemistry techniques in a regulatory environment require consistency in instrument conditions across all calibration, quality control, and sample analyses. Select variations may adversely affect the representativeness of instrument data and are only allowed on a case-by-case basis, typically for a specific matrix that impedes instrument response. Any adjustments made for a parameter must consistent in the calibration, quality control and sample analyses. Steep Hill's calibration and sample analysis procedures involve gathering data for a quality control parameter from a UV wavelength different from the UV wavelength used for potency parameters (e.g. THC, CBD) data. One way to assess the impact of this deviation is to recalculate Steep Hill's result using a calibration model similar to that of CannTest. This alternate calculation demonstrated differences in results ranging from 8-10% of their reported results.

Calibration Checks. Robust regulatory programs and methods, such as EPA-published or approved methodologies, require a verification of the accuracy of a calibration curve to a specified criteria prior to

sample analysis. Typically, this assessment is performed by analyzing a reference standard from a different vendor than that used for the instrument calibration standards. If a second vendor is not available, it is also permissible to use material from a different production lot from the same vendor that is different from the lot of the calibration standards. A recovery is calculated from the analysis of this standard and compared to acceptance criteria. The laboratories do not perform this calibration accuracy verification as part of their instrument calibration regimen.

The same methods require a stability assessment of the instrument calibration, determined through the use of a calibration verification standard, called a "continuing calibration verification" or CCV. CannTest's SOP instructs to analyze a CCV at the beginning and ending of the analysis sequence. CCV acceptance criteria are specified in the lab's SOP. For the CannaBanana sample analysis, the original beginning and ending analysis sequence CCVs had insufficient volume in the autosampler tube, so their chromatograms are simply baselines (i.e. no usable data obtained). A single remedial CCV was analyzed 12 hours after the analysis sequence was completed. The remedial CCV values did not pass the QC criterion for all constituents. For this situation and others where the CCV criteria for potency parameters were not met, no evidence was provided that corrective action was performed, that a reanalysis occurred, or that the data were flagged or a discussion of the outlier was included as part of the report.

The CCV criteria CannTest employs is more stringent than similar analytical organic chemistry methods in EPA-published methods; perhaps more conservative than needed as evidenced by results that are outliers to the criteria. Consideration for the widening of the criteria is warranted.

For each analysis sequence, Steep Hill submitted CCV results analyzed just before the samples, which met the QC criteria requirement in their SOP, criteria that are similar to like EPA-published methods. However, a CCV was not analyzed at the end of any of the analysis sequences, bringing into question the stability of the calibration curve during the entire sample analysis sequence or batch. The SOP should be amended to include a closing CCV at end of the run.

For both labs, a CCV minimally every 20 injections and at the end of each analytical run or batch is recommended, adding instrument calibration accuracy assessments, and re-examining acceptance criteria for achievability without being too generous.

Blanks. An instrument blank (IB) is a portion of the same solvent used to introduce a sample onto the instrument. Analysis of an IB demonstrates that the instrument system itself is not contributing to the concentrations of the target constituents. A method blank (MB) is a portion of "clean" material (e.g. lab water, "clean" solid matrix similar to the sample matrix and shown to be "clean") that accompanies a batch of samples, undergoing the same sample preparation and analysis steps as the samples. The MB demonstrates the cleanliness of reagents, materials, and handling protocols used during the sample preparation and analysis steps.

CannTest and Steep Hill prepared IBs, which are not extracted, to demonstrate the instrument was not a source of contamination. Both laboratories demonstrated that their analysis processes were not sources of influence on sample result concentrations. Both laboratories also prepared MBs that were extracted and analyzed with the samples, successfully demonstrating that the sample preparation was not a source of contamination. The exact matrix material used for the MBs is unknown.

Accuracy. Data review for potency testing was unable to assess the accuracy (i.e. degree to which results represent the THC/CBD content of the matrix) of the potency methods. The laboratories did not provide quality control (QC) data of spiked control or spiked sample matrices to assess the effectiveness of the combined extraction and analysis procedures implemented at each laboratory. Consequently, the testing accuracy for the full sequence of events of each laboratory is unknown.

The CannTest SOPs cites the use of a laboratory control sample (LCS), described as a sample previously analyzed by CannTest. An LCS is intended as an overall accuracy assessment of the sample preparation and analytical methods on a per test batch basis. Even though stipulated in the SOP, CannTest did not prepare and analyze an LCS in conjunction with the prep and analysis of the samples.

Steep Hill's SOPs describe the use of a surrogate (δ-tocopherol), a compound chemically similar to the constituents of interest. Steep Hill only qualitatively evaluates the surrogate for dilution verification because the instrument calibration does not include δ-tocopherol and therefore a means for quantitating is not available. The surrogate is identified on the chromatogram and Steep Hill solely uses it to assess stability in the elution order of constituents and as an indicator if instrument performance drift may be occurring. Addition of the surrogate to Steep Hill's instrument calibration protocol would allow for an assessment of the sample extraction performance information needed to evaluate overall method accuracy.

Analysis of two of the samples analyzed by CannTest yielded results that exceeded the calibration curve for total THC. The sample extract should have been diluted and reanalyzed to bring the instrument measurement within the calibration range in order to obtain an accurate measurement. Data representing a dilution analysis was not available, when requested. Conversely, Steep Hill analyzed samples at set dilutions as high as 1:20 for the initial analysis of each sample, possibly missing detections (i.e. false negatives) of minor cannabinoids that were detected by CannTest.

Each laboratory participated in a proficiency test (PT) event offered by Emerald for potency, a vendor specialized towards providing reference and PT materials for cannabis testing services. The PT sample consists of an acetonitrile matrix that was fortified with known amounts of THC and CBD constituents. This matrix does not require employing sample homogenization or preparation/extraction techniques required for a plant or edible matrix. Consequently, results generated for the PT study only assess the accuracy of the instrumental technique, not the full sequence of events employed at the laboratory for a plant or edibles matrix. Consequently, the overall analysis performance was not assessed by this study.

For the Fall 2017 Emerald PT event, both laboratories passed all five analytes offered in the potency study. Each analyte had an acceptable variability, as determined by the study's statistical analysis. All reported acceptance values were within ±20% of the true value. (According to Emerald, 41 laboratories participated in the Fall 2017 study and 36 passed all analytes.)

Precision. The entire sample is not used up in the analysis, so the design of subsampling and homogenization procedures are critical towards obtaining representative results. Documentation of the subsampling and homogenization procedures beyond the SOP discussions was not provided for either laboratory, so could not be verified as occurring and to what extent, if any, these procedures may contribute to variability in results between the two labs. Both laboratories indicated they followed their sample preparation SOPs, as written. However, the SOPs submitted by Steep Hill and CannTest for review by the EHL were dated "December 2017" and "1/26/2018", respectively, which is after the prep/analysis dates of the CannaBanana samples. For CannTest, the SOP is dated after the analysis of the edible and plant.

CannTest generated two duplicate samples by subsampling a second portion of two samples from another client (other than the AMCO samples). The processing of these duplicate samples yielded relative percent differences (RPDs) of 1.7% and 2.3%. However, it is unknown if the sample matrix of the CannTest duplicates is similar to that of the CannaBanana, edible, or plant samples, bringing into question the applicability of the these duplicate measurements.

Steep Hill generated duplicate samples of one AMCO sample in each submission, which yielded RPDs of 0.4% and 0.7% for THC.

EPA-published methods for similar instrument techniques allows for a maximum sample duplicate RPD of 20%.

Sample Preparation. The sample extractions the two laboratories described in their SOPs are similar in principle, but with differences. Both laboratories describe breaking up the entire sample to homogenize it prior to taking a representative sample of about 2 g, depending on the matrix. The differences are in the extraction solvents and the physical extraction techniques employed by each laboratory. Since neither laboratory generates quality control samples assessing the accuracy of the entire process, it is not possible to assess the existence, direction or bias for either technique or make a comparison of the techniques.

Microbial

Methodology. CannTest analyzes for *Aspergillus species* by quantitative polymerase chain reaction (qPCR) assay using a Medicinal Genomics qPCR system. Steep Hill analyzes for *Aspergillus species* using cultural techniques from the US FDA's Bacteriological Analytical Manual (BAM) and Larone, D.H., 1995, "Medically Important Fungi: A Guide to Identification", 3rd ed. ASM Press, Washington D.C. CannTest also uses the qPCR method to analyze for *Salmonella* and *E.coli,* while Steep Hill uses cultural and antibody methods to analyze for *Salmonella* and *E.coli.*

Records necessary to fully evaluate adherence to each laboratory's Standard Operating Procedure (SOP) were not available upon request for both laboratories; however, the records provided did not indicate either laboratory varied from their SOP for the analysis of *Salmonella* or *E.coli.* Both laboratories have SOPs containing modifications to either an equipment manufacturer's procedure or the BAM methods. CannTest did not make available method validation studies demonstrating the effects, if any, of these changes. Steep Hill submitted validation studies with their license application documenting the changes employed for their methods.

Specificity – The qPCR assay utilized by CannTest identifies a species of bacteria or fungi based on a DNA (or RNA) sequence, which is specific to a species.

The cultural procedure employed by Steep Hill, for *Aspergillus* species, incubates the sample on Dichloran Rose Bengal Chloramphenicol (DRBC) plates, a selective media. A selective media is designed to encourage the growth of a targeted species and/or inhibits the growth of competing species. DRBC encourages the growth of more than one genus and species. This scenario allows for the possibility of non-target species to grow on plates, which if the morphology is similar, leaves open the possibility of misidentification. If growth occurs the analyst will look at macroscopic and microscopic morphology to identify the species.

Cultural techniques vary greatly in specificity; for example, Steep Hill uses a chromogenic agar medium, called HardyCHROM Salmonella, which produces colonies of a defined color in the presence of a specific enzyme. This type of media is far more specific than the type of media they use for Aspergillus testing, though still not as specific as molecular testing (e.g. qPCR).

Listed below, are some considerations for deciding if one type of method should be used over another for microbial testing.

- Specificity: There are many issues to consider when considering how target-specific the method should be. Is it better to have a test that will only confirm for selected organisms, or should it be able to detect similar organisms as well?
    - Similar organisms may or may not have similar physiological effects.
    - Does misidentification of a non-target species lead to higher pesticide/herbicide use?
- Sensitivity: A comparison study by Medicinal Genomics, which manufactures PCR testing kits, indicates PCR analysis identified contaminants more often than plating methods for Total Yeast Mold counts, when running the same sample.
- Living vs. dead organisms: Another consideration is if regulations are only concerned with living organisms. Cultural methods will only identify viable organisms, i.e. only those living organisms healthy enough to grow and reproduce. qPCR will identify DNA so organisms that are not viable or not living will still produce positive results. This consideration takes into account whether the concern is the organism itself, or possible toxins the organism produces. If the organism itself is the main concern, it is reasonable to only test for viable organisms. If toxins are the concern, testing for dead organisms may be more appropriate

Microbial Analysis. Documentation - Neither laboratory provided sufficient documentation of sample preparation protocols upon request, so review of the data assumes the laboratories followed SOP. Use of reagents, incubation temperature, or incubation time different than stipulated in the SOPs could have an adverse effect on microbial testing results. Upon request, the laboratories provided documentation of critical parameters; however, some of the documentation (e.g. incubation times and temperatures) was insufficient for demonstrating adherence to SOPs throughout the testing process.

Positive Control. Positive controls are used to show the method will result in positive identification if a target organism is present. CannTest runs a positive control with each batch. The data CannTest provided demonstrated passing positive controls, indicating the testing was in control and the reported negative results were not a result of a faulty testing method.

Steep Hill runs positive controls. Data provided for what the lab described as the most recent positive controls for *Aspergillus* were run June 27, 2017 and demonstrated acceptable results. The analysis time separation of the controls relative to the sample analysis submitted by AMCO is lengthy in comparison to other industry laboratories (e.g. drinking water) operating under a regulatory environment. Additionally, Steep Hill is using *Aspergillus brasiliensis* as its positive control in the analysis for *Aspergillus niger*.

Review of the positive control data provided by each laboratory did not suggest any deviations to the SOPs.

Extraction Control. An extraction control is used with qPCR (CannTest) to show the sample extraction was done properly. Extraction controls are not usually run with cultural methods (Steep Hill).

CannTest runs an extraction control with each sample set, using actual plant material vs. the isolated Single Copy Cannabis Gene (SCCG) recommended by the qPCR manufacturer (Medicinal Genomics). Positive results were achieved for related extraction controls, indicating the samples were properly extracted. This difference is believed to not have an adverse effect on the extraction control results; however, a validation study incorporating the change was not made available by CannTest.

Negative Control. Negative controls are run to demonstrate the analysis was free from contamination, which may cause false positives. Both laboratories ran negative controls with every batch for Aspergillus.

CannTest's negative controls were negative. Steep Hill's negative controls were also negative, indicating the so-identified *Aspergillus niger* growth identified for some samples was not a result of contamination introduced during the testing process. Negative controls for all microbial analyses were not submitted, but since both laboratories reported negative results for *Salmonella* and *E.coli*, there is no impact on the evaluation of the data.

III. CONCLUSIONS

Potency Analysis. This review was able to reproduce the qualitative and quantitative aspects of the reported results. Demonstration of intra-laboratory precision was evident for both laboratories, indicating each laboratory's data is reproducible. One goal of this review was determining the reason for the variability or precision values for the inter-laboratory comparison of results. This determination could not be made based on data because of a lack of accuracy checks throughout the entire preparation and analytical processes at both laboratories. Differently stated, this review could not determine which set of lab results best represents the true potency content of the matrices submitted by AMCO because the level of accuracy assessment is insufficient at both laboratories.

Microbial Analysis. The instrument results provided by CannTest demonstrate the absence of *Aspergillus niger* for all samples.

There was insufficient macroscopic and microscopic data (pictures and analyst observational notes) provided by Steep Hill to confirm the reported results. Macroscopic and microscopic pictures were provided by Steep Hill; however, the microscopic pictures were date stamped three weeks past the report date, which does not reflect observations at time of analysis. Other species of *Aspergillus*, like *Aspergillus brasiliensis*, have been noted to have a similar appearance to *Aspergillus niger* upon microscopic examination and may also grow on the selective media used by Steep Hill. In the absence of photographs or written observations from Steep Hill's examination, it cannot be determined if misidentification occurred due to mischaracterization or presence of another species with highly similar morphology to *Aspergillus niger*.

Given the lack of documentation on sample preparation and reagent information from either laboratory, adherence to SOPs could not be confirmed and sample preparation cannot be ruled out as a cause for the varying *Aspergillus niger* results. Some false negatives have been demonstrated for Total Yeast Mold analysis by qPCR if the wrong reagents are used in preparing the sample. Although the reagents actually used by

CannTest could not be confirmed beyond the SOP narrative, their controls demonstrated an appropriate response. For either methodology, spore clumping may lead to target organisms not being taken up in the analyzed aliquot, resulting in a false negative. While this cannot be ruled out or confirmed in this case, the available information indicates the samples contained an organism other than *Aspergillus niger*, rather than clumping leading to false negatives in three separate samples. Neither lab provided results of blind PTs for *Aspergillus*, so the ability of either lab to differentiate between *Aspergillus niger* and another organism has not been fully demonstrated.

Based on the available data, a factor in the difference of *Aspergillus niger* results between the two laboratories may arise from the varying specificity between the two methods.

Edibles and Flower Samples

Select elements used to evaluate the reported results for the edible and flower samples are missing. The results from both laboratories coincidentally agree.

IV. RECOMMENDATIONS

The initial audit by A2LA for both laboratories occurred before obtaining their operational licenses. A follow-up audit is recommended for verifying implementation of their SOPs, corrections implement in response to the A2LA audit report, institution of accuracy controls, and in the case of Steep Hill, incorporation of an *Aspergillus niger* positive control into the testing process. Additionally, several questions arose during the data review (e.g. nature of the materials used to create lab blanks), for which documentation was not made available by the labs upon request. The missing information hinders any assessment for completely determining adherence to SOPs.

Oversight of laboratories in Alaska State Regulations is limited to mention of AMCO specifying an entity to audit a laboratory as a condition to receiving a business license. Revising the regulations to include the option for AMCO to request follow-up audits once a lab is operational is recommended, both on a periodic (e.g. annually and for special purpose. The audits should establish and verify on an ongoing basis through audit activities, a list of methods that the auditing entity deems a laboratory has a demonstrated capability to report scientifically valid and defensible data.

Medicinal Genomics has published a new protocol (for 1g sample size) called the "California Protocol". If CannTest does not have a validation study for the current protocol, it is recommend they switch to the "California Protocol".

Alaska regulations should require laboratories develop quality assurance activities to characterize the accuracy, precision, and representativeness of all reported data. It is acknowledged the availability of reference material for cannabis constituents is limited. However, alternative methods of demonstrating quality parameters include, but are not limited to, the following:

- Use of compounds similar to the constituents of interest,
- Self-characterizing matrix material to use as a reference source, and
- Utilizing reference materials from more than a single vendor.