



# **SAMPLING AND TESTING TECHNICAL GUIDANCE FOR MARIJUANA PRODUCTS**

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## **Version 5.1**

This information is intended for use by licensed safety compliance facilities and licensed marihuana safety compliance facilities, collectively defined as laboratories, regulated by the Cannabis Regulatory Agency (MRA).

*This version of the technical guide combines and replaces all prior iterations published under the purview of MMFLA, MRTMA, MTA and the Administrative Rules.*

*This information does not constitute legal advice and is subject to change. Licensees are encouraged to seek legal counsel to ensure their operations comply with the Medical Marihuana Facilities Licensing Act and associated Administrative Rules.*

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## INTRODUCTION

The Cannabis Regulatory Agency (MRA) is committed to evidence-based decision-making when implementing technical guidance for licensed laboratories. As research into marijuana use, safety, and testing advances, this guide may be revised and updated to reflect these changes where appropriate. Upon licensing of a safety compliance facility/marihuana safety compliance facility, licensees must comply with all applicable statutes, administrative rules.

### **Rule (1) in the Marihuana Sampling and Testing Rule Set R 420.305 (1)**

A laboratory shall become accredited for all required safety tests in at least 1 matrix to the International Organization for Standardization (ISO), ISO/IEC 17025:2017, by an International Laboratory Accreditation Cooperation (ILAC) recognized accreditation body or by an entity approved by the agency within 1 year after the date the laboratory license is issued and agree to have the inspections, reports, and all scope documents sent to the agency.

### **Rule (2) in the Marihuana Sampling and Testing Rule Set R 420.305 (2)**

A laboratory shall use analytical testing methodologies for the required safety tests in subrule (3) of this rule that are based upon published peer-reviewed methods, have been validated for cannabis testing by an independent third party, and have been internally verified by the licensed laboratory according to Appendix J or K of Official Methods of Analysis authored by the Association of Official Analytical Collaboration (AOAC) International, with guidance from published cannabis standard method performance requirements where available.

### **Rule (3) in the Marihuana Sampling and Testing Rule Set R 420.305 (3)**

A laboratory must conduct the required safety tests specified in subdivisions (a) to (i) of this subrule on marihuana product that is part of the harvest batch or production batch as specified in R 420.303, except as provided in subrule (4) of this rule. The minimum testing portions to be used in compliance testing must be consistent with the testing portions used during method validation.

The sampling and analysis described in this guide must be conducted by a laboratory licensed by the MRA and accredited to ISO/IEC 17025:2017 by an ILAC recognized accreditation by 1 year after the date the license is issued. A laboratory must use analytical testing methodologies that are based upon published, peer-reviewed methods which have been validated for cannabis testing by an independent third party and have been internally verified by the laboratory. Please refer to administrative rules for additional guidance and clarification.

Analytical testing of marijuana for safety and potency is increasingly recognized as a critical and necessary component of the industry for several reasons <sup>[12]</sup>: Moreover, laboratory testing minimizes the risk of exposure to pesticides, microbes, heavy metals,

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molds, and residual solvents by providing consumers with information about the products they are purchasing and helps to prevent consumption by sensitive populations.

- Quantification of cannabinoid profiles and potency is available to the consumer to inform decision making and aids in determining appropriate dosing for individual use.
- Laboratory testing provides transparency and promotes a sense of reassurance to consumers as well as ensuring the quality of the marijuana product and that it is safe for consumers.

## **REQUIRED SAFETY TESTS AND ACTION LIMITS**

The MRA has established action limits for all required safety tests based on the most current literature. The laboratory must report the results of the testing by indicating “pass” or “fail” in the Metrc system and on the Certificate of Analysis (COA). The results in the Metrc system and the COA should be identical aside from the reported results of testing not required by the agency (i.e., terpenes). All results will be reported in parts per million (ppm) and to 3 decimal places unless otherwise specified.

- When reporting results for any analytes that were detected below the analytical method Limit of quantitation (LOQ), include the numerical LOQ and indicate “<LOQ” in the notes in the Metrc system.
- All products should be tested “as is” with minimal manipulation to the sample which could cause the results to no longer be representative of the product that will go to the consumer.

## **SAMPLING**

### **Rule (2) in the Marijuana Sampling and Testing Rule Set R 420.304 (3)**

A laboratory shall collect samples of a marijuana product from another marijuana business, and that marijuana business shall not interfere or prevent the laboratory from complying with all of the following requirements: (n) The agency may publish guidance that must be followed by marijuana businesses for chain of custody documentation.

The laboratory chain of custody must include the **complete** Metrc identification number, name of product, type of product, number of containers, weight of both the source package and test sample.

## **ACTION LIMITS, LOQ’s AND MINIMUM TEST PORTIONS**

### **Rule (2) in the Marijuana Sampling and Testing Rule Set R 420.304 (3)**

The agency shall publish a list of action limits for the required safety tests in subrule (3) of this rule, except for potency. A marijuana sample with a value that exceeds the

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published action limit is a failed sample. A marijuana sample that is at or below the action limit is a passing sample.

## POTENCY

Please refer to R. 420.305 (9) and R. 420.305(3)(a). Laboratories may analyze additional cannabinoids with approval from the agency based on an acceptable method validation and successful accreditation from ISO.

The laboratory must analyze a minimum 0.5 gram representative sample of marijuana product to determine the cannabinoid profile. The laboratory must establish a limit of quantitation (LOQ) of 1.0 mg/g or lower **for all required cannabinoids** analyzed and reported.

## RESIDUAL SOLVENTS

The laboratory must analyze a minimum 0.25 gram representative sample to assess the presence of residual solvents in accordance with their associated action limits (Table 1).

**Table 1. Action Limits for Residual Solvents.**

Solvent	CAS Number	Action Limit for Inhaled Products (ppm)	Action Limit for Non-inhaled Products (ppm)
1,2-Dichloroethane	107-06-2	2.00	5.00
Acetone	67-64-1	750	5000
Acetonitrile	75-05-8	60.0	410
Benzene	71-43-2	1.00	2.00
Butanes all isomers*	106-97-8	800	5000
Chloroform	67-66-3	2.00	60.0
Ethanol	64-17-5	1000	5000
Ethyl acetate	141-78-6	400	5000
Ethyl ether	60-29-7	500	5000
Ethylene oxide	75-21-8	5.00	50.0
Heptane	142-82-5	500	5000
Hexanes all isomers^	110-54-3	50	290
Isopropyl alcohol	67-63-0	500	5000
Methanol	67-56-1	250	3000
Methylene chloride	75-09-2	125	600
Pentanes all isomers+	109-66-0	750	5000
Propane	74-98-6	2100	5000
Trichloroethylene	79-01-6	25.0	80.0
Toluene	108-88-3	150	890
Total xylenes (ortho-, meta-, para-)	1330-20-7	150	2170

\* Butane isomers include 2-methylpropane or isobutane CAS Number 75-28-5.

+ Pentane isomers include isopentane (methylbutane) CAS Number: 78-78-4 & neopentane (dimethylpropane) CAS Number: 463-82-1 Y



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^ Hexane isomers 2,2-Dimethylbutane CAS Number: 75-83-2, 2,3-Dimethylbutane CAS Number: 79-29-8, 2-Methylpentane CAS Number: 107-83-5, 3-Methylpentane CAS Number: 96-14-0

## HEAVY METALS

The laboratory must analyze a minimum 0.5 gram representative sample of marijuana products to assess the presence of heavy metals in accordance with their associated action limits based on a 5 gram/day consumption of marijuana (Table 2).

The LOQ for heavy metals must be  $\frac{1}{2}$  the action limit.

**Table 2. Heavy Metal Action Limits.**

Heavy metal	Action Limit for Inhaled Marijuana flower (ppm)	Action Limit for Inhaled Marijuana concentrates (ppm)	Action Limit for other Marijuana products (ppm)
Lead	1.00	0.500	0.500
Inorganic Arsenic	0.400	0.200	1.500
Mercury	0.200	0.100	3.000
Cadmium	0.400	0.200	0.500
Total Chromium	1.200	0.600	2.000
Nickel*	1.000	0.500	N/A
Copper*	N/A	3.000 <sup>+</sup>	N/A

+ Copper is required for vaping products only.

## FOREIGN MATTER ANALYSIS

The laboratory should perform foreign material testing on not less than 30% of the total representative sample of intact buds and flower material prior to sample grinding or milling.

1. The buds should be separated into no less than 10 increments. The results from which can be averaged together as total foreign matter contamination.
2. Dissection of nodes should be done whenever physically possible.
3. If dissection of distinct nodes is deemed unnecessary, due to the small and compact nature of the buds (“popcorn” buds), the buds then should be examined in their entirety and additionally cut in half to observe the inside portion.
4. In the case of marijuana trim, kief, concentrate or infused product, the calculation to determine 30% of the sampling batch should be included in the SOP.
5. Quantitation of filth should be done as a total surface area calculation. The laboratory-derived calculation should be included in the SOP.
6. If a sample fails for foreign matter, the laboratory should include a note in Metrc listing all contaminants identified.

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When the laboratory performs foreign material testing, the laboratory must do all of the following:

1. Develop a procedure and associated training documents for the identification of powdery mildew, pests, mold and other foreign material which requires agency approval before testing. The training document must include a reference text for common cannabis contaminants.
2. Examine both the exterior and interior of the sample. Samples must be viewed on both high and low power magnifications. Pests and other foreign contaminants typically do not sit on the outside of the material and will require the technician to dissect the material while viewing under a microscope.
3. All quantitation must be done on high power magnification and the laboratory must develop a grading scale which clearly dictates a failed sample.

#### **Example grading scale for pests:**

0-2 pests per HPF (high power field) = mild

2-4 pests per HPF = moderate

4+ pests per HPF = severe

All samples graded as severe are automatic failures.

0. All failures should be documented photographically.

#### **Analysis for Organic Matter**

The action limit for crude marijuana is not more than 5.0% of stems and not more than 2.0% of other foreign matter. All failures should be documented photographically. It is recommended that the laboratory clearly delineate calculation guidelines and ranges for total surface area contamination. Foreign matter analysis should be performed prior to all other testing, aside from microbials. The material remaining after foreign matter analysis is acceptable for all chemical testing, but should not be used for microbial testing. The amount of marijuana or marijuana product used for testing should be no less than 30% of the total gram weight or total sample lot obtained for compliance testing.

In the case of marijuana flower, the allotted 30% should come from separate, intact buds.

1. The buds should be separated into no less than 10 increments. The results from which can be averaged together as total foreign matter contamination.
2. Dissection of nodes should be done whenever physically possible.
3. If dissection of distinct nodes is deemed unnecessary, due to the small and compact nature of the buds ("popcorn" buds), the buds then should be examined in their entirety and additionally cut in half to observe the inside portion.
4. In the case of marijuana trim, kief, concentrate or infused product, the calculation to determine 30% of the sampling batch should be included in the SOP.

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5. Filth analysis should be performed at a low-power magnification.
6. Quantitation of filth should be done as a total surface area calculation. The laboratory-derived calculation should be included in the SOP.
7. If a sample fails for foreign matter, the laboratory should include a note in Metrc listing all contaminants identified.

### **Analysis for Inorganic Matter**

For these purposes, inorganic matter includes, but is not limited to, any material that would not normally be found on a living organism (plant) and includes materials such as glass, metal shavings, or synthetic fibers. In this case, the presence of any inorganic matter on any marijuana plant, concentrate, or infused product would result in an automatic failure for foreign matter. The observed matter should be documented in the results note included in Metrc.

### **WATER ACTIVITY ( $a_w$ )**

- A marijuana sample passes water activity testing if the water activity does not exceed 0.65  $a_w$ .
- An **edible** marijuana-infused product passes water activity testing if the water activity does not exceed 0.85  $a_w$ .
- Non-edible marijuana-infused products are **not** subject to water activity testing.
- Marijuana infused beverages are **not** subject to water activity testing with approval from the agency.

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## MICROBIALS

Any detection that exceeds the published action limit is considered a failure based on the criteria for acceptability (Table 3).

**Table 3. Microbial Screening Action Limits (CFU/g).**

	<b>Bud, shake/trim, and kief from Harvest Batch (CFU/g)</b>	<b>Marijuana-Infused Product (CFU/g)</b>
<b>Medical Total Yeast &amp; Mold Count</b>	10,000	10,000
<b>Adult-Use Total Yeast &amp; Mold Count</b>	100,000	10,000
<b>Total Coliform</b>	1000	100
<b>Shiga toxin-producing <i>E. coli</i> (STEC)</b>	Not detected in 1 gram	Not detected in 1 gram
<b><i>Pathogenic Salmonella spp.</i></b>	Not detected in 1 gram	Not detected in 1 gram
<b><i>Aspergillus flavus, fumigatus, niger &amp; terreus</i></b>	Not detected in 1 gram	Not detected in 1 gram

## CHEMICAL RESIDUE

### Rule 5(11) in the Marijuana Sampling and Testing Rule Set R 420.305 (11)

For chemical residue and target analyte testing, the agency shall publish a list of quantification levels. Any result that exceeds the action limit is a failed sample.

The laboratory must analyze a minimum 0.5-gram representative sample for analysis of chemical residues.

**Table 4. List of Banned Chemical Ingredients: Action Limit and LOQ <sup>[11]</sup>.**

<b>Analytes</b>	<b>CAS Number</b>	<b>Action Limit (ppm)</b>	<b>LOQ (ppm)</b>
Abamectin	71751-41-2	0.5	0.25
Acephate	30560-19-1	0.4	0.2
Acequinocyl	57960-19-7	2	1
Acetamiprid	135410-20-7	0.2	0.1
Aldicarb	116-06-3	0.4	0.2
Azoxystrobin	131860-33-8	0.2	0.1
Bifenazate	149877-41-8	0.2	0.1
Bifenthrin	82657-04-3	0.2	0.1
Boscalid	188425-85-6	0.4	0.2



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Carbaryl	63-25-2	0.2	0.1
Carbofuran	1563-66-2	0.2	0.1
Chlorantraniliprole	500008-45-7	0.2	0.1
Chlorfenapyr	122453-73-0	1	0.5
Chlorpyrifos	2921-88-2	0.2	0.1
Clofentezine	74115-24-5	0.2	0.1
Cyfluthrin	68359-37-5	1	0.5
Cypermethrin	52315-07-8	1	0.5
Daminozide	1596-84-5	1	0.5
DDVP (Dichlorvos)	62-73-7	1	0.5
Diazinon	333-41-5	0.2	0.1
Dimethoate	60-51-5	0.2	0.1
Ethoprophos	13194-48-4	0.2	0.1
Etofenprox	80844-07-1	0.4	0.2
Etoxazole	153233-91-1	0.2	0.1
Fenoxycarb	72490-01-8	0.2	0.1
Fenpyroximate	134098-61-6	0.4	0.2
Fipronil	120068-37-3	0.4	0.2
Fonicamid	158062-67-0	1	0.5
Fludioxonil	131341-86-1	0.4	0.2
Hexythiazox	78587-05-0	1	0.5
Imazalil	35554-44-0	0.2	0.1
Imidacloprid	138261-41-3	0.4	0.2
Kresoxim-methyl	143390-89-0	0.4	0.2
Malathion	121-75-5	0.2	0.1
Metalaxyl	57837-19-1	0.2	0.1
Methiocarb	2032-65-7	0.2	0.1
Methomyl	16752-77-5	0.4	0.2
Methyl parathion	298-00-0	0.2	0.1
MGK-264	113-48-4	0.2	0.1
Myclobutanil	88671-89-0	0.2	0.1
Naled	300-76-5	0.5	0.25
Oxamyl	23135-22-0	1	0.5
Paclobutrazol	76738-62-0	0.4	0.2
Permethrins*	52645-53-1	0.2	0.1
Prallethrin	23031-36-9	0.2	0.1
Phosmet	732-11-6	0.2	0.1
Propiconazole	60207-90-1	0.4	0.2
Propoxur	114-26-1	0.2	0.1
Pyridaben	96489-71-3	0.2	0.1
Pyrethrins+	8003-34-7	1	0.5
Spinosad	168316-95-8	0.2	0.1
Spiromesifen	283594-90-1	0.2	0.1
Spirotetramat	203313-25-1	0.2	0.1
Spiroxamine	118134-30-8	0.4	0.2
Tebuconazole	80443-41-0	0.4	0.2
Thiacloprid	111988-49-9	0.2	0.1

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Thiamethoxam	153719-23-4	0.2	0.1
Trifloxystrobin	141517-21-7	0.2	0.1

\* Permethrins should be measured as cumulative residue of cis- and trans-permethrin isomers (CAS numbers 54774-45-7 and 51877-74-8).

+ Pyrethrins should be measured as the cumulative residues of pyrethrin 1, cinerin 1 and jasmolin 1 (CAS numbers 121-21-1, 25402-06-6, and 4466-14-2 respectively)

## TARGET ANALYTES

“Target Analyte” is defined as non-marihuana inactive ingredient designated for analysis.

Vitamin E Acetate has been identified as a target analyte by the MRA. All vape cartridges must be tested for Vitamin E Acetate.

**Table 5. Target Analytes for Marihuana Products Intended for Inhalation.**

Target Analyte	(CAS) Number	LOQ (ppm)
Vitamin E Acetate	58-95-7	100

## MYCOTOXINS

### Rule (3) in the Marihuana Sampling and Testing Rule Set R 420.306 (3)

Products that failed testing for Aspergillus may be remediated after subsequent testing for mycotoxins in accordance with R 420.305(3)(h).

The laboratory must analyze a minimum 0.5-gram representative sample for analysis of mycotoxins.

### Rule (20) in the Marihuana Sampling and Testing Rule Set R 420.305 (20)

The agency may request mycotoxin testing. A marihuana sample with a value that exceeds the published acceptable level is a failed sample. A marihuana sample that is below the acceptable value is a passing sample.

**Table 6. List of Required Mycotoxins and Accepted Detection Limits.**

Mycotoxin	PPB
Aflatoxin B1	<20
Aflatoxin B2	<20
Aflatoxin G1	<20
Aflatoxin G2	<20
Ochratoxin A	<20

## HOMOGENEITY

Homogeneity testing or the process of homogeneous sampling, is when all items in a sample are chosen at random to be representative of product batch so they have similar

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or identical traits. **To perform homogeneity testing each dose or serving must be treated as a separate individual sample and a total of 10 doses or servings must be sampled at random.** The weight and concentration of Delta-9 THC must be recorded and the variability of weight and concentration of Delta-9 THC among servings in a single package must not exceed +/- 15%.

This can be determined by first calculating the Standard Deviation (SD) among samples and subsequently calculating the Relative Standard Deviation (RSD) for both weight and concentration of Delta-9 THC.

The SD is calculated using the following formula:

$$SD = \sqrt{\frac{(sample1 - mean) + (sample2 - mean) , \dots , (sample10 - mean)}{total\ number\ of\ samples - 1}}$$

The RSD is calculated using the following formula:

$$RSD = \frac{SD}{mean} \times 100$$

The MRA has determined that the laboratories should complete potency testing based on the information provided by the processors at the time of the sampling event. If a product is sampled as an individual serving/dosage, the facility will report total THC by serving/dose. If the product is sampled in final form, total THC content for the package will be reported.

It is not incumbent on the laboratory to determine if the product will meet the package labeling requirements. The laboratory testing results provided on the package will report the calculated THC as the product was submitted for testing and is not required to replicate the processor designated package label for THC content. The processor designated package label will be considered the target THC.

## pH

pH is a quantitative measure of the acidity or basicity of aqueous or other liquid solution and is a required test for marijuana infused beverages. Any laboratory seeking approval to test marijuana infused beverages must follow AOAC Method 945.10 and should conduct analyses and validations in accordance with all manufacturer specifications.

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**Table 7. Marijuana Testing Requirements.**

	<b>Raw Plant Material</b>	<b>Non-Solvent Concentrate <sup>a</sup></b>	<b>Inhalable Concentrate (Solvent based)</b>	<b>Vape Concentrate</b>	<b>Inhalable Compound Concentrate Products <sup>c</sup></b>	<b>Marijuana -Infused Product</b>
<b>Vitamin E Acetate</b>				√		
<b>Homogeneity</b>						√
<b>Potency Analysis</b>	√	√	√	√	√	√
<b>Foreign Matter Inspection</b>	√	√			√	√
<b>Microbial Screen</b>	√	√			√	√
<b>Water Activity</b>	√				√	√ <sup>b</sup>
<b>Heavy Metal Screen</b>	√	√	√	√	√	√
<b>Residual Solvents</b>			√	√	√	√
<b>Chemical Residue Analysis</b>	√	√	√	√	√	√

<sup>a</sup> Extraction using ice water, rosin press or dry ice

<sup>b</sup> Not required for non-edible marijuana product or beverages with CRA approval.

<sup>c</sup> Moonrock, Caviar joint, infused pre-roll, tarantula etc.



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## RECOMMENDATIONS FOR QUALITY CONTROL

Rule (5)(b) in the Marijuana Sampling and Testing Rule Set R 420.305b(5) A laboratory must prepare a written description of its quality control activities, included as part of a quality control manual.

All of the following items should be addressed in the quality control manual:

- (a) Daily, weekly, monthly, and annual requirements.
- (b) An analytical testing batch.
- (c) All analytical testing runs must be bracketed with quality controls. (6)

Method specific quality control acceptance criteria, which must be followed.

## RECOMMENDATIONS

### Analytical Batch

- Should be clearly defined as every 20 samples.

### Laboratory Reagent Blank

- Checks for background contamination should be the first sample of the analytical run; a blank should also be run before and after a calibration check (CC), initial calibration verification (ICV) /continuing calibration verification (CCV) and at the end of the run.

### ICV/CCV

- Initial Calibration Verification (ICV) – the ICV is performed by analyzing a test solution of known analyte concentration(s) after calibration, but before sample testing on the initial day of the calibration. The ICV should be a standard that is not from the same vendor/lot that is used for the calibration curve.
- Continuing Calibration Verification (CCV) – the CCV is performed by analyzing a test solution of known analyte concentration(s) prior to sample testing on each testing day and continued periodically during the analytical batch run, no less frequently than once after each set of 20 samples. The CCV should be a standard that is not from the same vendor/lot that is used for the calibration curve **IF** calibration is maintained for more than one week.

### Laboratory Fortified Matrix (LFM) / Laboratory Fortified Matrix Duplicate (LFMD)

- Analyze a sample with a known amount of standard added (spike).
- For the LFMD, spike the same sample a second time (duplicate).

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- Calculate Relative Percent Difference (RPD) between spiked sample and spiked duplicate, target value should be close to the first value and have a small RPD (less than 20%).
- Spike volume should be less than 1% of the volume. Example: spike with 1 mL of 1000 mg/L into 100 mL sample will equal a 10 mg/L increase in concentration.

### **Duplicate**

- Analyze the same sample twice, this must be two separate preparations. The sample should be chosen at random and run together on the same analytical run.
- Calculate the relative percent difference (RPD) between first sample and replicate, target value should be close to the first value and have a small RPD (less than 20%).
- Variability may be introduced during sample preparation. To account for this, if more than one staff member is prepping samples, a sample duplicate for each set of prepared samples must be run.
- All validated approved methods have been demonstrated to be precise, therefore laboratories are **NOT** permitted to run samples in duplicate and average the results.

## **ANNUAL REQUIREMENTS**

### **Demonstration of Capability (DOC)**

- Each analyst must have a DOC which includes documentation that they can accurately run each test.
- Documentation that an analyst has read and understands all appropriate SOPs and methods.
- Backup analysts should do this once a year or any time there is a reason to question competence.
- Competency assessments should be completed not less than annually including having staff run a previously reported sample from sample preparation through result reporting to assure all staff are following the written SOPs.

### **Method Detection Limit (MDL)**

- Run at least seven samples at low levels following procedure outlined below, i.e., daily requirements.

### **Corrective Action**

- Corrective actions must be included in the SOPs for each method and should include what to do if QC tests fail or are out of range. For example, if standards fail, then recalibrate and run test again.

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## QC Acceptance

- Include in the SOP for each method the acceptance ranges for standards, duplicates, spikes, etc., and verify that they match the method requirements.

## QC ACCEPTANCE CRITERIA\*

\*unless otherwise specified in reference method.

- LRB < MDL
- ICV/CCV  $\pm$  10%
- LFM/LFMD  $\pm$  20%
- RPD < 20%
- Reporting limit = MDL Calculations
- % Recovery for LFB =  $\frac{\text{LFB Result} \times 100\%}{\text{Expected Concentration}}$
- RPD – relative percent differences for duplicates and LFM/LFMD

$$\text{RPD} = (|\text{Num1}-\text{Num2}| / ((\text{Num1}+\text{Num2}) / 2)) \times 100$$

Where:

Num1= Original Number

Num2= Second Number

- % Recovery for LFM – when using less than or equal to 1% spike volume compared to sample volume

$$\% \text{ Recovery} = \frac{\text{LFM Result} - \text{Sample Result}}{\text{Sample Result}} \times 100\%$$

## DEFINITIONS

A variety of definitions relating to detection limits and quantitation limits are published in reference literature and by government agencies, however, universally accepted procedures for calculating these limits do not exist. The definitions below attempt to clarify the meaning of these terms as recognized by the CRA.<sup>[13]</sup>

**Analytical batch size:** An analytical batch is defined as 20 samples excluding QC.

**Calibration check (CC):** Should be analyzed prior to sample analysis and every 10 – 20 samples thereafter (or after a 12-hour period, should less than 20 samples be analyzed). The CCV controls are generally created from the same source as the calibration material. The laboratory must outline acceptance criteria as relevant to the method; ideally, the CCV should fall within at least  $\pm$  15% of the spike value.

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**Calibration requirements:** Calibration must occur not less than monthly. At the beginning of each day samples are to be analyzed, a calibration curve composed of four or more points including all target analytes should be generated according to the approved SOP. Where the determinative time is extensive and the instrument is stable, the calibration curve should be initially developed; thereafter, each day that samples are to be analyzed, this curve should be verified by analysis of a calibration check (CC) following the requirements listed below. The check must be +/- 10% of the known value.

**Continuing calibration verification (CCV):** This verification should be done at both the beginning and end of the analyses, including at least one standard for each of the target analytes at the expected concentration range. It is recommended that a calibration standard of one component of a multicomponent analyte also be analyzed each day or work shift. All checks must be within 10% of the known value or the instrument is to be recalibrated as specified in the calibration requirements.

**Demonstration of capability (DOC):** Each analyst should be assigned a file in which records are maintained that demonstrate the capability of the individual to perform analyses for which they are responsible on frequent or infrequent basis. At a minimum, the analyst should calibrate and analyze four standards to demonstrate their ability to run each test. Additionally, the file should contain documentation (signed form) that the analyst has read and understands all assigned SOPs and methods. Backup analysts should do this once a year. The primary analyst should recalculate DOCs when a change in the method, analyst, or instrument is made which could affect precision, accuracy, or sensitivity. Minor changes in methodology should prompt a check to verify that the precision, accuracy, and sensitivity have been maintained.

**Initial demonstration of capability (IDOC):** Before beginning the analysis of samples, an IDOC must be performed. The IDOC includes a demonstration of the ability to achieve a low background, the precision and accuracy required by the method, and determination of the limit of detection (LOD) (see below). An IDOC should be performed for each instrument. It is also recommended that an IDOC be performed by each analyst. In addition, it is recommended that the IDOC also addresses the variability introduced if more than one sample preparation analyst is used. Precision, accuracy, and LOD should be comparable for each technician.

**Laboratory fortified sample matrix:** Laboratory fortified sample matrix requirements stated in the methods must be met. If there are no laboratory fortified sample matrix requirements in the method, the following guidelines are to be used: The laboratory should add a known quantity of analyte(s) to a percentage (to be described in the approved SOP) of the routine samples to determine sample matrix interference; the fortified concentration should not be less than the concentration of the sample selected for fortification unless specified by the method; if the sample concentration is unknown or less than detectable, the analyst should choose an appropriate concentration (e.g., a percentage of the published action limit or mid-point in the calibration range); over time, samples from all routine sources should be fortified; the procedure should be described



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in the SOP; if any of these checks are not within the criteria specified in the method or control limits specified in this document, and the laboratory performance is in control, the result for that sample should be flagged to inform the data user that the results are suspect due to matrix effects.

**Laboratory reagent blank (LRB):** An LRB should be carried through the full analytical procedure with every sample batch. In general, results from LRBs should not exceed the laboratory's minimum reporting limit (MRL), the lowest concentration of standard used for quantitation.

**Limit of detection (LOD) or detection limit:** The lowest concentration level that can be determined to be statistically different from a blank (99% confidence). The LOD is typically determined to be in the region where the signal to noise ratio is greater than five. Limits of detection are matrix, method, and analyte specific. The LOD is approximately equal to the MDL for those tests which the MDL can be calculated.

**Limit of quantitation (LOQ):** The minimum concentration or mass of an analyte in a target matrix that can be reported as a quantitative result. For all analyses aside from Target Analytes, the LOQ must be at a minimum 1/2 of the published action limit when the LOQ is not specified in the SMPR. The analyst should determine LOQs when any change is made which could affect the LOQs, or more frequently if required by the method. In addition, the analyst must demonstrate low-level capability on an ongoing basis through an LOQ determination or repeated low-level analyses.

**Linear calibration range (LCR):** The region of a calibration curve within which a plot of the concentration versus the response of a particular analyte remains linear, and the correlation coefficient of the line is approximately 1 (0.995 for most analytes). The plot may be normal-normal, log-normal, or log-log when allowed by the analytical method.

**Low level quantitation:** The laboratory's minimum reporting limits (MRL) should be reported to the client along with the data. The reporting limit must be below the CRA's published action limits. Laboratories should run an LFB at their MRL every analysis day and should not report contaminants at levels less than the level at which they routinely analyze their lowest standard.

**Method detection limit (MDL):** The minimum concentration of a substance that can be measured and reported with 99% confidence that the analyte concentration is greater than zero and is determined from analysis of a sample in a target matrix containing the analyte. The MDL procedure is designed to be a straightforward technique for estimation of the detection limit for a broad variety of physical and chemical methods. The procedure requires a complete, specific, and well-defined analytical method. It is essential that all sample processing steps used by the laboratory be included in the determination of the MDL. To calculate MDLs, please follow this [procedure](#).

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