California and Cannabis

Paul B. Kimsey Ph.D.
State Public Health Laboratory Director
California Department of Public Health

Olivera Marjanovic-Peyron Ph.D.
Research Scientist
Food and Drug Laboratory Branch

Agenda

- Introduction to CA Cannabis Law, State Licensing Authorities and CDPH role and responsibilities
- Introduction to Cannabis Testing Section (CTS)
- Cannabis testing developments in response to CA regulations
- Overview of microbiologic testing plan in CTS, method validation data
- Overview of chemical testing plan in CTS, method validation data
- ISO 17025 Accreditation

Progression of CA Cannabis Law

1996	• Proposition 215, Compassionate Use Act
2003	• Senate Bill 420, Medical Marijuana Program (MM ID Card)
2015	• Medical Cannabis Regulation & Safety Act (MCRSA)
2016	• Adult Use of Marijuana Act (Prop. 64)
2017	• Medicinal and Adult-Use Cannabis Regulation and Safety Act (MAUCRSA) - SB 94 and AB 133

CDPH

State Licensing Authorities

CA Department of Food & Agriculture

CalCannabis Cultivation Licensing

Cultivators

Track-and-Trace

CA Department of Public Health

Manufactured Cannabis Safety Branch (MCSB)

Manufacturers

Bureau of Cannabis Control (Bureau)

Retailers

Distributors

Testing Labs

Microbusinesses

CDPH – Role and Responsibilities

Role: Protect public health by promoting product and workplace safety

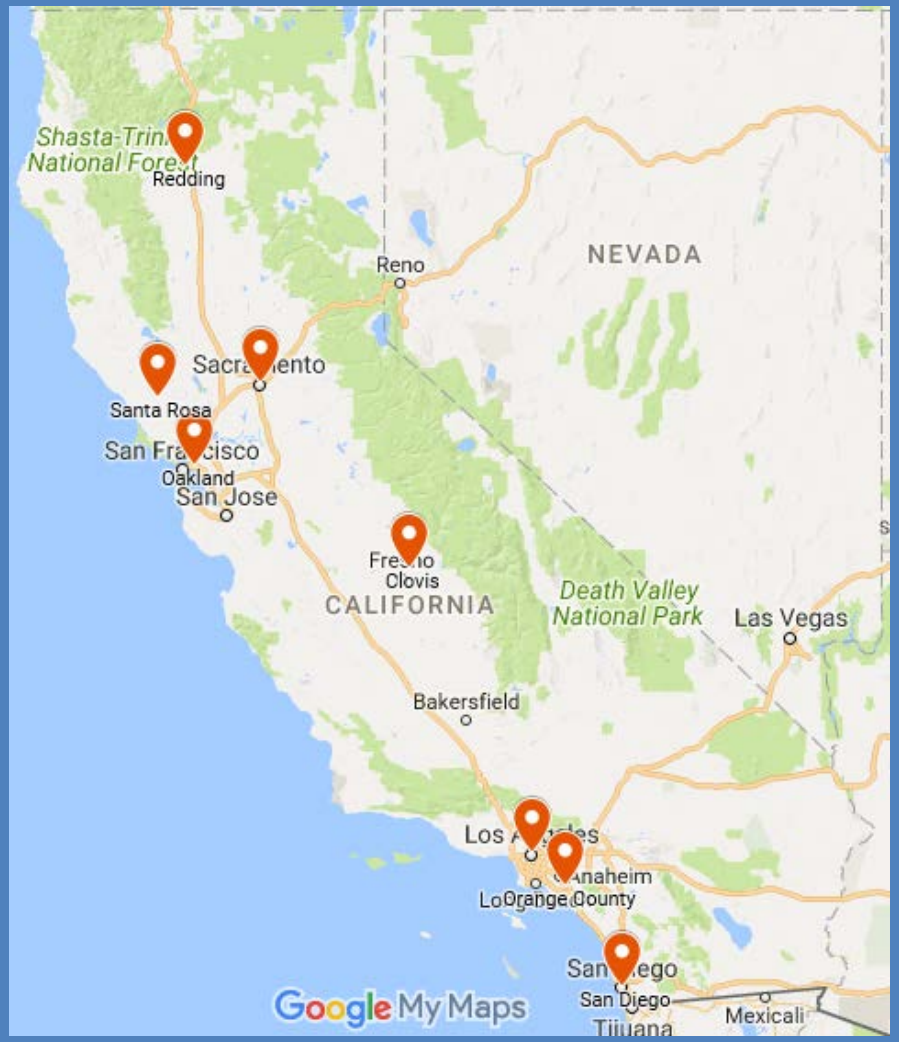
Manufactured Cannabis Safety Branch

- Regulations
- Licensing
- Compliance

CDPH - Cannabis Regulations Evolution

• Starting in September of 2016, pre-regulatory workshops were held throughout CA (Redding, Sacramento, Santa Rosa, Oakland, Fresno, Los Angeles, Orange County, San Diego) to gain feedback on regulatory concepts, and to help inform public, industry and local communities on the regulations.

• Advisory Committee formed to advise licensing authorities (CDPH, BCC, CDFA) on the development of regulations that help protect public health and safety while reducing the illegal market for cannabis.



Cannabis Testing Section (CTS)

- Established in May of 2016, part of Food and Drug Laboratory Branch
- Staff were hired to assist the Bureau of Medical Cannabis Regulation (DCA) in development of regulations for licensing of 3rd party testing laboratories, and to test medical cannabis safety through microbiological and chemical analyses
- CTS's main function is to support CDPH's Manufactured Cannabis Safety Branch (MCSB). This includes analytical support of routine inspections, investigations and response to public health outbreaks.
- Purchasing equipment and supplies, training staff, developing and validating methods, planning for ISO 17025 accreditation
- Still in process of remodeling laboratory space ~2,000 sq ft
- Equipment (Chemistry, Filth and Microbiological testing) purchased and installed.
- Training – ongoing, Method Development in progress, Method Validations for critical methods scheduled to be completed by July 2018

§ 5714. Required Testing

CALIFORNIA CODE OF REGULATIONS TITLE 16 DIVISION 42. BUREAU OF CANNABIS CONTROL

- **Cannabinoids** - Tetrahydrocannabinol (THC), Tetrahydrocannabinolic Acid (THCA), Cannabidiol (CBD), Cannabidiolic Acid (CBDA), Cannabigerol (CBG), Cannabinol (CBN).
- Foreign material
- Heavy metals
- Microbial impurities
- Mycotoxins
- Moisture content and water activity
- Residual pesticides
- Residual solvents and processing chemicals
- Terpenes
- Homogeneity

BUREAU OF CANNABIS CONTROL
CALIFORNIA

ALL CANNABIS HARVESTED ON OR AFTER 1/1/2018 AND ALL CANNABIS PRODUCTS MANUFACTURED ON OR AFTER 1/1/2018, SHALL BE TESTED ACCORDING TO TITLE 16 OF THE CALIFORNIA CODE OF REGULATIONS, SECTION 5715, AND THE REGULATIONS THAT FOLLOW.

PHASE-IN OF REQUIRED LABORATORY TESTING	INHALABLE CANNABIS	INHALABLE CANNABIS PRODUCTS	OTHER CANNABIS & CANNABIS PRODUCTS
JANUARY 1, 2018			
Cannabinoids Testing	✔	✔	✔
Moisture Content Testing	✔		
Category II Residual Solvents and Processing Chemicals Testing		✔	✔
Category I Residual Pesticides Testing	✔	✔	✔
Microbial Impurities Testing (A. fumigatus, A. flavus, A. niger, A. terreus)	✔	✔	
Microbial Impurities Testing (Escherichia coli and Salmonella spp.)	✔	✔	✔
Homogeneity Testing of Edible Cannabis Products			✔
JULY 1, 2018			
Category I Residual Solvents and Processing Chemicals Testing		✔	✔
Category II Residual Pesticides Testing	✔	✔	✔
Foreign Material Testing	✔	✔	✔
DECEMBER 31, 2018			
Terpenoids Testing	✔	✔	✔
Mycotoxins Testing	✔	✔	✔
Heavy Metals Testing	✔	✔	✔
Water Activity Testing of Solid or Semi-Solid Edibles	✔		✔

STATE OF CALIFORNIA
dca
DEPARTMENT OF CONSUMER AFFAIRS

Bureau of Cannabis Control
1625 North Market Boulevard, Suite 202-S
Sacramento, CA 95834
(800) 952-5210

For the latest updates, follow the Bureau on social media

Current Microbiology Work

- Completed validations:
  - Detection of *Salmonella* in environmental swabs using a real-time PCR and culture based method (modified FDA BAM method)
  - Detection of *Salmonella* in chocolate using a real-time PCR and culture based method (modified FDA BAM method)
  - Detection of Shiga-toxin producing *E.coli* (STEC) in environmental swabs using a real-time PCR and culture based method (modified FDA BAM method).

- Developing methods or planned validations:
  - Detecting of *Salmonella* in plant material, various edibles, oils, etc.
  - Detecting Shiga-toxin producing *E. coli* (STEC) in plant material, various edibles, etc.
  - Detecting *Aspergillus niger, fumigatus, flavus,* and *terreus* in inhalable cannabis and cannabis products by real-time PCR
  - Bacterial and fungal strain identification by MALDI-TOF
  - Developing fungal culturing and detection methods by microscopy

Current Microbiology Work

- Methods Training/Evaluation:
  - Modified FDA BAM methods for detecting *Salmonella* and Shiga-toxin producing *E. coli (STEC)*
  - MALDI-TOF method for bacterial and fungal cell preparation for sample identification analysis
  - qPCR based kits for detection of pathogenic *Aspergillus* from cannabis and cannabis containing products

- Equipment training:
  - Real time qPCR instruments
  - MALDI-TOF
  - Commercial platforms for pathogen detection and confirmation

- Purchasing of equipment, supplies, reagents/kits in anticipation of meeting the sample workload requirements

- Hiring new staff

- ISO 17025 training and implementation

Microbiology Method Validation Requirements

- **§ 5713. Validation of Test Methods**
- (a) The laboratory may use a nonstandard, amplified, or modified test method or a method that is designed or developed by the laboratory to validate the methods for analyses of samples.

- (b) The laboratory shall follow the guidelines set forth in the US Food and Drug Administration's Guidelines for the Validation of Methods for the Detection of Microbial Pathogens in Foods and Feeds, 2nd Edition, 2015, incorporated herein by reference, to validate test methods for the microbial analysis.

Criteria	Requirement
Number of target organisms; inclusivity	5
Number of non-target organisms; exclusivity	5
Number of analyte levels per matrix: Qualitative methods	3 levels: high and low inoculum levels and 1 uninoculated level
Number of analyte levels per matrix: Quantitative methods	4 levels: low, medium and high inoculum levels and 1 uninoculated level
Replicates per food at each level tested	2 or more replicates per level

Microbial Pathogen Validation Workflow

Salmonella Validation Data for Swabs and Chocolate

	Organism	Strain	PCR Screen (%)[1]	Confirmed (%)[2]
Targets	**Salmonella enterica**	ATCC 8324 (low)	100	100
	Salmonella enterica	ATCC 8324 (high)	100	100
	Salmonella enterica	ATCC 13311 (low)	100	100
	Salmonella enterica	ATCC 13311 (high)	100	100
	Salmonella enterica	ATCC 13314 (low)	100	100
	Salmonella enterica	ATCC 13314 (high)	100	100
	Salmonella enterica	FDA-A49 (low)	100	100
	Salmonella enterica	FDA-A49 (high)	100	100
	Salmonella enterica	F15M02184 (low)	100	100
	Salmonella enterica	F15M02184 (high)	100	100
Non-Targets	**Escherichia coli**	ATCC 25922	0	-
	Klebsiella pneumoniae	ATCC 13882	0	-
	Pseudomonas aeruginosa	ATCC 27853	0	-
	Staphylococcus aureus	ATCC 33591	0	-
	Serratia marcescens	ATCC 14756	0	-
	Enterobacter aerogenes	ATCC 13048	0	-
	Media Control		0	-

[1]Percent positive out of two replicates. [2]Only samples testing positive in the screen were plated.

Salmonella* was detected, recovered and confirmed in 100% of all samples spiked with *Salmonella

CDPH

STEC Validation Data for Swabs

	Organism	Strain	PCR Screen (%)[1]	Confirmed (%)[2]
Targets	**Escherichia coli O157:H7**	ATCC 43888 (low)	100	100
	Escherichia coli O157:H7	F1701010-004 (low)	100	100
	Escherichia coli O157:H7	ATCC 43894 (low)	100	100
	Escherichia coli O157:H7	F06M00948-10 (low)	100	100
	Escherichia coli O157:NM	F1703020-001 (low)	100	100
	Escherichia coli O26:H11	CDC-272 (low)	100	100
	Escherichia coli O45:H2	CDC-267 (low)	100	100
	Escherichia coli O103:H2	CDC-269 (low)	100	100
	Escherichia coli O111:NM	F1702010-001 (low)	100	100
	Escherichia coli O121:H19	CDC-268 (low)	100	100
	Escherichia coli O157:H7	ATCC 43888 (high)	100	100
	Escherichia coli O157:H7	F1701010-004 (high)	100	100
	Escherichia coli O157:H7	ATCC 43894 (high)	100	100
	Escherichia coli O157:H7	F06M00948-10 (high)	100	100
	Escherichia coli O157:NM	F1703020-001 (high)	100	100
	Escherichia coli O26:H11	CDC-272 (high)	100	100
	Escherichia coli O45:H2	CDC-267 (high)	100	100
	Escherichia coli O103:H2	CDC-269 (high)	100	100
	Escherichia coli O111:NM	F1702010-001 (high)	100	100
	Escherichia coli O121:H19	CDC-268 (high)	100	100
Non-Targets	**Escherichia coli**	ATCC 25922	0	--
	Salmonella enterica	ATCC 8324	0	--
	Enterobacter aerogenes	ATCC 13048	0	--
	Pseudomonas aeruginosa	ATCC 27853	0	--
	Staphylococcus aureus	ATCC 33591	0	--
	Media Control		0	--

[1]Percent positive out of two replicates.
[2]Only samples testing positive in the screen were plated.

STEC was detected, recovered and confirmed in 100% of all samples spiked with STEC

CDPH

Current Chemistry Work

- Cannabinoid profile determination by UHPLC-DAD
  - THC, THCA, CBD, CBDA, CBG, CBN, THCV, CBC
  - Validation completed for Hemp Oil
  - Planning validation for cannabis concentrates and edibles

- Residual solvents and Terpenes tests using Headspace GC-MS/FID
  - Method developed for 20 residual solvent compounds listed in CA regulation and currently undergoing method validation
  - Method developed for 21 terpenes and currently undergoing method validation

- Contaminants Screening test using LC-MS
  - Method for synthetic cannabis screening from natural cannabis products developed and currently undergoing method validation

- Equipment Installation/Training
  - ICP-MS for Cadmium, Lead, Arsenic, and Mercury listed in CA regulation;
  - ELISA reader for Mycotoxin detection (total of aflatoxin B1, B2, G1, and G2, and ochratoxin A)

Chemistry Method Validation Requirements

- **§ 5713. Validation of Test Methods**
- (c) The laboratory shall follow the guidelines set forth in the US Food and Drug Administration's *Guidelines for the Validation of Chemical Methods for the FDA FVM Program*, 2nd Edition, 2015, incorporated herein by reference, to validate test methods for chemical analysis of samples.

- (1)The laboratory shall include and address the criteria listed below to validate test methods for chemical analyses of samples.

- (A) Accuracy; (B) Precision; (C) Linearity and range; (D) Calibration standard; (E) Sensitivity and selectivity; (F) Limit of detection and limit of quantitation; (G) Recovery; (H) Reproducibility; and (I) Robustness.

- (d) If available, the laboratory shall use cannabis reference materials or certified reference materials to validate test methods.

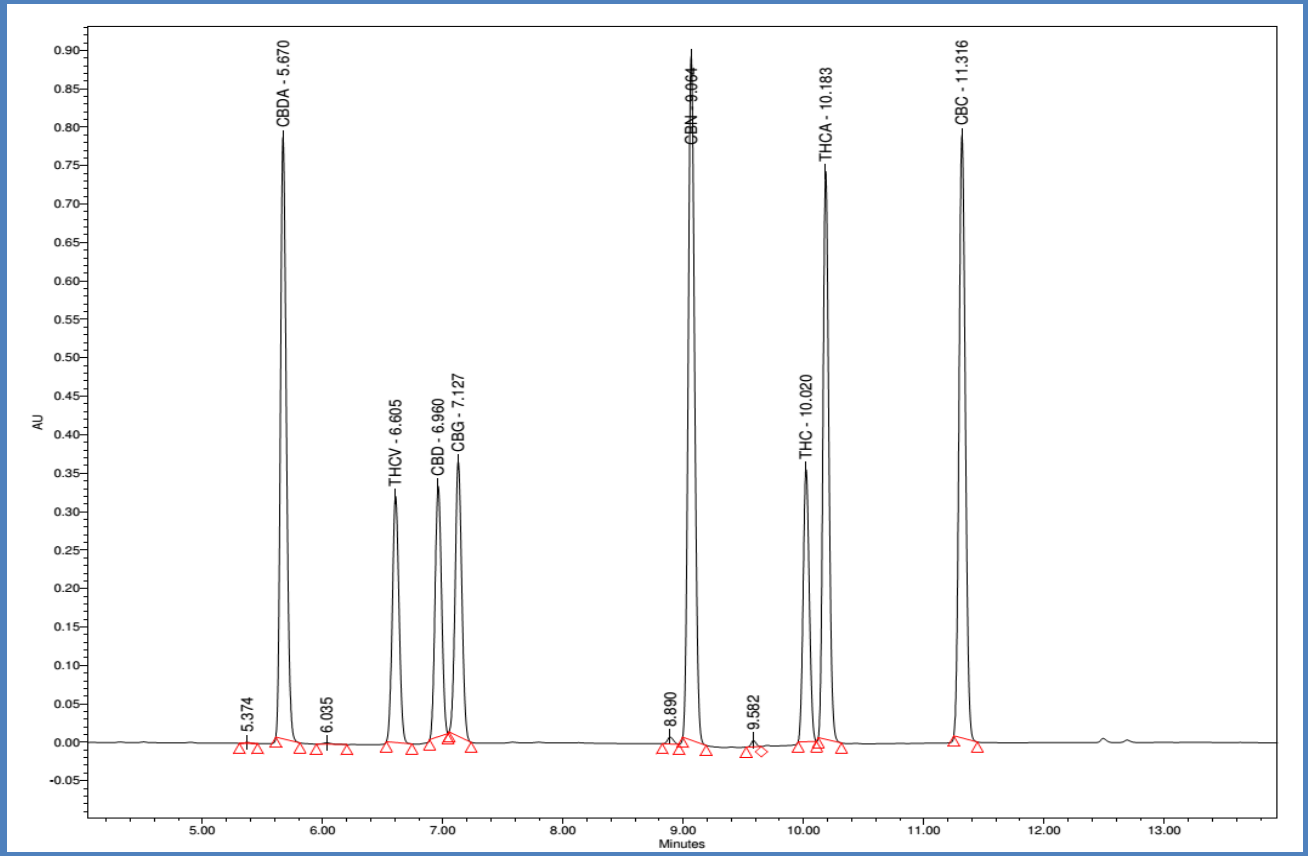
Cannabinoids Concentration Test using UHPLC-DAD

- Modified method based on Elizabeth Mudge, et al. "**Leaner and greener analysis of cannabinoids**" (Analytical and Bioanalytical Chemistry (2017) 409(12):3153-3163) - https://www.ncbi.nlm.nih.gov/pubmed/28233028#
- Column: Phenomenex Kinetex C18 100mm x 3.0 mm, 1.7 um with guard cartridge
- Mobile phase A: 10 mM ammonium formate, pH 3.6
- Mobile phase B: Acetonitrile
- Gradient Program:

Time (min)	Flow rate (mL/min)	% Mobile Phase A	% Mobile Phase B
0	0.8	48	52
8.0	0.8	34	66
8.5	0.8	30	70
13	0.8	20	80
15	0.8	20	80
15.1	0.8	48	52
22	0.8	48	52

- Flow Rate: 0.8 mL/min
- Detection: 228 nm
- Run time: total 22 min: 15 min + 7 min column re-equilibration
- Column Temperature: 25°C
- Autosampler Temperature: 4°C

Cannabinoids Concentration Test using UHPLC-DAD



Chromatogram of 100 ppm cannabinoids mix standards

Method Validation on Hemp Oil

Cannabinoids concentration in three different hemp oil samples

	Concentration in sample (ug/g)							
	CBDA	THCV	CBD	CBG	CBN	THC	THCA	CBC
Hemp Oil #1	42.7	10.1	7.87	ND	ND	ND	3.24	ND
Hemp Oil #2	ND	ND	11.1	ND	ND	ND	ND	ND
Hemp Oil #3	29.4	5.98	9.38	ND	ND	ND	2.05	ND

Inter-day precision (3-day RSD)

	RSD of Concentration in sample (ug/g)							
	CBDA	THCV	CBD	CBG	CBN	THC	THCA	CBC
Hemp Oil #1	2.8%	7.6%	2.8%	N/A	N/A	N/A	2.9%	N/A
Hemp Oil #2	N/A	N/A	6.3%	N/A	N/A	N/A	N/A	N/A
Hemp Oil #3	4.6%	7.6%	4.3%	N/A	N/A	N/A	2.6%	N/A

Method Validation on Hemp Oil – Spike Recovery

- Spike 1: spike 30 ug of CBD to hemp oil #2

Hemp Oil #2	Recovery
Spike 1	30 ug CBD
Day 1	80.0%
Day 2	78.9%
Day 3	74.6%

- Spike 2: spike 20 ug of each cannabinoids to hemp oil #2

Hemp Oil #2	Recovery							
Spike 2	CBDA	THCV	CBD	CBG	CBN	THC	THCA*	CBC
Day 1	93.0%	104.9%	86.8%	89.8%	93.7%	108.9%	67.7%	90.5%
Day 2	91.7%	104.2%	87.1%	89.2%	94.0%	110.0%	68.4%	90.4%
Day 3	86.4%	97.3%	79.9%	83.8%	87.3%	110.0%	64.1%	84.4%

* THCA recovery low may be due to matrix effect

Path to ISO 17025 Accreditation

- SOP writing
- Forms, recordkeeping
- Quality manual
- ISO 17025 compliance implementation in laboratory operations
- ISO 17025 standard training

How to reach us

Gordon A. Vrdoljak, Ph.D.
Cannabis Testing Section Chief, Food & Drug Lab Branch
CA Dept. of Public Health | 850 Marina Bay Pkwy, G365 | Richmond, CA 94804
Office: +1 510 412 3958 | Fax: +1 510 412 6280 | Gordon.Vrdoljak@cdph.ca.gov
Website: http://www.cdph.ca.gov/programs/DFDRS/Pages/FDLB-Contact.aspx

Microbiology contact:
Olivera Marjanovic-Peyron, Ph.D., PHM
Research Scientist III
Cannabis Testing Laboratory Section
Food and Drug Laboratory Branch
850 Marina Bay Parkway, G-365
Richmond, CA 94804
Office: +1 510 620 2894 | Fax: +1 510 412 6280 | Olivera.Marjanovic@cdph.ca.gov

Thank you

Resources:

Manufactured Cannabis Safety Branch

www.cdph.ca.gov/mcsb

mcsb@cdph.ca.gov

Cannabis Portal

www.cannabis.ca.gov