CANNABIS TESTING GUIDE

Organism	Medium	Cat. no.
Microbial Indicator Tests:		
General Culture	Tryptic Soy Agar (TSA), USP	G60
	Blood Agar Plate, 5% Sheep Blood in Tryptic Soy Agar (TSA) Base	A10
	Tryptic Soy Agar (TSA) Deep, USP	Q58
Aerobic Plate Count (APC)/ Total Plate Count (TPC)	Standard Methods Agar (Plate Count Agar/Tryptone Glucose Yeast (TGY) Agar)	G43
	Standard Methods Agar (Plate Count Agar/Tryptone Glucose Yeast (TGY) Agar) Deep	Q21
	Compact Dry™ TC, Total Plate Count,	54081
Yeast & Mold, Total Count	Compact Dry™ YM, Yeast and Mold	54083
	Compact Dry™ YMR, Yeast Mold Rapid	54084
	SabDex (Sabouraud Dextrose) Agar, USP	W70
Aspergillus spp., *Mucor* spp., *Penicillium* spp., and Thermophilic Actinomycetes	Potato Dextrose Agar, USP	W60
	Corn Meal Agar with Tween® 80, for chlamydospores	W10
	V9 Agar (V8 juice and potato flakes)	G98
	MycoVue™ Slide Culture System, Potato Flake Agar	MV1
Bile Tolerant Bacteria	EE Broth Mossel, USP	U291
	Violet Red Bile with Glucose Agar (VRBGA), USP	G178
Clostridium botulinum	Reinforced Clostridial Medium, USP	U172
	Egg Yolk Agar, Modified	G215
Coliforms, Total	Compact Dry™ EC, *Escherichia coli* and Coliforms	54082
	EMB Levine Agar (Eosin Methylene Blue)	G25
	HardyCHROM™ ECC, a chromogenic medium for *E. coli* and Coliforms	G303
	m Endo LES Agar	G28
	MacConkey Broth, USP	U125

Escherichia coli (STEC)	EC Broth with MUG (methylumbelliferyl glucuronide)	K64
	SHIBAM Agar	A146
	MacConkey Agar with Sorbitol (SMAC), for E. coli O157	G36
	CT-SMAC with Cefixime and Tellurite, for *E. coli* O157	G129
	HardyCHROM™ O157	G305
	Stx (Shiga-Toxin) Induction Broth	K274
Klebsiella spp.	MacConkey Agar, USP	G35
Pseudomonas aeruginosa	Cetrimide Select Agar, USP	G18
	Pseudomonas Agar F	G198
	Pseudomonas Agar P	G201
	Pseudomonas Isolation Agar	G219
Salmonella spp.	Rappaport-Vassiliadis Broth, USP	K246
	XLD Agar, USP	G65
	HardyCHROM™ Salmonella	G309
	Compact Dry™ SL, Salmonella	54085
	BioLine Salmonella ELISA Test Kit	CHB0081
Staphylococcus aureus	Compact Dry™ X-SA, *Staphylococcus aureus*	54086
	HardyCHROM™ Staphylococcus aureus	G311
	Mannitol Salt Agar (MSA), USP, for *Staphylococcus*	G40
	Vogel and Johnson Agar	G193
	Baird Parker Agar, for *Staphylococcus*	G96
Streptococcus spp.	Selective Strep Agar (COBA Medium)	A70
Dilution Buffers:		
Buffered Dilution Blanks	Dilu-Lok II™, Phosphate Buffer with Magnesium Chloride	D690
	Dilu-Lok II™, Phosphate Buffer with Magnesium Chloride	D699
	Dilu-Lok II™, Butterfield's Buffer	D590
	Dilu-Lok II™, Butterfield's Buffer	D599
Deionized Water	Water, Deionized	K259
	Water, Deionized	U85
	Dilu-Lok II™, Deionized Sterile Water	D090
	Dilu-Lok II™, Deionized Sterile Water	D099
Buffer Solutions	Buffered Sodium Chloride-Peptone Solution, USP	U255
	Phosphate Buffer pH 7.2	U438
Environmental Monitoring:		
	Tryptic Soy Agar (TSA) with Lecithin and Tween® 80,	P34

Surface Sampling	USP	
	SabDex (Sabouraud Dextrose) Agar	P36
	Malt Extract Agar with Lecithin and Tween®	P93

INTENDED USE

Hardy Diagnostics Cannabis Testing Guide is a general document regarding microbiological media and testing procedures for cannabis and cannabis products. Laboratories should check their current state requirements for a complete list of testing procedures for raw or processed cannabis to maintain compliance with local regulations. It is the responsibility of each laboratory to be fully aware of their state's current guidelines in order to meet and maintain appropriate regulatory requirements and/or licensure, where applicable.

This guideline is not intended to be used for the diagnosis of human disease.

SUMMARY

Cannabis, or marijuana as it's more commonly known, is a genus of flowering plant in the *Cannabaceae* family often used to make hemp products, used for medicinal or neutraceutical purposes, used to make extracts, tinctures and oils, or used as a psychoactive drug for recreational use. Cannabis plants produce cannabinoids, which are psychoactive substances responsible for the mental and physical side effects associated with cannabis use. Cannabis, along with tobacco and alcohol, is one of the most widely consumed drugs in the world.

As medical and recreational marijuana legalization at the state level increases, there is an increasing need for regulation of the plant and its by-products in order to ensure the safety of its use. Although every state differs slightly regarding testing regulations, many states do share basic testing specifications and pathogen screening requirements with existing regulatory bodies due to the potential impact caused by contaminants. Below are a few of the testing criteria used to assess the safety of cannabis or cannabis products. Please note, there may be other testing criteria besides microbial listed on state guidelines, such as chemical (i.e. cannabinoid), solvents, heavy metals, pesticide contamination, etc. not covered in this document. Please refer to the applicable state guideline for a complete list of testing requirements.

Bacterial Indicator Tests– Tests designed to determine the total aerobic plate count (TPC or APC), Gram-negative bacterial level, and total coliform level to detect the general contamination level of cannabis.

Fungal Indicator Tests – Tests designed specifically to assess fungal load and to detect the general contamination level caused by yeast and mold of cannabis.

Bacterial and Fungal Spores – Tests designed to assess the presence of bacterial and fungal spores that may impact patient health and safety, particularly for immunocompromised medicinal users.

***Escherichia coli* and *Salmonella* spp.** – Tests designed for pathogen detection. Because cannabis is a plant, it is susceptible to these potential environmental contaminants that may cause disease.

Mycotoxins – Tests to detect toxin levels. Mycotoxins are secondary metabolites produced by naturally occurring fungi that readily colonize crops or are commonly found in the environment on decomposing matter. The major groups of mycotoxins include aflatoxin, ochratoxin, citrinin, ergot, patulin, and fusarium. Aflatoxins are probably the most common major group, as well as the most toxic and carcinogenic. Detecting the presence of mycotoxins in cannabis products, especially in the context of medicinal cannabis, is vital to patient health and safety as they can be extremely toxic to humans.

Environmental Monitoring – Tests designed to assess the environmental quality of processing facilities and laboratories. Environmental monitoring of the processing plant or laboratory is also required in most states in order to meet quality assurance standards and to show evidence of good manufacturing and/or good laboratory practices.

Method Suitability – Testing laboratories should be conscious of the final delivery route (i.e. inhalation vs. ingestion, etc) of the cannabis product to ensure compliance with minimum testing standards. For example, edible products may need to be tested using guidelines for microbial food testing. Medicinal or nutritional products may follow more stringent guidelines using pharmaceutical standards. These types of sampling guidelines can be found in the state guidelines, or in supplemental reference materials such as the Food and Drug Administration Bacteriological Analytical Manual (FDA-BAM), or United States Pharmacopeia (USP) chapters <61>, <62>, and <2021>. Other USP chapters or reference materials may also apply as new standards and regulations are developed. The supplemental materials help outline some of the basic testing procedures for pathogens and contaminants, and suggest culture media and quality control procedures that may be useful for testing.

PRODUCT SPECIFICATIONS AND QC TESTING

Please refer to the Instructions for Use for the specific item on the Hardy Diagnostics online catalog for the product-specific specifications, storage, and QC testing criteria.

SUGGESTED PROCEDURES

Refer to the appropriate state or other regulatory guidelines for more information on the procedures listed below, or refer to the product-specific Instructions for Use on the Hardy Diagnostics online catalog, where applicable.

Below are examples of general sampling guidelines. There are a variety of procedures that may be utilized depending upon the organism of interest or product being tested. Please note it is the lab's responsibility to verify and validate the correct testing procedures to conform to their regulatory guidelines and/or licensure, where applicable.

Procedure for General Sample Preparation:

1. Place 10.0g of the sample in 100.0mL of sterile deionized water or appropriate buffer, and place the sample in a paddle blender (i.e. Stomacher, WhirlPak, or Blender bags). Mix the sample according to procedure to liberate microorganisms from the sample.

2. Take a 1.0mL aliquot of this suspension and pipet the sample to a separate dilution flask.

3. Make three subsequent 10X dilutions with fresh sterile deionized water or buffer such as Butterfield's Buffer (e.g. Cat. no. D599). Make a 1/10, 1/100, and 1/1000 dilution from the original suspension. NOTE: if colony counts exceed a countable number from these dilutions, further dilutions may be needed to achieve a countable range.

4. Plate the dilutions to three separate plates of the same media using the spread plate technique, or streak the sample to achieve isolated colonies, and incubate at the indicated temperature and duration.

Procedure for Inducing Sporulation:

1. Place 10.0g of the sample in 100.0mL of deionized water (e.g. Cat. no. U85).

2. Place a 10.0mL aliquot of this dilution into a separate sterile, screw cap, 20x150mm tube.

3. Place the sample in a water bath at 95°C to 100°C for fifteen minutes to heat-shock the cells.

4. Remove the tube and cool rapidly in an ice bath (0°C to 4°C).

5. For a 10^5 to 10^6 population, vortex the heat-shocked tube for at least ten seconds.

6. Transfer two 1.0mL aliquots to two primary dilution tubes containing 9.0mL of sterile water (e.g. Cat. no. K259).

7. Vortex the primary dilution tube for at least ten seconds.

8. Transfer 1.0mL to a second dilution tube containing 9.0mL of sterile water (repeat this step one more time for a

10 population) and vortex this tube for at least ten seconds.

9. Perform the spread plate technique using 1.0mL aliquots plated on Tryptic Soy Agar (e.g. Cat. no. G60) to confirm colony counts. Alternately, use the pour plate method (e.g. Cat. no. Q58) to confirm colony counts.

10. Incubate plates at 55-60°C for 48 hours.

11. After incubation, count the number of CFU/plate. Plates with counts between 30-300 CFUs should be used, but not less than 6 CFU per USP guidelines.

LIMITATIONS

It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on colonies from pure culture for complete identification.

Processing of cannabis products could introduce human pathogens. Use of gloves and appropriate disinfection procedures should be mandatory to reduce the chance of product contamination.

The delivery route of cannabis products should be taken into account when selecting a sampling and testing method. Method suitability testing is advised.

Fresh, unprocessed cannabis will require additional microbial or spore testing. This includes testing for *Pseudomonas aeruginosa*, *Clostridium botulinum*, and toxigenic *E. coli*.

Cannabis extracts must be tested for *Aspergillus* if they are intended for direct inhalation. There are four species of *Aspergillus* that should be tested: *A. flavus*, *A. fumigatus*, *A. niger*, and *A. terreus*.

Refer to the document "Limitations of Procedures and Warranty" for more information.

REFERENCES

1. Daley, P., et al. 2013. Testing Cannabis for Contaminants. *Botec Analysis Corporation*. Lafayette, CA.

2. Gonzaga de Freitas Araujo, M., et al. 2012. Microbial Quality of Medicinal Plant Materials. *InTech.* Araraquara, Brazil.

3. Holmes, M., et al. 2015. Microbiological Safety Testing of Cannabis. *Cannabis Safety Institute.*

4. *United States Pharmacopeia and National Formulary* (USP-NF). Rockville, MD: United States Pharmacopeial Convention.

IFU-10322[B]

HARDY DIAGNOSTICS

1430 West McCoy Lane, Santa Maria, CA 93455, USA
Phone: (805) 346-2766 ext. 5658
Fax: (805) 346-2760
Website: www.HardyDiagnostics.com
Email: TechService@HardyDiagnostics.com
Ordering Information

Distribution Centers:

The Hardy Diagnostics manufacturing facility and quality management system is certified to ISO 13485.