How Accurate is Potency Testing?

By Dale Gieringer and Arno Hazekamp

RING TEST: A standard procedure in the analytical testing industry for external quality control assurance, in which identical samples are sent to a variety of testing facilities in order to compare results.

Want to know the potency of your medicine? How much, if any, CBD, does it contain? Has it been sprayed with mold or bacteria? The only way to answer these questions for sure is to have it tested by an analytical lab.

Many labs offer testing services for the medical cannabis industry. How do you know the results they provide are accurate?

Our investigation was launched in the winter of 2010/11. An identical set of samples was submitted to 10 labs. The purpose of this set was to test THC, CBD, and CBN, the three major cannabinoids for which testing is generally available. To encourage participation, the identity of the labs was kept confidential; in this report, they are identified by their participation in a ring test (L, 2, and so on).

In most cases, lab results were consistent to within 20% of each other. To some degree, the differences in results might be explained by natural variations in the consistency of the cannabis samples used; to some degree, by differences in lab procedures. In certain cases, there were glaring discrepancies suggesting laboratory error. Three of the 10 labs reported results that were a full order of magnitude off the mark.

Ring tests are conducted to identify the strengths and weaknesses of each lab. The results can be used to improve the methods of testing in the future.

SAMPLES

Labs were asked to examine six different samples: four herbal cannabis samples (A - D), and two liquid (tincture) extracts (E - F).

The cannabis samples were taken from herbal material homogenized in a blender to minimize variations in potency. Samples were stored in a cool, dark room in tightly closed containers until sent to the labs. All testing was completed within 12 months of sending the samples; this allowed the labs to develop protocols, to determine productivity, and to improve their analytical methods.

Samples A, C, and D: replicates

These samples were exactly identical, and intended to check the reproducibility of participating labs, including their extraction protocol, sample preparation, and analytical methodology. Samples consisted of roughly one gram of THC-rich marijuana cannabis that had been homogenized in a kitchen blender, followed by manual stirring.

Sample D: high CBD

This sample consisted of one gram of a similarly prepared mixture of CBD-rich herbal cannabis. The sample was intended to check for lab’s capability of identifying and reporting on CBD.

Samples E and F: extracts

Samples F and G were alcohol tinctures of about one milliliter each. Both were prepared from a single CBD-rich herbal sample. Material in Sample F was decarboxylated by heating in a closed glass jar at 100°C for 90 minutes before soaking in alcohol. Sample G was prepared from the same material, but unheated. Samples were extracted in Sample E for 12 hours, filtered in cheesecloth, then a coffee filter. These samples were intended to evaluate the testing methodology of the participating labs, by removing the need for extraction and sample preparation.

METHODOLOGIES

Different kinds of lab equipment were used to test the samples. Five labs employed gas chromatography (GC), in which the sample is first vaporized under heat. The result is subsequently analyzed by a mass spectrometer (GC-MS, used by one).

For the samples with high levels of THC, HPLC (High Pressure Liquid Chromatography) analyzes cannabinoids in the chemical form in which they are actually present in the sample. For fresh herbal material, labs report the sum of THCA (as "THC-A") and THCA (as "THC"), as well as of "spontaneous" decarboxylation during harvest, processing, and storage. Consequently, labs using HPLC reported the following:

- THC and THCA.
- THCA.
- THCA.
- THCA.
- THCA.
- THCA.
- THCA.
- THCA.

In principle, when the majority of labs report similar values, and only a few labs have found significantly different results, it should be the outliers that need to take a closer look at their methodology.

Because we do not know what the true potency values were for our test samples (we would need a reliable lab to tell us), but evaluating reliability is the purpose of our study), we cannot say with certainty which lab gave more accurate results. We can, however, look at the reproducibility of the same sample in a single lab and the consistency of results between labs. In principle, when the majority of labs report similar values, and one or a few labs have found significantly different results, it should be the outliers that need to take a closer look at their methodology.

Although the samples A, C, and D consisted of the same homogenized cannabis material, the results ranged from 4.16% to 14.3%. The wide variation was mainly due to several factors. One was that the samples might account for some degree of the variance.

In order to convert their measurements to total weight, labs varied in their calibration standards. This could lead to significant differences, as a result of different expansion of solvent in the chromatographic standards, in contrast, would not be affected by this. Another explanation could be problems in calibrating the equipment to the relevant weight of the cannabinoids, which was significantly lower than that of normal herbal samples.

Although CBN is not produced by the cannabis plant itself, it is commonly formed by degradation of THC under the influence of light, storage, and heat. It is therefore not surprising that all samples contained at least some CBN. Levels were generally very low, on average around 0.6% or less in all samples. However, reported CBN values in Sample G varied from 0 to 1.44%. Such a wide variation in results is unacceptable for quality control.

Because most labs were informed that they were participating in a ring test, they were able to give these samples extra attention. Several labs revised or corrected results after initially misreporting them. In a couple of cases, this wasty appears to have been due to simple mislabeling of current methods for testing tinctures and other extracts.

One potential explanation could be that our sample extracts were made in a different solvent (ethanol) than labs were used to. This might cause some of the variance, but not the calibration standards in this. It could lead to significantly different outcomes, as a result of different expansion of solvent in the chromatographic standards.

In contrast, the differences in results were not significantly less than that of normal herbal samples.

An examination of the reported data provides some insight into technical issues that play a role in the laboratory. For example, fresh material might normally be expected to contain substantially higher amounts of acidic cannabinoids (THCA, CBDVA) than neutral ones (THC and CBD).

However, a couple of labs reported the opposite in certain tests (e.g. lab 3 for CBD and lab 7 for THC and CBD in samples A, C and D). Based on reported separation issues in HPLC, this might be caused by an overlap of CBD with other cannabinoids. This has to be due to simple mislabeling of the two cannabinoid analyzed. The same applies to the commonly found cannabinoids Cannabichromene (CBC), Tetracydrocannabinol (THC) and THCA, as well as their acidic precursors.

Even though care was taken to homogenize the herbal samples, there is always a chance that one or more showed some deviation in cannabinoid content. Therefore, liquid samples might contain very low levels of CBD, THC, or other cannabinoids, making the need for extraction and have already been fully homogenized before distributing the samples. Analysis of these liquid samples therefore purely depends on the accuracy of the methodology applied. Liquid samples F and G were prepared from homogenized herbal material like the other
Consumer who need accurate results should consider doing back-up tests with different labs, at least every once in a while. With sufficient care, it is possible to get a good handle on the potency of well-mixed cannabis to be used in the early stages of the methodology. However, it remains unclear how to assure similar accuracy with liquid samples or edibles.

Even while labs struggle to establish industry standards for potency testing, many are already expanding their services to other and potentially more challenging tests. Some commercial labs offer testing for terpenes, known as CBC, CBG, and THCV, which occasionally occur in significant quantities with uncertain effects on the user. Labs are already beginning to develop tests for terpenoids (or terpenes), the components that give cannabis its characteristic smell and are thought to contribute significant, additional “entourage” effects to the medical and psychoactive effects of cannabinoids.

Although we did not attempt to evaluate testing for biological or chemical contaminants such as molds, bacteria or pesticides, these are important services for clients concerned about health and safety. Labs have already reported finding traces of Aspergillus mold and E. coli bacteria in a fraction of samples submitted. Aspergillus can cause serious lung infections, especially in immune-compromised patients. Although the overall risk is modest, there have been a handful of case reports published in the medical literature about illnesses or death due to contaminated cannabis.

Conflict of Interest: The authors undertook this study on behalf of Project CBD and California NORML, and have no stakes in the participating labs. Some members of the participating panel in the past, but not subsequent to the initiation of this study.

10 Questions to ask your cannabis scientist

1. What training or expertise do you have to be able to perform cannabis analysis?

2. Which cannabinoids do you test for? Do you have reliable reference standards for all of them?

3. How is CBN related to THC, and why is it important to test for it?

4. What kind of samples do you test (flowers, edibles, tincture)? Have you optimized your extraction and analysis protocol for each kind of sample?

5. Are you aware of acidic cannabinoids? In samples such as edibles and tinctures, are there significant amounts present at high levels. How do you deal with that?

6. What is your analytical methodology for testing cannabis (HPLC, GC, TLC, others)? What are the limitations of your selected method?

7. What is the average THC/CBD content you find in a sample, and how does it vary? Is there variability that you can be sure they are fully reliable. Have you done this already, and how did you do this. Did it include a third party? If you didn’t do it yet, how can I be sure my results will be accurate?

8. Did your lab ever test the same cannabis twice, with very different results? What was the explanation for that, and what has been changed to prevent it from happening again?

9. Analytical methods need to be “validated” before you can be sure they are fully reliable. Have you done this already, and how did you do this. Did it include a third party? If you didn’t do it yet, how can I be sure my results will be accurate?

10. What do you do with left-over samples?

—Samantha Miller et al.