First of its Kind Lateral Flow Device to Detect Capsaicin

Introduction:



Feeling hot, hot, hot? Capsaicin is a naturally occurring botanical irritant in chili peppers, synthetically derived for pharmaceutical formulations. Capsaicin is also often used as a topical analgesic and exists in many formulations of cream, liquid, and patch preparations of various strengths; however, it may also be found in some dietary supplements. The measure of hotness of a chili pepper or anything derived from chili peppers, such as hot sauce, is measured using the Scoville scale. The scale is a measure of the concentration of capsaicin, which is what gives chili peppers their heat. The Scoville Scale is measured by what is called Scoville Heat Units (SHU). These units range on a spectrum from 0 (no heat at all) to 16,000,000+ (pure capsaicin). The range of SHU is typically used to call a pepper mild, medium, hot, or extra hot and these will range from mild (50 to 2,500), medium (2,500 to 30,000), hot (30,000 to 100,000), and extra hot (100,000 to 500,000). Anything above that is extreme. For example, a bell pepper would have zero SHU because it does not contain capsaicin. On the other hand, pure capsaicin would have 16 million SHU.

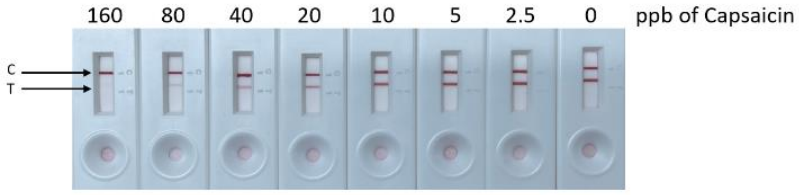
Determining Scoville heat units classically involves diluting a pepper extract with sugar water and then asking human testers to identify the heat level. The more sugar water is required to negate the heat, the higher the number of Scoville Heat Units that pepper contains. So, for example, if you have a pepper with 100,000 SHU, the extract from it would need to be diluted 100,000 times which would then allow the testers to no longer detect any spiciness. While this method is clearly useful, it was also obviously quite subjective since it relies on human testers. This method has since been replaced with expensive and time-consuming methods such as Gas Chromatography (GC), Gas Chromatography-Mass Spectrometry (GC-MS) and high-performance liquid chromatography (HPLC) to become more quantitative. Clearly, the quantitative determination of Scoville heat units is cumbersome and requires skilled personnel. There has got to be an easier way to determine how much to 'spice up your life', no?

To make the SHU determination process easier and readily available for hot sauce manufacturers of all sizes, Attogene has recently developed a sensitive and rugged lateral flow assay for detecting the concentration of capsaicin in a hot sauce sample with minimal laboratory equipment or training. Once the capsaicin concentration is determined, it can be converted into Scoville heat units using a simple conversion factor.

Results:

The lateral flow kit we developed is a competitive assay in which the more capsaicin present in the sample, the less bright the test line. In our test, a capsaicin protein conjugate (test line) and goat anti mouse (control line) are sprayed onto a nitrocellulose membrane. Next, anti-capsaicin antibody is applied to our in-house manufactured colloidal gold (AU2014) using optimized and specialized conditions. Gold conjugates are then deposited onto a conjugate pad, assembled onto the nitrocellulose, and placed within a lateral flow cassette. Using our optimized

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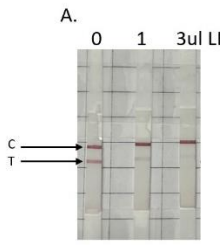


Concentration of Capsaisin	Control Line	Test Line	Ratio
160 ppb	1193564	23296	0.02
80 ppb	1074569	164326	0.15
40 ppb	1322762	401944	0.3
20 ppb	988811	570541	0.58
10 ppb	1158916	949000	0.82
5 ppb	1116224	1014275	0.91
2.5 ppb	1139626	1100004	0.97
0ppb	1067261	1096337	1.03

Figure 1. Evaluation of the limit of detection of Attogene's capsaicin lateral flow assay. A serial dilution of capsaicin was added into running buffer at the indicated final concentrations. The cassettes were run for 15 minutes and then analyzed using a lateral flow reader. These data indicate the test can detect as low as 10ppb using a lateral flow reader.

proprietary running buffer and lateral flow materials, we verified the detection limit of this capsaicin lateral flow test (Figure 1). A standard dilution series of capsaicin placed into running buffer was evaluated using our Attogene kit AU2046. Briefly, a stock solution of capsaicin was made and diluted to the final concentrations shown in Figure 1. The solution was then added to the sample port of the lateral flow cassettes and run for 15 minutes. After being run for 15 minutes a picture was taken and the results of the lines were analyzed using a lateral flow reader. These data demonstrate that this test can detect down to at least 10ppb of capsaicin using the lateral flow reader. Most importantly, these data also establish that this test is rapid and easy to perform and could be an alternative to the subjective Scoville method and lengthy and difficult highly technical HPLC/GC/MS techniques.

Now let's move our test into real world samples: To test the ability of our capsaicin lateral flow assay to analyze capsaicin levels in hot sauces, we purchased sauces at opposite ends of the Scoville spectrum. The sauce LHS has a published SHU of 450 while the extreme sauce, TE has a published SHU of 7 million. In our tests, we first added 1ul or 3ul of LHS hot sauce directly into the running buffer, applied our test strips and ran the assay for 15 minutes. Both 1 and 3ul of LHS decreased the level of the test line like that in the range of 40-80ppb as shown in Figure 1.



B.

Sample	Control Line	Test Line	Ratio	
0ppb Capsaicin	751388	729837	0.97	
1ul L Hot Sauce	1044525	203608	0.19	Claim roughly 450 SHU
3ul L Hot Sauce	1024377	78753	0.08	
1ul TE Hot Sauce	703596	3549	0.01	
1:1,000 1ul TE Hot Sauce	780549	1875	0	Claim roughly 7 million SHU
1:10,000 TE Hot Sauce	832526	87097	0.1	
300ppb Capsaicin	846359	78737	0.09	

Figure 2. Evaluation of hot sauces using the Attogene Capsaicin Lateral Flow assay kit. Panel A shows the impact of a mild hot sauce on the test line of the lateral flow test. It can be easily visualized that both 1 and 3 ul of the LHS drastically reduced the test line indicating the presence of capsaicin in the sample. This intensity of the test line is like that observed with a capsaicin sample of 40ppb. We next tested an extremely hot – hot sauce (TE) and needed to significantly dilute the sample to start to observe a similar range as the LHS. Our estimation is that the TE hot sauce has at least 10,000x more capsaicin than the LHS. These data demonstrate the utility of using the capsaicin lateral flow test to detect and quantify capsaicin levels in hot sauces.

It is important to note that no sample preparation was needed to generate this capsaicin level determination. Next, we compared LHS with an extreme sauce claiming 7 million SHU. In this experiment, the lateral flow reader results are shown in Panel B. Even upon dilution of the TE hot sauce 10,000-fold, a significant level of capsaicin was detected. Our results suggest that TE hot sauce is roughly 10,000-fold

higher than the LHS and is in line with the estimates of SHU listed for them on the internet. A capsaicin spike sample of 300ppb was also included as a reference in these studies.

Discussion:

In conclusion, Attogene has developed a rapid, user friendly lateral flow assay for detection of capsaicin. This technique can be used by the hobby or industrial hot sauce maker to understand, screen and evaluate the levels of capsaicin in their products during or after manufacturing. The levels of capsaicin can then be readily translated into SHU units. Just as Chili Pepper X recently dethroned the Carolina Reaper as the hottest pepper, our assay might just be able to dethrone other more classical ones in this field.