Matrix Extension Study of the TEMPO® EC (*E. coli*) Method for Dried Cannabis Flower

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Introduction

The testing requirements in the cannabis industry are evolving and vary from state-to-state. The presence of *E. coli* in food is used as an indicator for fecal contamination or unsanitary processing [1]. Detection and enumeration of *E. coli* can help improve the safety and quality of cannabis products.

TEMPO® EC is used with the TEMPO® system for enumeration of *E. coli* in 22-27 hours in food products and environmental samples. The TEMPO® EC test kit consists of a vial of culture medium and a card which are specific to the test. The culture medium is inoculated with the sample to be tested. The TEMPO® Filler instrument is used to transfer the inoculated vial of culture medium to the corresponding card. Each card contains 3 sets of 16 wells (small, medium, and large wells) with each set of wells containing a one log difference of volume. The card is designed to simulate the Most Probable Number (MPN) method. Following the filling process, the card is hermetically sealed to avoid risk of contamination.

Based on β-glucuronidase activity, *E. coli* present in the card reduce the substrate in the culture medium during incubation and cause a fluorescent signal to appear. This fluorescent signal in detected by the TEMPO® Reader instrument and depending on the number and type of positive wells, the TEMPO® software calculates the number of *E. coli* present in the original sample according to an MPN calculation.

Purpose

This study evaluated the effectiveness of the TEMPO® EC method in the enumeration of *E. coli* in dried cannabis flower (>0.3% delta 9-tetrahydrocannabinol (THC)) at a 10 gram test portion size.

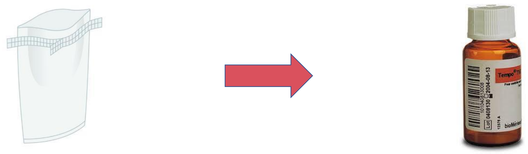
Method

Dried cannabis flower (>0.3 THC) was artificially inoculated with *E. coli* at three levels of contamination: low, medium, and high. Five replicates were tested for each level, and all samples were tested in a paired manner utilizing the VRBA + MUG plating method outlined in FDA/BAM Chapter 4 as the reference method. To each 10 g test portion, 90 mL of BPW was added and homogenized, 1 mL of sample was transferred to TEMPO® EC vials and serial dilutions were plated to VRBA + MUG. TEMPO® EC cards were filled from the inoculated vials using the TEMPO® Filler instrument and then incubated at 35 °C for 22 hours. Following incubation, cards were reading using TEMPO® Reader instrument and an MPN value was generated.

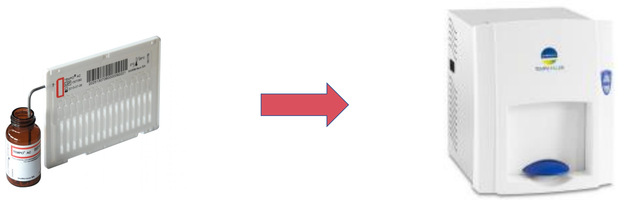
References

1. FDA-BAM Bacterial Analytical Manual Chapter 4 (2020), *Enumeration of Escherichia coli and the Coliform Bacteria*, https://www.fda.gov/food/laboratory-methods-food/bam-chapter-4-enumeration-escherichia-coli-and-coliform-bacteria (Accessed July 2022)
2. Official Methods of Analysis (2019) 21st Ed., Appendix J: AOAC INTERNATIONAL, Rockville, MD, http://www.eoma.aoac.org/app_j.pdf (Accessed December 2021)

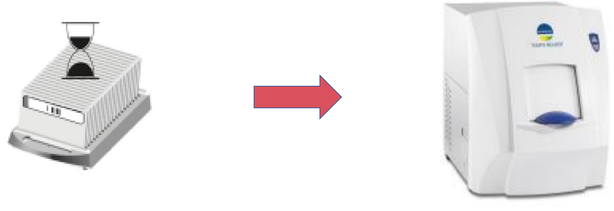
Methodology



Test portions are diluted 1:10 and 1 mL is transferred to a reconstituted TEMPO® EC vial, representing a 1:40 dilution (used to provide a 10 to 49,000 CFU/g value).



Vials and cards are organized into a filling rack and inserted into the TEMPO® Filler instrument. Instrument which automatically transfers liquid from vials into the wells of the cards.



Cards are organized into a reader rack and then incubated. Following incubation, the card rack is inserted into the TEMPO® Reader and cards are analyzed using the fluorescent signal in the individual wells to provide an MPN value.

Results

The MPN values from the TEMPO® EC and FDA/BAM Chapter 4 methods were logarithmically transformed. Log values were averaged for each contamination level and the mean difference was calculated with 90% and 95% confidence intervals using the Paired Statistical Analysis for Equal Variances.

Data analysis comparing the 90% confidence interval of the difference of means between the two methods demonstrated equivalence for each contamination level. Comparison at the 95% confidence interval did not find a statistical difference between the two methods for the Low and Medium inoculation levels. The High level of inoculation did show a statistical difference despite a low mean difference.

Table 1: TEMPO® EC method vs. FDA/BAM Chapter 4 – Dried Cannabis Flower, 10 g

Inoculation Level	Sample Replicate	TEMPO® EC				FDA/BAM Chapter 4				Mean Difference[c]	90% CI[d]	95% CI[e]
		CFU/g	Log10	Log10 Mean[a]	SD[b] (S_i)	CFU/g	Log10	Log10 Mean	SD[b] (S_i)			
Low	1	86	1.94	1.19	1.26	40	1.60	1.04	1.19	0.147	(-0.023, 0.317)	(-0.074, 0.368)
	2	0	-1.00			0	-1.00					
	3	21	1.32			10	1.00					
	4	44	1.64			50	1.70					
	5	110	2.04			80	1.90					
Medium	1	330	2.52	2.40	0.123	220	2.34	2.27	0.176	0.126	(0.028, 0.223)	(-0.002, 0.253)
	2	180	2.26			120	2.08					
	3	280	2.45			320	2.51					
	4	310	2.49			210	2.32					
	5	190	2.28			130	2.11					
High	1	710	2.85	2.79	0.183	600	2.78	2.65	0.135	0.135	(0.055, 0.215)	(0.030, 0.240)
	2	500	2.70			420	2.62					
	3	730	2.86			430	2.63					
	4	330	2.52			280	2.45					
	5	990	3.00			590	2.77					

[a]Log10 Mean = Average of five replicates logarithmically transformed with the equation $\text{Log}_{10}(\text{CFU/g} + 0.1)$
[b]SD = Standard deviation
[c]Mean Difference = Candidate Log Mean – Reference Log Mean
[d]90% CI = If the confidence interval does not fall between -0.50 and 0.50, then the methods would not be considered equivalent
[e]95% CI = If the confidence interval does not contain the point 0, then the methods would not be considered statistically similar

Conclusion

Results from this study provide evidence that the TEMPO® EC method is effective in the enumeration of *E. coli* in dried cannabis flower after 22 hours of incubation. Use of a single vial avoids the need to prep serial dilutions as well as helps easily differentiate *E. coli* from coliform bacteria. Based on the study, the TEMPO® EC method was granted AOAC PTM approval for dried cannabis flower.

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