AOAC Validation of Multiple TEMPO Methods for the Enumeration of Quality Indicator Organisms in Cannabis Flower: AOAC Performance Tested Method℠ 041001, 121204, 060702, 080603 and 050801

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INTRODUCTION

The TEMPO® system is an AOAC® Performance Tested Method℠ (PTM) approved for the automated enumeration of yeasts and molds (YM), total aerobic count(AC), coliform count(CC), *Escherichia co*li (EC) and Enterobacteriaceae(EB) in cannabis flower. The method utilizes a selective dehydrated culture medium and an enumeration card containing 48 wells across three dilutions for the automatic determination of the Most Probable Number (MPN).

PURPOSE

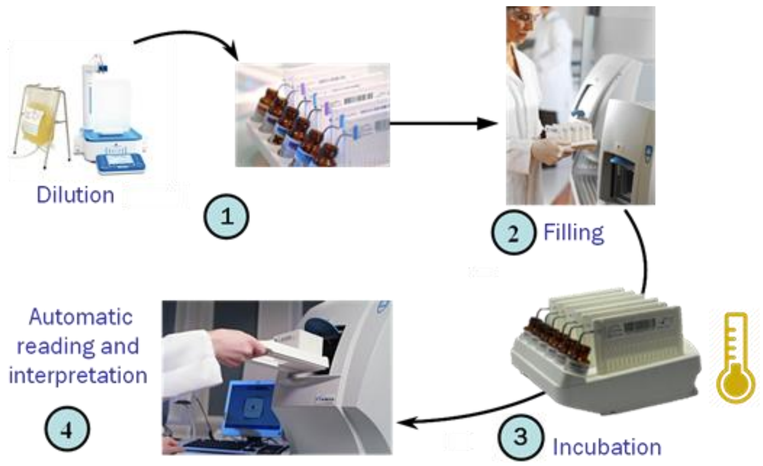
As part of the AOAC® Research Institute's Emergency Response Validation (ERV) Program and the Performance Tested Method (PTM) program, the alternative methods were compared to the FDA Bacteriological Analytical Manual (BAM) to add cannabis flower (delta 9-tetrahydrocannabinol >0.3%; 10 g test sample) as a PTM matrix extension.

Figure 1. TEMPO System

PRINCIPLE OF THE TEST

The culture medium is inoculated with the sample to be tested. The inoculated medium is transferred from the TEMPO Filler into the card containing 48 wells of three different volumes. The card contains three sets of 16 wells (small, medium, and large wells) with a 1 log difference in volume for each set of wells. The card is designed to simulate the most probable number (MPN) method. The card is then hermetically sealed in order to avoid any risk of contamination during subsequent handling. Target organisms present in the card reduce, acidify or cleave the substrate in the culture medium during incubation and create a fluorescent signal to appear, which is detected by the TEMPO Reader. After incubation, the cards are transferred to an automated card reader that detects the fluorescent signal. Depending on the number and type of the positive wells, the TEMPO system calculates the number of yeasts and molds present in the original sample according to a calculation based on the MPN method.

Figure 2. TEMPO Workflow



METHODS

The TEMPO methods were evaluated for one cannabis flower and the results were compared to traditional plating. Cannabis test portions were screened for target organisms prior to the study. Five replicate test portions were evaluated for each of the 3 levels of contamination. Individual 10 g test portions from each contamination level were prepared in sterile filter Whirl-Pak bags. In addition to the candidate methods, all test portions were enumerated using traditional plating agar. All testing was performed using paired test portions. TEMPO cards and agar plates were prepared for each contamination level. TEMPO cards were incubated per instructions for use for each respective card type. Traditional agar plate were incubated per BAM procedures.

RESULTS

The 90% confidence interval of the difference between the means of each TEMPO method (YM, AC, CC, EC and EB) and the corresponding reference method obtained in the comparison study demonstrated statistical equivalence between the candidate methods and the confirmation platings. Inclusivity and exclusivity results showed all TEMPO methods are highly specific in discriminating target organisms found in cannabis flower from non-target organisms.

CONCLUSION

The TEMPO methods successfully all target organism in cannabis flower. In the method comparison study, the mean log counts for the TEMPO methods were consistent with those obtained by the plating agar. Each level produced equivalent results between the candidate presumptive and confirmed results. In the inclusivity and exclusivity evaluations, all inclusivity organisms were correctly identified, and all exclusivity organisms were correctly excluded.
The AC, YM, CC, EC and EB TEMPO methods are a rapid, easy-to-use automated alternatives to traditional plating procedures for the enumeration of quality indicator organisms in cannabis flower.

SIGNIFICANCE

- The statistical data, within their uncertainty, indicate that the TEMPO system is suitable for the enumeration of yeasts and molds, total aerobic count, coliform count, Enterobacteriaceae, and *Escherichia coli* in cannabis flower.
- TEMPO provided a faster turnaround time, elimination of all subjectivity and automated traceability when compared to standard plating methods.
- The TEMPO system provides an automated, standardized, accurate method for the enumeration of Yeast and Mold, total aerobic count, coliform count, Enterobacteriacea and *Escherichia coli* in cannabis flower.