

**To: Scott Williams**

**Date: September 27, 2017**

**From: Elisabeth Miller**

**Subject: Copenhagen Snuff Fine Cut Total Aerobic Microbial Count, Total Yeast and Mold Count**

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**Objective:**

To determine the stability of microbial populations in the following product:  
Copenhagen Snuff Fine Cut

**Materials and Methods:**

**Table 1: Samples and Parameters Tested**

Sample Name	Shelf Life Sample Point (Weeks)
Copenhagen Snuff Fine Cut	0, 2, 4

Moist Smokeless Tobacco (MST) samples were collected and stored at room temperature (22°C to 23°C) until tested. The products were tested for Tobacco Specific Bacteria (TSB), Total Aerobic Microbial Count (TAMC), Total Yeast and Mold Counts (TYMC) and *E. coli*/coliforms. (b) (4)

(b) (4). The following modifications were made to the above referenced method for TAMC and TYMC:

1. Total colony counts were performed for each sample instead of cocci counts.
2. Nitrate reduction testing was not applicable and not performed.

The products were tested for *E. coli*/coliforms in accordance with the method “*Compendium of Methods for the Microbiological Examination of Foods, Fourth Edition.*” Method sections 3.541- 3. 543.”

See Table 2 for testing parameters.

**Table 2. Testing Parameters**

Test	Process and Analysis Method	Agar Type	Incubation Temperature	Incubation Length
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(b) (4)

**Results:**

The cocci counts varied from 1.46E+07 to 1.87E+07 during the 4 week shelf life, and the rod counts varied from 3.00E+02 to 1.10E+03. The TN18 total count (cocci and rods) did not increase because of the small amount of rod bacteria compared to the cocci bacteria.

(b) (4)

All samples were below the limit of detection for TYMC and *E. coli*/coliform.

(b) (4)

Sample	Sample Time Point (Weeks)	Counts	Counts	TN18	TN0	TSA SB	DRBC <sup>3</sup>	Coliform <sup>4</sup>
		CFU/g	CFU/g	CFU/g	CFU/g	CFU/g	CFU/g	CFU/g

(b) (4)

**Discussion:**

TAMC is a generic test that does not differentiate organisms that grow aerobically at mesophilic temperatures (between 20-45°C). Samples were plated on non-selective Tryptic Soy Agar supplemented with 0.1% potassium nitrate (TN0) for accompanying bacterial nitrate reduction testing when applicable, and non-selective Tryptic Soy Agar supplemented with 5% sheep's blood (TSA SB). Overall, the populations of bacteria are stable over shelf life in all products tested. The populations vary slightly, which could be due any one of the following:

1. Sample to sample variation
2. Inhibitory effects of the ingredients added during the finishing process

Samples were plated on selective Dichloran Rose-Bengal Chloramphenicol (DRBC) for TYMC. DRBC agar is selective for yeast and mold. The levels of mold and yeast were lower than the detectable limit of 5 CFU/g throughout shelf life.

Samples were plated on selective PetriFilm™ for *E. coli*/coliform counts. PetriFilm™ is selective for *E. coli* and coliforms. The levels of *E. coli* and coliforms were lower than the detectable limit of 5 CFU/g throughout shelf life.

The TAMC, TYMC, *E. coli*/coliform counts demonstrate the products tested are stable throughout shelf life in regards to bacterial populations, and that moist smokeless tobacco products are inherently non-supportive to the proliferation of yeasts and molds (Kotrola, J., 2011), (Corry, J. E. L., 1987) and (Ray, B. & Bhunia, A., 2004).

(b) (4)

**References:**

Altria Client Services (ALCS) (2015). Quantitation of Tobacco Specific Bacteria, Rev. 1.0 (Rep. No. ST-TM-950-104).

Corry, J. E. L. (1987). Relationships of Water Activity to Fungal Growth, p. 51-99. In L. R. Beuchat (ed.), *Food and Beverage Mycology*, 2nd ed. AVI/Van Nostrand Reinhold, New York, N. Y.

Downes, F. P., and K. Ito (ed.), Method sections 3.541- 3.543. *Compendium of Methods for the Microbiological Examination of Foods, Fourth Edition*.

Kotrola, J. (2011). RD&E Interoffice Correspondence. *Mold absence verification study – Final Report* January 2011.

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