FINAL REPORT

Germicidal Spray Efficacy
Of
Nanopiur NTS Concentrate

Order Number: 552004309

PREPARED FOR
Nanopiur | 4900 Jean-Talon Ouest Suite 230
Montreal, QC H4P 1W9

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5/14/2020

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CERTIFICATE OF ANALYSIS

CLIENT: NANOPIUR

CONTACT: FRANK SALTARELLI

PROJECT:

GERMICIDAL SPRAY EFFICACY

PRODUCT: NANOPIUR NTS CONCENTRATE

SAMPLE RECEIVED: 04/23/2020

REPORT DATE: 05/14/2020

CHALLENGE BACTERIA:

Listeria monocytogenes

I. EXPERIMENTAL SUMMARY

The testing procedure was designed after discussions between EMSL Canada Inc. and Frank at Nanopiur. The testing procedure is based on AOAC 961.02, and conducted on the Nanopiur NTS Concentrate for its ability to cause reduction of *Listeria monocytogenes* (ATCC 19111) for exposure times of 60 seconds and 5 minutes for two different concentrations as specified by client. The testing was conducted in EMSL Mississauga Microbiology Laboratory.

II. PROCEDURE

Culture preparation:

A pure culture of *L.monocytogenes* (ATCC 19111) was streaked on to TSA (Tryptic Soy Agar, Oxoid Canada) and incubated at 35°C for up to 24 hours. A single isolated colony was taken and inoculated in 10 mL of TSB (Tryptic Soy Broth, Hardy Diagnostics) at 35°C for 24 hours. Culture purity and biochemical reactions for *L.monocytogenes* were confirmed. An aliquot of this broth culture was transferred again to another tube of TSB and incubated for another 24 hours at 35°C. A final transfer was done a day before testing was conducted. 50µL of this TSB solution was then inoculated on to individual sterile glass slides (carriers). Each slide was placed in an empty sterile Petri dish and allowed to dry at room temperature inside a biological safety cabinet (~45-60 minutes).

Product Preparation:

Nanopiur NTS Concentrate was delivered to EMSL Mississauga Lab. Two concentrations were tested as per client's specifications. The preparations were made by adding sterile water as follows:

Concentration A:
1 part Nanopiur + 9 parts Sterile Water (1:10 dilution)
Concentration B:
1 part Nanopiur + 12 parts Sterile Water (1:13 dilution)

The final working solutions for each concentration were added to appropriate individual spray bottles. These were used within an hour of preparation for the spray studies.

Test Procedure:

For determination of starting population, three dried inoculated glass carriers were selected as controls. These were not exposed to spray treatment. Each was placed into 10 mL of Butterfields buffer solution and vortexed for 30 seconds. 1 mL aliquots were taken and serial dilutions performed were plated on TSA plates (Tryptic Soy Agar, Oxoid Canada). The plates were incubated at 35°C for up to 24-48 hours.
Simultaneously, three glass carriers were selected as test carriers. Each of these was individually sprayed with a fine mist of Concentration A of the Nanopiur solution from a distance of 10-12 inches for exposure time of 60 seconds. Following exposure, the carriers were picked up with sterile tweezers; excess solution was allowed to drain off and placed into 10 mL D/E neutralization buffer. After vortexing, serial dilutions were plated on to TSA plates incubated at 35°C for 24-48 h.
Same procedure was repeated again for Concentration A solution - exposure time 5 minutes, Concentration B - exposure times 60 seconds and 5 minutes for three inoculated carriers each. Following incubation, colonies on respective control and test plates were enumerated. All tests and procedures were performed in a Class II Biosafety Cabinet.

III. EXPERIMENTAL RESULTS

Quantitative Counts for *L.monocytogenes* exposed to Concentrations A & B of Nanopiur NTS Concentrate:

Concentration A	Control Average CFUs	Test Average CFUs	% Reduction	Log Reduction
60 seconds	1.57×10^5	8.16×10^2	99.480%	2.284
5 minutes	1.57×10^5	1.00×10^2	99.936%	3.195

Concentration B	Control Average CFUs	Test Average CFUs	% Reduction	Log Reduction
60 seconds	1.57×10^5	2.5×10^3	98.407%	1.797
5 minutes	1.57×10^5	1.00×10^2	99.936%	3.195

% Reduction: Percent difference between starting population and exposed population.
Log Reduction: Log difference between starting population and test carrier.

IV. CONCLUSIONS/OBSERVATIONS

Nanopiur NTS concentrate was diluted to two different concentrations and tested for *Listeria monocytogenes* against exposure times of 60 seconds and 5 minutes. Blank media was tested to confirm sterility of media used during testing.

For the exposure time of 60 seconds, Concentration A caused a reduction of 99.480% and proved to be more effective than Concentration B on *L.monocytogenes.* Both concentrations were effective for the 5 minute exposure duration causing a reduction of >99.9% reduction of *L.monocytogenes*.

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