



Certified Food Safety Center, Inc.

Food Safety and Process Technology

Validation of Neo-Pure Food Safety System

- Alfalfa Seed -

BY

CERTIFIED FOOD SAFETY CENTER

A Division of Certified Laboratories

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Almond Board Approved Process Authority

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Subject: Validation of Agri-Neo's Food Safety System Using Neo-Pure Solution

Date of Testing: May 18, June 1 and June 13, 2017

Product: Alfalfa Seeds

Date of Report: July 31, 2017

1.0 BACKGROUND

The mission of Agri-Neo Inc. is to help safely feed the world. Founded in 2009, Agri-Neo is headquartered in Toronto where it assembles its Food Safety Systems for Neo-Pure, and conducts food safety science done by chemists, microbiologists, and engineers. To accomplish their vision they have promulgated new food safety standards for seeds, grains, and nuts, by utilizing the Neo-Pure System for pathogen control.

Recent regulations in Canada (Safe Food for Canadians Act) and the United States (Food Safety Modernization Act) are demanding preventative controls for pathogens. With increasing health awareness and consumer fondness for organic food, Agri-Neo's new organic sanitizer, Neo-Pure, provides instantaneous contact control of pathogens on the surface and inside raw food products. The sanitizer then biodegrades completely, leaving no residues, which means that the taste, smell, appearance and nutritional profile of the raw food products remain unchanged. Neo-Pure sanitizer is approved by Health Canada, the United States Environmental Protection (EPA) Agency and the United States Food and Drug Administration (FDA). Neo-Pure is also approved by halal, kosher and organic certifiers.

This report summarizes a validation treatment on May 18, June 1 and June 13, 2017 for bulk inoculation of alfalfa seeds using *Enterococcus faecium* NRRL B-2354 as a surrogate for *Salmonella* species. The validation was conducted using two treatment rates of 40 and 60 L/t, held for 24 hours and dried at 140°F.

2.0 METHODS

- All microbiological inoculations and enumerations were conducted by Agri-Neo Laboratory in Toronto, Canada under the direction of Dr. Amir Hamidi.
- By using two 4.5 quart Kitchen Aid Mixers with a wire whisk (model K45SS) 100 x 1 kg samples of alfalfa seed were inoculated with 30 milliliters (10^8 cfu/ml) of overnight *E. faecium* inoculum grown in TSB at 35°C (for each kg; total of 2 liters on 100 kg). After mixing for two minutes each kg of inoculated alfalfa seed was



transferred to a CentreFlow Buckhorn container (100 kg or 220 pounds) and left overnight to equilibrate for moisture. The seeds were then thoroughly mixed with a sterile ladle and 20 x 50 g samples were taken from multiple locations of inoculated alfalfa seeds.

- Inoculated alfalfa seeds were then treated at 40 liters/ton, held for 24 hour post treatment, and dried at 140 °F until the moisture content was similar to the original moisture content.
- Twenty x 50 gram composite samples were taken after drying.
- Each 50 gram sample was mixed with 100 milliliters Buffered Peptone Water (1:2 ratio) in a filtered stomacher bag, and stomached for 2 min at 230 rpm.
- Three aliquots of 333 µL from stomacher bag were plated across 3 Bartley plates (total of 1 ml) for a detection limit of 2 CFU/g (0.30 log CFU/g).
- Dilutions plated were -4 (untreated inoculated controls) and 0 (after drying).
- Plates were incubated at 35°C for 48 hours followed by enumeration.
- All above steps are repeated for treatment rate of 60 liters/ton.

3.0 NEO-PURE TREATMENT

Prior to treatment the Neo-Pure applicator was thoroughly cleaned with Ora detergent and sanitized with AciQuat sanitizer and cleaning process was verified by ATP swabbing.

Fresh Neo-Pure was prepared by mixing the two ingredients (Neo-Carrier and Neo-Pure active) which was then transferred into the stainless steel mixing tank to be used for treatment.

The Buckhorn container was lifted into place by fork lift and the applicator was set to a treatment rate of 40 and 60 liters per ton of Neo-Pure solution. This entire process is automatically controlled by the PLC once the recipe is activated. As the inoculated alfalfa seeds pass through the atomizer, they are sprayed on with the Neo-Pure solution and are thoroughly mixed with the Neo-Pure solution as they travel the length of the rotary drum. At the exit portion of the drum, the treated alfalfa seeds are deposited into a clean lined Buckhorn container in which it is held for 24 hours before drying.



Fig. 1: A, Inoculated alfalfa seed inside a CentreFlow Buckhorn container lined with a FDA-approved food-grade plastic bag; B, Neo-Pure Applicator; C, Neo-Pure Dryer



A

B

C

4.0 DRYING IN NEO-PURE DRYER

Neo-Pure dryer is used to remove the moisture added during application and improve the organoleptic properties of the alfalfa seed. Prior to drying, the dryer was cleaned with the same solutions as used for the Neo-Pure Applicator.

The capacity of the dryer is larger than the supply of inoculated/treated seeds, so a system was designed to allow the drying of small quantities of inoculated/treated seeds. The circular dryer was filled up to approximately 10 inches from the top with treated/un-inoculated alfalfa seeds. A rubber gasket was installed on this level and the treated inoculated alfalfa seeds were filled on the top to a depth of approximately 10 inches. The alfalfa seeds were dried for approximately 50 minutes using 140°F air at a flow rate of 5,000 CFM. Moistures were checked frequently by QC to determine the correct moisture level of the inoculated/treated samples and the drying was ended when the moisture content was close to the original moisture content.

5.0 SAMPLING

After drying 20 x 50g samples were taken directly from multiple locations of the dryer. Enumeration was conducted following the procedures in section 2.0.



6.0 TEST RESULTS

The summary results of the two treatment rates are shown in Table 1 after the Neo-Pure application, hold time of 24 hours and drying at 140°F. Treatment at 40 and 60 liters/ton showed all values to be >4 and >5 log reduction, respectively, after drying.

Table 1: Results of Two Treatment Rates

Treatment Rate (liters/ton)	After 24 hours hold, and dried at 140°F, Avg. Minimum Log Reduction (Log CFU/g)
40	4.22
60	6.05

The data for log reductions achieved at 40 and 60 L/t rates after drying at 140°F are also presented in tables 2 and 3, respectively.



Table 2: Log reduction after 40 L/t Neo-Pure application, 24 hour hold, and drying at 140 °F

Treated	0	CFU/g	Log CFU/g	Untreated Control Minimum Log CFU/g	Minimum Log Reduction Log CFU/g	STD. Dev Minimum Log Reduction Log CFU/g
1	86	172	2.24	6.45	4.21	0.19
2	148	296	2.47	6.45	3.98	
3	191	382	2.58	6.45	3.87	
4	77	154	2.19	6.45	4.26	
5	78	156	2.19	6.45	4.26	
6	71	142	2.15	6.45	4.30	
7	88	176	2.25	6.45	4.20	
8	54	108	2.03	6.45	4.42	
9	73	146	2.16	6.45	4.29	
10	62	124	2.09	6.45	4.36	
11	43	86	1.93	6.45	4.52	
12	46	92	1.96	6.45	4.49	
13	80	160	2.20	6.45	4.25	
14	95	190	2.28	6.45	4.17	
15	142	284	2.45	6.45	4.00	
16	127	254	2.40	6.45	4.05	
17	63	126	2.10	6.45	4.35	
18	121	242	2.38	6.45	4.07	
19	178	356	2.55	6.45	3.90	
20	46	92	1.96	6.45	4.49	
AVG.	89.0	186.9	2.23	6.45	4.22	N/A



Table 3: Log reduction after 60 L/t Neo-Pure application, 24 hour hold, and drying at 140 °F

Treated	0	CFU/g	Log CFU/g	Untreated Control Minimum Log CFU/g	Minimum Log Reduction Log CFU/g	STD. Dev Minimum Log Reduction Log CFU/g
1	1>	<2	0.3	6.35	6.05	0.22
2	1>	<2	0.3	6.35	6.05	
3	1>	<2	0.3	6.35	6.05	
4	1>	<2	0.3	6.35	6.05	
5	1>	<2	0.3	6.35	6.05	
6	1	2	0.3	6.35	6.05	
7	1	2	0.3	6.35	6.05	
8	1>	<2	0.3	6.35	6.05	
9	1>	<2	0.3	6.35	6.05	
10	1>	<2	0.3	6.35	6.05	
11	1	2	0.3	6.35	6.05	
12	1>	<2	0.3	6.35	6.05	
13	1>	<2	0.3	6.35	6.05	
14	1>	<2	0.3	6.35	6.05	
15	1>	<2	0.3	6.35	6.05	
16	1>	<2	0.3	6.35	6.05	
17	10	20	1.3	6.35	5.05	
18	1>	<2	0.3	6.35	6.05	
19	1>	<2	0.3	6.35	6.05	
20	1	2	0.3	6.35	6.05	
AVG.	1.6	2.9	0.3	6.35	6.05	N/A



7.0 CRITICAL PARAMETERS ALFALFA SEED

When the Critical Parameters shown in Table 4 and 5 are followed, the Neo-Pure System (applicator + dryer) is capable of producing >4 and >5 log destruction of *Salmonella*, respectively. Using lower values than indicated in Table 4 and 5 have not been validated and no conclusion to lethality can be made for this report.

Table 4: Critical Parameters to achieve 4 log reduction in *Salmonella*

Critical Parameter	Minimum
Neo-Pure Solution (liters/ton)	40
Post Treatment Time (Hours)	24
Drying Temperature (°F)	140

Table 5: Critical Parameters to achieve 5 log reduction in *Salmonella*

Critical Parameter	Minimum
Neo-Pure Solution (liters/ton)	60
Post Treatment Time (Hours)	24
Drying Temperature (°F)	140

8.0 CONCLUSIONS

The Neo-Pure System (applicator + dryer) is capable of a >4 and >5 log destruction of *Salmonella* organisms when used at 40 and 60 liters per ton, respectively, and held for 24 hours followed by drying at 140°F.

Drying is accomplished for quality reasons and is required to achieve >5 log lethality. However, it is important to reduce moisture content and return the alfalfa seed back to the original moisture content as measured before treatment.

The inoculation procedure for each treatment rate of 40 and 60 liters/ton was very consistent varying by only 0.07 and 0.08 standard deviations, respectively.

Based on the data presented above, as a Process Authority, I am confident that when used according to the Critical Parameters the Neo-Pure System (applicator + dryer) will provide >4 or > 5 log destruction of *Salmonella* on alfalfa seeds, depending on the applied Neo-Pure rate.

Almond Board of California Approved Process Authority