THE EFFECTIVENESS OF THE FIREFLY RAPID METHOD IN DETERMINING SANITATION PROCEDURES AT A TUNA PROCESSING PLANT

by

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Abstract

The first steps in the implementation of food safety regulations are often the sanitation procedures. The main objective of this study was to determine the level of surface contamination at different points in a tuna processing plant using microbiological evaluation (Log CFU/mL) and then correlating them to the relative light units (RLU) values from the firefly rapid method. The second objective of this study was to assess and compare how changing the sampling surface area (2"x4", 4"x4", and 6"x4") of the microbiological testing procedure would affect the results. The surface areas that were evaluated were the stainless steel production tabletops at the point of entry (E), middle (M) and as the fish were leaving (L) the processing line, along with the trays that the tuna were carried into and out of the work area. The time intervals that were studied were after production (AP), after sanitation (AS), and before production (BP). Total aerobic plate counts (APC's) were obtained and enumerated using 3M Petrifilm^{TM} and incubating them at 35°C for 48 hours. No significant difference ($P > 0.05$) was found between AS' and BP's APCs. Overall, high APC's were found at the AP time, as surface areas had not yet been sanitized. Even though a laboratory calibration curve for Log CFU's and RLU's was highly correlated ($r^2 = 0.9527$), the plant's CFU and RLU values were found not to correlate ($r^2 = 0.139$).

Resumen

Los primeros pasos en la implementación de las regulaciones de seguridad en los alimentos a veces suelen ser los procesos de sanitización. El objetivo principal de esta investigación fue determinar el nivel de contaminación superficial en diferentes puntos de una planta procesadora de atún usando evaluación microbiológica (Log UFC/ml) para luego correlacionar estos valores con valores de unidades relativas de luz (RLU) del método rápido "firefly". El segundo objetivo de esta investigación fue evaluar y comparar cómo se afectaban los resultados de las pruebas microbiológicas al cambiar las áreas de superficie (2"x4", 4"x4", y 6"x4") de muestreo. Las áreas de superficie evaluadas fueron los topes de mesas de producción hechas de acero inoxidable a la entrada (E), la mitad (M), y la salida (L) de la línea de producción, junto con las bandejas donde el atún era transportado hacia dentro y afuera del área de trabajo. Los intérvalos de tiempo estudiados fueron: después de producción (AP), después de sanitización (AS) y antes de producción (BP). Los conteos aeróbicos totales fueron obtenidos y enumerados usando 3M Petrifilm^{TM} e incubándolos a 35°C por 48 horas. No se encontró diferencia significativa ($P > 0.05$) entre los conteos aeróbicos totales AS y BP. En general, los conteos aeróbicos totales para AP fueron altos, ya que las áreas de superficie en este tiempo aun no habían sido sanitizadas. Aunque las curvas de calibración hechas en el laboratorio para Log UFC's y RLU's tuvieron una correlación alta ($r^2 = 0.9527$), los valores UFC y RLU obtenidos en la planta no se correlacionaron entre sí ($r^2 = 0.139$).

Dedication

I will like to dedicate this work to my family:

(María Teresa, Edwin Román, Kervin Román, Irwin Román, María Socorro, Nuria Mariel, Ulianis Nicol, Sol María and Juana Rivera).

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Introduction

The tuna *Thunnus spp.* is a great tasting fish and civilizations all over the world have prized this magnificent animal as a great delicacy. Incas and other South American civilizations along the Pacific called it Xatunkama, while ancient Greeks and other Mediterranean's knew of it as Thunnos (Tuna for Life). Tuna belongs to the Scombroid family along with the mackerel and swordfish. The tuna is nicely streamlined, having a sharp pointed nose, tapering tail and crescent shaped fins. They have strong, firm, round bodies and are swift swimmers having speeds of up to 67 kph (Tuna for Life).

The commercial tuna industry has been around since the early 1900's and over 60,000 people have been employed directly or indirectly by the tuna industry. Approximately five million pounds of fish are processed and consumed annually worldwide, therefore making it one of the largest industries in America (California Seafood Council, 1997; Fernandes, 1997). Tuna is one of the most important seafood products widely traded in the international market, accounting for around eight per cent of the global fish imports by value worth more than 5 billion US dollars (World Tuna Trade Conference and Exhibition, 2004). Here in Puerto Rico the tuna fish industry has been around since 1977 and currently Bumble Bee Acquisition is the only active working tuna processing plant, where tuna is canned and distributed to the US and other countries. Current problems that the tuna industry faces are those of lowering their operating costs in order to compete with their competition and due to this, many of the fishing fleets are being sold and canneries being closed world wide. These actions in return cause a problem for the fishermen who depend on regular tuna sales to make a living. (Stuart 2001, Casamar 2002).

Fish in general are a highly perishable food product. Many microorganisms can grow on fish, as it is a rich source of nutrients for bacterial growth. Microorganisms come in many forms and can be either good or bad for fish products. There are bacteria that deteriorate/spoil the fish until it is no longer fit for consumption, and there are harmful pathogenic bacteria that can cause illnesses and death upon consumption of the fish. Histamine formation caused by the decarboxilation of L-histidine due to time and temperature abuse can cause an allergic reaction in tuna consumers (Díaz, 1988, Salas, 1995, Hernández, 1996).

Microbial growth in food products is largely avoided by having standard operating procedure for sanitation properly functioning in the plant. The sanitation process of any processing plant must be taken seriously as it can be a critical control point for microbial proliferation. There are many traditional ways in which sanitation can be monitored in a plant and at the same time it can take a lot of time to verify if sanitation was properly conducted. As technologies have become available and inexpensive, they have been incorporated into business operations. As such has been the case for rapid methods, which, as their name implies, were invented with the sole purpose of shortening the time that an item is quarantined until results demonstrate its safety. A very reliable and well-known sanitation monitoring system is the firefly rapid method, which measures sanitation effectiveness upon light emission.

Adenosine Triphosphate (ATP) bioluminescence technique is a rapid convenient method for hygiene monitoring in the food industry. This technique has been used in the food industry for over 24 years to quickly access and monitor microbial contamination on surfaces (Madl, 1997). The “Firefly” rapid method is a portable ATP bioluminescence

hygiene monitoring system that is a qualitative tool that can effectively monitor onsite microbial contamination on surfaces after sanitation procedures have been carried out. The technology of ATP bioluminescence for hygiene monitoring has become increasingly useful because of its real time capabilities, ease of use, and affordability (ArtecPro).

Swabs and plate counts are tedious, expensive and cannot generate timely results; therefore, the ATP system has become very popular. This rapid method cannot replace microbiology testing but does provide a real time indicator of the efficacy of the cleaning and sanitation procedures. Due to expense as well as time constraints, most companies do not have monitoring or final testing procedures to determine when a surface has been sufficiently sanitized. By placing this technology in the hands of trained personnel, they will be able to monitor the sanitation performance and evaluate microbial contamination.

Since the molecule adenosine triphosphate (ATP) is found in all living cells, both eukaryotic and prokaryotic, its detection is indicative of living material still being present once the surface has been cleaned by removing soils. ATP can be detected rapidly by light emission through the combined use of the enzyme luciferin luciferase and a luminometer (Stanley *et al*., 1989; Bautista *et al*., 1994); it is this compound that is necessary for the light reaction to occur in the firefly method, therefore getting its name. ATP is a highly stable compound that can remain long after the cell has died. This compound combines and reacts with an enzyme resulting in the release of light. The light emitted is measured using a luminometer. This output of light is proportional to the amount of ATP present on any given surface and indicates the presence of microorganisms (Madl, 1997). Thus the light units can be used to estimate contamination

on the sampled surface. Sometimes, the reaction can be used to estimate the biomass of cells in a sample (Fung and Matthews, 1991).

Literature Review

Rapid methods and their automation is a dynamic area in applied microbiology dealing with the study of improved methods in the isolation, early detection, characterization, and enumeration of microorganisms and their products in clinical, food, industrial, and environmental samples. In the past 20 years, this field has emerged into an important subdivision of the general field of applied microbiology and is gaining momentum nationally and internationally as an area of research and application to monitor the numbers, kinds, and metabolites of microorganisms related to food spoilage, food preservation, food fermentation, food safety, and foodborne pathogens (Fung, 1992, 2002).

As the field of rapid methods continues to develop, so does the instrumentation and diagnostic tests that are available. Instruments are needed that can indirectly monitor changes in a population using such indices as ATP levels, specific enzymes, pH, heat generation, among many other factors. It is important to know that for information to be useful these factors must be related to viable cell counts for the same sample readings (Fung, 2002). In general, the larger the number of viable cells in the sample, the shorter the detection time of these systems.

ATP bioluminescence is one of the most rapid emerging technologies used for rapid microbiological analysis. The ATP bioluminescence assay has been used for a variety of applications in the dairy and food industries (Samkutty *et al*., 2001; Gregg, 1991; Griffiths, 1991, 1993). The most widely used current application of ATP bioluminescence in the United States is for the estimation of surface cleanliness (Griffiths, 1991, 1993).

All living things utilize ATP, which functions as the major source of chemical energy in all cells (Lehninger *et al*., 1993). Bioluminescence is the light producing reaction catalyzed by enzymes in a living organism (Velazquez and Feirtag, 1997). In the presence of luciferin luciferase, oxygen and magnesium ions, ATP will facilitate the reaction to generate light. The amount of light generated by this reaction is proportional to the amount of ATP present in the sample. Thus, the light units can be used to estimate the biomass of cells in a sample (Stanley *et al*., 1989; Madl, 1997; Kiss *et al*., 1998; Green *et al*., 1999; Fung, 2002). Studies have demonstrated that this hygiene-monitoring rapid method is a dependable tool when it comes to sampling surfaces for contamination.

Kiss *et al*. (1998) proved the efficiency of detecting microorganisms after sanitation had taken place in a poultry slaughterhouse. La Duc *et al*. (2004) used the firefly rapid method in the International Space Station to asses any environmental contamination in a spacecraft and discovered it provided results more rapidly than using traditional microbial methodology. Leon and Albrecht (2004) used the same rapid method technique to measure levels of microbial loads on plastic food contact surfaces and, they too agreed upon the efficiency that the firefly rapid method had when it came to monitoring hygiene and sanitizing procedures. Jones *et al*. (2003) and Musgrove *et al*. (2004) conducted two very similar studies on egg processing plants and their similar results reinforced those of Leon and Albrecht (2004) proving again the effectiveness of the rapid methods.

The main goal of this research was to prove that sanitation procedures are being conducted properly at a tuna processing plant and that the surface areas where the tuna is processed will not result in a problem further down the production line. As such, if as

stated above is true, then the firefly rapid method will be a supporting tool used when evaluating Standard Sanitation Operation Procedures (SSOP's) and Good Manufacturing Practices (GMP's).

Hypothesis

The firefly rapid method is capable of determining the efficacy of sanitation procedures at a tuna processing plant.

Materials

- 3M Aerobic Count Plate (AC) Petrifilm™ (St. Paul MN) distributed by Fisher Scientific
- 3M Coliform Petrifilm™ (St. Paul MN) distributed by Fisher Scientific
- Fisher brand Polystyrene Disposable Serological Pipettes (individually packed)
- BD Bacto™ Peptone Enzymatic Digest of Protein distributed by Fisher Scientific
- PocketSwab Plus ® distributed by Charm Sciences Inc. and Ecolab
- Firefly ATP Monitor Analyzer distributed by Charm Sciences Inc. and Ecolab
- Whirl-Pack Meat/Turkey Carcass Sampling Kit distributed by Nasco
- 6"x 4" Glass Frame made by La Casa del Cristal located in Mayagüez, Puerto Rico
- Kimax ® USA Test Tubes and Pyrex ® USA Test Tubes (#9860, #9800, #9820)
- ThermoSpectronic Spectronic 20 D+ Spectrophotometer, Model 333183
- Fisher Scientific Isotemp Incubator, Model 5370
- Bantex Colony Counter, Model 920A

Facilities

- Tuna Processing Plant located in Mayagüez, Puerto Rico
- Food Microbiology Laboratory UPR Mayagüez, Piñero Building 014, Mayagüez, Puerto Rico

Methodology

Table and tray surface areas of a tuna processing plant were sampled for microbial growth. Three surface areas (2"x4", 4"x4", and 6"x4") were used as swabbing areas with the ATP PocketSwab Plus and the Whirl-Pack Meat/Turkey Carcass Sampling Kit. The production area of the tuna processing plant was sampled three times. These times were: after production had finalized around 7:00 pm, after sanitation of the processing area had finalized around 1:00 am and, just before production started at around 4:00 am. Samples were gathered on Thursdays.

The surface areas of interest in this study are the stainless steel production tabletops along with the trays where the tuna is carried to and from work areas. The production table is 38.71 meters long and 1.52 meters wide with a conveyor belt running along its length with a width of 40.64 cm. Three randomly selected distances were chosen to be sampled using the three different surface areas (Bottom of table, Middle of table, and Top of table). Two out of five trays in the transportation cart were randomly chosen to be swabbed as well. Each tray is 81.92 cm long, 39.37 cm wide and 10.16 cm deep. The three surface areas were also swabbed in the trays to assay bacterial growth. The water used for washing/rinsing the tabletops was tested to confirm that is was ATP free (Griffith *et al*., 1994).

To determine the amount of ATP present in the sampled areas, a calibration curve was conducted using the procedure described by Maturing and Peeler (1998). This allowed correlation of the number of aerobic colony forming units (CFU) in relation to the number of relative light units (RLU) given by the luminometer.

To collect each sample a sterile dry sponge pad 3" x 1.5" was moistened in a Whirl-Pack bag (NASCO, Modesto, CA) containing 25 mL of sterile peptone water. The sponge pad was gently squeezed to remove excess moisture and then used to aseptically sample predefined areas at each sample site. After sampling, the sponge pad was returned to the bag containing the peptone water and sealed. A clean pair of gloves was used when handling each sponge pad. Samples were transported on ice back to the laboratory and refrigerated at 4°C until plated.

Serial dilutions 10^1 through 10^6 of each sample were made in 1/100 concentration with sterile peptone water. Total aerobic plate counts (APC's) were determined by plating 1.0 mL on Petrifilm™ Aerobic Count Plates (3M Microbiology Products, St. Paul, MN) and incubated at 35°C for 48 hours. All dilution samples were plated in duplicate. (The samples taken after production were plated within 24 hours. The samples taken after sanitation had taken place were plated before 48 hours and the samples taken before production had taken place were plated before 72 hours).

The experimental design was a 5 x 3 x 3 factorial arrangement of treatments in a completely randomized design with three repetitions done in duplicate. The factors were: area on the production line (stainless steel production table tops and tuna trays), surface area size (2"x4", 4"x4", and 6"x4") and position in relation to the stainless steel production table tops (Bottom of table, Middle of table, and Top of table). Results were analyzed using the analysis of variance option and means were separated using the Tukey variance analysis test at a significance of 95% using InfoStat (2004).

Sanitizers Used in the Sanitation Process

Eco Care 250- One step hand cleaner sanitizer used by the brewery, beverage, dairy and food processing plant personal. Contains triclosan as its active ingredient.

Enforce LP- Self-foaming, chlorinated, alkaline detergent for cleaning process equipment, in the dairy, beverage, and food processing industries. Contains sodium hydroxide (caustic soda) and sodium hypochlorite. To be used on stainless steel surfaces no more than fifteen minutes.

Evapoo Kleen- Acid detergent for cleaning stainless steel only (No foam). For cleaning only high temperature short time units and evaporators in the dairy and food industry. Contains nitric acid; to be used on stainless steel surfaces no more than fifteen minutes.

Quorum Purple- Self-foaming detergent for the food processing industry. Contains phosphoric acid, dodecylbenzenesulfonic acid, and anionic and nonionic surfactants. Used to shine stainless steel surfaces for a period of no more than fifteen minutes.

Quorum Brown- Heavy-duty alkaline cleaner for the food processing industry. Contains sodium hydroxide (caustic soda) and chelating agents. To be used in the pipeline system no more than fifteen minutes.

All the sanitizers are distributed by Ecolab Food and Beverage Division, Ecolab Center, St. Paul, Minnesota

Results and Discussion

This study was conducted to evaluate the effectiveness of surface sanitation procedures at a tuna processing plant. Table 1 shows that the APC values between the AS and BP times were not significantly different (P> 0.05). However, a significant difference in Log CFU/mL (P< 0.05) was found between the AP value and the AS and BP values. This expected difference was due to the fact that the AP samples were taken before the surface areas sampled were cleaned and sanitized, therefore they contained the tuna particles/residue and all its associated microorganisms from a whole days production. Even though the AS and BP values were not significantly different, the BP values were greater than the AS values indicating an increase in microbial loads during the 3-hour interval between the samplings.

Table 1: Average Log CFU/mL for after production (AP), after sanitation (AS), and before production (BP) for all three surfaces tested.

Time	Means	
AP	6.31	B
AS	3.84	A
BP	4.48	A

Different letters indicate significant difference (p<=0.05)

Figures 1, 2 and 3 show how a large number of Log CFU/mL were found after production, how the Log CFU/mL decreased significantly after sanitation procedures had taken place, and how the Log CFU/mL increased again during the time before production restarted. Although graphically a difference does exist between after sanitation and before production in Log CFU/mL (Figures 1, 2 and 3), statistically no difference exists among them.

Figure 1 Average Log CFU/mL from 2” x 4” Surface Areas at All Times

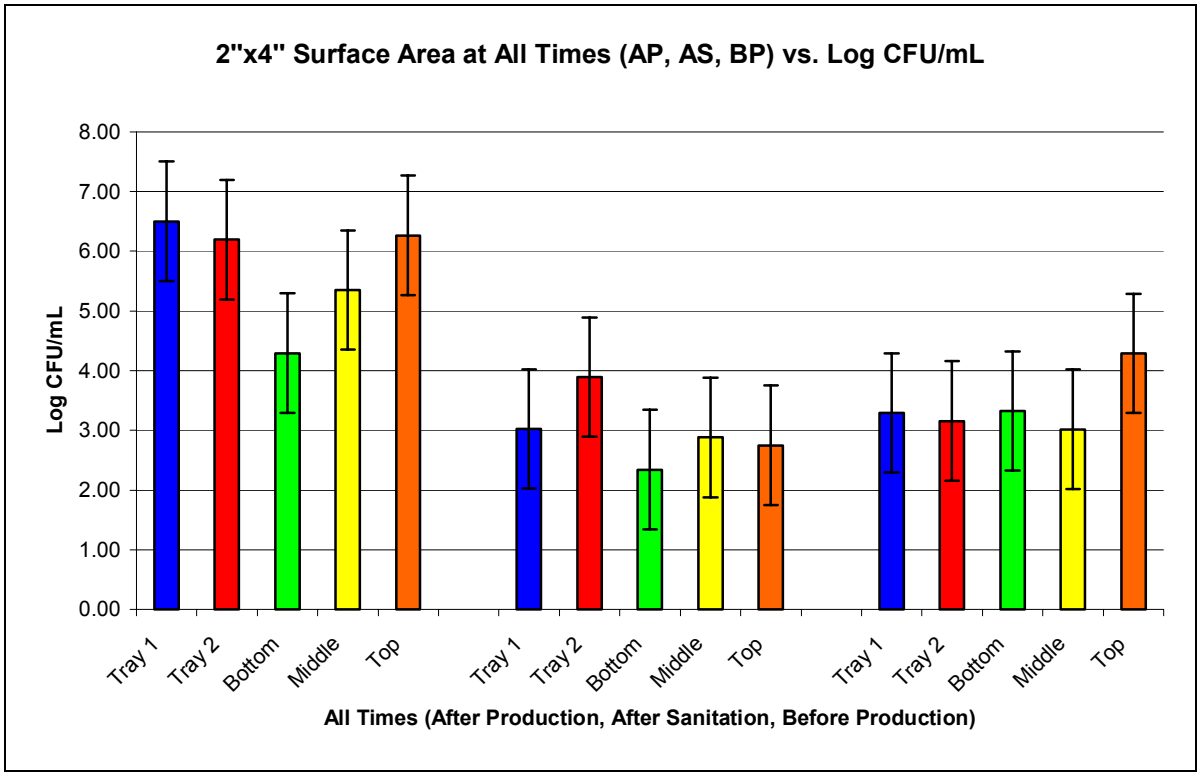


Figure 2 Average Log CFU/mL from 4” x 4” Surface Areas at All Times

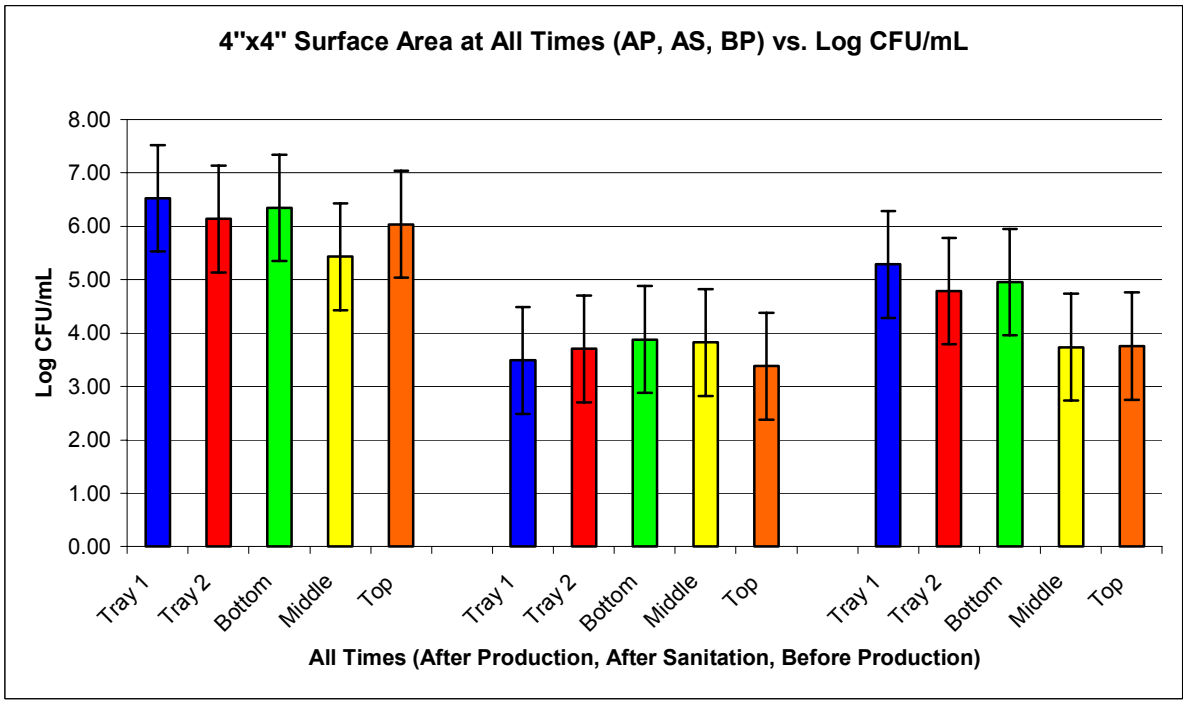


Figure 3 Average Log CFU/mL from 6” x 4” Surface Areas at All Times

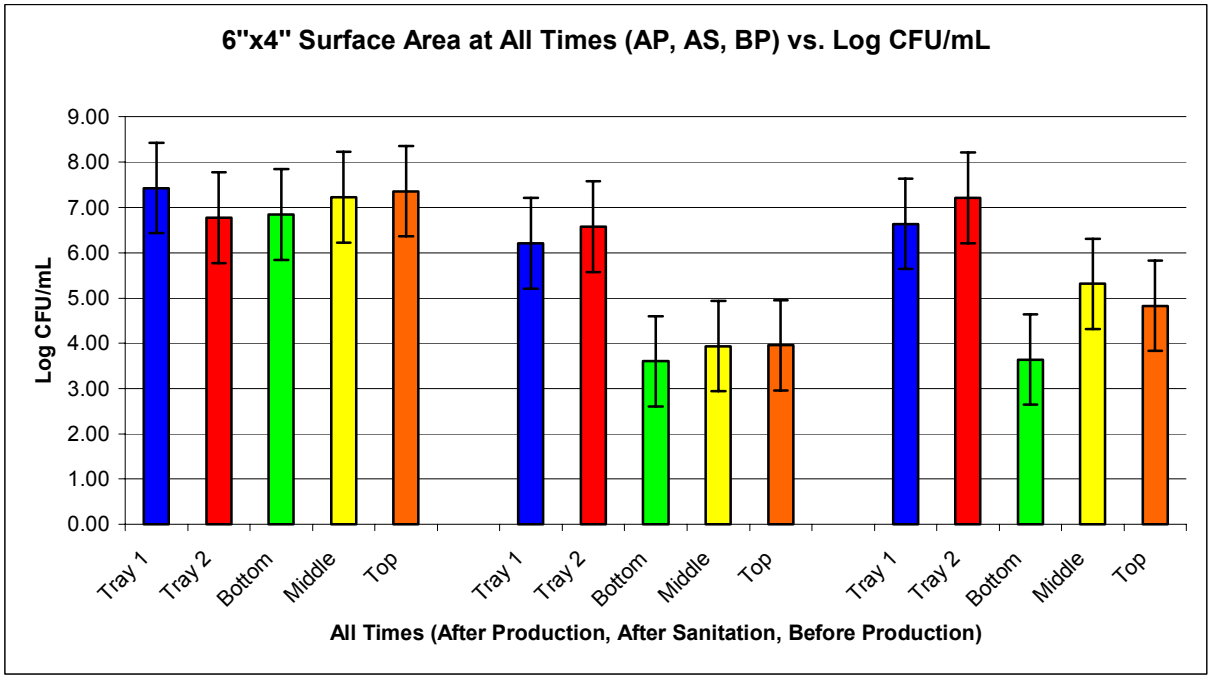


Table 2 shows significant differences (P< 0.05) between all three surface areas (2”x 4”, 4”x 4”, 6”x 4”). This considerable increase is in all likelihood due to the extra amount of space sampled in the surface area of interest. But on a square inch basis the values decreased from 0.505 Log CFU/mL for the 8 in^2, to 0.298 Log CFU/mL for the 16 in^2, and 0.243 Log CFU/mL for the 24 in^2. This could indicate that the smaller the swab used the more efficient is the lifting of the material off of the surface due to a greater hand pressure or friction.

Table 2: Average Log CFU/mL for the three different size swab areas (2”x 4”), (4”x 4”), and (6”x 4”) for the five sites tested.

Area	Means	.
2”x 4”	4.04	A
4”x 4”	4.76	B
6”x 4”	5.84	C

Different letters indicate significant difference (p<=0.05)

While Table 3 for the five places sampled at the tuna processing plant (Tray 1, Tray 2, Entering (E), Middle (M) and, Leaving (L) the Processing Table) does not show any significant differences in microbial loads (P> 0.05), between them definite trends are seen. The Log CFU/mL increased as one moved down the processing line and the two tray values were identical to each other and greater than any of the line values. One possible explanation for the higher tray values is probably due to the large number of cracks in their surfaces making them harder to clean resulting in higher microbial loads (Jones *et al*., 2003; Joseph *et al*., 2001).

Table 3: Average Log CFU/mL for the five sites tested: two trays carrying loins to processing line (Tray 1, Tray 2), entering processing line (E), middle of the processing line (M), and leaving the processing line (L).

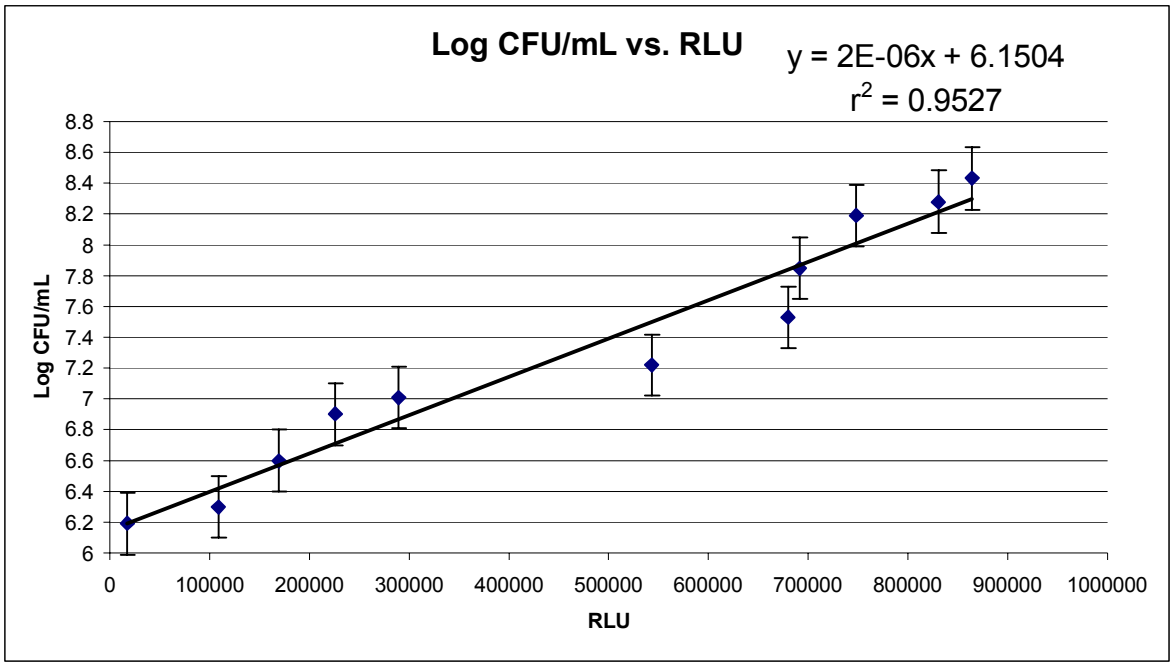
Places	Means	.
Tray 1	5.38	A
Tray 2	5.38	A
E	4.38	A
M	4.52	A
L	4.74	A

Different letters indicate significant difference (p<=0.05)

Through the use of calibration curves it was possible to correlate Log CFU/mL with Relative Light Units (RLU) and then the RLU values with the effectiveness of sanitation. Graph 4 shows the linear regression calibration curve with a high correlation of $r^2 = 0.9527$. By using the linear equation given by Figure 4 $\mathbf{y=2x10^{-6} X + 6.1504}$, and applying the anti logarithm variable (anti-Log), a colony forming unit (CFU) value can be calculated by plotting in the relative light unit (RLU) value in the X axis. National and international organizations have all demonstrated that a Log of 10^5 CFU/mL or less is an acceptable value for aerobic plate counts of raw, breaded, and

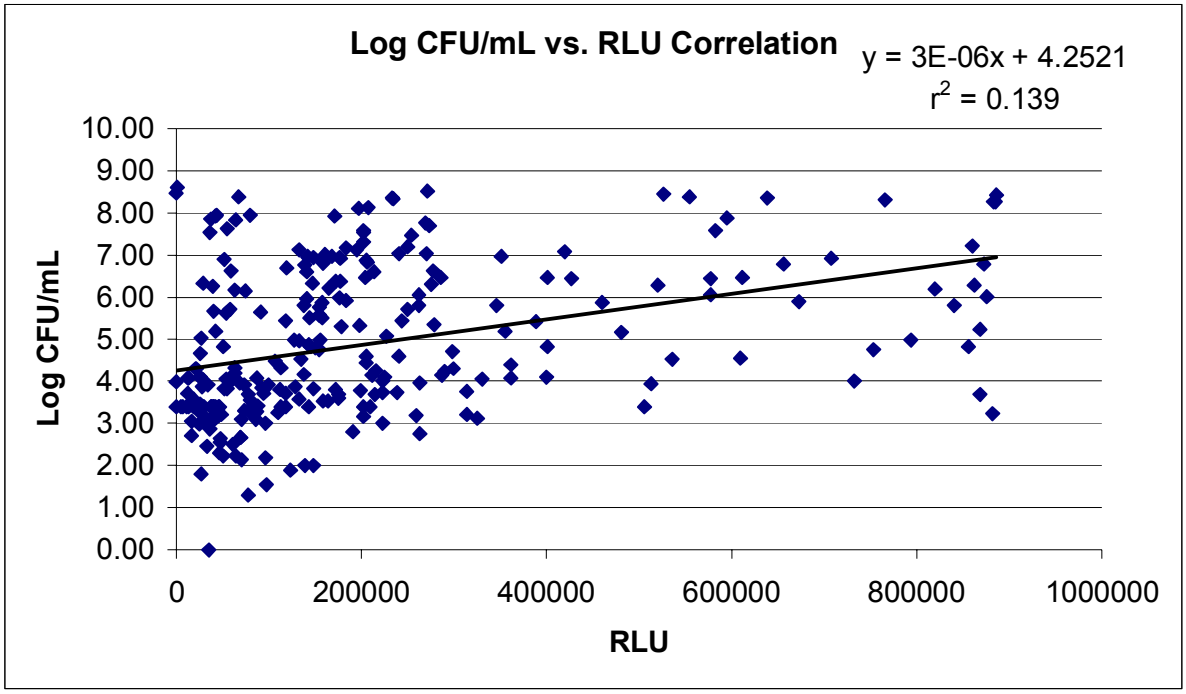
frozen fish at 35°C under which the tuna fish at the processing plant falls under (APHA, 1992; Microbiological Reference Criteria for Food, 1995) so the surface areas fall out of compliance as was expected.

Figure 4 Linear Regression Log CFU/mL vs. RLU Calibration Curve



However, a calibration curve of the plant's CFU and RLU values did not correlate as expected, having a $r^2 = 0.139$ as shown by Figure 5. This inaccurate correlation can be due to the fact that many other factors were involved at the processing plant that were not present in a laboratory setting. Factors such as: a controlled environment, completely dry surfaces where the ATP swabs were used, an environment free of flying insects like flies and moths, free of rust and pollution among other factors.

Figure 5 Linear correlation of processing plants Log CFU/mL vs. RLU values



By working and sampling at a tuna processing plant the areas of interest had to endure harsh treatments and handling. Among those treatments was the sanitation process where the trays and stainless steal surface tabletops were exposed to several sanitizers. Active ingredients like sodium hypochlorite, self-foaming chlorinated alkaline and no foaming acid detergent, and sodium hydroxide (caustic soda) have proven to indicate false positives and/or negatives as found in studies by Velazquez and Feirtag (1997) and Green *et al.* (1998, 1999). With the above mentioned, one must take into consideration the fact that those active ingredients were present at the time of sanitation and the RLU values could have been quenched or enhanced by the presence of any of the sanitizers. According to Velazquez and Feirtag (1997), a quencher is a substance or compound that withdraws or reduces the light signal, and an enhancer is a substance or compound that increases or amplifies the light signal.

Velazquez and Feirtag (1997) and Green *et al*., (1998, 1999) both demonstrated how with most cleansers and sanitizers, the lowest concentrations produced signal enhancements. While the higher concentrations produced quenching effects on the light signal emitted by the luminometer. The results of Velazquez and Feirtag (1997) and Green *et al*., (1998, 1999) could be a possible explanation as to why the Log CFU/mL vs. RLU correlation presented in Graph 5 was so poor. When performing the calibration curve in a laboratory setting no sanitizers of any kind were present when using the luminometer, while at the tuna processing plant, the surface areas of interest were swabbed while possible residues of sanitizers may have been present, therefore affecting the luminometers bioluminescent reading.

Another factor that must be taken into consideration when samples were being collected was the water present on the stainless steel production tabletops. By the surface being uneven or not leveled, the water formed a puddle and did not wash away like in some other parts of the table. By water being essential for microbial growth, microbial niches can develop when wet cleaning is performed and surfaces are not completely dried (Musgrove *et al*., 2004, Gregerson, 2005). The presence of those water puddles may have also affected in some way both the CFU values and RLU readings. The CFU values may have been affected by showing an increase in colony growth in the Petrifilm, while the RLU readings may have been affected by the light signal being enhanced and /or quenched with a possible mixture of water and sanitizer.

Table 4: Bacterial reduction values attained using after production (AP) and after sanitation (AS) CFU/mL values ([$10^{AP} - 10^{AS}$) / 10^{AP}] x 100).

	4x4	2x4	6x4
Tray 1	99.9077	99.9665	94.0434
Tray 2	99.6327	99.4988	37.6265
Entry (E)	99.4930	98.8780	99.9425
Middle (M)	97.5453	99.6572	99.9487
Leaving (L)	99.7012	99.9695	99.7015
Average	**99.272**	**99.594**	**98.409***

**Omitting Tray 2 value*

According to the Code of Federal Regulations (21 CFR 178.1010), bacterial reduction for sanitizers of non food-contact surfaces is 99.9% and 99.999% for food-contact surfaces. With the above said, Table 4 shows that while using the recommended 4"x 4" swab areas that the values obtained for Tray 1 was the only one that met non food-contact surface requirements, while none met the requirements for food contact surfaces indicating that sanitation procedures are not fully complying with the federal regulation standards.

Conclusions

The study conducted here proved that the firefly rapid method is indeed an effective rapid method to determine if sanitation procedures are being conducted accurately at the tuna processing plant only if interfering agents are not introduced into the evaluation process.

The stainless steel tabletops and trays which are used appear to be clean after sanitation has taken place but in reality are still contaminated with microorganisms which are later detected both as a bioluminescent signal and as an actual bacterial colony forming unit due to the cracks that have developed. This proves that over time management has to incorporate testing procedures that will deal with the physical changes in the plant so that the sanitation crew is correctly sanitizing the tuna processing plant once production has been completed.

As expected, the size of the swabbed surface, (2”x4”, 4”x4”, and 6”x4”) did make a significant difference in the amount of material obtained. The larger the area size, the more sample gathered, therefore a higher Log CFU/mL as the sizes increased. However, since the amount of material obtained per square inch decreased with increasing surface area swabbed may have demonstrated that efficiency in obtaining material from the surface increases with a decreasing swab area. When it came to RLU values the surface area was irrelevant even though the luminometer’s instructions manual says that 4”x4” is the preferred area size.

Recommendations

To determine if the firefly rapid method could be implemented to monitor surface cleanliness and sanitation in this plant, a series of studies should be conducted to analyze how different sanitizers and their corresponding components interfere with the firefly rapid method. Once this information is known it should be used to develop procedures that could be implemented into the processing plant while allowing the use of the firefly rapid method.

The last step of sanitation procedures for food contact surfaces is sanitizing because a food contact sanitizer needs no rinsing after it's applied (21 CFR 178.1010). A food contact surface should be allowed to drain and should not be rinsed because this not only meets FDA regulations but eliminates the possibility of quenching and/or enhancing the luminometers light signal due to water contamination of the swab.

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Appendix

Appendix 1

Results from Statistical Variance Analysis ANOVA using Infostat 2004

Variable	N	R^2	R^2Aj	CV
Log CFU/mL	**90**	**0.78**	**0.57**	**22.65**

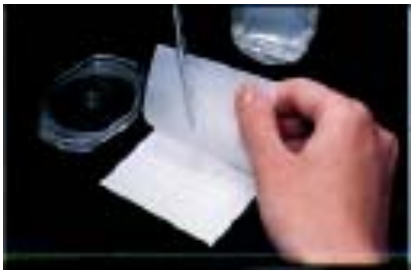
Log CFU/mL Variance Analysis

F.V.	SC	df	CM	F	p Value
Model	200.06	44	4.55	3.72	<0.0001
Time	98.83	2	49.42	40.47	<0.0001
Area	49.20	2	24.60	20.15	<0.0001
Places	16.18	4	4.05	3.31	0.0184
Time*Area	1.73	4	0.43	0.35	0.8397
Time*Places	3.87	8	0.48	0.40	0.9167
Area*Places	14.04	8	1.75	1.44	0.2077
Time*Area*Places	16.20	16	1.01	0.83	0.6476
Error	54.95	45	1.22		
Total	255.00	89			

Appendix 2

3M Petrifilm™ Total Aerobic Plate Count Enumeration

A



B

C

D

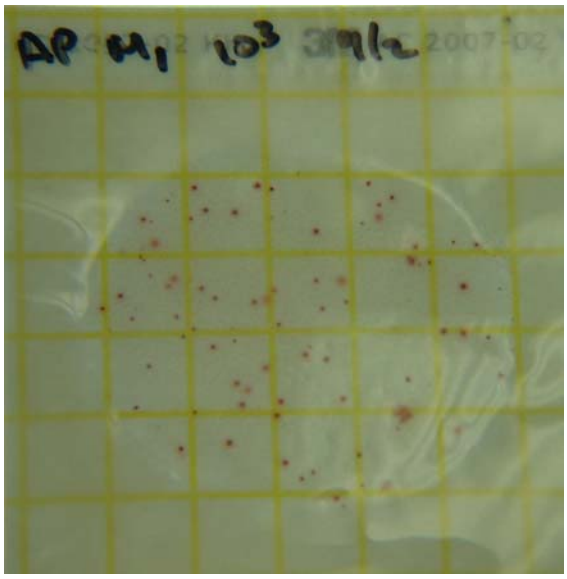
E

F

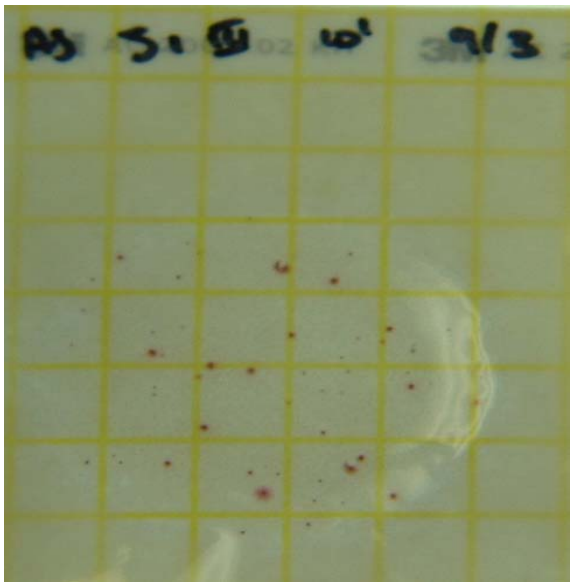
G

Appendix 3

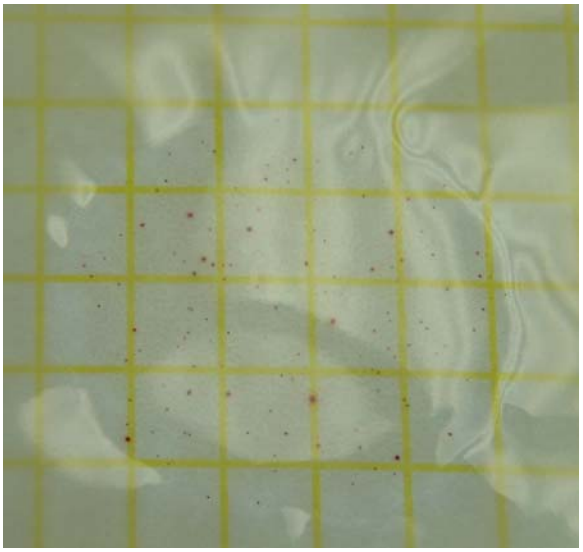
Photos of Plate Count Enumeration During the AP, AS, and BP Times



After Production
Plate Count Enumeration



After Sanitation
Plate Count Enumeration



Before Production
Plate Count Enumeration