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## **Optimization of the Celsis ATP Bioluminescence Assay for the Detection of Microbial Contamination in Triclosan and High Fluoride Dentifrices**

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### **Abstract**

The Celsis ATP Bioluminescence method was optimized and validated to detect the presence of microbial contamination in High Fluoride and Triclosan dentifrice formulations. Several enrichment broths were evaluated by using a 24 - 27 hour incubation period. The ATP concentrations of the enrichment broths were found to range from 0.012 to 0.040 nM. None of the tested enrichment broths were found to exhibit any sample inhibition/enhancement effects on the ATP Bioluminescence reaction. Dentifrice suspensions were inoculated with bacteria, yeast, and mold. All test microorganisms were detected within a 24 - 27 hour incubation period by using TAT Broth Base enrichment broths containing different concentrations of the following ingredients: Tween 20, neopeptone, dextrose, Triton X-100, sodium thiosulfate, sodium dibasic phosphate, and glycine.

### **Introduction**

Quality control of personal care products typically includes microbiological analysis by standard procedures which require 5 to 7 days to complete. Furthermore, these procedures are time consuming, and labor intensive.

Several new technologies have been developed for the rapid detection of microorganisms in food, cosmetic, and pharmaceutical products (Fung, 1994). Bioluminescence is one of the new technologies for the rapid detection of microbial activity in environmental and product samples (De Weger et al. 1991; Siragusa and Cutter 1995). The Celsis ATP Bioluminescence assay is based upon the enzyme luciferase which hydrolyzes ATP to produce light. ATP is present in all living microorganisms and can be used to ascertain the presence or absence of microbial contamination in product samples. English et al. (1995) showed a 24 hour detection of microbial contamination in regular dentifrices after enrichment in TAT Broth Base containing 4% Tween 20. However, no other studies have been reported regarding the use of ATP Bioluminescence for quality control of High Fluoride and Triclosan dentifrices.

The purpose of this investigation was to optimize and validate the use of the Celsis ATP Bioluminescence Assay for the detection of microbial contamination in Triclosan and High Fluoride Dentifrices for faster product release and quality evaluation.

### **Materials and Methods**

#### **Microbial Cultures**

Test microorganisms were cultured for 24 - 48 hours in Trypticase Soy Broth (TSB) or Sabouraud Dextrose Broth (SDB). The reference strains were *Escherichia coli* ATCC

8739, *Pseudomonas aeruginosa* ATCC 9027, *Staphylococcus aureus* ATCC 6538, *Salmonella typhimurium* ATCC 13311, *Candida albicans* ATCC 10231, and *Aspergillus niger* ATCC 16404.

#### **Enrichment Broth Preparation**

Three enrichment broths were evaluated: TAT Broth Base with 4% Tween 20, R Broth (TAT Broth Base with 4% Tween 20, 1% neopeptone, 1% dextrose, and 0.25% sodium thiosulfate), Modified R (MR) Broth (TAT Broth Base with 10% Tween 20, 1% neopeptone, 1% dextrose, 1% glycine, 1% Triton X-100, 0.5% sodium dibasic phosphate, and 0.50% sodium thiosulfate).

#### **ATP Bioluminescence Method Evaluation**

Product samples were added to the broth (1% w/v) and mixed thoroughly. The effects of the samples on the ATP Bioluminescence reaction were determined as previously described (English et al. 1995).

To determine the sensitivity of the ATP Bioluminescence assay to detect microbial contamination, suspensions of microorganisms were serially diluted and added to the different dentifrice suspensions. Inoculated and uninoculated dentifrice suspensions, along with enrichment broth controls were incubated at 35°C, with shaking at 200 r.p.m., for 24 - 48 h. From each incubated sample, duplicate 50 ml aliquots were pipetted into separate cuvettes. Each cuvette was analyzed for the presence of ATP using the Personal Care Products Kit and Optocomp Luminometer (both from Celsis I Lumac, Evanston, IL). The results were printed in relative light units (RLU).

If a test sample result was two times the reading of the uninoculated enrichment broth, it was considered positive. After analysis, samples were streaked on Trypticase Soy Agar (TSA), Sabouraud Dextrose Agar (SDA), and Potato Dextrose Agar (PDA) to confirm the presence or absence of bacteria, yeast, and mold growth, respectively.

#### **Results and Discussion**

The ATP concentrations of the various enrichment broths and the tested dentifrice suspensions are shown in Table 1. The products did not contain significant ATP.

The effects of the dentifrice suspensions on the ATP Bioluminescence reaction are shown in Table 1. The response of High Fluoride and Triclosan dentifrices suspensions to ATP, expressed as a percentage of the response to broth alone, ranged from 97.5% to 113.9%. These values are within the range recommended by the supplier for an acceptable response. In general, all tested enrichment broths and sample suspensions demonstrated

low endogenous ATP, and no enhancement/inhibition effect on the ATP bioluminescence reaction.

The final step in validating the Celsis ATP Bioluminescence Assay is to add various levels of different types of microorganisms to the 1% dentifrice suspensions to ensure detection of microbial growth. After a 24 hour incubation period, bacterial and yeast contamination of the High Fluoride dentifrice suspensions were detected in TAT

Broth Base containing 4% Tween 20 (Table 2). However, *A. niger* was not detected until 48 hours. Although English et al. (1995) reported the detection of *A. niger* in toothpaste suspensions after a 24 hour incubation period, the different chemical composition of the High Fluoride dentifrices required a longer incubation period.

**Table 1 ATP Concentration Values and Inhibition/Enhancement Effects on the ATP Bioluminescence Reaction**

Test Sample	Broth	ATP Concentration of Broth alone (nanomolar)	ATP Concentration of Sample Suspension (nanomolar)	Response of Product Suspension to ATP (%)	
High Fluoride Dentifrices	TAT Broth w/4% Tween 20	0.014	0.014	98.1	
		0.030	0.030	107.9	
			0.012	0.012	105.0
	R Broth	0.020	0.020	97.5	
		0.030	0.030	113.9	
			0.020	0.020	103.2
Triclosan Dentifrice	Modified R	0.040	0.040	103.0	

To reduce the detection time for mold contamination in High Fluoride dentifrice a new enrichment broth, R Broth, was formulated by the addition of dextrose, neopeptone, and sodium thiosulfate. Test results showed that mold contamination was detected within a 27 hour incubation period (Table 2). The addition of dextrose and neopeptone, known ingredients for the isolation and cultivation of molds, to the TAT Broth with 4% Tween 20 provided more nutrients for the mold to grow, while sodium thiosulfate provided further neutralization of antimicrobial agents by chemical inhibition.

Test results using the R Broth with Triclosan dentifrice suspensions demonstrated a 24 hours detection of the following microorganisms: *E. coli*, *P. aeruginosa*, *S. typhimurium*, *C. albicans*, and *A. niger*. However, *S. aureus* was not detected until 48 hours (data not shown). This was due to the low minimal inhibitory concentration value (MIC) of Triclosan against *S. aureus* which indicated that it was necessary to further neutralize the Triclosan and also provide some additional nutrients for the *S. aureus* cells to grow (Russell 1995).

**Table 2 Detection Times of Microbial Contamination in High Fluoride and Triclosan Dentifrice Suspensions using Different Enrichment Broths**

Sample	Broth	Microorganism	Detection Time (Hours)
High Fluoride Dentifrice	TAT w/4% Tween 20	<i>Escherichia coli</i>	24
		<i>Pseudomonas aeruginosa</i>	24
		<i>Staphylococcus aureus</i>	24
		<i>Salmonella typhimurium</i>	24
		<i>Candida albicans</i>	24
		<i>Aspergillus niger</i>	48
		<i>Escherichia coli</i>	24
	R Broth	<i>Pseudomonas aeruginosa</i>	24
		<i>Staphylococcus aureus</i>	24
		<i>Salmonella typhimurium</i>	24
		<i>Candida albicans</i>	24
		<i>Aspergillus niger</i>	27

Triclosan	MR Broth	<i>Escherichia coli</i>	24
Dentifrice		<i>Pseudomonas aeruginosa</i>	24
		<i>Staphylococcus aureus</i>	24
		<i>Candida albicans</i>	24
		<i>Aspergillus niger</i>	27

To enhance the recovery of *S. aureus*, different enrichment broths were analyzed. The R Broth was modified (MR) by increasing the concentration of Tween 20 from 4% to 10%, and adding Triton X-100, glycine, and sodium dibasic phosphate. The Triton X-100 provided additional neutralization of the preservative system while glycine is a known ingredient for the isolation and cultivation of *Staphylococcus* spp. which is also found in Vogel Johnson and Baird Parker media. Test results of Triclosan dentifrice suspensions in MR Broth showed the detection of *S. aureus* contamination after 27 hours (Table 2).

With each High Fluoride dentifrice formulation tested, we were able to detect an inoculum of between 1 to 10 Colony Forming Units (CFU)/per gram of

toothpaste while the sensitivity for the Triclosan dentifrice was found to range between 1 to 8 CFU/gram of toothpaste (See Tables 3 and 4). All samples showing a positive response by the ATP Bioluminescence method were confirmed as positive by showing growth on TSA, SDA, and PDA media.

Because of the different chemical composition of regular, High Fluoride, and Triclosan dentifrice formulations, it can be concluded that optimization of the ATP bioluminescence test method will require the continuous modification of the enrichment broth in order to detect the presence of microbial contamination. Rapid analysis of High Fluoride and Triclosan dentifrices was performed which will allow faster quality evaluation, product release and resource maximization.

**Table 3 Detection of Microorganisms in a High Fluoride Dentifrice by Using R Broth**

	Mean RLU of Broth	Approx. CFU per Inoculum	Mean RLU of Sample in Broth post-incubation	Detection by ATP Bioluminescence	Growth on Agar
<i>P. aeruginosa</i> ATCC 9027	2,161	29	20,500,000	Y	+
		9	20,100,000	Y	+
		2	20,400,000	Y	+
		<1	1,990,000	Y	+
		<1	3,139	N	-
<i>S. aureus</i> ATCC 6538	2,161	44	5,309,677	Y	+
		21	7,143,769	Y	+
		7	6,161,864	Y	+
		3	581,185	Y	+
		2	270,208	Y	+
<i>E. coli</i> ATCC 8739	2,095	45	3,294,931	Y	+
		14	5,489,070	Y	+
		2	7,211,048	Y	+
		<1	3,445	N	-
		<1	3,168	N	-
<i>S. typhimurium</i> ATCC 13311	3,096	61	5,880,750	Y	+
		12	10,291,926	Y	+
		3	12,442	Y	+
		<1	3,376	N	-
		<1	3,158	N	-
<i>C. albicans</i> ATCC 10231	2,221	40	8,919,016	Y	+
		10	1,701,841	Y	+
		1	4,109	N	-
		<1	3,924	N	-
		<1	4,697	N	-
<i>A. niger</i> ATCC 16404	2,670	20	58,779	Y	+
		11	32,508	Y	+
		7	17,433	Y	+
		4	9,275	Y	+
		2	7,328	Y	-

Table 4 Detection of Microorganisms in a Triclosan Dentifrice by Using MR Broth

	Mean RLU of Broth	Approx CFU per Inoculum	Mean RLU of Sample in Broth post-incubation	Detection by ATP Bioluminescence	Growth on Agar
<i>P. aeruginosa</i> ATCC 9027	1,365	17	387,825	Y	+
		4	312,845	Y	+
		<1	1,616	N	-
		<1	2,333	N	-
		<1	1,567	N	-
<i>S. aureus</i> ATCC 6538	1,365	23	218,886	Y	+
		7	46,254	Y	+
		<1	9,081	Y	+
		<1	1,587	N	-
		<1	1,640	N	-
<i>E. coli</i> ATCC 8739	1,111	29	4,695,722	Y	+
		8	685,643	Y	+
		<1	1,705	N	-
		<1	1,473	N	-
		<1	1,524	N	-
<i>S. typhimurium</i> ATCC 13311	924	30	11,430,634	Y	+
		13	7,704,608	Y	+
		2	2,517,238	Y	+
		<1	1,488	N	-
		<1	1,693	N	-
<i>C. albicans</i> ATCC 10231	924	11	188,786	Y	+
		4	37,410	Y	+
		<1	2,783	Y	+
		<1	1,512	N	-
		<1	1,545	N	-
<i>A. niger</i> ATCC 16404	942	9	8,339	Y	+
		2	3,978	Y	+
		<1	1,565	N	-
		<1	1,533	N	-
		<1	1,465	N	-

## References

- DE WEGER, L.A., DUNBAR, P., MAHAFFEE, W.F., LUGTENBERG, B.J.J., and SAYLER, G.S. 1991. Use of bioluminescence markers to detect *Pseudomonas spp.* in the rhizosphere. *Applied and Environmental Microbiology* 57, 3641-3644.
- ENGLISH, D., IGNAR, R., JIMENEZ, L., and REID, W. 1995. Rapid release of end-products using the Celsis PCP system - a novel microbial assay. *Cosmetics and Toiletries Manufacture Worldwide Supplement*, 246-255.
- FUNG, D.Y.C. 1994. Rapid methods and automation in food microbiology. A review. *Food Reviews International* 10, 357 - 375.
- RUSSELL, A.D. 1995. Mechanisms of bacterial resistance to biocides. *International Biodeterioration and Biodegradation* 36, 247 - 265.
- SIRAGUSA, G.R., and CUTTER, C.N. 1995. Microbial ATP bioluminescence as a means to detect microbial contamination on artificially contaminated beef carcass tissue. *Journal of Food Protection* 58: 764 - 769.