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The Evaluation, Validation and Implementation of ATP Bioluminescence Technology for the Microbiological Examination of Personal Care Products

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Abstract

Standard microbiological techniques for the analysis of personal care products take a minimum of 5 days to complete. An ATP Bioluminescence system was evaluated with the objective of reducing this testing time to 24 hours.

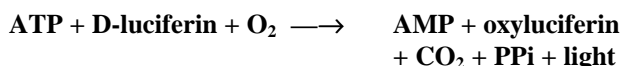
All products tested to date have proved compatible with the assay, showing no significant effects on the bioluminescence reaction. Two enrichment broths, Tryptone Azolectin Tween (TAT) and Letheen have been evaluated, showing minimal difference in response. Letheen broth was selected for regulatory aspects and to maintain consistency within the company. Good correlation (between 89 and 98%) was achieved when various formulations of personal care products were artificially contaminated with low levels of different organisms, including *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis*, and tested by both the standard agar plate method and ATP bioluminescence.

Validation of the system followed standard protocols, demonstrating that the specificity, limits of detection and limits of quantitation of the technology were acceptable. Installation qualification demonstrated the function and reliability of the equipment.

Successful evaluation and validation of this system has enabled the introduction of ATP bioluminescence into routine use within the microbiology laboratory. This has provided a rapid assessment of product quality, resulting in faster throughput and resource maximisation.

Introduction

Finished product is subject to a number of quality checks before it can be released by the manufacturing plants. Although the results from the chemical analyses are available within one day, the conventional microbiological procedures require five days to complete, thus delaying the process and incurring significant warehousing costs. This has put increasing pressure on the microbiology departments to change to more rapid methods which allow positive release of product. With the objective of reducing testing time to 24 hours, we have evaluated a system based on ATP bioluminescence. This is a sensitive technique which detects micro-organisms by measuring the light emitted when their ATP reacts with a reagent cocktail of firefly luciferase and luciferin.



The test sample is first enriched in broth for 24 hours to allow any micro-organisms present to multiply to a detectable level. This provides a rapid assessment of product quality and will allow the vast majority of product batches to be released the next day.

In order to evaluate the suitability of ATP bioluminescence to the analysis of mouthwashes and toothpastes, the following parameters were investigated:

- Selection of enrichment broth
- The effects of the products on the Bioluminescence Assay
- The detection of low levels of micro-organisms
- The level of agreement with the SmithKline Beecham traditional method.

Materials and Methods

The ATP Bioluminescence assays were performed using the Celsis I Lumac Personal Care Products System, including the reagents and protocol of the Personal Care Products Kit (PCPK) and the Optocomp II Luminometer. The Letheen broth and Tryptone Azolectin Tween (TAT) broth base were from Difco Laboratories and prepared according to the manufacturer's instructions.

Assay Protocol

1. A sample of product was dispersed in broth to give a 10% (w/v) suspension (1g in 9 ml or 10g in 90 ml).
2. This suspension was further diluted by removing 1ml and adding it to a further 9ml of the same broth
3. The suspensions were incubated at 30 °C, shaking at 250 r.p.m., for 24 hours.
4. Duplicate aliquots of 50 ml were pipetted into separate cuvettes and placed into the Optocomp II Luminometer. The assays were performed automatically as follows:
 - 200 ml ATP Releasing Agent
 - 10 second delay
 - 100 ml Bioluminescence Reagent
 - 10 second integration
 - Result printed in relative light units (RLU)
5. Interpretation: An sample with an RLU of more than twice the RLU for broth alone is positive.

Selection of enrichment broth

The variation in RLU within a batch of two preservative-neutralising enrichment broths, TAT and Letheen, was investigated. For each broth a bulk amount of broth was prepared which was divided into ten separate bottles and autoclaved. The response in the bioluminescence assay was determined following the protocol above; no enrichment was involved. The mean RLU and coefficient of variation for duplicate assays were recorded. The selected broth was then used for all subsequent work.

Determination of the Effects of Products on the Bioluminescence Assay

Products may contain components which contain ATP, or which either enhance or inhibit the bioluminescence reaction. These effects were investigated for a total of 8 formulations of mouthwash and 13 different formulations of toothpaste. In order to establish the extent of reproducibility of the baseline signal between batches of product, three separate suspensions of product from each of three different production dates were prepared in Letheen broth (1% w/v). Assays were performed in duplicate on the following (no incubation was involved):

50 ml product suspension

50 ml product suspension + 10 ml ATP Positive Control (final ATP concentration 16nM)

The results were used to calculate both the ATP concentration of the product suspensions and to determine the response of the suspensions to ATP. The response to ATP was reported as a percentage of the response to the same concentration of ATP in broth alone, that is, in the absence of product.

Detection of Micro-organisms

1. Spiking Studies

A representative range of mouthwash and toothpaste formulations were selected for further study in order to confirm that low inocula levels of typical contaminating organisms were detected.

Serial dilutions of overnight broth cultures of *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis* were prepared to give inocula ranging from approximately 1 to 104 CFU/ml. These inocula (0.1ml) were used to artificially contaminate 10ml aliquots of a 1% suspension of product in Letheen broth. All suspensions were then incubated and assayed according to the Assay Protocol. The inocula levels were confirmed by the agar plate technique. In addition to assay by bioluminescence, the enriched samples were streaked on to Tryptone Soya Agar (TSA) plates and incubated at 30 °C for 48 hours to assess whether or not the organism had grown.

2. Parallel Testing of Routine Samples

A total of 140 samples, comprised of 21 different product formulations, which came into the laboratory for routine

testing were analysed by both bioluminescence and the SmithKline Beecham conventional pour-plate method. The agreement of the two methods was assessed.

Results and Discussion

Selection of enrichment broth

Table 1 shows the within-batch variation of a batch each of TAT and Letheen broth.

Table 1 Within-batch variation of RLU for TAT and Letheen Broths

Bottle	Letheen		TAT	
	Mean RLU	CV of duplicates (%)	Mean RLU	CV of duplicates (%)
1	9196	1.8	9493	0.54
2	9317	1.0	11102	2.8
3	8662	1.6	10519	2.1
4	9671	1.0	10018	1.9
5	9981	0.34	10925	0.69
6	8797	3.5	10074	0.16
7	8479	0.85	10986	3.7
8	8978	1.1	11780	3.1
9	8686	8.6	9499	1.6
10	8801	1.0	10035	1.7
Mean	9058		10446	
S.D.	479		750	
CV	5.3%		7.2%	

Letheen broth was found to give a lower baseline RLU than TAT, with more consistent results. As Letheen complies with the existing validated methods and the regulatory requirements, this broth was selected for use in the subsequent evaluation work.

Determination of the Effects of Products on the Bioluminescence Assay

Table 2 shows that of the 21 formulations tested, only 3 showed interference with the bioluminescence reaction. Macleans Whitening, Aquafresh Whitening and Aquafresh Tartar Control Formula (TCF) demonstrated an inhibition of the luciferase reaction resulting in a low response to ATP (10.8 - 57.3% of that for the broth control). Further work will be required to establish the most appropriate protocol modification for this particular formulation.

Table 2 Effect of Products on the Bioluminescence Assay

Product 1% in Lethen Broth	Mean ATP conc. (nM)	SD (nM)	Mean Response to ATP (%)	SD (%)
Lethen broth Control	0.054	0.014	100.0	
Mouthwashes				
Macleans Mouthgard - Green	0.053	0.004	114.5	5.2
Macleans Mouthgard - Blue	0.072	0.023	125.9	21.5
Odol Mouthwash - Red	0.094	0.020	105.2	13.3
Odol Mouthwash - Light Blue	0.066	0.006	106.7	7.8
Odol Mouthwash - Green	0.059	0.015	106.13	19.5
Odol Mouthwash - Junior	0.049	0.004	100.5	4.1
Odol Mouthwash - Dark Blue	0.054	0.005	99.3	2.3
Corsodyl Original Mouthwash	0.085	0.023	98.4	7.6
Toothpastes				
Macleans Whitening	0.341	0.088	55.4	7.6
Macleans Bicarbonate of Soda	0.050	0.007	84.4	8.2
Macleans Sensitive	0.058	0.015	92.6	6.8
Macleans Coolmint	0.056	0.011	97.6	9.9
Macleans Extrafresh	0.046	0.007	98.3	5.8
Macleans Freshmint	0.049	0.005	96.5	9.2
Aquafresh Fresh n Minty. Tube	0.051	0.011	97.5	3.7
Aquafresh Fresh n Minty. Pump	0.063	0.020	93.9	3.6
Aquafresh Mild n Minty. Tube	0.049	0.008	99.7	4.9
Aquafresh Mild n Minty. Pump	0.050	0.012	97.8	5.9
Aquafresh Bicarbonate of Soda	0.042	0.003	93.1	4.0
Aquafresh Whitening	0.283	0.107	57.3	4.2
Aquafresh Tartar Control Formula	0.219	0.032	10.8	0.6

Detection of Micro-organisms

1. Spiking Studies

Table 3 summarises the results of the detection of micro-organisms which had been added to product suspensions. The bioluminescence method and the agar plate method gave good agreement of between 89 and 100%.

Table 3 Detection of Artificial Contamination in Product Samples

Micro-organism	No. Samples tested	No. Giving same result with both methods	Agreement (%)
Mouthwashes			
<i>P. aeruginosa</i>	20	18	90
<i>S. aureus</i>	12	12	100
<i>B. subtilis</i>	12	12	100
Toothpastes			
<i>P. aeruginosa</i>	55	49	89
<i>S. aureus</i>	56	54	96
<i>B. subtilis</i>	48	47	98

2. Parallel Testing of Routine Samples

Of the 140 samples tested:

- 100 samples were negative by bioluminescence and <10 CFU/g by traditional method.
- One sample was contaminated above the acceptable limit of 100 CFU/g. This was clearly detected by both methods, giving an RLU of over 1 million in the bioluminescence assay.
- 5 samples were positive by bioluminescence and detected as < 100 CFU/g by traditional method.
- The remaining 34 samples were found to be positive by either bioluminescence or the traditional method (10-90 CFU/g). To confirm positive results, a 10g sample was enriched in 90ml of Lethen broth for 48 hours followed by agar plating.

All 8 of the bioluminescence positives were confirmed, but only 4 samples which were positive by the traditional method were confirmed by 10g enrichment. This could be due to one of the following:

1. The extremely low level of organisms in the product such that not all of the sub-samples taken were contaminated.
2. The low levels of organisms were rapidly eliminated by the formulation.
3. Poor aseptic technique in the traditional method.

Of the 139 samples which were within specifications, 13 were positive by bioluminescence. Therefore this data shows that 90% of product could have been released after a contamination screen by bioluminescence.

Summary

- A wide range of mouthwash and toothpaste formulations can be rapidly screened for contamination using the PCPK system.
- The system uses Lethen broth which maintains consistency within SmithKline Beecham laboratories.
- Low levels of micro-organisms have been detected, showing good correlation with the standard agar plate method.
- Mouthwash and toothpaste formulations with no microbial contamination gave consistently negative results.
- We will be able to release approximately 90% of product within 30 hours of receipt of the samples by the laboratory, based on a days production.