

Fast and Sensitive Swab-based Bioluminescent detection method for meat and chicken microbiological contamination using *Amydetes vivianii* firefly luciferase

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SUPPLEMENTARY INFORMATION

Table S1. Luminescence intensity values (cps) for each ATP concentration tested over three days for *E. coli* X11-Blue diluted cultures.

Day	Luminescence Intensities (cps)				
	1000	100	10	1	0.1
1	1.70 ^x 10 ⁶	2.10 ^x 10 ⁵	3.89 ^x 10 ⁴	1.53 ^x 10 ⁴	9.22 ^x 10 ³
2	1.95 ^x 10 ⁶	1.79 ^x 10 ⁵	3.09 ^x 10 ⁴	1.34 ^x 10 ⁴	1.01 ^x 10 ⁴
3	1.18 ^x 10 ⁶	2.25 ^x 10 ⁵	2.98 ^x 10 ⁴	1.23 ^x 10 ⁴	1.07 ^x 10 ⁴
Average (cps)	1.61 ^x 10 ⁶	2.05 ^x 10 ⁵	3.32 ^x 10 ⁴	1.36 ^x 10 ⁴	1.00 ^x 10 ⁴
SD	3.89 ^x 10 ⁵	2.37 ^x 10 ⁴	4.97 ^x 10 ³	1.54 ^x 10 ³	7.60 ^x 10 ²
Precision%	24.2	11.6	15.0	11.3	7.6

Table S2. Luminescence intensity values (cps) for each concentration tested over three days for *E. coli* X11-Blue diluted cultures using swabs.

Day	Luminescence Intensities (cps)				
	1000	100	10	1	0.1
1	4.90 ^x 10 ⁵	1.01 ^x 10 ⁵	2.88 ^x 10 ⁴	1.47 ^x 10 ⁴	9.03 ^x 10 ³
2	5.93 ^x 10 ⁵	1.07 ^x 10 ⁵	1.92 ^x 10 ⁴	1.06 ^x 10 ⁴	7.75 ^x 10 ³
3	5.97 ^x 10 ⁵	7.17 ^x 10 ⁴	2.14 ^x 10 ⁴	8.76 ^x 10 ³	5.81 ^x 10 ³
Average (cps)	5.60 ^x 10 ⁵	9.32 ^x 10 ⁴	2.32 ^x 10 ⁴	1.13 ^x 10 ⁴	7.53 ^x 10 ³
SD	6.05 ^x 10 ⁴	1.89 ^x 10 ⁴	5.02 ^x 10 ³	3.04 ^x 10 ³	1.62 ^x 10 ³
Precision%	10.8	20.2	21.7	26.8	21.5

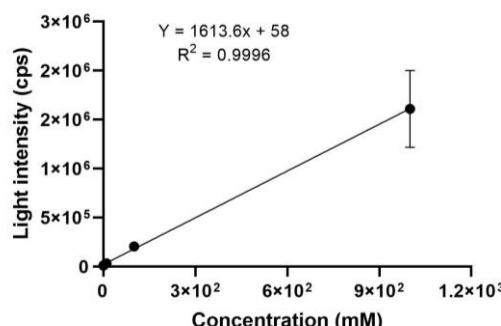
Table S3. Luminescence intensity values (cps) for each ATP concentration tested over three days.

Day	Luminescence Intensities (cps)					
	ATP concentration (μ L/mL)	5.00 ^x 10 ⁻³	5.00 ^x 10 ⁻⁴	5.00 ^x 10 ⁻⁵	5.00 ^x 10 ⁻⁶	5.00 ^x 10 ⁻⁷
1	1.94 ^x 10 ⁶	3.15 ^x 10 ⁵	3.53 ^x 10 ⁴	4.86 ^x 10 ³	1.68 ^x 10 ³	
2	2.48 ^x 10 ⁶	2.35 ^x 10 ⁵	2.48 ^x 10 ⁴	4.16 ^x 10 ³	1.82 ^x 10 ³	
3	2.02 ^x 10 ⁶	2.41 ^x 10 ⁵	3.21 ^x 10 ⁴	5.44 ^x 10 ³	2.27 ^x 10 ³	
Average (cps)	2.02 ^x 10 ⁶	2.41 ^x 10 ⁵	3.21 ^x 10 ⁴	4.86 ^x 10 ³	1.82 ^x 10 ³	
SD	2.89 ^x 10 ⁵	4.45 ^x 10 ⁴	5.39 ^x 10 ³	6.42 ^x 10 ²	3.08 ^x 10 ²	
Precision%	14.3	18.5	16.8	13.2	16.9	

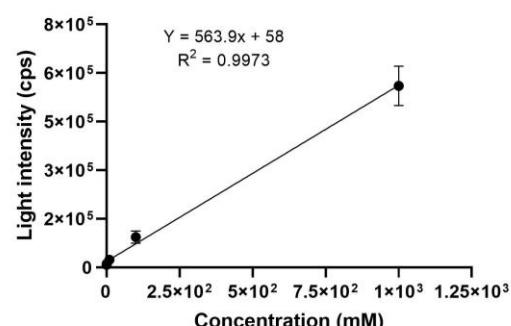
Table S4. Values from the equation of the lines over three days of linearity evaluation.

	Dilution			Swab		
Day	Intercept	Slope	R ²	Intercept	Slope	R ²
1	58.00	1701.10	0.9997	58.00	495.63	0.9926
2	58.00	1945.20	0.9998	58.00	598.02	0.9955
3	58.00	1194.50	0.9931	58.00	597.91	0.9998
N	3	3	3	3	3	3
Average	58.00	1613.60	0.9975	58.00	563.85	0.9960
SD	0	382.92	0.00	0	59.08	0.00
Precision%	0	23.73	0.38	0	10.48	0.36

(A)



(B)

**Figure S1.** Linearity graphs of the ATP extracted (A) from the diluted culture, and (B) from the culture in swab of five concentrations and their respective results in cps.**Table S5.** Luminescence intensity values (cps) for solutions without ATP.

Luminescence intensity (cps)		
Measurement	Dilution	Swab
Average (cps)	58.20	45.20
SD	5.54	9.93
Precision%	10%	22%

Table S6. Luminescence intensity results (cps) in the interday evaluation of the *E. coli* Xl1-Blue diluted culture.

Luminescence intensity (cps) of dilutions						
Day	Standard value*	Average of high value*	Standard value*	Average of the average value*	Standard value*	Average of low value*
1		1.43 ^x 10 ⁶		2.54 ^x 10 ⁴		1.89 ^x 10 ⁴
2	2.02 ^x 10 ⁶	1.56 ^x 10 ⁶	3.21 ^x 10 ⁴	2.69 ^x 10 ⁴	3.87 ^x 10 ³	1.24 ^x 10 ⁴
3		2.18 ^x 10 ⁶		3.27 ^x 10 ⁴		1.10 ^x 10 ⁴
N		15		15		15
Average (cps)		1.72 ^x 10 ⁶		2.83 ^x 10 ⁴		1.41 ^x 10 ⁴
SD		3.86 ^x 10 ³		4.37 ^x 10 ³		3.88 ^x 10 ⁵
Precision%		27.4		15.4		22.5

Table S7. Luminescence intensity results (cps) in the interday evaluation of the *E. coli* Xl1-Blue diluted culture in swab.

Luminescence intensity (cps) using swab						
Day	Standard value*	Average of high value*	Standard value*	Average of median value*	Standard value*	Average of low value*
1		3.86 ^x 10 ⁵		1.60 ^x 10 ⁴		6.48 ^x 10 ³
2	2.02 ^x 10 ⁶	4.29 ^x 10 ⁵	3.21 ^x 10 ⁴	2.00 ^x 10 ⁴	3.87 ^x 10 ³	7.77 ^x 10 ³
3		4.51 ^x 10 ⁵		1.88 ^x 10 ⁴		6.27 ^x 10 ³
N		15		15		15
Average (cps)		4.22 ^x 10 ⁵		1.83 ^x 10 ⁴		6.84 ^x 10 ³
SD		9.56 ^x 10 ⁴		3.21 ^x 10 ³		1.57 ^x 10 ³
Precision%		22.6		17.6		22.9

Table S8. Luminescence intensity results (cps) in the intraday evaluation of the *E. coli* X11-Blue diluted culture.

Luminescence intensity (cps) of dilutions						
Measurements	Standard value*	Average of high value*	Standard value*	Average of median value*	Standard value*	Average of low value*
Average (cps)	2.02 ^x 10 ⁶	1.43 ^x 10 ⁶	3.21 ^x 10 ⁴	2.54 ^x 10 ⁴	3.87 ^x 10 ³	1.89 ^x 10 ⁴
N		5		5		5
SD		2.13 ^x 10 ⁵		1.24 ^x 10 ³		1.32 ^x 10 ³
Precision%		14.9		4.9		7.0

Table S9. Luminescence intensity results (cps) in the intraday evaluation of the *E. coli* X11-Blue diluted culture in swab.

Luminescence intensity (cps) using swab						
Measurements	Standard value*	Average of high value*	Standard value*	Average of median value*	Standard value*	Average of low value*
Average (cps)	1.92 ^x 10 ⁶	3.86 ^x 10 ⁵	3.21 ^x 10 ⁴	1.60 ^x 10 ⁴	3.87 ^x 10 ³	7.25 ^x 10 ³
N		5		5		5
SD		6.38 ^x 10 ⁴		9.87 ^x 10 ²		4.77 ^x 10 ²
Precision%		16.5		6.2		6.6

Table S10. Luminescence intensity results (cps) of the matrix effect evaluation in two standard ATP concentrations (5.00 x10⁻³ and 5.00 x10⁻⁷ mM).

Measurements	Culture with standard ATP without extraction buffer		Swab with water and standard ATP without extraction buffer	
ATP concentration (mM)	5.00x10⁻³	5.00x10⁻⁷	5.00x10⁻³	5.00x10⁻⁷
Average	3.76 ^x 10 ⁶	4.55 ^x 10 ⁴	3.95 ^x 10 ⁶	5.57 ^x 10 ⁴
SD	3.64 ^x 10 ⁵	8.11 ^x 10 ³	3.87 ^x 10 ⁵	1.05 ^x 10 ⁴
Precision%	10%	18%	10%	19%
Accuracy%	71%		70%	

Table S11. Luminescence intensity of four conditions under which the solution was evaluated in for stability over four different time periods.

Measurements	With Luciferin -20°C			Without Luciferin -20°C			With Luciferin 5°C			Without Luciferin 5°C		
Concentration	5.00 $\times 10^{-3}$	5.00 $\times 10^{-5}$	5.00 $\times 10^{-7}$	5.00 $\times 10^{-3}$	5.00 $\times 10^{-5}$	5.00 $\times 10^{-7}$	5.00 $\times 10^{-3}$	5.00 $\times 10^{-5}$	5.00 $\times 10^{-7}$	5.00 $\times 10^{-3}$	5.00 $\times 10^{-5}$	5.00 $\times 10^{-7}$
Average 15 days	1.46 $\times 10^7$	1.52 $\times 10^5$	6.01 $\times 10^3$	1.15 $\times 10^7$	1.39 $\times 10^5$	6.65 $\times 10^3$	1.01 $\times 10^7$	1.29 $\times 10^5$	7.22 $\times 10^3$	1.32 $\times 10^7$	1.44 $\times 10^5$	1.60 $\times 10^5$
SD	1.83 $\times 10^6$	1.49 $\times 10^4$	2.55 $\times 10^2$	1.27 $\times 10^6$	1.14 $\times 10^4$	7.01 $\times 10^2$	3.62 $\times 10^5$	6.85 $\times 10^3$	7.29 $\times 10^2$	5.40 $\times 10^5$	3.80 $\times 10^4$	4.88 $\times 10^5$
Precision%	12%	10%	4%	11%	8%	11%	4%	5%	10%	4%	26%	30%
Average 1 month	1.89 $\times 10^7$	1.92 $\times 10^5$	2.04 $\times 10^4$	2.04 $\times 10^7$	2.14 $\times 10^5$	2.68 $\times 10^4$	3.33 $\times 10^6$	4.77 $\times 10^4$	4.89 $\times 10^3$	1.89 $\times 10^7$	2.66 $\times 10^5$	2.82 $\times 10^4$
SD	1.88 $\times 10^6$	1.52 $\times 10^4$	1.47 $\times 10^3$	1.35 $\times 10^6$	3.19 $\times 10^4$	2.22 $\times 10^3$	2.86 $\times 10^5$	4.47 $\times 10^3$	7.28 $\times 10^2$	1.38 $\times 10^6$	5.76 $\times 10^4$	4.07 $\times 10^3$
Precision%	10%	8%	7%	7%	15%	8%	9%	9%	15%	7%	22%	14%
Average 3 month	1.58 $\times 10^7$	1.98 $\times 10^5$	1.13 $\times 10^4$	2.98 $\times 10^7$	2.58 $\times 10^5$	1.68 $\times 10^4$	4.54 $\times 10^3$	1.58 $\times 10^2$	2.74 $\times 10$	2.37 $\times 10^6$	2.74 $\times 10^4$	2.81 $\times 10^3$
SD	1.79 $\times 10^6$	1.92 $\times 10^4$	2.20 $\times 10^3$	3.87 $\times 10^6$	2.47 $\times 10^4$	1.68 $\times 10^3$	4.91 $\times 10^2$	3.08 $\times 10$	5.37	7.02 $\times 10^5$	8.29 $\times 10^3$	7.81 $\times 10^2$
Precision%	11%	10%	19%	13%	10%	10%	11%	19%	20%	30%	36%	28%
Average 6 month	9.38 $\times 10^6$	2.59 $\times 10^5$	1.01 $\times 10^4$	1.91 $\times 10^7$	2.69 $\times 10^5$	1.17 $\times 10^4$	3.01 $\times 10^3$	2.43 $\times 10^3$	2.46 $\times 10^3$	6.76 $\times 10^4$	1.62 $\times 10^3$	2.51 $\times 10^2$
SD	6.69 $\times 10^5$	6.77 $\times 10^3$	1.26 $\times 10^3$	9.57 $\times 10^5$	1.36 $\times 10^4$	2.14 $\times 10^3$	9.46 $\times 10^2$	1.05 $\times 10^3$	1.46 $\times 10^3$	1.19 $\times 10^4$	3.42 $\times 10^2$	4.50 $\times 10$
Precision%	7%	3%	12%	5%	5%	18%	31%	43%	59%	18%	21%	18%

Table S12. Analysis of the assay solution stability at different storage conditions and time periods by comparison using test-t of Student's with time zero.

Luminescent activity comparison (%)				
Incubation period	With Luciferin -20°C	Without Luciferin -20°C	With Luciferin 5°C	Without Luciferin 5°C
15 days	84%	96%	97%	88%
1 month	72%	69%	56%	72%
3 month	80%	70%	41%	51%
6 month	94%	72%	41%	41%

Table S13. Average results of triplicate analysis in CFU/mL at three different optical densities of five different dilutions.

Dilution (μL)	CFU/mL			Total Average	SD
	Average OD ₆₀₀ =1.08	Average OD ₆₀₀ =1.25	Average OD ₆₀₀ =1.17		
1000	3.10×10^8	1.49×10^8	4.10×10^8	2.90×10^8	1.32×10^8
100	5.30×10^7	2.34×10^7	6.30×10^7	4.65×10^7	2.06×10^7
10	3.70×10^6	1.42×10^6	2.60×10^6	2.57×10^6	1.14×10^6
1	6.80×10^5	1.76×10^4	5.30×10^5	4.09×10^5	3.47×10^5
0.1	5.30×10^4	1.21×10^3	2.50×10^4	2.64×10^4	2.59×10^4

Table S14. Average results of triplicate analysis in luminescence intensity (cps) using *A. vivianii* assay at three different optical densities of five different dilutions.

Luminescence intensity (cps) using <i>A. vivianii</i> luciferase					
Dilution (μL)	Average OD ₆₀₀ =1.08	Average OD ₆₀₀ =1.25	Average OD ₆₀₀ =1.17	Total Average	SD
1000	1.23×10^9	1.46×10^9	1.87×10^9	1.52×10^9	3.24×10^8
100	1.47×10^8	1.24×10^8	1.42×10^8	1.37×10^8	1.21×10^7
10	2.02×10^7	1.86×10^7	1.40×10^7	1.76×10^7	3.22×10^6
1	3.04×10^6	5.50×10^6	3.85×10^6	4.13×10^6	1.25×10^6
0.1	3.71×10^6	3.66×10^6	3.89×10^6	3.75×10^6	1.21×10^5

Table S15. Average results in luminescence intensity (cps) using a commercially available swab (3M) at three different optical densities of five different dilutions.

Luminescence intensity (cps) using 3M commercial swab					
Dilution	Average	Average	Average	Total	SD

(μ L)	$\text{OD}_{600}=1.08$	$\text{OD}_{600}=1.25$	$\text{OD}_{600}=1.17$	Average	
1000	1.25×10^8	9.53×10^7	1.43×10^8	1.21×10^8	2.41×10^7
100	2.83×10^7	2.47×10^7	3.55×10^7	2.95×10^7	5.50×10^6
10	2.71×10^6	2.07×10^6	5.62×10^6	2.47×10^6	1.89×10^6
1	5.07×10^5	4.10×10^5	1.00×10^6	5.07×10^5	3.16×10^5
0.1	2.71×10^5	2.56×10^5	6.51×10^5	2.93×10^5	2.24×10^5

Table S16. Luminescence intensities (cps) of food matrix effect evaluation at two standard ATP concentrations (5.00×10^{-3} and 5.00×10^{-7} mM).

Luminescence intensity (cps)						
Sample	Meat		Chicken		Milk	
[ATP] (mM)	5.00×10^{-3}	5.00×10^{-7}	5.00×10^{-3}	5.00×10^{-7}	5.00×10^{-3}	5.00×10^{-7}
Average	1.07×10^6	4.80×10^4	1.21×10^6	1.58×10^5	3.64×10^6	1.83×10^5
SD	6.12×10^4	9.00×10^3	3.68×10^5	3.78×10^4	3.00×10^5	3.47×10^4
Precision%	6%	19%	30%	24%	8%	19%
Accuracy%	73%		80%		70%	

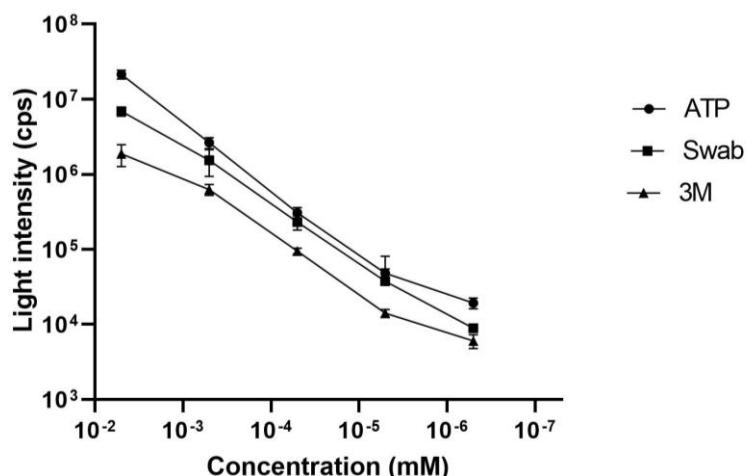


Figure S2. Linearity curves constructed for standard ATP (ATP), ATP extracted from the cultures in the swab (Swab) and the commercial swab (3M) at five different concentrations along with their respective luminescence intensities.