

# ATP Bioluminescence a Means for Assessing Trailer Cleanliness

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## SUMMARY

Proper washing and disinfection of swine transport trailers is an important step in maintaining biosecurity. This study examined the feasibility of using adenosine triphosphate (ATP) bioluminescence as a rapid and effective swine trailer cleanliness assessment tool. Samples were taken from newly-cleaned, dry trailers using an ATP swab by swabbing an area of 10 cm x 10 cm and were tested for microbial contamination level using an ATP bioluminescence meter.

The results obtained from ATP testing were compared to the co-located samples taken using standard microbiological techniques with MacConkey and R2A agar contact plates (diameter  $\varnothing = 60$  mm). From a total of more than 500 samples collected from 16 commercial swine transport trailers across Saskatchewan, a significant correlation ( $r = 0.206$ ;  $p=0.001$ ) was found between ATP bioluminescence method and standard microbiological technique using R2A agar plates. Lower correlation ( $r = 0.154$ ;  $p=0.002$ ) was observed between ATP method and MacConkey agar plate counts. Unlike R2A that detects a wider group of bacteria, MacConkey agar supports only the growth of selected gram-negative bacteria while ATP bioluminescence detects ATP from both microbial and organic sources.

*“Visual inspection of newly-cleaned transport trailers is not sufficient in assessing trailer cleanliness.”*

Assessing the effectiveness of swine transport trailer cleaning protocol using ATP bioluminescence method threshold values were established with readings of less than 430 RLU per 100 cm<sup>2</sup> as ‘Pass’ while higher than 850 RLU per 100 cm<sup>2</sup> as ‘Fail’ or has high risk of disease propagation. With these assessment criteria, ATP bioluminescence method can be used as a supplementary tool for monitoring surface cleanliness of transport trailers in a rapid, simple, inexpensive and reliable way, to complement the procedures specified in CSHB (2011) guidelines.

## INTRODUCTION

Animal transportation is widely recognized as a significant risk for transmission of swine diseases. With the recent outbreaks of Porcine Epidemic Diarrhea (PEDv), rigorous effort has been exerted to ensure that transport trailers are properly washed, disinfected and inspected for organic debris and microbial contamination prior to use. However, confirmation of the cleanliness of trailers after washing/disinfection/drying procedures is presently carried out mainly by visual inspection, with occasional microbiological testing using culture method (CSHB, 2011).

Visual inspection is not a reliable assessment, while traditional microbiological culture method involves the use of plated media which need to be incubated and analyzed to obtain an indication of the contamination of the sampled surfaces. This approach can cause significant down-time for trailer operations and delays implementation of corrective actions while waiting for test results. A rapid, easy to use and reliable way of monitoring surface cleanliness of swine transport trailers is needed for practical industry applications.



Over the last decade, adenosine triphosphate (ATP) bioluminescence method has been used in other industries (i.e., food, hospitals, cattle) for monitoring surface cleanliness and microbial contamination. This method uses bioluminescence as an indicator of the level of residual ATP present on swabbed surfaces. Once the surface is swabbed, the sample is exposed to an ATP-releasing agent (lysis buffer) and an ATP-activated light-producing substrate and enzyme (luciferin and luciferase). The amount of ATP present on the tested surfaces can then be quantified by the amount of light emitted during the enzymatic reaction (in terms of relative luminescence units, RLU) (Green et al., 1999). The intensity of light is proportional to the amount of ATP and therefore the degree of contamination (Davidson et al., 1999). The ability to provide results within minutes, as opposed to days for traditional microbiological testing, makes ATP bioluminescence a good alternative tool for monitoring surface cleanliness of swine transport trailers. If proven effective, rapid monitoring of surface cleanliness of swine transport trailers can be done on the large number of trailers being used across the industry at a relatively low cost, thus enhancing the effectiveness of biosecurity measures.



Six sampling locations were identified for each trailer: lower deck floor and wall, upper deck floor and wall, loading ramp and partition panels, and trailer exterior. At each sampling location, five pairs/sets of co-located samples, one for each method, were gathered. Overall, 536 swab samples for ATP bioluminescence and 626 microbiological samples (389 using MacConkey Agar and 237 using R2A agar), were collected.

## RESULTS AND DISCUSSIONS

Based on the criteria for assessing whether a newly-washed trailer can be categorized as 'Pass', 'Critical/Caution', or 'Fail' using microbiological testing with MCA plates as specified in the "Quality Control of Wash/Disinfect/Dry Protocols for Live-Hog Transport Vehicles" document from the Canadian Swine Health Board (CSHB, 2011), the equivalent threshold limits for ATP and R2A readings were derived using the relationships established from the paired data collected using both methods. The data values which were normalized to cfu/cm<sup>2</sup> and RLU/cm<sup>2</sup> during statistical analysis, were converted to their original units of RLU per 10 cm x 10 cm for ATP bioluminescence and cfu/per contact plate (A = 28.3 cm<sup>2</sup>) for both MCA and R2A, to make them directly comparable with the CSHB guidelines shown in Table 1. Expressed in these units, the original data set for ATP bioluminescence ranged from 0 to 3500 RLU per 10cm x 10cm sampling area.

## MATERIALS AND METHODS

A total of sixteen (16) commercial swine transport trailers were assessed for surface cleanliness after washing and disinfection using two methods: microbiological control method using two formulations of contact agar plates, and using the ATP bioluminescence meter. Sampling was done on dry trailers after undergoing washing and disinfection procedures in their respective truck washes.

**Table 1.** Threshold values in assessing effectiveness of swine transport trailer washing/ disinfection/drying protocol using MCA, ATP bioluminescence and R2A

Assessment criteria from CSHB, 2011		Threshold Values		
Category	Remarks	MacConkey agar[a]	ATP bioluminescence[b]	R2A agar[c]
Pass	Maintain wash, disinfection and drying protocols.	0 – 10	0 – 430	0 – 140
Critical	Risk of disease propagation, improve protocols. Room for improvement.	11 – 50	431 – 850	141 – 625
Fail	High risk of disease propagation. Identify problem and correct the wash, disinfect and drying protocol and its observance.	>50	>850	>625

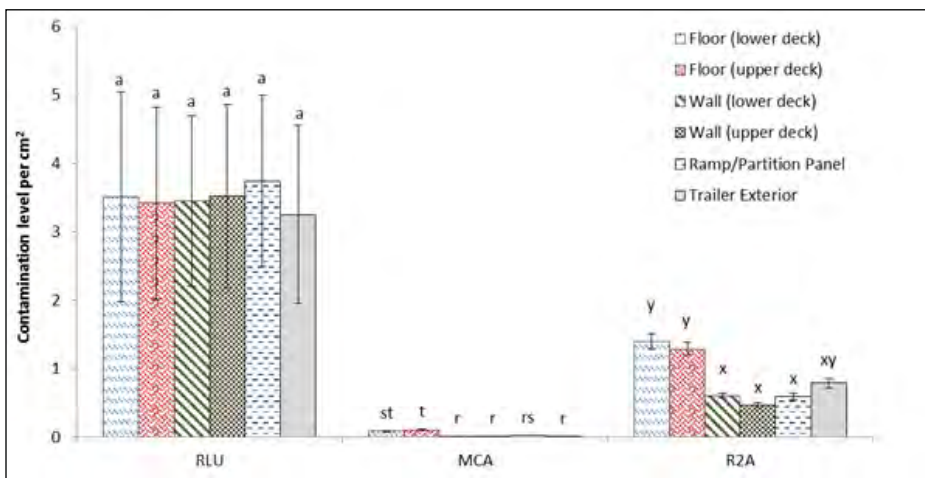
a Values are in colony forming units (cfu) per agar contact plate (ϕ = 60 mm). Source: CSHB Quality Control document 2011.

b Relative light unit (RLU) per 10 cm x 10 cm sampling area. Range of values obtained from regression equation derived from the paired data set.

c Values are in colony forming units (cfu) per agar contact plate (ϕ = 60 mm).

### Sampling location

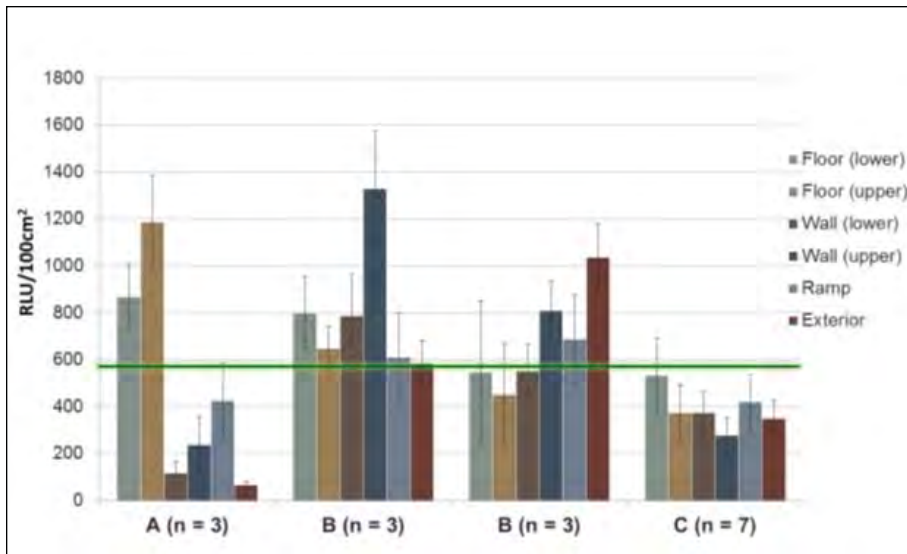
To identify the critical areas for cleaning (i.e., 'hot spots') the trailers, five co-located samples using the ATP bioluminescence meter and either or both MCA or R2A were randomly gathered per sampling area. Figure 1 shows the mean value of RLU per cm<sup>2</sup> and CFU per cm<sup>2</sup> for each sampling location for all 16 trailers. Using the ATP bioluminescence meter, no clear 'hot spots' were identified since the RLU levels were comparable among the different sampling locations for all trailers sampled. However, significant difference (p<0.05) in microbial contamination levels was observed among the different sampling locations when tested using R2A and MCA plates. Specifically, microbial contamination for all trailers was significantly high on floors. Aside from corrugations on floors which make cleaning relatively challenging, accumulation of water on floor surfaces before complete drying may have contributed to this observation.



**Figure 1.** Mean (±SE) contamination levels (n = 16) of different sampling locations in the trailers as detected by the ATP bioluminescence meter (in RLU per cm<sup>2</sup>), and MCA and R2A agar plates (in CFU per cm<sup>2</sup>). Means with the same letters are not significantly different (p>0.05).

Figure 2 shows the plot of contamination levels in different locations in trailers washed from three different truck wash facilities (A, B, and C). Trailer floors from truck wash A had significantly higher RLU values compared to other sampling locations, while truck wash B needs to pay special attention to the walls during washing of trailers. However, microbial contamination was found concentrated on floors which may suggest careful cleaning and extra disinfection need to be applied on floors.

Trailers washed in truck wash C had mean RLU values below the suggested pass threshold level for RLU readings, indicating that the cleaning and disinfecting procedures in this facility was adequate.



**Figure 2.** Mean (±SE) RLU readings from trailers washed at different truck wash facilities. Horizontal green line represents suggested RLU pass threshold.

**CONCLUSIONS**

- ATP bioluminescence method can be used as a tool for rapid assessment of surface cleanliness of swine transport trailers, complementing the procedures specified in CSHB (2011). Dirty areas in trailers can be rapidly identified using ATP method hence, corrective actions on the current washing/disinfection protocol can be made.
- ATP method had moderate correlation with the standard microbiological method using R2A agar plates; no readily apparent relationship was observed between ATP method and MacConkey agar (MCA) plate counts.
- Results from this study have confirmed that visual inspection of newly-cleaned transport trailers is not sufficient in assessing its surface cleanliness because significant levels of ATP and microbial loads were detected on trailer surfaces after cleaning.
- Regardless of the method of assessment used, trailer floors posed the highest risk of microbial contamination among all the six critical areas tested.

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