# ATP bioluminescence method as a rapid tool for assessment of cleanliness of commercial animal transport trailers

Alvin C. Alvarado[1], Jingjing Cabahug[2] and Bernado Z. Predicala[1,*]

[1]*Prairie Swine Centre Inc., PO Box 21057, 2105 - 8th St. East, Saskatoon, SK S7H 5N9 Canada*

[2]*Department of Chemical and Biological Engineering, University of Saskatchewan, Saskatoon, SK S7N 5A9 Canada*

*Corresponding Author: bernardo.predicala@usask.ca*

**ABSTRACT**

Animal transportation is widely recognized as a significant risk for disease transmission. At present, cleanliness of animal transport trailers is mostly assessed through subjective visual inspection (i.e., 'white-glove' test), which may sometimes be supplemented by microbiological testing with results obtained after at least 2-3 days. In this study, the feasibility of using adenosine triphosphate (ATP) bioluminescence method as a rapid tool for objectively assessing animal transport trailer cleanliness was investigated. Eighteen newly-cleaned transport trailers were tested using both ATP bioluminescence and microbiological techniques. In each trailer, six (6) locations including floors, walls, ramps, partitions and trailer exterior surfaces were sampled using an ATP meter, and MacConkey and R2A agar contact plates. From a total of more than 500 paired samples collected from all the sampled trailers, significant correlation was found between ATP bioluminescence method and standard microbiological technique using R2A agar ($r$ = 0.206; $p$ = 0.001) and MacConkey agar ($r$ = 0.154; $p$ = 0.002) plates. Using a threshold or 'Pass' limit of 390 RLU per 100 cm2 of trailer surface, ATP method was able to provide a good objective measure of the surface cleanliness, thus may be considered as an additional tool available for rapid and less costly assessment of trailer surface cleanliness.

**KEYWORDS**

ATP bioluminescence; contact agar plates; microbiological testing; surface cleanliness; animal trailer.

**RÉSUMÉ**

Le transport des animaux est généralement reconnu comme un risque important de transmission de maladies. Actuellement, la propreté des remorques de transport d'animaux est principalement évaluée par une inspection visuelle subjective (c.-à-d. le test du « gant blanc »), qui peut parfois être complétée par des tests microbiologiques dont les résultats sont obtenus au moins 2 ou 3 jours plus tard. Dans cette étude, la faisabilité de l'utilisation de la méthode de bioluminescence de l'adénosine triphosphate (ATP) a été étudiée comme outil rapide pour évaluer objectivement la propreté des remorques de transport d'animaux. Dix-huit remorques de transport nettoyées ont été testées à la suite du nettoyage en utilisant à la fois la bioluminescence de l'ATP et des techniques microbiologiques. Dans chaque remorque, six (6) endroits, dont les planchers, les murs, les rampes, les cloisons et les surfaces extérieurs de la remorque, ont été échantillonnés à l'aide d'un compteur ATP et de plaques de contact en gélose MacConkey et R2A. Sur un total de plus de 500 échantillons appariés prélevés dans chacune des remorques, une corrélation significative a été observée entre la méthode de bioluminescence ATP et les techniques microbiologiques standard utilisant des plaques de gélose R2A ($r$ = 0,206 ; $p$ = 0,001) et MacConkey ($r$ = 0,154 ; $p$ = 0,002). En utilisant un seuil ou une limite de « réussite » de 390 RLU par 100 cm2 de surface de remorque, la méthode ATP a été capable de fournir une mesure objective adéquate de la propreté de la surface. Par conséquent, elle peut être considérée comme un outil additionnel disponible pour une évaluation rapide et moins coûteuse de la propreté des surfaces des remorques.

**MOTS CLÉS**

Bioluminescence ATP; plaques de contact de gélose; tests microbiologiques; propreté des surfaces; remorque pour animaux.

**CITATION**

Alvarado, A.C., J. Cabahug and B.Z. Predicala. 2020. **ATP bioluminescence method as a rapid tool for assessment of cleanliness of commercial animal transport trailers.** Canadian Biosystems Engineering/Le génie des biosystèmes au Canada 62: 5.1-5.7. https://doi.org/10.7451/CBE.2020.62.5.1

## INTRODUCTION

Animal transport vehicles have been identified as a significant risk factor for transmission of pig diseases. Rigorous effort has been exerted to ensure that transport trailers are properly washed, disinfected, and inspected for presence of organic debris and microbial contamination prior to use. However, in many practical situations, the confirmation of the cleanliness of trailers after washing-disinfection-drying procedures is mainly carried out by visual inspection (i.e., 'white glove' test wherein the cleaned surface is wiped with a white cloth glove or cotton swab and then visually observed for any residue) of various trailer surfaces, and in certain circumstances may be supplemented by microbiological testing using culture method on contact agar plates (CSHB 2011).

Visual inspection is a subjective and not very 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. As such, this can cause significant down-time for trailer operations and can delay the implementation of corrective actions while waiting for test results. Hence, a rapid, easy to use, and objective means for evaluating surface cleanliness of swine transport trailers is needed for practical industry application.

The adenosine triphosphate (ATP) bioluminescence method has been used for assessing surface cleanliness and microbial contamination in hospitals (Aycicek et al. 2006; Boyce et al. 2009; Davidson et al. 1999), food facilities (Azizkhan 2014; Poulis et al. 1993), dairy industry (Vilar et al. 2008), among others. This method uses bioluminescence as an indicator of ATP present in all organic materials such as microorganisms and animal excretions, which are among the typical contaminants found in transport trailers. The intensity of bioluminescent light produced is proportional to the amount of ATP present in the sample, and therefore can be correlated with the degree of contamination (Davidson et al. 1999). The method has added benefit of providing results within minutes (Azizkhan 2014; Poulis et al. 1993; Davidson et al. 1999) compared to a few days for microbiological culture method, thus making ATP bioluminescence a good alternative tool for assessing surface cleanliness of animal transport trailers.

The overall goal of this study was to investigate the validity and reliability of rapid ATP bioluminescence monitoring as an alternative tool for assessment of surface cleanliness of animal transport trailers after they underwent washing-disinfection-drying procedures. Specific objectives of the study were to examine the correlation between ATP measurements and the standard microbiological culture counts on trailer surfaces, to evaluate cleanliness of surfaces at different locations in a trailer, and to assess the potential application of ATP technology as an objective indicator of the effectiveness of current cleaning procedures employed for animal transport trailers.

## MATERIALS AND METHODS

### Sample collection and testing

A total of sixteen (16) commercial animal transport trailers owned by various swine producers and a trucking company in Saskatchewan, Canada, were assessed for surface cleanliness after washing and disinfection using two methods: microbiological culture method and ATP bioluminescence method. Sampling was done on dry trailers after undergoing the routine washing and disinfection procedures in their respective truck wash facilities. Prior to sample collection, the cleaned trailers were visually inspected to ensure that the sampled surfaces were clean and free of any organic matter residue (i.e., passed the 'white glove' test).

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 (all of which were aluminum alloy surfaces), five pairs/sets of co-located samples, one for each method (i.e., ATP vs. culture method), were collected.

### Microbiological culture method

Two agar formulations of prepared contact plates (Ø = 60 mm, area = 28.3 $cm^2$) were used for microbiological testing: MacConkey Agar (MCA) and Reasoner's 2A Agar (R2A) (Hardy Diagnostics, Santa Maria, CA, USA). The Canadian Swine Health Board (CSHB) recommended the use of MacConkey Agar (MCA) in the microbiological assessment of cleanliness of swine transport trailers (CSHB 2011), while R2A agar permits the recovery of more bacteria from water or wet environments than conventional media (Swan et al. 2016). At each designated sampling point, the agar plate was pressed firmly against the target test surface for 10 seconds. Collected sample plates were stored in a cooler with ice pack and transported back to the laboratory. The exposed plates were incubated for 48 hours at 37°C, and the colonies that appeared on the plates were manually counted. To establish microbiological reference control for the plate media, for every sampling period a control plate (clean) was exposed by opening the plate lid at the trailer site without contact to any surface for 10 seconds, while another control plate (contaminated) was applied on another surface known to be contaminated to verify the ability of the plates to detect contaminants present on the surface. Both control plates were then incubated and counted similar to the trailer sample plates.

### ATP bioluminescence method

Bioluminescence is a biological reaction that produces light, similar to those in fireflies. Once the target surface was swabbed, the sample was exposed to an ATP-releasing agent (lysis buffer) and an ATP-activated light-producing substrate and enzyme (luciferin and luciferase). The quantity of light emitted during the enzymatic reaction is expressed in terms of relative luminescence or light units (RLU) which directly correlates with the amount of ATP present and, thus with the level of biologic load on the sampled area (Deshpande 2001).

A luminometer (EnSURE) and ATP swabs (SuperSnap) from Hygiena LLC (Camarillo, CA, USA) were used. The ATP swabs are comprised of a sterile swab in a clear plastic tube with liquid luciferin/luciferase reagent on a snap bulb. Sampling was done by pulling the swab out of the tube and swabbing in a zigzag pattern in one direction and again in a perpendicular direction within a 10 cm x 10 cm sampling template placed on the identified sampling area. The swab was then re-inserted into its tube and activated by bending the tube to break the snap valve and expelling the liquid reagent to the swab. The tube was shaken for 5 - 10 seconds to thoroughly moisten the swab and then the tube was placed into the luminometer chamber to take the RLU reading. Prior to each trailer testing, the luminometer was subjected to a calibration procedure wherein a Negative and a Positive control rod were used to confirm Zero RLU reading for the Negative rod (equivalent to a sterile sample) and a pre-defined reading (80-160 RLU) for the Positive rod.

**Statistical analysis**

Data on colony counts from the microbial culture method were originally obtained in colony forming unit (cfu) per contact plate area (cfu/28.3 $cm^2$) while data from ATP bioluminescence were expressed in relative light unit (RLU) per 100 $cm^2$ sampling area (RLU/100 $cm^2$). A statistical test showed that both the RLU data and cfu counts were not normally distributed, and were converted to $log_{10}$ values to normalize the data prior to further statistical analyses.

Analyses were done on the log-adjusted RLU and cfu values using Pearson correlation coefficients to compare ATP measurements with colony counts by type of agar (MCA and R2A) and by sampling location. Tests of normality, correlation and comparison of means were carried out using SPSS software version 20.0.

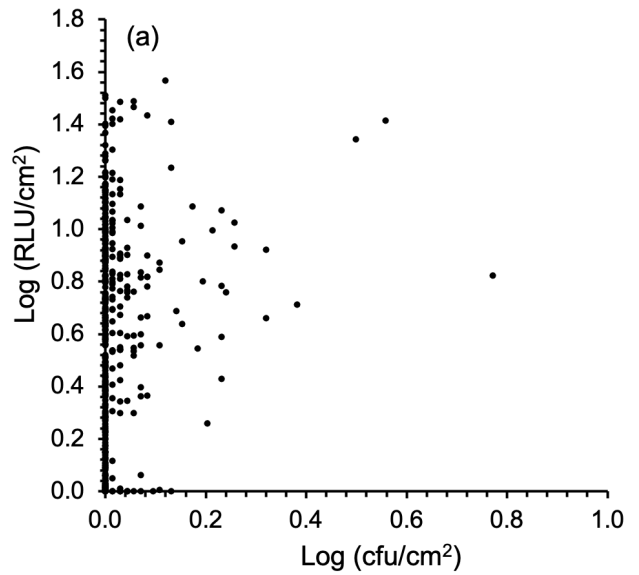
## RESULTS

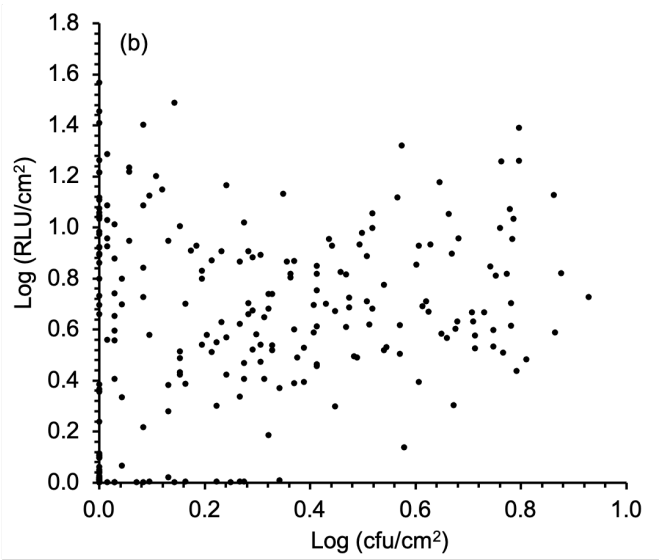
For all the samples collected in this study, the ATP readings ranged from 0 to 3,579 RLU per 100 $cm^2$ of trailer surface ($n$ = 536 swab samples). Colony counts using MCA ($n$ = 389) ranged from 0 to 139 cfu per contact plate area (28.3 $cm^2$) and 0 to 212 cfu per contact plate for R2A ($n$ = 238). Figure 1 shows the scatter plots of ATP measurements vs. colony counts using MCA and R2A agar plates. As shown in Fig. 1a, no readily discernable relationship was apparent between ATP method and the conventional microbiological method using MCA due to the prevalence of zero cfu values even when the equivalent RLU values were greater than zero. Compared to MCA, the plot between ATP method and the colony counts using R2A agar plates was more randomly scattered and more apparent as shown in Fig. 1b. This has led to a relatively higher correlation coefficient between ATP bioluminescence and R2A readings ($r$ = 0.206; $p$ = 0.001) than between ATP and MCA readings ($r$ = 0.154; $p$ = 0.002). Table 1 shows the means (and standard deviation) of the log adjusted ATP readings and colony counts at different trailer sampling locations. Using MCA, significant correlation with ATP readings was found on samples from lower deck floors ($r$ = 0.263; $p$ = 0.036) and upper deck walls ($r$ = 0.299; $p$ = 0.015). Slightly higher correlations with ATP results were obtained using R2A on lower deck floors ($r$ = 0.586; $p$ < 0.001) and upper deck floors ($r$ = 0.516; $p$ <0.001). Moreover, evaluation of paired samples taken using both MCA and R2A yielded a significant correlation ($r$ = 0.315; $p$ < 0.001; $n$ = 150) between the two microbial plates. All the correlation coefficients between ATP and cfu units were deemed to be categorically low to moderate (Mukaka 2012), albeit statistically significant.

To identify the critical areas for cleaning the trailers (i.e., 'hot spots'), RLU measurements and cfu counts

**Table 1. Correlations between log adjusted ATP bioluminescence results vs log adjusted colony counts detected at different sampling locations using (a) MacConkey and (b) R2A contact agar plates.**

| Contact agar plate | Sampling location | n | Mean log cfu (± SD) | Mean log ATP reading in RLU (± SD) | Pearson correlation coefficient ($p$ – value) |
|---|---|---|---|---|---|
| MCA | Floor (lower deck) | 64 | 0.35 (0.51) | 2.17 (1.12) | 0.263 (0.036) |
| | Floor (upper deck) | 65 | 0.41 (0.44) | 2.18 (1.05) | 0.095 (0.449) |
| | Wall (lower deck) | 65 | 0.18 (0.31) | 2.39 (0.67) | 0.237 (0.057) |
| | Wall (upper deck) | 65 | 0.14 (0.31) | 2.36 (0.84) | 0.299 (0.015) |
| | Ramp/Partition Panel | 65 | 0.19 (0.34) | 2.25 (0.89) | -0.038 (0.763) |
| | Trailer Exterior | 60 | 0.10 (0.20) | 2.43 (0.54) | 0.143 (0.276) |
| | All Samples | 389 | 0.23 (0.39) | 2.30 (0.88) | 0.154 (0.002) |
| R2A | Floor (lower deck) | 40 | 1.16 (0.98) | 2.02 (1.18) | 0.586 (<0.001) |
| | Floor (upper deck) | 40 | 1.21 (0.90) | 2.00 (1.05) | 0.516 (<0.001) |
| | Wall (lower deck) | 39 | 1.19 (0.57) | 2.48 (0.52) | -0.004 (0.981) |
| | Wall (upper deck) | 40 | 1.08 (0.59) | 2.30 (0.85) | -0.086 (0.597) |
| | Ramp/Partition Panel | 40 | 1.03 (0.75) | 2.31 (0.93) | 0.209 (0.195) |
| | Trailer Exterior | 39 | 1.05 (0.81) | 2.65 (0.37) | -0.614 (<0.001) |
| | All Samples | 238 | 1.12 (0.78) | 2.29 (0.89) | 0.206 (0.001) |




among the different sampling locations for all 16 trailers were compared. When using the ATP meter readings, it was not possible to readily identify any 'hot spots' clearly because the RLU levels were mostly within close range among the different sampling locations for all sampled trailers. This could be due to the high sensitivity of ATP bioluminescense method to detect ATP from all microbial and other organic residues present on the sampled surfaces, regardless of whether the contaminant material was already deactivated or not. However, using the values obtained from the R2A and MCA plates, significant difference ($p$<0.05) in microbial contamination levels was observed among the different sampling locations, indicating potential 'hot spots' that may require close attention during washing. Specifically, microbial contamination for all trailers as detected by the contact plates was significantly high on floors. Aside from corrugations on floor surfaces which make cleaning the floor relatively challenging, accumulation of water draining to the floor surfaces during the drying process (after cleaning) may have contributed to this observation.

**DISCUSSION**

Sixteen commercial swine transport trailers were sampled in this study to evaluate the applicability of ATP bioluminescence as a tool for rapid assessment of cleanliness of newly-washed and disinfected trailers. Although widely accepted in assessing cleanliness of environmental surfaces in healthcare and other settings, the usefulness of ATP method in this particular application has not been investigated yet. The results of this study indicated that ATP measurements had better correlation with R2A agar plates than with MCA plates. This can be observed in the difference in patterns of the scatterplots shown in Fig. 1, which could be due to the more selective nature of MCA compared to both ATP meter and R2A. Formulation of MCA and R2A are different in that MCA is considered a selective and differential medium for isolation of Gram (–) negative bacteria while inhibiting growth of most Gram (+) positive organisms (Hardy Diagnostics, 2020a). On the other hand, R2A is recommended for enumeration of a wider range of organisms (heterotrophic) and its formulation enhances recovery of many stressed and chlorine-resistant bacteria (Hardy Diagnostics, 2020b). ATP bioluminescence detects ATP from both microbial (non-selective) and organic sources.

In addition, a low to moderate (but statistically significant) positive correlation between ATP bioluminescence and colony count using MCA and R2A contact plates was observed. These results are comparable to the findings of Poulis et al. (1993) which reported a correlation coefficient of $r$ < 0.4 between ATP bioluminescence and microbial testing of food factory surface cleanliness using a contact (Rodac) plate. Larson et al. (2003) and Willis et al. (2007) reported poor correlations between ATP measurements and aerobic colony counts with correlation coefficients of -0.03 (hand samples), 0.04 (table samples) and 0.15 (hospital sites). On the contrary, other previous studies reported strong correlation between the two methods; Chen and Godwin (2006) reported $r$ values from 0.823 to 0.895 when evaluating cleanliness of household refrigerators. However, they used a microbial ATP bioluminescence (mATP) assay compared against aerobic plate count and psychotropic plate count, which likely contributed to the observed high correlation values. In addition, Azizkhan (2014) found improved correlation between ATP values and microbial counts (aerobic plate

count) after cleaning of food-contact surfaces ($r$ = 0.001 – 0.600 before cleaning to 0.72 - 0.99 after cleaning). The high correlation was likely due to the removal of food residues from the food processing equipment during the cleaning process, thus ATP method mostly detected the microorganisms present on surfaces similar to the traditional microbial counts.

The low to moderate correlation between ATP bioluminescence and colony counts observed in this study can be attributed to a number of factors. One is the high sensitivity of the ATP bioluminescence assay to detect ATP from different sources, i.e., all microbial and other organic residues present on the sampled surfaces, regardless of whether the contaminant material was already deactivated (hence, not culturable) or not. According to Green et al. (1999) and Mulvey et al. (2011), the fluctuating ATP measurements could also be caused by the presence of chemicals and other materials used for cleaning such as disinfectants. Results from the study of Green et al. (1999) demonstrate that commercial sanitizers and cleansers may either suppress or increase RLU measurement readings when the chemical comes into direct contact with the ATP bioluminescence reagents. While this is not considered a device or technique limitation in surface cleanliness monitoring, it may have caused the low correlations to viable microbial counts detected using either MCA or R2A.

Another potential reason is the difference in the capacity of the applied sampling methods to extract the contaminants from the surfaces. ATP method utilized a moistened swab that can easily access both flat and irregular features on the target sampling surface thereby recovering high percentage of the contaminants present on the surface, while use of the contact agar plates for direct sampling can lead to difficulty in making good contact with irregularities or uneven features on the target surface. Use of agar contact plates or impression plate method can only enumerate bacteria recovered from the points that actually made contact with the plate, and may have missed out those that accumulated in critical spots such as crevices and corrugations in the target surface. While the sampling surfaces chosen in this study were aluminum alloy surfaces that were mostly flat, some surfaces like the floors and ramps have corrugated anti-slip patterns on the surface.

The difference in reported correlation coefficients between the two methods from previous investigations and this study can be further attributed to the variation in the sensitivities of the different models of commercial ATP hygiene monitoring systems used (Colquhoun et al. 1998; Omidbakhsh et al. 2014; Willis et al. 2007), and also to the microbial monitoring techniques and analysis applied. For a number of models of ATP bioluminescence monitoring systems presently available, measurements are given in 'relative light units', thus results may not be directly comparable from one brand of luminometer and ATP swab to another (Willis et al. 2007). It has been reported that some ATP kits yielded considerably high measurements compared with other kits (Cooper et al. 2007; Lewis et al. 2008; Moore et al. 2010).

Despite the low correlation with the microbial method obtained in this study, a threshold or 'Pass' limit of 390 RLU per 100 $cm^2$ of trailer surface was established for assessing surface cleanliness in animal trailers using ATP bioluminescence method. As recommended by the manufacturer of the ATP meter (Hygiena LLC, Camarillo, CA, USA), this 'Pass' threshold limit was determined as the average of all RLU measurements when the equivalent colony counts using MCA and R2A agar plates were zero. As such, a 'Pass' reading obtained using the ATP method for the evaluation of animal trailer surface cleanliness, would yield equivalent result if the trailer is assessed by visual inspection and microbiological testing.

The group of Griffith et al. (2000) has recommended an integrated cleanliness monitoring program originally for healthcare facilities, which included an initial assessment of surface cleanliness carried out through visual inspection; if the surface is visually clean but considered as high risk, then ATP monitoring is recommended and remedial action is implemented where necessary. This same integrated cleanliness monitoring approach can also be applied for assessing trailer cleanliness, effectively utilizing the advantages of the ATP monitoring system.

The poor statistical correlation between RLU readings and contact plate counts seems to arise from the high sensitivity of ATP method for detecting the presence of both viable and deactivated microbial and organic residues on trailer surfaces, and conversely the selective nature and variable sensitivity of plate count method. However, regardless of the assessment method employed, any RLU readings above the 'Pass' limit or substantial counts on contact plates tested on key trailer surfaces would raise flags on the overall cleanliness of the trailer, necessitating careful deliberation before proceeding to use the trailer for transport. Hence, the low value calculated for a statistical parameter ($r$) should not preclude the use of ATP method as a valuable tool available to pig farmers for rapid and less costly means for checking trailer biosecurity before shipping their pigs.

The results from the conventional microbiological assessment techniques using R2A and MCA agar plates showed that there are certain areas in the sampled trailers that may require close attention during cleaning. After employing improved washing protocols, a rapid and economical assessment method such as the ATP bioluminescence test will be valuable in verifying that these critical spots are thoroughly cleaned prior to use of the trailer for swine transport, thereby facilitate subsequent efforts on improving the washing and disinfection protocols.

## CONCLUSIONS

ATP bioluminescence method had statistically significant correlation with the standard microbiological method using R2A and MacConkey agar (MCA) plates. Based on this relationship, a threshold 'Pass' limit of 390 RLU per 100 $cm^2$ of trailer surface was established, which will enable the use of the ATP method for conducting an objective

assessment of the overall cleanliness of an animal transport trailer to determine its suitability for use in transporting animals. In conjunction with current practices that employ visual inspection and microbiological testing, the priority areas in trailers that require close attention during washing can be conveniently and rapidly identified, and the efficacy of corrective actions on the current washing and disinfection protocol can be objectively verified using the ATP method. Results from this study have also shown that trailer floors posed the highest risk of microbial contamination among all the six critical areas tested, which indicated the need for extra vigilance in cleaning and disinfecting this part of the trailer, thereby ensuring the biosecurity of transport trailers used in the swine industry.

## ACKNOWLEDGEMENT

The authors would like to acknowledge the financial support provided by the Saskatchewan Ministry of Agriculture to this research project. The authors also acknowledge the strategic funding provided to Prairie Swine Centre by the Saskatchewan Pork Development Board, Alberta Pork, Manitoba Pork Council, Ontario Pork and the Saskatchewan Ministry of Agriculture. The authors also acknowledge Roberge Transport, Fast Genetics and Olysky Farms for their support by entrusting their cleaned trailers for sampling. Assistance provided by Jamie Neufeld and Richard Baah in gathering samples is also acknowledged.

## REFERENCES

Aycicek, H., U. Oguz and K. Karci. 2006. Comparison of results of ATP bioluminescence and traditional hygiene swabbing methods for the determination of surface cleanliness at a hospital kitchen. *International Journal of Hygiene and Environmental Health* 209:203-206. https://doi.org/10.1016/j.ijheh.2005.09.007

Azizkhan, Z. 2014. Comparison between ATP bioluminescence technique and traditional microbiological method to detect contamination within food facilities in Saudi Arabia (Jeddah). *Public Health Front* 3:11-18. https://doi.org/10.5963/PHF0301003

Boyce, J., N. Havill, D. Dumigan, M. Golebiewski, O. Balogun and R. Rizvani. 2009. Monitoring the effectiveness of hospital cleaning practices by use of an adenosine triphosphate bioluminescence assay. *Infection Control and Hospital Epidemiology* 30:678-684. https://doi.org/10.1086/598243

Canadian Swine Health Board. 2011. Quality control of wash/disinfect/dry protocols for live-hog transport vehicles. http://www.cpc-ccp.com/biosecurity/publications.php (accessed on 15 June 2020).

Chen, F. and S. Godwin. 2006. Comparison of a rapid ATP bioluminescence assay and standard plate count methods for assessing microbial contamination of consumers' refrigerators. *Journal of Food Protection* 69:2534-2538. https://doi.org/10.4315/0362-028X-69.10.2534

Colquhoun, K.O., S. Timms and C.R. Fricker. 1998. A simple method for the comparison of commercially available ATP hygiene monitoring systems. Journal of Food Protection 61:499-501. https://doi.org/10.4315/0362-028X-61.4.499

Cooper, RA, C.J. Griffith, R.E. Malik, P. Obee and N. Looker. 2007. Monitoring the effectiveness of cleaning in four British hospitals. *American Journal of Infection Control* 35:338-341. https://doi.org/10.1016/j.ajic.2006.07.015

Davidson, C.A., C.J. Griffith, A.C. Peters and L.M. Fielding. 1999. Evaluation of two methods for monitoring surface cleanliness - ATP bioluminescence and traditional hygiene swabbing. *Journal of Luminescence* 14:33-38. https://doi.org/10.1002/(SICI)1522-7243(199901/02)14:1<33::AID-BIO514>3.0.CO;2-I

Deshpande, S.S. 2001. Principles and applications of luminescence spectroscopy. *Critical Reviews in Food Science and Nutrition* 41:155-224. https://doi.org/10.1080/20014091091797

Green, T.A., S.M. Russel and D.L. Fletcher. 1999. Effect of chemical cleaning agents and commercial sanitizers on ATP bioluminescence measurements. *Journal of Food Protection* 62:86-90. https://doi.org/10.4315/0362-028X-62.1.86

Griffith, C.J., R. Cooper, J. Gilmore, C. Davies and M. Lewis. 2000. An evaluation of hospital cleaning regimes and standards. *Journal of Hospital Infection* 45:19-28. https://doi.org/10.1053/jhin.1999.0717

Hardy Diagnostics. 2020a. Information for Use. https://catalog.hardydiagnostics.com/cp_prod/Content/hugo/CRITN-MacConkeyAgar.htm (accessed on 18 March 2021).

Hardy Diagnostics. 2020b. Information for Use. https://catalog.hardydiagnostics.com/cp_prod/Content/hugo/R2AAgar.htm (accessed on 20 March 2021)Larson, E., A. Aiello, C. Gomez-Duarte, S. Lin, L. Lee, P. Della-Latta and C. Lindhardt. 2003. Bioluminescence ATP monitoring as a surrogate marker for microbial load on hands and surfaces in the home. *Food Microbiology* 20:735-739. https://doi.org/10.1016/S0740-0020(03)00041-8

Lewis, T., C. Griffith, M. Gallo and M. Weinbren. 2008. A modified benchmark for evaluating the cleaning of some hospital environmental surfaces. *Journal of Hospital Infection* 69:156-163. https://doi.org/10.1016/j.jhin.2008.03.013

Moore, G., D. Smyth, J. Singleton and P. Wilson. 2010. The use of adenosine triphosphate bioluminescence to assess the efficacy of a modified cleaning program implemented within an intensive care setting. *American Journal of Infection Control* 38:617-622. https://doi.org/10.1016/j.ajic.2010.02.011

Mukaka, M.M. 2012. A guide to appropriate use of correlation coefficient in medical research. *Malawi Medical Journal* 24(3):69-71.

Mulvey, D, P. Redding, C. Robertson, C. Woodall, P. Kingsmore, D. Bedwell and Dancer, SJ. 2011. Finding a benchmark for monitoring hospital cleanliness. *Journal of Hospital Infection* 77:25-30. https://doi.org/10.1016/j.jhin.2010.08.006

Omidbakhsh, N., F. Ahmadpour and N. Kenny. 2014. How reliable are ATP bioluminescence meters in assessing decontamination of environmental surfaces in healthcare settings? *PLoS ONE* 9(6), e99951. https://doi.org/10.1371/journal.pone.0099951

Poulis, J.A, M. De Pijper, D.A.A. Mossel and P.P.A Dekkers. 1993. Assessment of cleaning and disinfection in the food industry with the rapid ATP-bioluminescence technique combined with the tissue-fluid contamination test and a conventional microbiological method. *International Journal of Food Microbiology* 20:109-116. https://doi.org/10.1016/0168-1605(93)90098-2

Vilar, M.J., J.L. Rodríguez-Otero, F.J. Diéguez, M.L. Sanjuán and E. Yus. 2008. Application of ATP bioluminescence for evaluation of surface cleanliness of milking equipment. *International Journal of Food Microbiology* 125:357-361. https://doi.org/10.1016/j.ijfoodmicro.2008.04.024

Willis, C., R. Morley, J. Westbury, M. Greenwood and A. Pallett. 2007. Evaluation of ATP bioluminescence swabbing as a monitoring and training tool for effective hospital cleaning. *British Journal of Infection Control* 8:17-21. https://doi.org/10.1177/1469044607083604