ATP bioluminescence assay for biosecurity verification

ATP (Adenosine triphosphate) is the basic energy component molecule of all plant and animal cells and is present in all micro-organisms and organic residues. Due to this, its presence on a surface can be used as an indicator of organic contamination.

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When ATP reacts with luciferin in the presence of luciferase, it produces a release of energy in the form of light. This is called bioluminescence: the same mechanism for firefly light in their abdomens.

The presence of ATP can be detected as a light output being emitted. Therefore, the measurement of light intensity provides a visual indicator of ATP present and of the total organic contamination from the surface tested.

ATP bioluminescence is used extensively in the food industry as a tool for monitoring and maintaining cleanliness.

ATP bioluminescence is a rapid and simple method to assess surface cleanliness and microbiological contamination in food facilities, the dairy industry, hospitals, animal transports, etc.

Table 1. Example Preliminary Results Reported in RLU Values for Farm 1, Employee 8: Raw Data (RLU Values from Skin Swabs).

Date	Pre-shower	Post-shower
02-Aug	543	914
04-Aug	746	5,430
05-Aug	825	66
06-Aug	1,026	8
10-Aug	252	97
11-Aug	186	78
12-Aug	908	75
19-Aug	413	33
26-Aug	2,695	414

ATP bioluminescence detects, among others, microbial cells, food residues, bacteria, fungi and yeast, which might persist after inadequate cleaning.

It is measured in Relative Light Unit (RLU) values, which is directly proportional to the amount of ATP present.

Several studies have demonstrated strong correlations between RLU values and bacterial microbe counts or surfaces judged to be dirty.

Personal hygiene, such as changing clothes and footwear as well as handwashing prior to entering a farm, are important biosecurity measurements to prevent the entering and spreading of pathogens.

Several studies, especially from the pork industry, show the effectiveness of personal hygiene.

Pathogens such as Escherichia coli, foot and mouth disease virus, classical swine fever virus, African Swine Fever (ASF), influenza A virus and porcine reproductive and respiratory syndrome virus have been shown to be transmitted indirectly through people from infected pigs to other pigs when no or little personal hygiene measures were implemented.

Therefore, the use of an ATP bioluminescence assay to control personal hygiene on farms would be a useful and fast method for biosecurity verification.

A short literature review and pilot studies on the application of ATP bioluminescence assays on farms and the suitability to control effective hand washing was implemented.

Results regarding RLU values, interpretation, and thresholds as well as possible limitations of the method are presented and discussed below.

Table 2. Example Preliminary Results Reported in RLU Values for Farm 1. Employee 7: Raw Data (RLU Values from Skin Swabs).

Date	Pre-shower	Post-shower
02-Aug	161	286
03-Aug	983	1,189
04-Aug	103	106
05-Aug	231	198
06-Aug	345	1,378
19-Aug	5	972
26-Aug	311	10

Materials and methods

BioChek, a Hygiena company, has undertaken field studies using the Hygiena EnSURE Touch luminometer and VetAssure Surface ATP test in the South African pork

Table 3. Example Preliminary Results Reported in RLU Values for Farm 1. Visitor: Raw Data (RLU Values from Skin Swabs).

Date	Pre-shower	Post-shower
04-Aug	591	38
23-Aug	499	38
23-Aug	627	23

Item	Post spray disinfectant	Post spray disinfectant + disinfectant wipe down
Lunchbox 2	2,933	105
Lunchbox 4	1,376	239
Semen Delivery Exterior Container	574	11
Interior Semen Cooler	338	84
Veterinary Delivery 1	88	23
Veterinary Delivery 2	417	58
Pest Control 2	63	16
Pest Control 3	253	11

Table 4. Example Preliminary Results Reported in RLU Values for Farm 1. Raw Data from Incoming Containers (RLU Values from Exterior Swabs).

industry. The purpose of these projects was to evaluate whether the BioChek ATP technology can be adopted at the farm level as a tool to test and validate on-farm hygiene protocols.

These projects officially started on August 1, 2021 at a genetic Nucleus in South Africa. Other indoor commercial farms then followed this initial farm with protocols and hygiene guidelines adjusted as experience with the products and the labour force was gained.

This study was undertaken to both show the potential of biosecurity verification and its effect on worker adherence to the protocols, plus to prove that the products can be effectively utilised in a indoor commercial farm environment. The studies focused on farm hygiene and biosecurity verification in the following areas:

- Employees and visitors pre-shower and post-shower.
- Employee lunch containers brought into facility.
- Outside containers of semen deliveries.
- Outside containers of pest control equipment and supplies delivered to the farm.
- Outside containers of veterinary products delivered to the farm.
- Waterers, feeders, fencing, and pen slats post cleaning and disinfection.

For each of the items or areas to be sampled detailed instructions with diagrams were presented to the employees and lead employees were trained to perform the sampling on a consistent basis.

A read out of all tests was then performed using the Hygiena EnSure Touch luminometer to measure light generated by ATP reacting with liquid stable luciferase/luciferin reagent and results were then reported in Relative Light Units (RLU).

RLU results provide information on the level of contamination within seconds using this protocol.

The higher the RLU number the more ATP present and the dirtier the surface tested.

Results

Partial results for the first farm can be found in Tables 1-4. This data shows how the ATP bioluminescence method of biosecurity verification can be utilised to show the organic material reduction effect of cleaning and disinfection and how this pre- and post-cleaning plus disinfection data can cause labour behavioural changes for the long-term.

Discussion

Several studies have shown that the ATP bioluminescence method is useful and efficient to evaluate the cleanliness of a variety of surfaces in farm environments. For example, to monitor the hygiene standards on dairy farms by taking samples of milking equipment. However, it is not possible to distinguish whether high RLU values originated from bacteria or other organic material, such as milk residues. High RLU values are indicative of inadequate cleaning as evidenced by the bacterial and other ATP detected on surfaces being monitored for biosecurity verification.

Furthermore, ATP bioluminescence testing has been applied to determine the cleanliness of surfaces like floors, feeders, enrichment material, nipple drinkers and walls from farms and animal transporters. These studies also show that ATP testing is a highly accurate and cost-efficient alternative to microbiological methods for rapid, on-farm assessments.

In addition to visual inspection, it can be used to determine the cleanliness status of farms and animal transporters and is useful to identify critical areas for subsequent washing and disinfection. ATP bioluminescence assays are also a simple and reliable method to test handwashing compliance. They are used in several areas to monitor handwashing, as for example in hospitals or food-processing companies.

It needs to be considered that skin is a living organ and therefore has natural levels of ATP, even when it is clean. It is thus impossible to achieve an ATP level of zero.

Furthermore, due to variations of skin characteristics from person to person, it is not possible to define universal baseline ATP levels for clean hands.

Thresholds for the differentiation between 'clean' and 'dirty' vary depending on the surface and the environment where the sample is being taken. A collection of studies indicating threshold values and

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Table 5. Methods to Define ATP RLU clean/dirty thresholds. (Literature review complied by SAFOSO) www.safoso.ch

Location	Tested Surfaces	Threshold	Method to define thresholds
Dairy Farms	Milking Equipment	Clean: < 150 RLU Dirty: > 300 RLU Acceptable but not good: 150 – 299 RLU	Based on recommendations by the manufacturer.
Dairy Farms	Milking Equipment	Teat cup rubbers = 152 RLU Teat dip containers = 242 RLU Milk receivers = 282 RLU Pipeline joints – 1,821 RLU	Reference values for each surface were established based on mean RLU values observed in class A farms. Class A farms are farms with a very low bacterial count (<10 x 10^3) in bulk tank milk.
Swine Livestock Trailer	Floors, walls, ramps, partitions, and trailer exterior surfaces	Pass (Clean): 430 RLU/100cm^2 Critical: 431 – 850 RLU/100cm^2 Fail: (Dirty): > 850 RLU/100cm^2	Defined by a microbial assessment through MacConkey agar contact plates. The thresholds for the definition of cleanliness through the microbiological assessment was taken from the guidelines of the Canadian swine health board. Based on those thresholds the researchers calculated the equivalent threshold values for RLU/100cm^2

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methods to define the thresholds are presented in Table 5. One limitation of the ATP bioluminescence test is that high ATP levels are not necessarily linked to a biosecurity risk. Materials such as milk residues, organic debris, epithelial cells or body secretions can also raise ATP values. All of these factors could carry a virus which could contribute to a biosecurity risk.

Furthermore, the ATP bioluminescence method is not able to differentiate living from dead cells or inactivated microbes. In addition, residues of cleaning and disinfecting agents can influence the result of an ATP test and lead to decreased ATP levels. One study showed that the ability of an ATP test to detect gram-negative bacteria is lower compared to gram-positives, due to insufficient cell lysis.

Many pathogens that pose a risk of introduction due to inadequate personal hygiene are viruses. ATP assays do not directly measure viruses.

However, some studies suggest that ATP measurements correlate significantly with reduced viral recovery.

ATP bioluminescence measurements can, therefore, be used to monitor the effectiveness of interventions taken to reduce viral contamination. More work needs to be done to establish this correlation in pork industry test environments.

Taking swabs in the weaner house.

Conclusion

Several studies have demonstrated that ATP bioluminescence is a fast and easy-to-use method to monitor microbial contamination and the effect of hygiene interventions for a variety of surfaces (for example, milking equipment, animal transporters, stable-surfaces, and hands). No general thresholds for the differentiation between clean and dirty exist; the differentiation needs to be defined based on the surface tested, the environment and the desired degree of cleanliness.

There is no standard method for the definition of thresholds. Methods include reference values based on objects/surfaces with a low bacterial count, calculation of RLU thresholds based on existing thresholds of microbiological assessments, or the relative drop of ATP levels after a hygiene intervention.

Limitations of the method mainly concern the specificity of the assay, since high ATP levels can also be caused by non-pathogenic or dead microbes, as well as interactions with certain disinfectant materials.

People and vehicles are major risk factors regarding the introduction of pathogens into a farm.

This risk can be reduced by certain biosecurity measures, such as proper hand washing and showering or cleaning and disinfecting vehicles before entering a farm. ATP bioluminescence is a suitable tool to monitor these measurements in real time.

Additionally, hand washing improves over time when ATP tests are implemented due to the increased awareness of the on-farm personnel. ■

References are available from the author on request