

## Title: An Overview of Rapid Hygiene Testing Using ATP Bioluminescence

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Common sense dictates that after cleaning, equipment should be free of visible product residues and look clean. Visual assessment is one of the tools used to judge if a surface is clean. It is a real time test but it is subjective and relatively insensitive.

Testing for microorganisms provides more information. However, this is related more to the effectiveness of **disinfection** than the cleaning. These microbiological tests require laboratory facilities and a relatively high skill level. Additionally, this information is not available in a time frame that allows for immediate re-cleaning of equipment prior to production if unacceptable results are obtained.

For rapid hygiene testing, the needs are for a quick and sensitive test that can detect if cleaning is not to the expected standard and that is safe to use in the production environment. The bioluminescence test for adenosine tri-phosphate (ATP) has been developed to meet these needs. It is relevant to the measurement of the effectiveness of **cleaning** as it measures ATP not only from microorganisms but also from product residues. Instruments and kits have been developed to provide an easy to use test that can be performed in the production environment by operatives with minimal training.

### What is ATP and how do we measure it?

**ATP** is the basic energy currency molecule of all types of living organisms and as such, is present in all microorganisms, plant and animal cells. The technique of using the enzyme and substrate of the firefly (luciferase and luciferin) to detect and measure this key metabolite by measuring the light output was first described in 1947. Since then the detailed mechanisms have been comprehensively investigated and many diverse applications described. The assay can be set up to give a linear relationship between the light output and the ATP concentration. The result is obtained within seconds. Measurement is in a sensitive luminometer with results usually expressed as Relative Light Units (RLU).

In early work, the reagents used required the use of fairly complex instruments where reagents were added via injection systems, as the light signal decayed too rapidly to allow the use of simple formats of testing. Even when this was resolved by optimizing the formulation, some of the chemical treatments to extract ATP from intact cells involved boiling solvents or strong acids which would cause the signal to decay rapidly and were not very user friendly.



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The use of detergents or cationic agents as extractants are more user friendly but still cause the signal to decay and also require some further development in neutralizing after extraction or protecting the enzyme from the extractant. During the last few years, therefore, the reagent chemistry and the instrumentation have been developed to the point that simple to use and portable tests are now possible.

### **What are the benefits of ATP?**

The range of applications for which ATP bioluminescence has been considered is wide and diverse, from detecting microorganisms in a wide variety of sample types to detecting life on the moon or Mars! There was a high level of interest in its use as a rapid method for the detection and enumeration of microorganisms in the 70's and 80's but with a few exceptions the applications have not been widely adopted. Generally they involved complex techniques to separate the microbial from the non-microbial ATP of the sample and the methods were not practical for routine use.

The presence of ATP in both food and viable microorganisms is a disadvantage in using the technology to detect microorganisms in food. However, it is of great benefit when one considers checking for cleanliness. Food residues contain large amounts of ATP either in intact cells or as free ATP, originating from those cells. By the late 80's this application was gaining acceptance and during the 90's, with the advent of the simple to use and portable tests, it has become widely adopted by many major food and beverage manufacturers.

### **How does it work?**

A range of products are available, but all work on similar principles. An ATP free swab is provided pre-moistened or is moistened by the user with an ATP free buffer, water or extractant. The extractant can help with sampling as it is effective at releasing ATP from the surface. Using portable instruments, testing of the swab is usually done immediately. However, with some products the swabs are stable for a number of hours allowing the user to return to the instrument at a 'workstation' if preferred.

How the sample is then processed and measured varies depending on which product is used. With some products the freeze dried luciferin/luciferase reagent is provided within the swab device and no preparation is required. With other test formats there is a need to reconstitute a bottle of freeze dried enzyme and dispense this using a dropper bottle or pipette.

The trend is for unit dosed 'single shot' tests with no requirement for any preparation steps for the user. Liquid stable luciferin/luciferase reagents are now available for some applications.

In addition to surface testing, the technique can be applied to rinse water samples to assess closed CIP systems. Again various products are available - some with reagents which are pipetted, others with unit dose single shot formats.

Nevertheless, whichever product is used, there is a need for some preliminary work to establish the relevant Pass/Fail limits for the test. This is usually done by collecting reference data after the normal cleaning procedures. The levels set will depend on the type and condition of the surface and the method of cleaning used.



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### Summary

ATP bioluminescence can be a valuable tool in conjunction with visual assessment to 'positively release' the production line after cleaning. Its use allows corrective action to be taken before production starts and reduces the risk of poor cleaning resulting in a product quality problem.

Other uses are to optimize cleaning regimes and so contribute to cost effective chemical use. However, ATP Bioluminescence is not a direct replacement for microbiological testing. Such testing should still be carried out for monitoring background flora or checking for the presence of specific spoilage or pathogenic organisms. If used in conjunction with other control measures, a proactive and effective hygiene management system can be developed, and with regular review of results, the system can evolve and improve.

### References

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