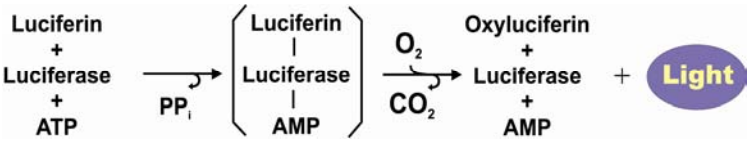
ATP Hygiene Monitoring

ATP (Adenosine Triphosphate) is present in all living cells and is therefore an indicator of biological contamination of e.g. human or bacterial origin. ATP can be detected rapidly by light emission through the combined use of the enzyme luciferase and a luminometer (Stanley, 1989). The measured light is proportional to the ATP level.



The technique has a detection limit of 1 pg ATP, which is equivalent to approximately 1000 bacterial cells.

ATP content of microorganisms in mol/cfu:

Spores:	0.01 x 10 - 18
Gram+:	2.3
Gram-:	12
Yeast:	500

The use of bioluminescent ATP detection for hygiene monitoring offers:

- Speed (in minutes, faster than colony counting)
- Convenience and
- The measurement of total hygiene.

Principle

For hygiene testing the total ATP content of the sample is determined. This will include eucaryotic and microbial ATP.

An ATP free swab is provided pre-moistened or is moistened by the user with an ATP free buffer, water or extractant. The use of extractant can help with sampling as it is effective at releasing ATP from the surface. Using portable luminometers like the BERTHOLD Junior LB 9509, testing 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. Therefore BERTHOLD TECHNOLOGIES is offering a variety of luminometers like the single tube luminometer Lumat LB 9507 and the automated tube luminometers AutoLumat LB 953 and Flash & Glow LB 955.

To determine the microbial ATP level selective extraction is used. First, non-microbial ATP is extracted with a non-ionic detergent (Triton X-100) and then destroyed by treating with a high level of potato ATPase for 5 minutes. Subsequently the microbial ATP is extracted using trichloroacetic acid (5%), an organic solvent (ethanol, acetone or chloroform) which will require subsequent dilution to avoid luciferase inhibition or cationic detergents. Careful timing of mixing and reading is required to allow for luciferase inhibition (Simpson & Hammond, 1991). Since the level of ATP in eucaryotic cells is three orders of magnitude greater than bacterial cells this procedure is difficult to achieve reliably.

Interpretation of results

Generally, clean surfaces show low levels of total ATP. Therefore, light output greater than 2 to 3 times background of the clean surface indicates that the area tested is contaminated with biological material. However, the method is very sensitive and in practise a threshold of 10 times background can be accepted. Nevertheless, whichever product is used, there is a need for some preliminary work to establish the relevant Pass/Fail limits for the test and this is usually done by collecting reference data after the normal cleaning procedures. The level set will depend on type and condition of the surface and the method of cleaning used.

The portable Luminometer Junior LB 9509 from BERTHOLD TECHNOLOGIES is best suited for this type of assay, since the instrument can be used in front of the machinery or working benches. 2000 results can be stored and later easily downloaded in the office. The instrument can be programmed according to the determined Pass/Fail limits, depending on the RLUs a green or a red LED will light up.

Selection of hygiene monitoring kits:

Luminescence technology:

Kit:
- ATP Hygiene Kit HS (BioThema)
- ATP Bioluminescence Assay Kit CLS II (Roche)
- ATP Bioluminescence Assay Kit HS II (Roche)
- ENLITEN® Total ATP Rapid Biocontamination Detection Kit (Promega)

BERTHOLD instruments:
Junior portable tube luminometer LB 9509

Publications:

Girotti et al. (1997): ATP Bioluminescence for the rapid assessment of the microbial content of wastewater and sludge; A practical guide to industrial uses of ATP-luminescence in rapid microbiology; Cara Technology Limited, Lingfield. ISBN 0952934507.

Griffiths (1996): The role of ATP bioluminescence in the food industry: new light on old problems; Food Technology.

Stanley PE (1989): A review of bioluminescent ATP techniques in rapid microbiology; J Biolumin Chemilumin. Jul; 4(1): 375-80.

Simpson WJ, Hammond JR (1991): The effect of detergents on firefly luciferase reactions; J Biolumin Chemilumin. Apr-Jun; 6(2):97-106.
Erratum in J Biolumin Chemilumin 1991 Jul-Sep;6(3):146.