

High Tech Detection Methods:

Hygiene Detection using ATP

Bioluminescence

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3A Standards Meeting

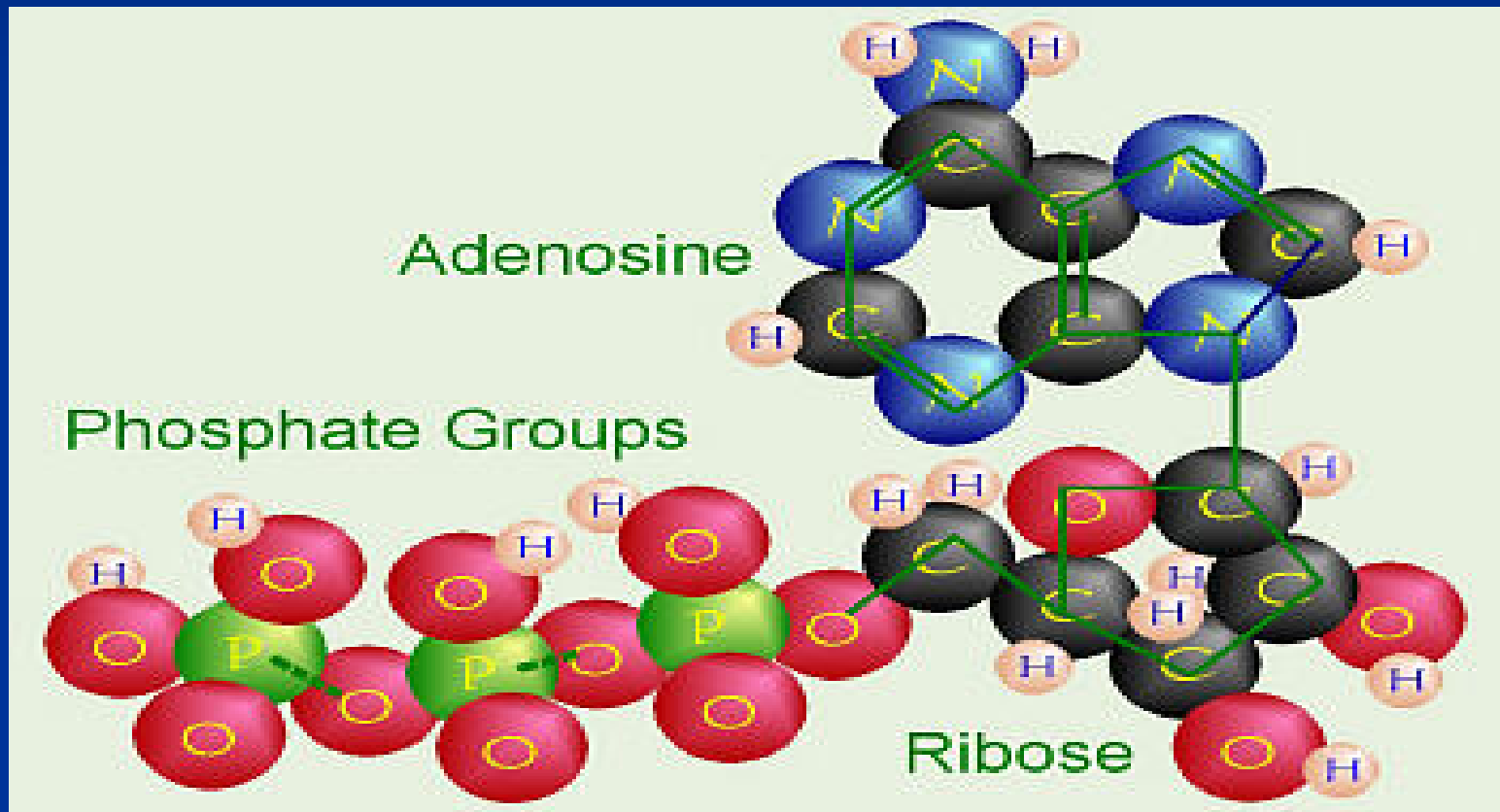
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ATP Bioluminescence

- What is ATP?
- What is bioluminescence?
- How does ATP bioluminescence work?
- What the results can show
- What the results cannot show

What is ATP?

Adenosine-5'-triphosphate



ATP

■ Adenosine-5'-triphosphate

- Source of energy that can be easily stored and used when needed for cellular functions.

- Found in all organic matter



- Release of phosphate groups cause release of energy.

What is Bioluminescence?

Bioluminescence

- **Origin:** Greek *bios* for "living" and the Latin *lumen* "light".
- The result of a chemical reaction-chemical energy is converted to light energy.
- Production and emission of light
- Light is produce in a cold reaction as opposed to incandescent light, which is produced with electricity and emits heat

How does ATP bioluminescence work?

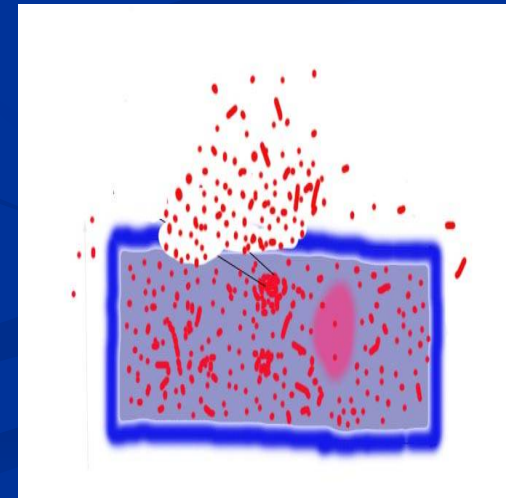
Bioluminescence principles

■ Based on detection of ATP in metabolically active cells

STEP 1: Wash and sanitize gloves/hands to remove ATP

STEP 2: Sponge surface area with ATP free sponge

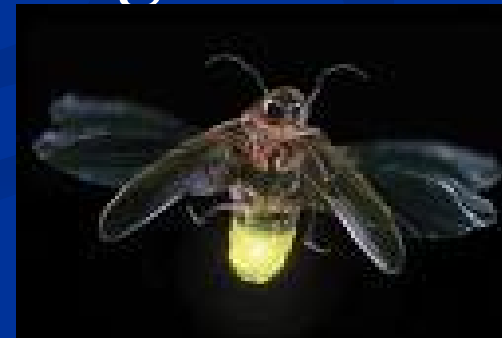
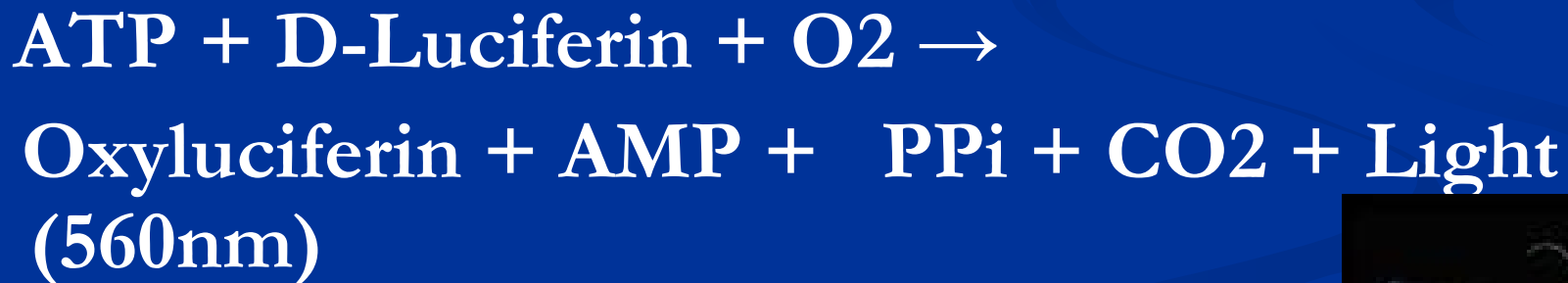
STEP 3: Place sponge in a solution that contains agents (trichloroacetic acid) that ruptures cell membrane and destroys enzymes that degrade ATP



Bioluminescence principles

- **STEP 4: Place swab in luminometer**

The following reaction is catalyzed by luciferase embedded in kit

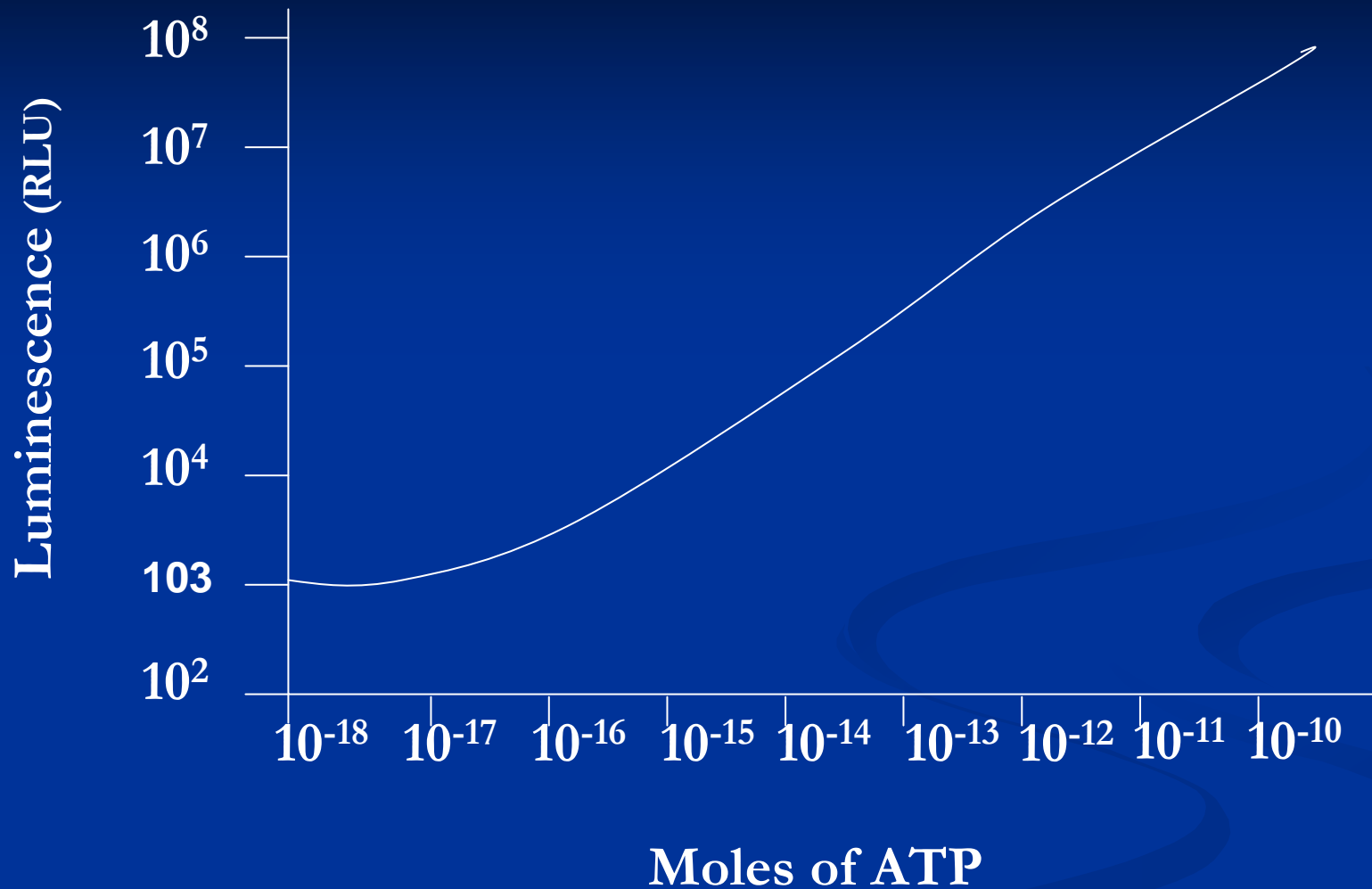


What the data can show

Data analysis

- Measurement of the light intensity
- Direct quantization of ATP
 - Light is quantified as Relative Light Units (RLU)
 - The intensity of the emitted light is proportional to the concentration of ATP.

ATP Standard Curve



What can data the show?

- **ATP detection is a useful tool when applied immediately after cleaning and before sanitization to assess cleaning effectiveness**
 - Indicate trouble spots
 - Reclean
- **Real time data**
- **Hygienic condition indicator of the amount of organic matter remaining on surfaces**
 - **Nutrients on surfaces may support the bacterial adherence process and biofilm formation**

Pre-operational sanitation assessment

■ Meat plants

- ATP used as a pre-operational release of equipment
- 50% of areas per line
- Each area sampled two or more times per week.

Pre-operational sanitation assessment

■ Action levels:

- 2 or more failures on the same line, same site on same day.
- Line is not released for production by Quality Assurance Department

Pre-operational sanitation assessment

Corrective actions:

- Reclean, sanitize and retest
- Intensified cleaning
 - Use cleaning/sanitizing agents at higher than normal concentrations
 - Use different cleaning and sanitization chemicals
- Equipment teardown

What the data cannot show

What the data cannot show

- **No correlation of bacterial surface counts to ATP**
 - **Non-direct relation between RLU and bacterial colony forming units (CFU/cm²).**
 - **Bacterial genus or species not known**

Disadvantages

- For thorough assessment of cleaning program both ATP and bacterial testing is needed.
- In meat plants *Listeria* testing is required by USDA on product contact surfaces

ATP \neq Bacteria

- Can have a very high count of bacteria with low light reading
- Can have a high light reading with low bacterial counts

What the data cannot show

- **Data decreases in effectiveness if time between production and sanitation is long**
 - **ATP disappears within 2 hrs of living matter death**

Disadvantages

Areas to analyze

- **Best use application is product contact areas**
 - Due to high signal to noise ratio
- **Most bacterial harborage areas are not on product contact areas**
 - Niches that are embedded within equipment
 - Hard to clean areas-cannot reach or see
 - Hard to disassemble pieces of equipment

Preparation work

Signal to noise ratio

- Measure of assay sensitivity
- Indicator of a kit's ability to distinguish between regular background (noise) generated from a surface and contaminated surfaces (signal)
- Any S/N value significantly above the control baseline indicates a contamination

Signal to noise ratio

- **Background noise is generated from non-microbial sources of ATP**
- **For some products, background levels are elevated**
 - **Decreases the sensitivity of the assay**

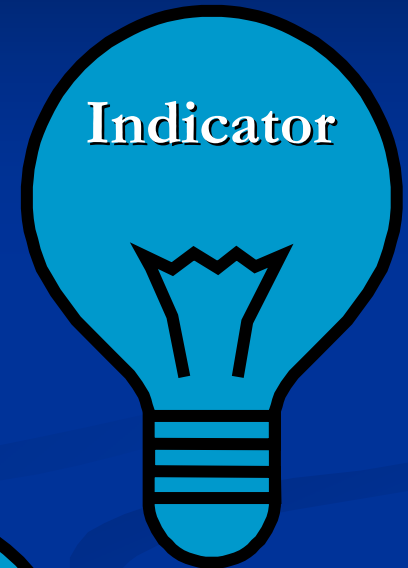
Allergens and ATP

- Allergenic protein can be up to 100 times the level of concern on surface and still be below detection level of ATP system
- Determination of ATP level from allergenic protein vs. other organic matter
- Setting low ATP limit may lead to over cleaning



Summary

ATP Bioluminescence



Thank you!