Snabb enzymatisk hygienkontroll
Rapid enzymatic hygiene control

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Technical considerations when choosing rapid hygiene control method

- When sampling surfaces the dirt releasing agent should be directly applied to the surface using a flat and flexible swab.
- Swab, reagents and reaction chamber should be contained in a single shot device.
- The assay should be possible to calibrate as remaining cleaning agents may inhibit the signal when using enzymes.
- If using an instrument it should be portable.

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What is the ideal biomolecule to detect?

- Universally present at similar concentrations in biological materials (cells)
- Easily quantified even at low levels with portable instrumentation
- Molecule should be stable
- Assay should be possible to calibrate

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Composition of an *E. coli* cell

macromolecule	percentage of total dry weight	weight per cell (fg)	characteristic molecular weight (Da)	number of molecules per cell
protein	55	165	3×10^4	3,000,000
RNA	20	60		
23 S rRNA		32	1×10^6	20,000
16 S rRNA		16	5×10^5	20,000
5 S rRNA		1	4×10^4	20,000
transfer		9	2×10^4	200,000
messenger		2	1×10^6	1,400
DNA	3	9	3×10^9	2
lipid	9	27	800	20,000,000
lipopolysaccharide	3	9	8000	1,000,000
peptidoglycan	3	9	$(1000)_n$	1
glycogen	3	9	1×10^6	4,000
metabolites and cofactors pool	3	9		
inorganic ions	1	3		
total dry weight	100	300		
water (70% of cell)		700		
total cell weight		1000		

composition rules of thumb

- carbon atoms ~10^{10}
- 1 molecule per cell gives ~1 nM conc.
- ATP required to build and maintain cell over a cell cycle ~10^{10}
- glucose molecules needed per cell cycle ~3×10^9 (2/3 of carbons used for biomass and 1/3 used for ATP)

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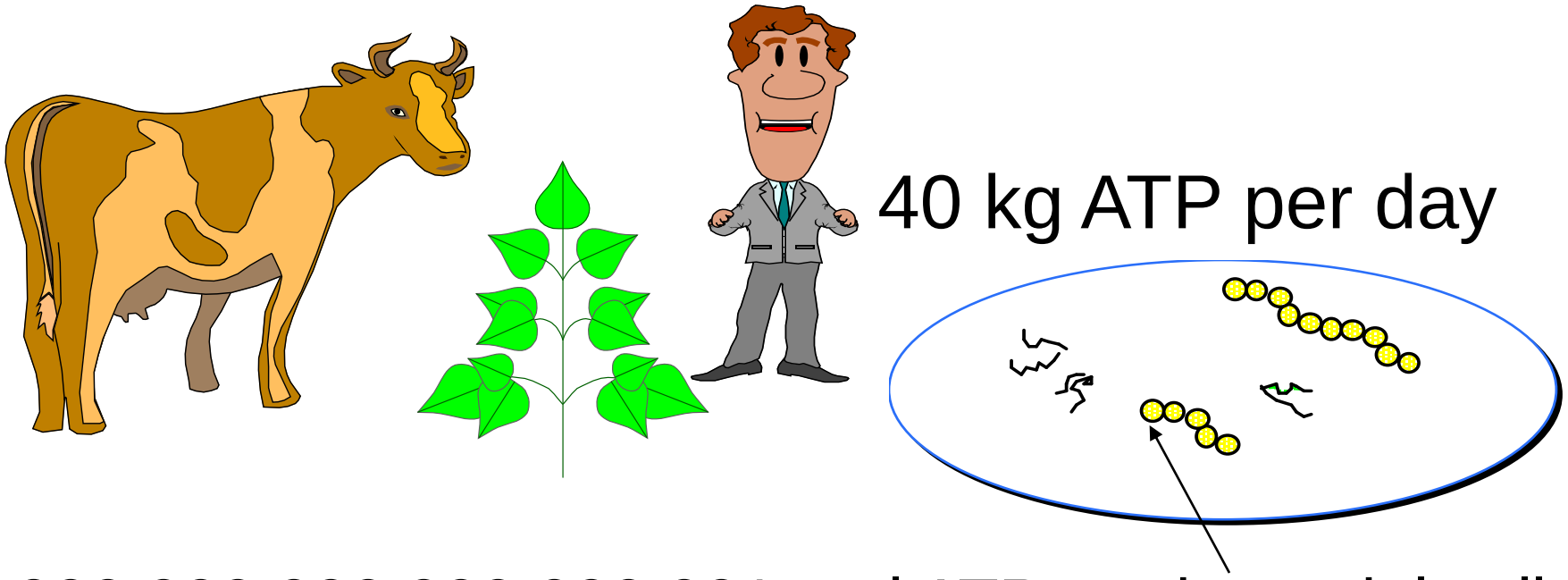
ATP is present in all living cells



0.000 000 000 000 000 001 mol ATP per bacterial cell

and >0.000 000 000 000 01 mol ATP per animal cell

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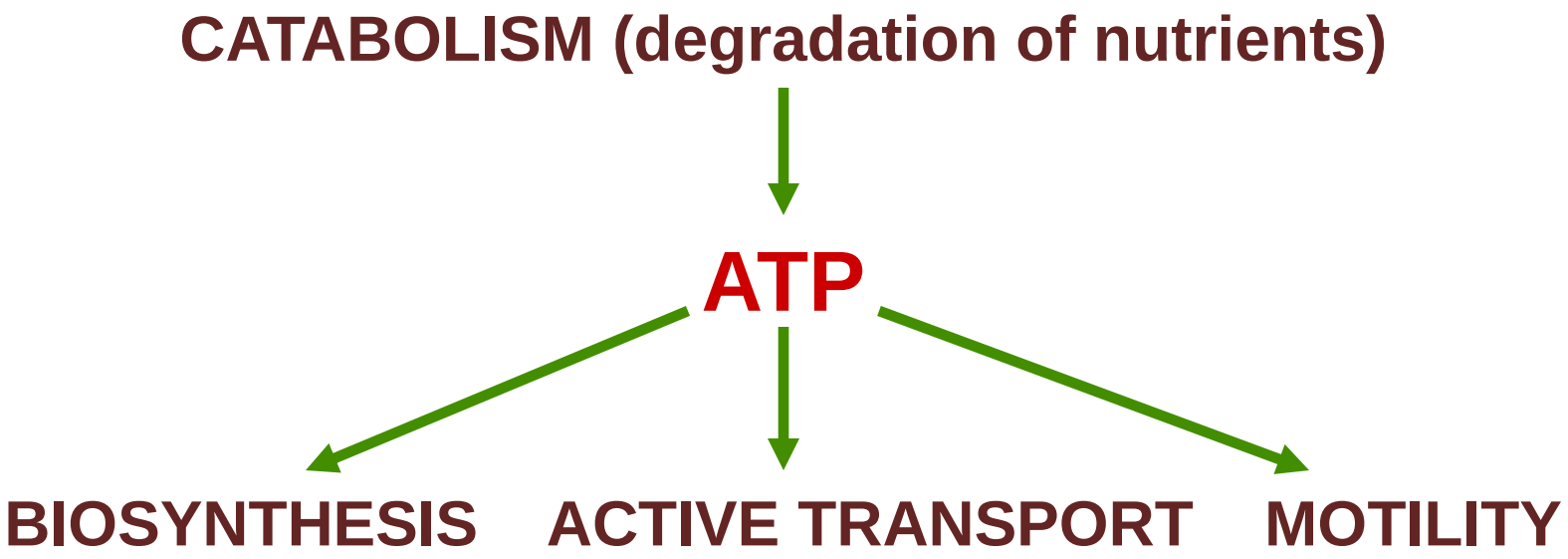
ATP (adenosine triphosphate)

the energy currency in all living cells



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What happens at cell death?

- ATP ⟹ ADP+Pi
- ADP ⟹ AMP+Pi
- $\mathrm{EC} = Energy\ Charge = \frac{ATP+0.5*ADP}{ATP+ADP+AMP}$
- EC is a measure of the energy status of living cells
- In healthy cells 0.85<EC<0.95
- In dying cells EC approaches 0.00

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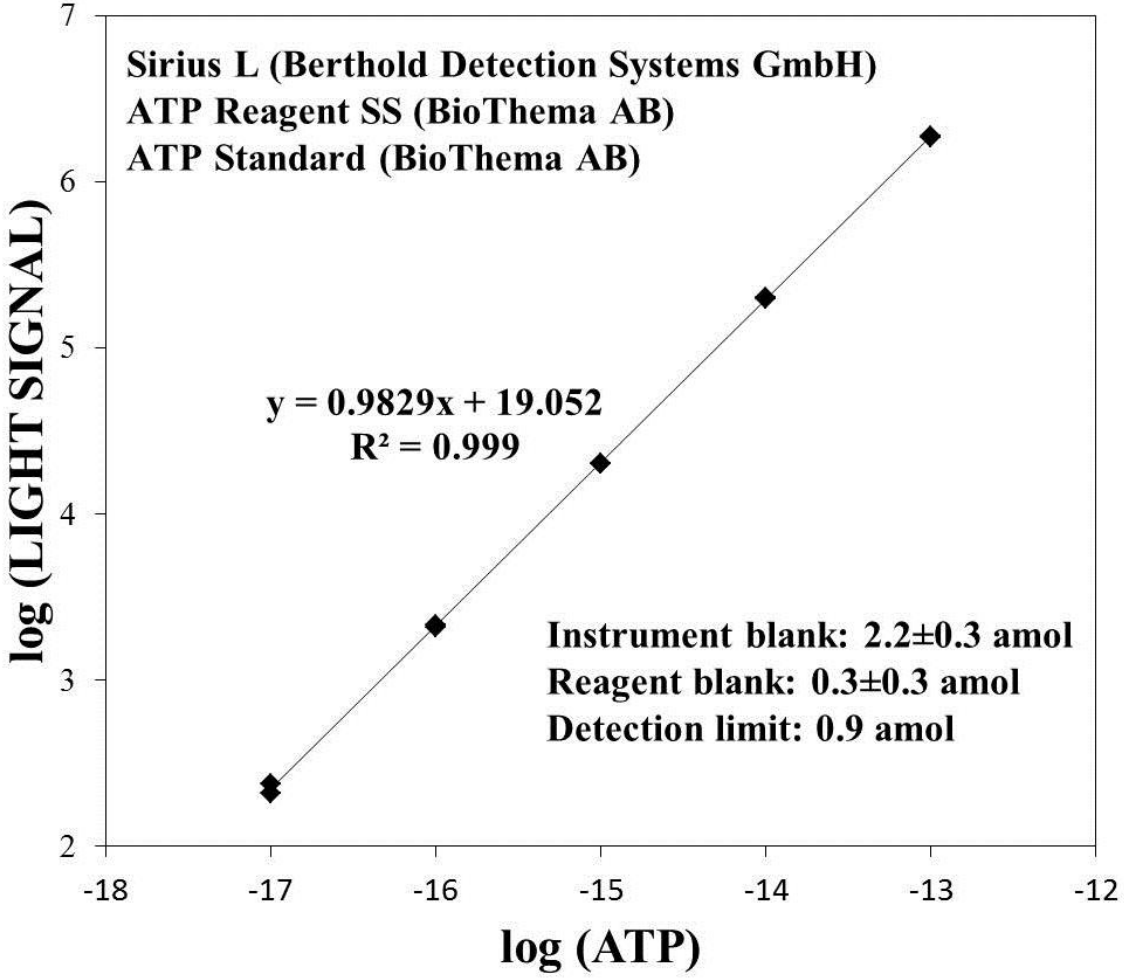
Detection limit of ATP assay



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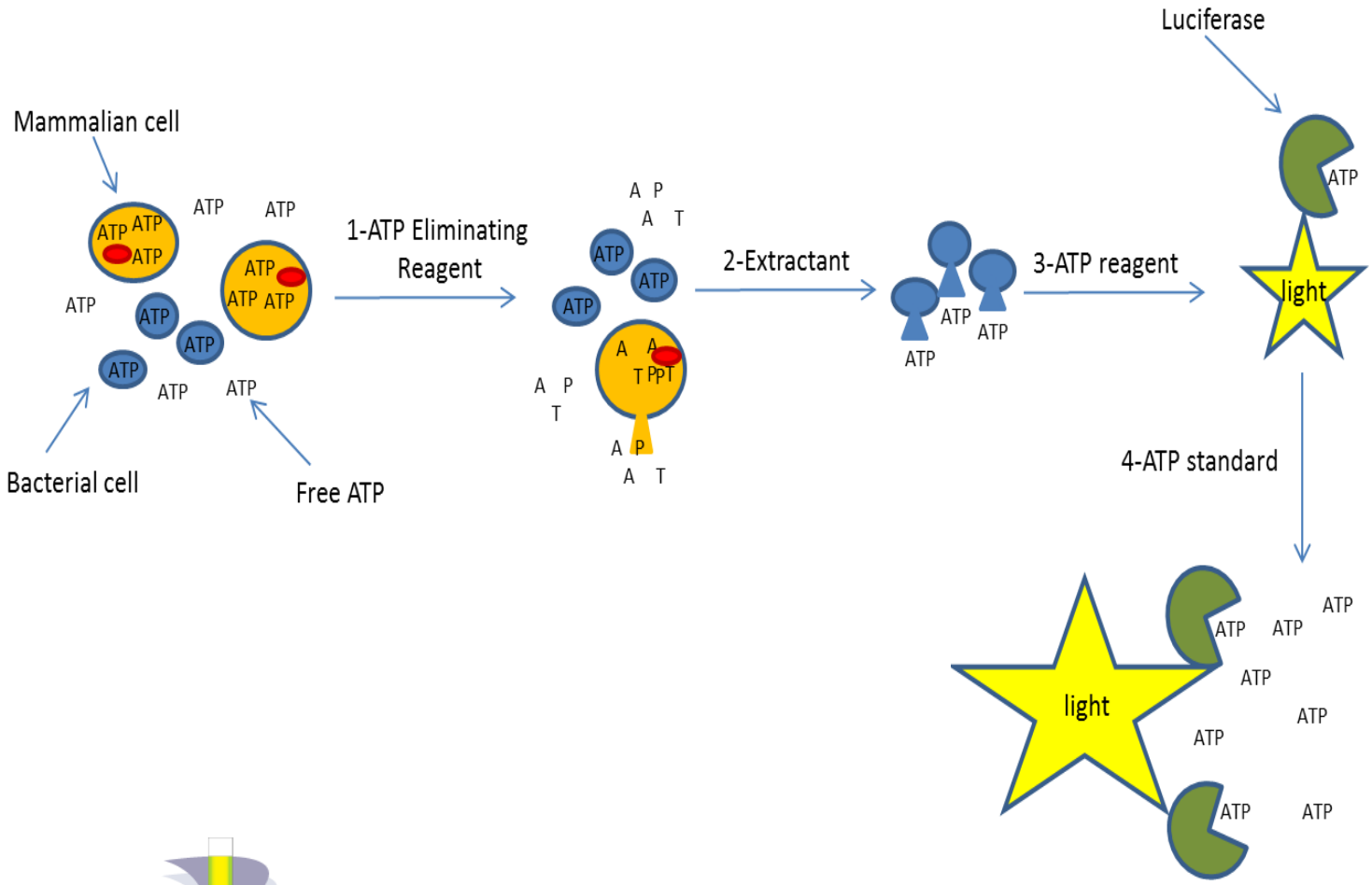
Measurement of bacterial ATP



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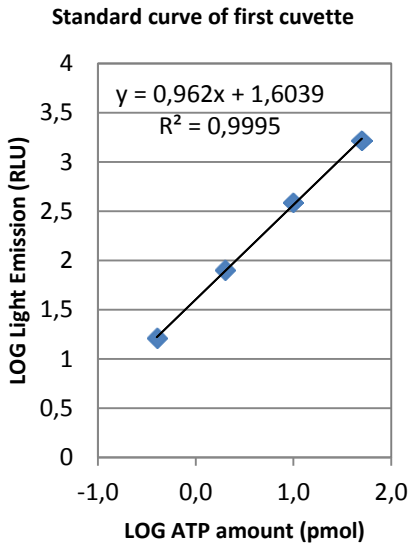
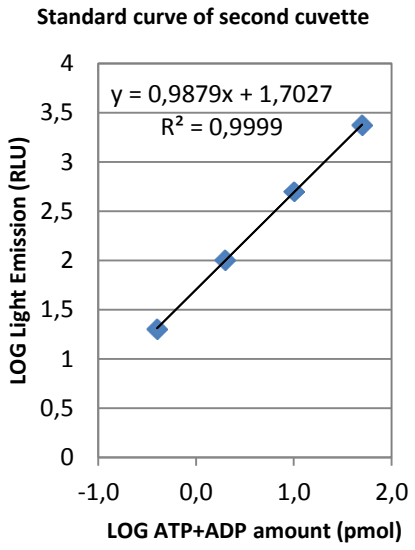
Assay of ATP, ADP and AMP

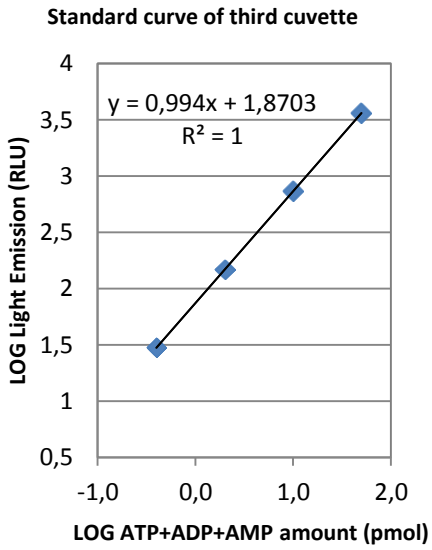
Luciferase: ATP+luciferin+O_2→AMP+PPi+oxyluciferin+CO_2+photon

Luciferase and pyruvate kinase: ADP+PEP →ATP+pyruvate

Luciferase and pyruvate, phosphate dikinase: AMP+PEP+PPi →ATP+pyruvate+Pi







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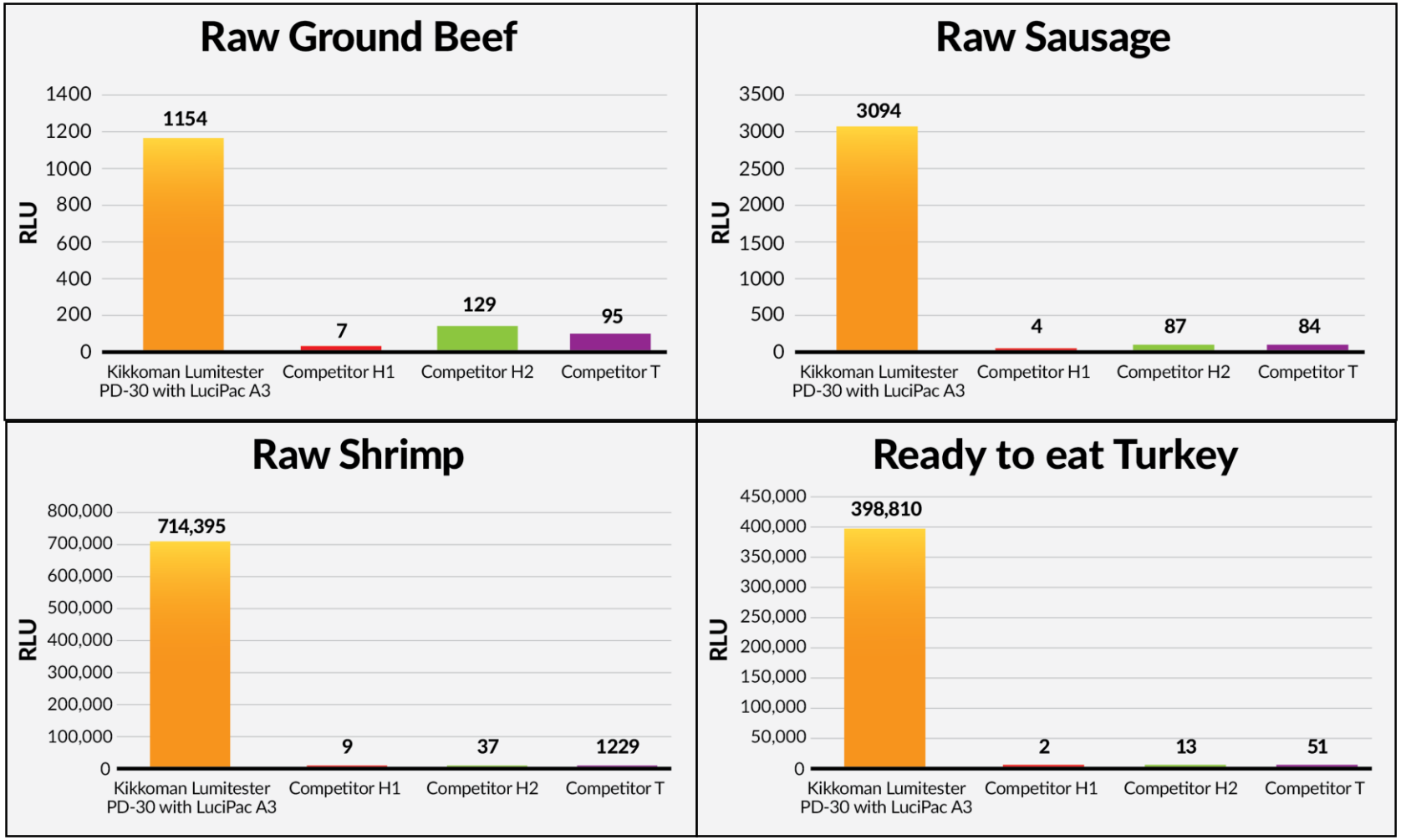
ATP+ADP+AMP is more sensitive than ATP



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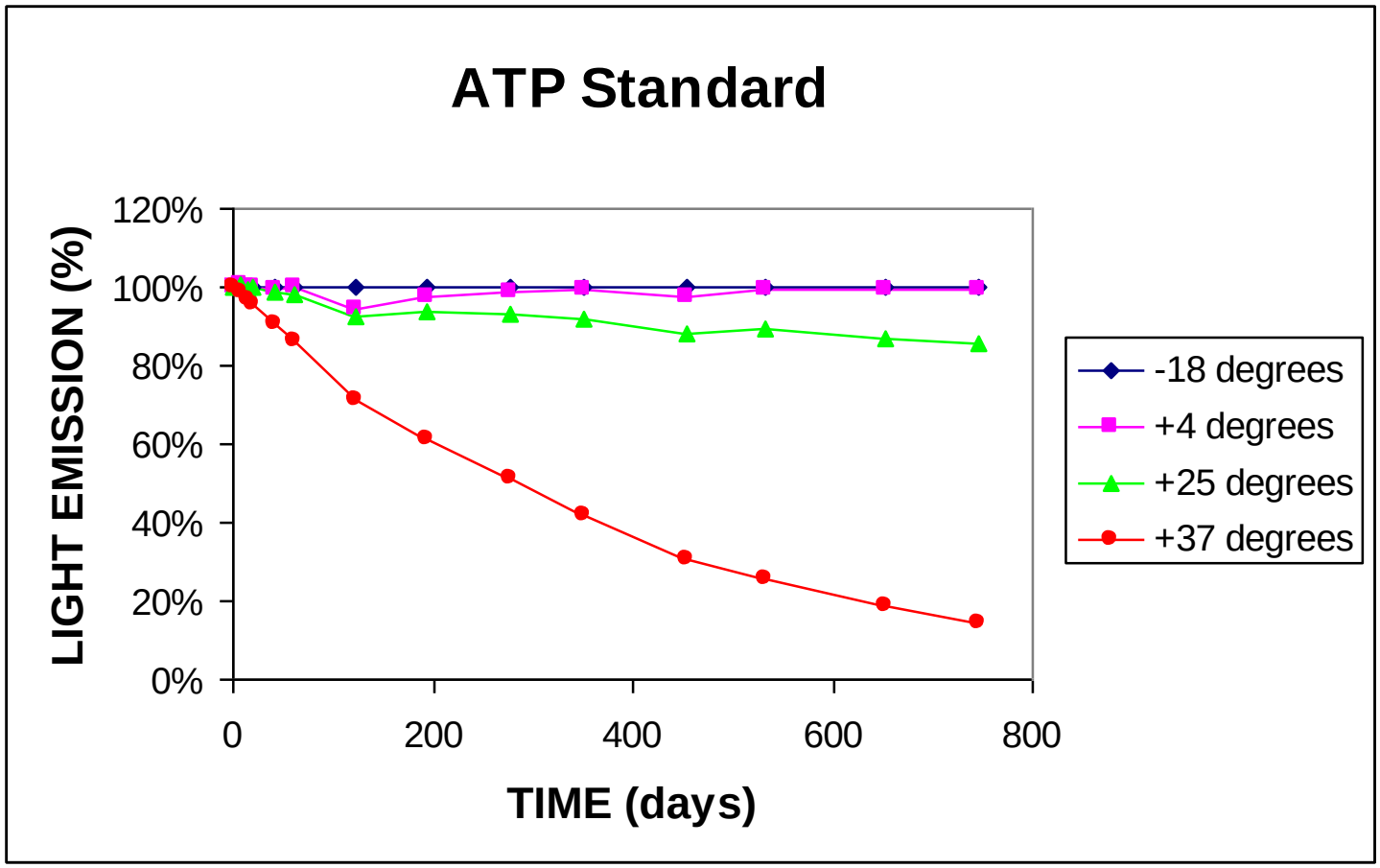
Stability of certified, liquid-stable ATP standard (10 µmol/L)



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Lundin, A, Inglis, R. and Touma, (2010) N. Luminescence 25: 159-161

Comparison between different hygiene tests

Test	Properties
• Cultivation	• Several days to obtain results. Poor sampling with rounded swab. Only bacteria measured.
• Protein swab	• Poor detection limit (3 µg) corresponding to $2x10^7$ *E.coli* cells. Poor sampling with rounded swab.
• PCR	• Bacterial species can be identified. Method does not differentiate between living and dead cells.
• ATP+ADP+AMP	• Simple and rapid test for biological contamination. Sampling with flat or rounded swab.
• Total ATP	• Rapid test of biological contamination regardless of type of cells. Sampling with flat or rounded swab.
• Bacterial ATP	• Rapid test on living bacteria from 10 till 100.000 cells. No identification of species.

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ATP and protein compared

	Protein	ATP
Time to result (min)	15*	<1
Detection limit (µg)	3*	0.000 000 000 5**
Detection limit (cfu)	20 000 000***	1**
Dynamic range	semiquantitative	5 orders
Instrument cost (SCrs)	7 200	10 000 – 20 000
Reagent cost (SCrs)	29	11

*http://multimedia.3m.com/mws/media/645040O/3m-clean-trace-surface-protein-allergen-brochure.pdf

** Application Note 2010/01 Berthold Detection Systems

***http://kirschner.med.harvard.edu/files/bionumbers/Composition%20of%20an%20average%20Ecoli%20Br%20cell.pdf

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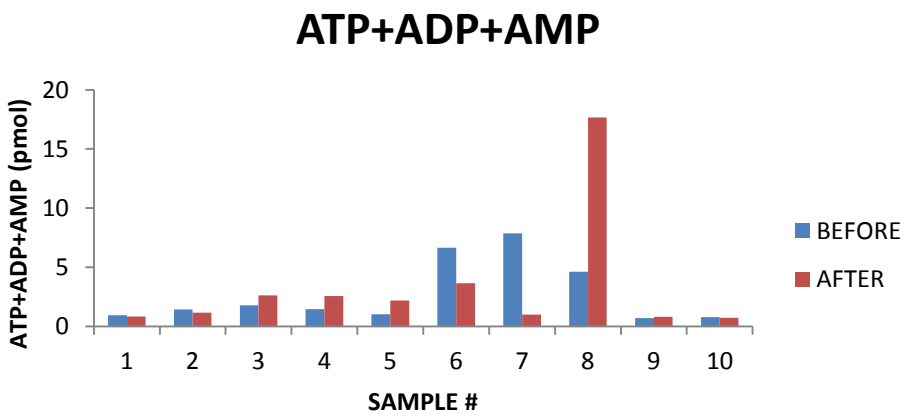
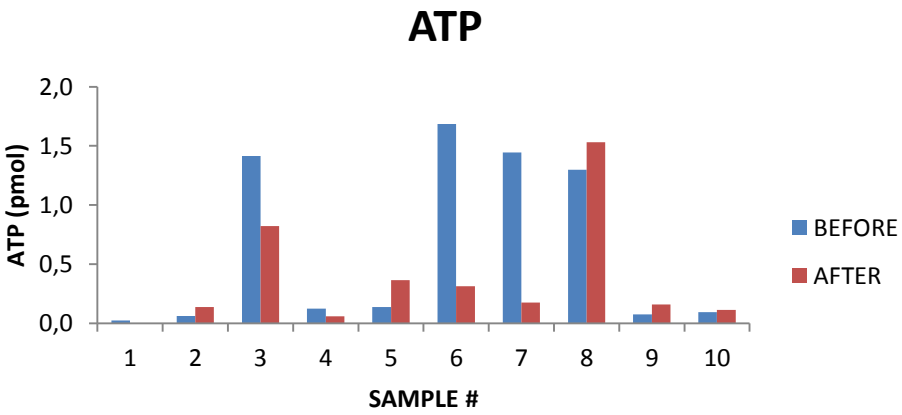
Cleaning efficacy at 10 sampling sites in a hospital

1. Toastöd
2. Lock soptunna
3. Spolknapp
4. Larmdosa
5. Toalock, framkant
6. Kran
7. Handfat, kant
8. Dörrhandtag
9. Väggfast duschstöd
10. Duschstol





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Summary

- ATP is sometimes, but not in general, a measure of living cells.
- ATP+ADP+AMP assays are a measure of biological contamination just like protein but is much more sensitive.
- ATP based assays can be performed at the sampling site with a portable instrument.
- ATP and ATP+ADP+AMP can be performed with single shot devices both on surfaces and in liquids.
- Which one to choose in a hospital environment remains to be shown in the Vinnova project “Clean care”.

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