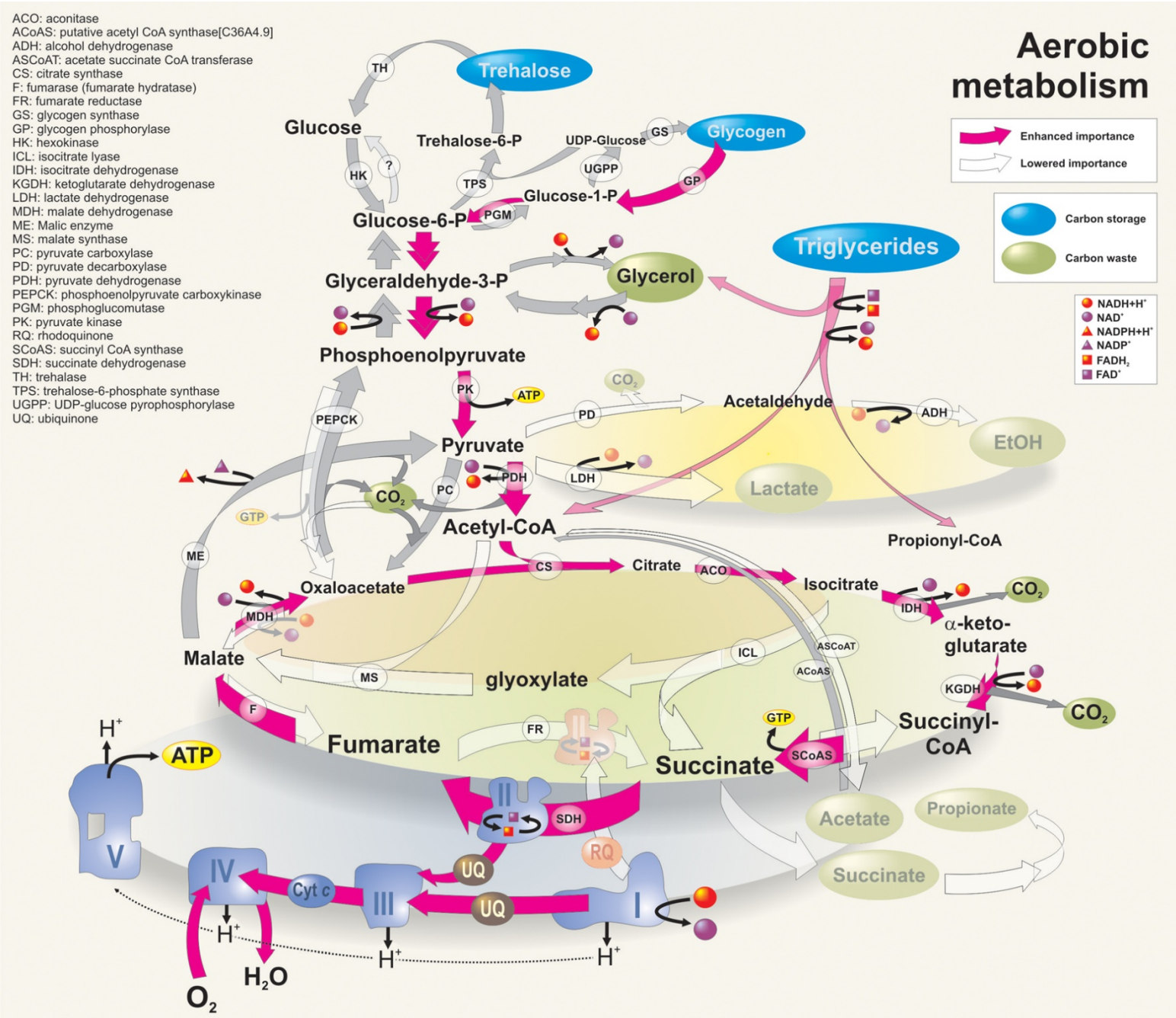
Assessing environmental contaminations in elderly homes with an ATP-based monitoring system: A pilot study

Our track

- Introduce the biomolecules: ATP, luciferase and luciferin
- The coupling reactions between these molecules
- Exploitation of these reactions in hygiene monitoring
- Introduce the working method of one of the commercial system

- Preliminary data for the usage of the system in monitoring hygiene status in elderly homes


ACO: aconitase
ACoAS: putative acetyl CoA synthase[C36A4.9]
ADH: alcohol dehydrogenase
ASCoAT: acetate succinate CoA transferase
CS: citrate synthase
F: fumarase (fumarate hydratase)
FR: fumarate reductase
GS: glycogen synthase
GP: glycogen phosphorylase
HK: hexokinase
ICL: isocitrate lyase
IDH: isocitrate dehydrogenase
KGDH: ketoglutarate dehydrogenase
LDH: lactate dehydrogenase
MDH: malate dehydrogenase
ME: Malic enzyme
MS: malate synthase
PC: pyruvate carboxylase
PD: pyruvate decarboxylase
PDH: pyruvate dehydrogenase
PEPCK: phosphoenolpyruvate carboxykinase
PGM: phosphoglucomutase
PK: pyruvate kinase
RQ: rhodoquinone
SCoAS: succinyl CoA synthase
SDH: succinate dehydrogenase
TH: trehalase
TPS: trehalose-6-phosphate synthase
UGPP: UDP-glucose pyrophosphorylase
UQ: ubiquinone
Aerobic metabolism
Enhanced importance
Lowered importance
Carbon storage
Carbon waste
NADH+H+
NAD+
NADPH+H+
NADP+
FADH2
FAD+
Trehalose
Glucose
Trehalose-6-P
UDP-Glucose
Glycogen
Glucose-1-P
Glucose-6-P
Glyceraldehyde-3-P
Glycerol
Triglycerides
Phosphoenolpyruvate
Pyruvate
Acetaldehyde
EtOH
Lactate
Acetyl-CoA
Propionyl-CoA
Citrate
Isocitrate
α-keto-glutarate
Oxaloacetate
Malate
glyoxylate
Fumarate
Succinate
Succinyl-CoA
Acetate
Propionate
Succinate
ATP
H2O
O2
CO2
TH
HK
TPS
PGM
GS
UGPP
GP
PK
PEPCK
PD
ADH
LDH
PDH
PC
ME
CS
ACO
IDH
MDH
ICL
ASCoAT
ACoAS
MS
F
FR
SCoAS
KGDH
SDH
RQ
UQ
Cyt c
I
II
III
IV
V

Energy cycle or metabolism

- The chemical reactions of a living organism can be divided into two main types
  1. *The chemical reactions by which the large molecules are constantly broken down into smaller ones* are called **catabolism**.
  2. *he chemical reactions by which the macromolecules are synthesised within the cell* are called **anabolism**.

- These two processes *i.e.*, degradation and synthesis are collectively called **metabolism**.
- Catabolism reactions are usually accompanied by *release of energy* (e.g. ATP >> ADP/AMP)

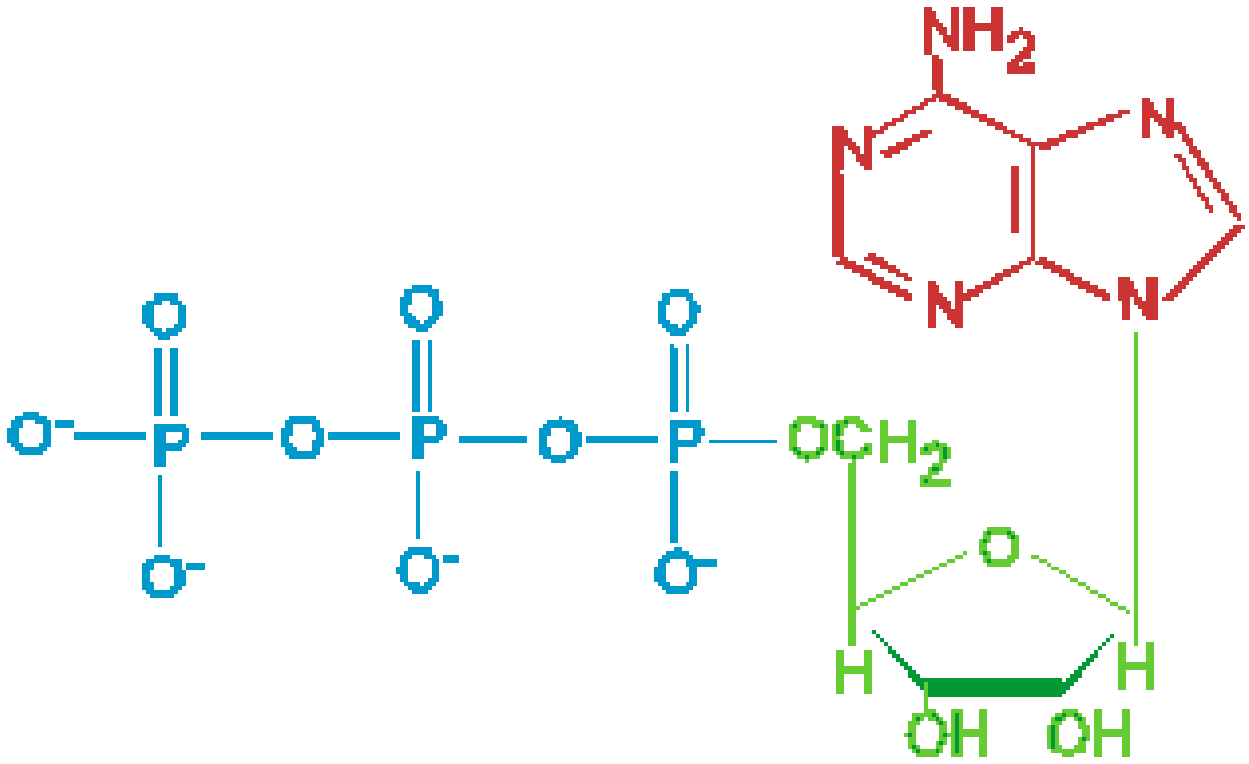
- The primary energy found in living cells is chemical energy
- Can be easily stored, transferred and transformed.

Adenosine triphosphate (ATP)

- Universal energy molecule found in all animal, plant, bacteria, yeast, mold and organic matters.

- ATP is regarded as **energy currency** of living cells because it can trap, store and release small packets of energy with ease.

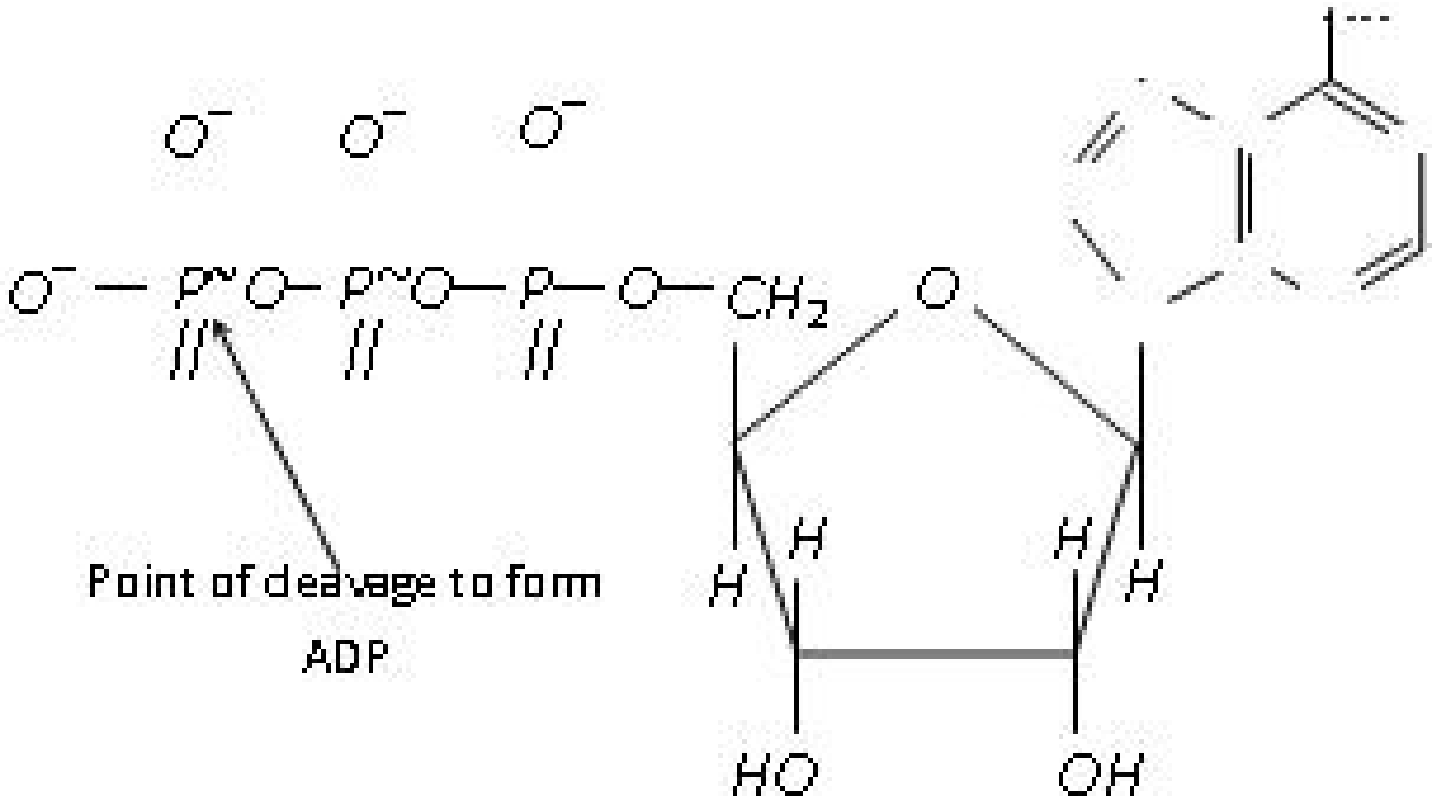
Adenosine triphosphate (ATP)



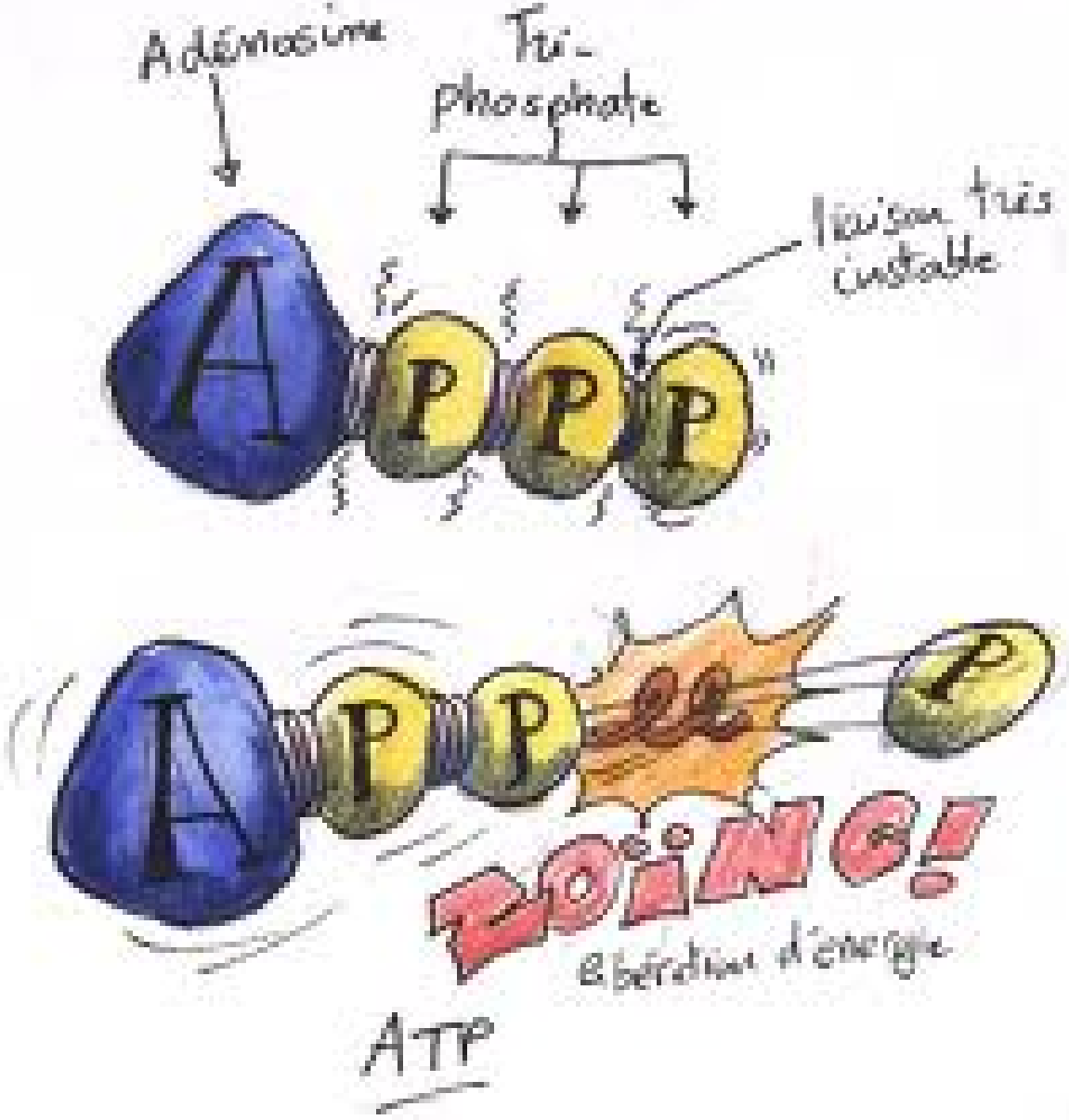
- The energy stored within the molecule is contained in the phosphate bonds.

Structure

- ATP consists of a purine base called **adenine** linked to a five carbon sugar named **ribose** which is further attached to **three molecules of phosphate.**


Point of cleavage to form
ADP

- ATP is energy rich molecule this is because of the presence of four negatively charged oxygen atom very close to each other.
- These four negatively charged *o*-atoms experience very high repulsive energy


Adénosine
Tri-
phosphate
liaison très
instable
A
P
P
P
A
P
P
P
BOING!
libération d'énergie
ATP

Luciferase

- Identified in firefly *Photinus pyralis* (1998)
- The most thoroughly studied luciferase is that of the Photinini firefly (*Photinus pyralis)*, which has optimal working pH at 7.8

- Steghens JP, Min KL, Bernengo JC (November 1998). Firefly luciferase has two nucleotide binding sites: effect of nucleoside monophosphat and CoA on the light-emission spectra. *Biochem. J.* **336 (Pt 1)**: 109–13..

- In luminescent reactions, light is produced by the oxidation of a luciferin (a pigment):

$$\text{luciferin} + O_2 \rightarrow \text{oxyluciferin} + \text{light}$$

- The rates of this reaction between luciferin and oxygen are extremely slow until they are catalyzed by luciferase
- Reactions mediated by the presence of cofactors such as calcium ions or ATP

- The reaction catalyzed by firefly luciferase takes place in two steps:

1. luciferin + ATP $\rightarrow$ luciferyl adenylate + PP_i
2. luciferyl adenylate + O_2 $\rightarrow$ oxyluciferin + AMP + light

Metabolism of ATP

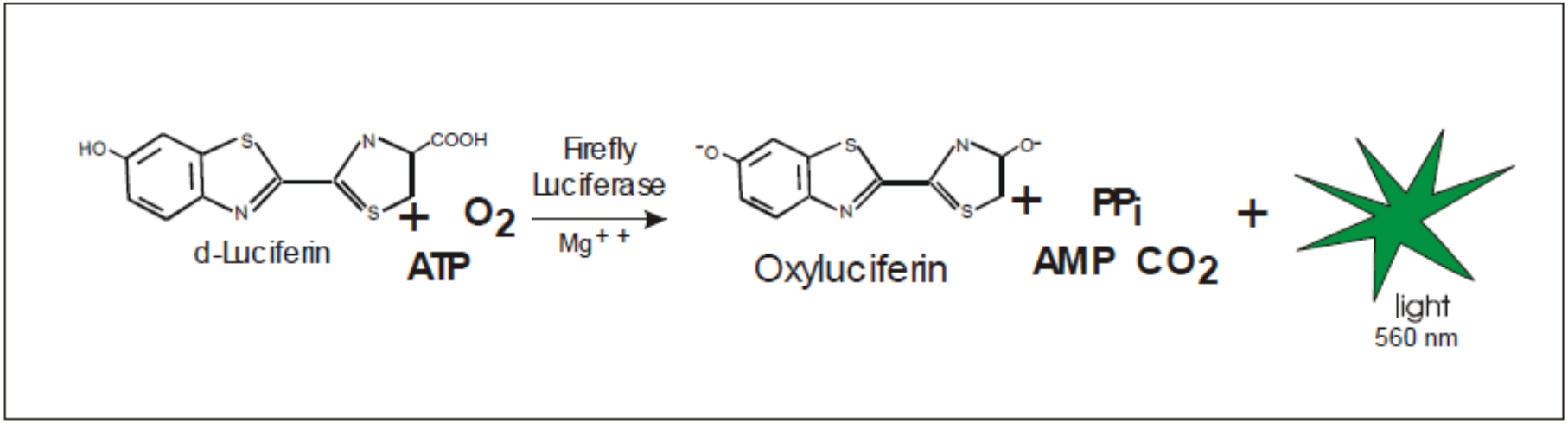


Figure 2. Bioluminescent Reactions Catalyzed by Firefly Luciferase. Firefly luciferase, using ATP, catalyses the two-step oxidation of luciferin to oxyluciferin, which yields light at 560 nm. In this reaction, ATP is hydrolyzed to AMP and pyrophosphate.

Luciferase assay

- Luciferase substrate D-luciferin
- Accepted by the enzyme as a substrate, capable of generating bioluminescence in the presence of oxygen, ATP and Mg^{2+}.

- In the assay, the presence of ATP could be detected by this D-luciferin metabolism (oxidization via Firefly luciferase activity with emission of light)
- The intensity should be proportional to the ATP concentration

Linearity of Luminescent Response to ATP

- Luminescent Determination of ATP Concentrations Using the Clarity™ Luminescence Microplate Reader
- 22nd June 2004

- Serial dilutions of ATP were made using ATP-free water as the diluent.
- The dilutions were then assayed using an ENLITEN kit (Promega, Madison, WI) and the subsequent luminescence plotted against ATP concentration.

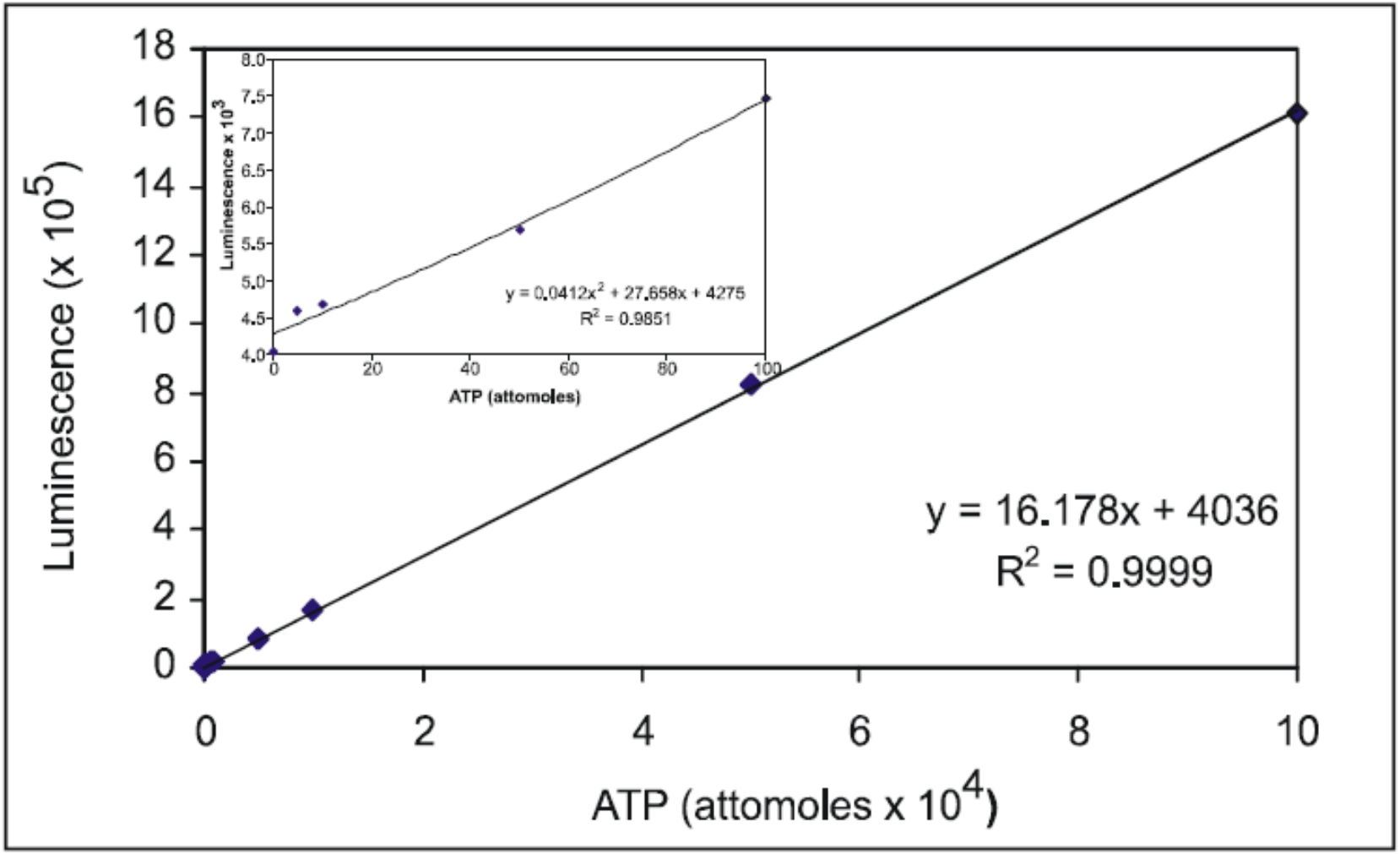


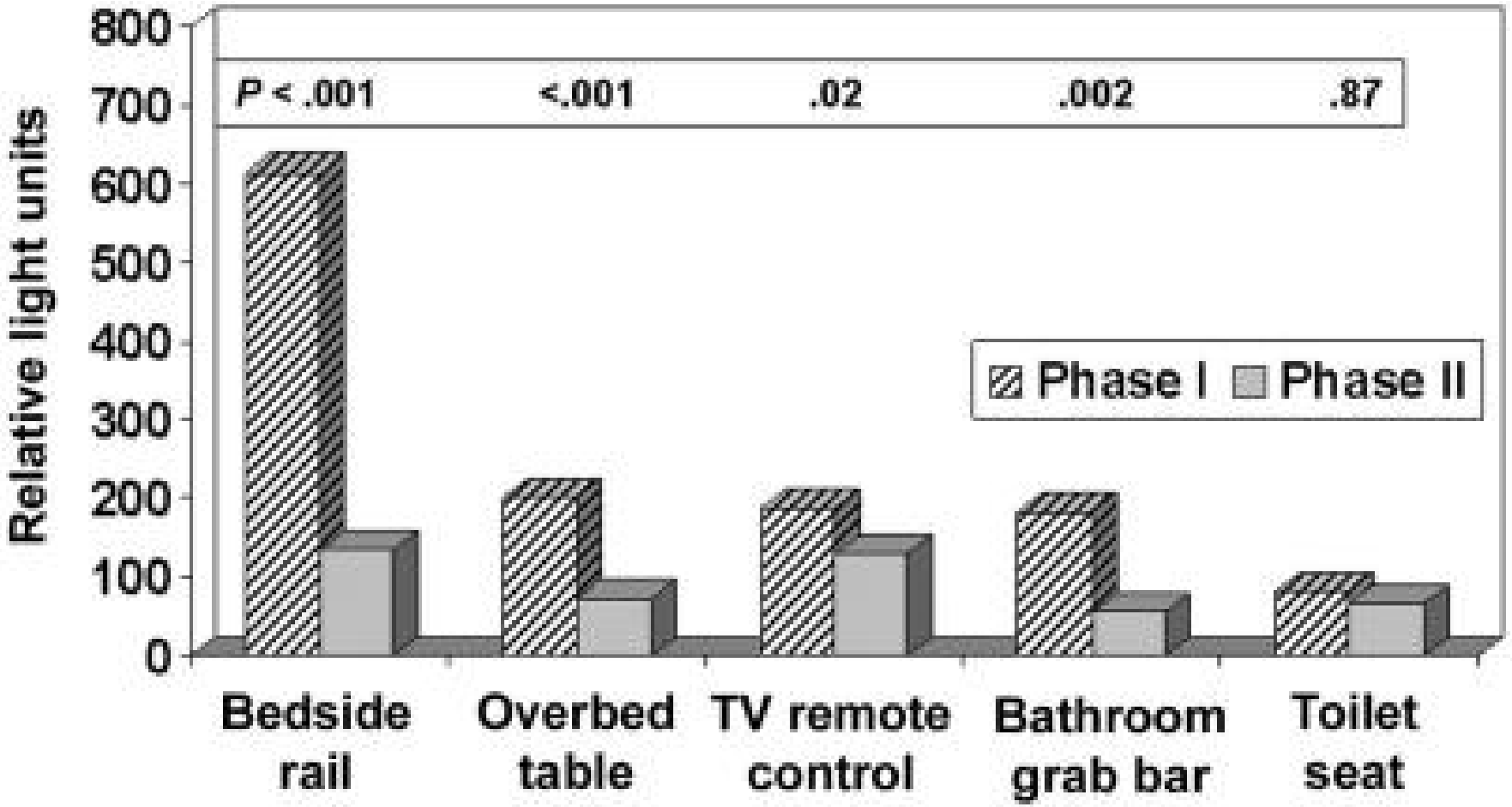
Figure 3. Linearity of Luminescent Response to ATP. Serial dilutions of ATP were made using ATP-free water as the diluent. The dilutions were then assayed using an ENLITEN kit (Promega, Madison, WI) and the subsequent luminescence plotted against ATP concentration. The inserted graph depicts the signal generated at low concentrations of ATP.

Commercial ATP-based monitoring systems

- Commercial handheld monitoring systems have been developed for detecting the light emission resulted from luciferase-mediated metabolism of ATP.
- The small amount of light produced would be converted to **Relative Light Units (RLU).**
- The value is proportional to ATP and in turn the organic materials or microbial number.

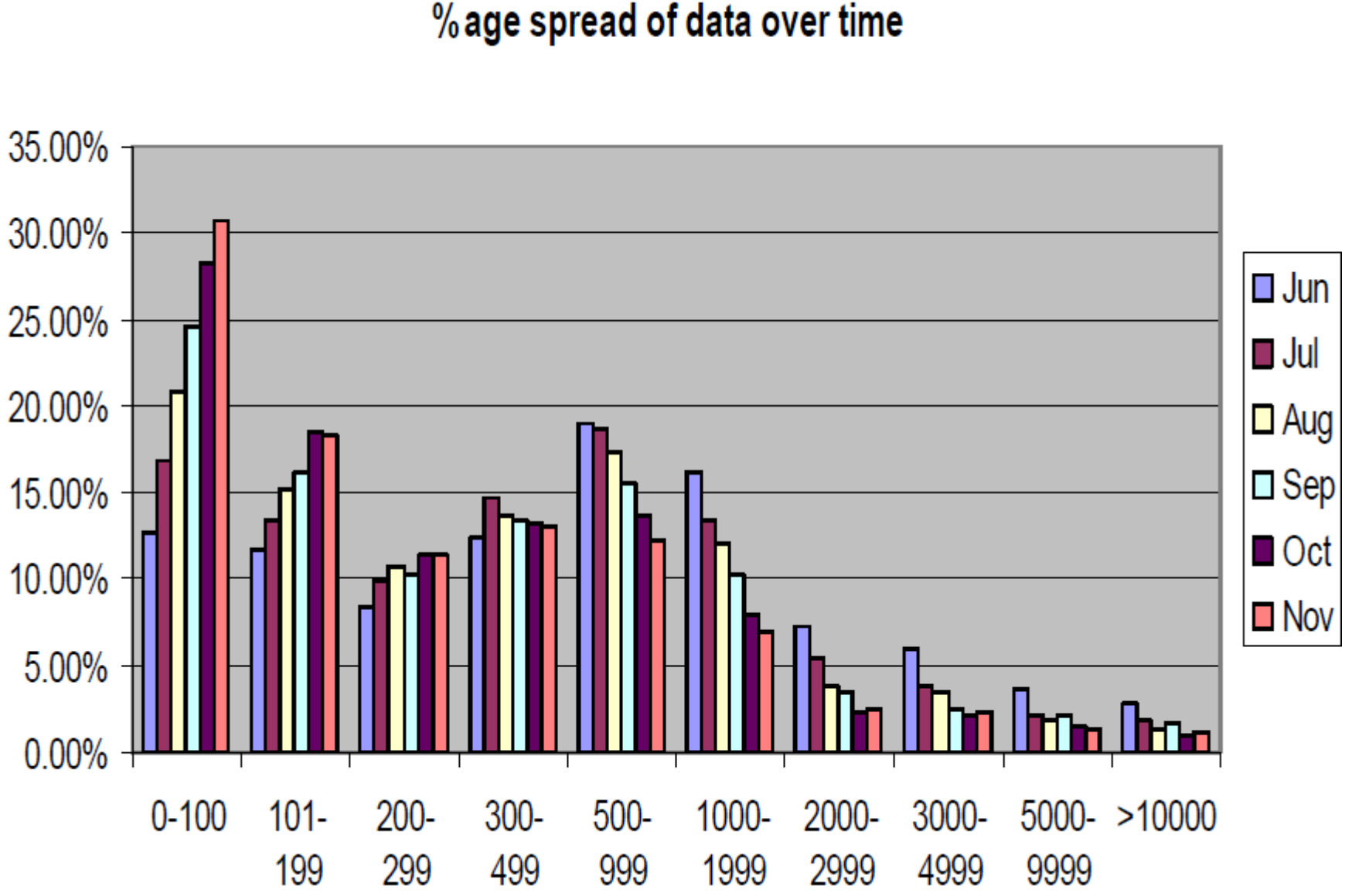
- The system allows one to indirectly estimate the amount of ATP, even from different kinds of organism (e.g. bacteria, mold, etc.)
- Assessing hygiene status of the target surfaces.

- Instant monitoring systems have been well adopted in food industry for on-site examination of bacterial contamination on different surfaces.
- Published reports on its application in health care settings and for infection control practice are increasing



Monitoring the Effectiveness of Hospital Cleaning Practices by Use of an Adenosine Triphosphate Bioluminescence Assay, *Infection Control and Hospital Epidemiology*, Vol. 30, No. 7 (July 2009), pp. 678-684

- NHS in UK
- The HCAI Technology Innovation Programme launched in January 2008, following “Clean, Safe Care – Reducing Infection and Saving Lives” (DoH, 2008).
- details available on www.clean-safe-care.nhs.uk


%age spread of data over time
35.00%
30.00%
25.00%
20.00%
15.00%
10.00%
5.00%
0.00%
0-100
101-199
200-299
300-499
500-999
1000-1999
2000-2999
3000-4999
5000-9999
>10000
Jun
Jul
Aug
Sep
Oct
Nov

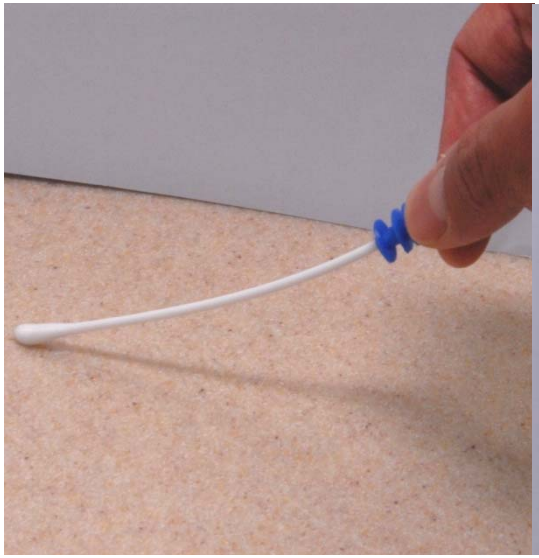
Local pilot study

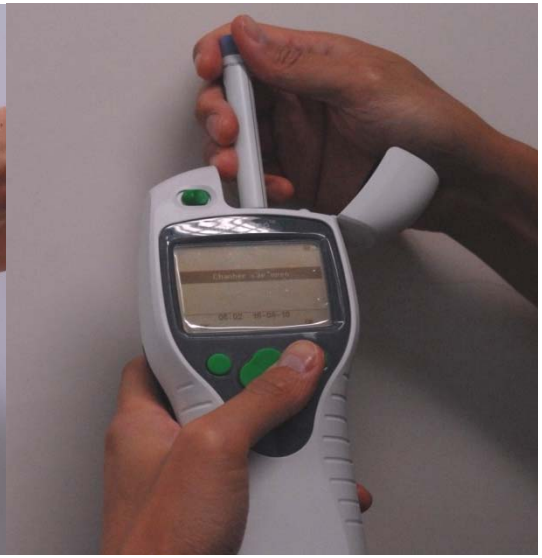
- One of the branded ATP monitoring systems was adopted for detecting bacterial contamination on different environments in elderly homes

Working processes

- Different high touch areas in elderly homes were sampled with swabs.
- The RLU would be indicated on the machine panel after measurement.

Procedures for environmental sampling





Optimal working conditions

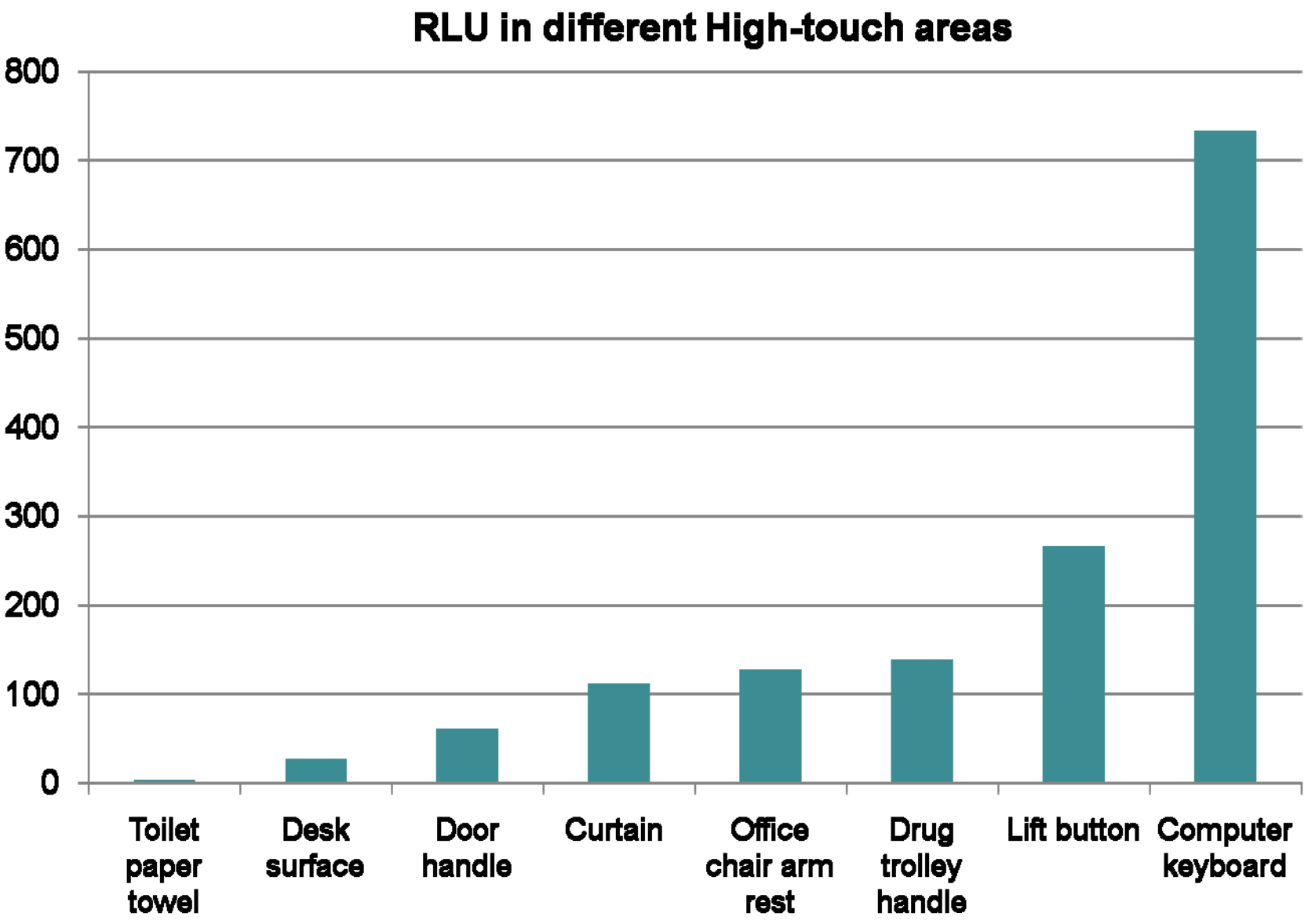
- All swabs should either be stored at:
  - 2 - 8 ◦C
  - 21 ◦C for 28 days at most

Manufacturer suggestions

- Put the swabs at room temperature for at least 10 minutes before use
- Swabbing target site (environmental surfaces)
- Activate within 4 hours after sampling
  1. Immerse the swab stick in a small vial containing reagents
  2. Hold vertically and shake for 5 seconds
- Measure RLU immediately after activation

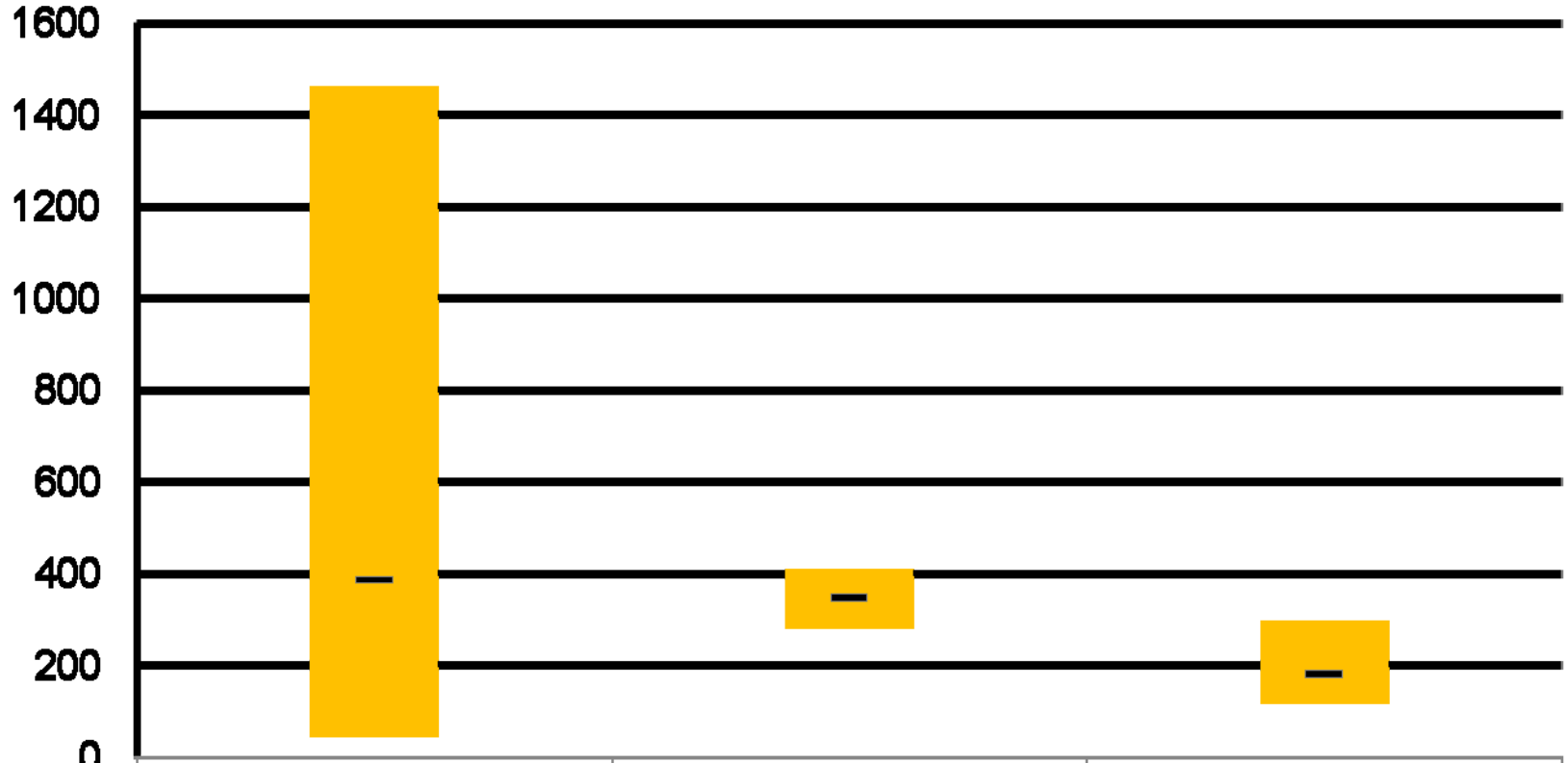
Results

Level of contamination (RLU) in some of the high-touch areas


RLU in different High-touch areas
800
700
600
500
400
300
200
100
0
Toilet paper towel
Desk surface
Door handle
Curtain
Office chair arm rest
Drug trolley handle
Lift button
Computer keyboard

Contamination of handrails and armrests

Contamination of handrails and armrests in RCHE



	Corridor handrail	Bed handrail	Wheelchair armrest
Maximum	1460	408	297
Minimum	47	283	118
– Average	385	346	179

Discussions

Our findings suggested that:

- different high-touched areas (including handrails and wheelchair armrests) in elderly homes were contaminated.

Conclusions

- The ATP monitoring system being tested provide instant monitoring tools on site
- System may serve as a supplementary tool for assessing environmental contamination level in different environments
- Observe the suggested working condition while using the system