Quantifying Sanitation

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ميفوسا ش.م.م
مستشارو السلامة الغذائية للشرق الأوسط وشمال افريقيا
MEFOSA S.A.R.L
MIDDLE EAST NORTH AFRICA FOOD SAFETY ASSOCIATES

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   b) How do we control bacterial growth
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II. Importance of Quantifying sanitation
   a) Internationally
      - Importance and usage of Culture independent diagnostic tests comparative study from 2013 to 2016 in the health care industry
   b) Nationally
      - Use of rapid test technologies in SME's for early detection in Lebanon

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I. Introduction:

Back to basics

a) What is bacterial life cycle

b) How do we control bacterial growth

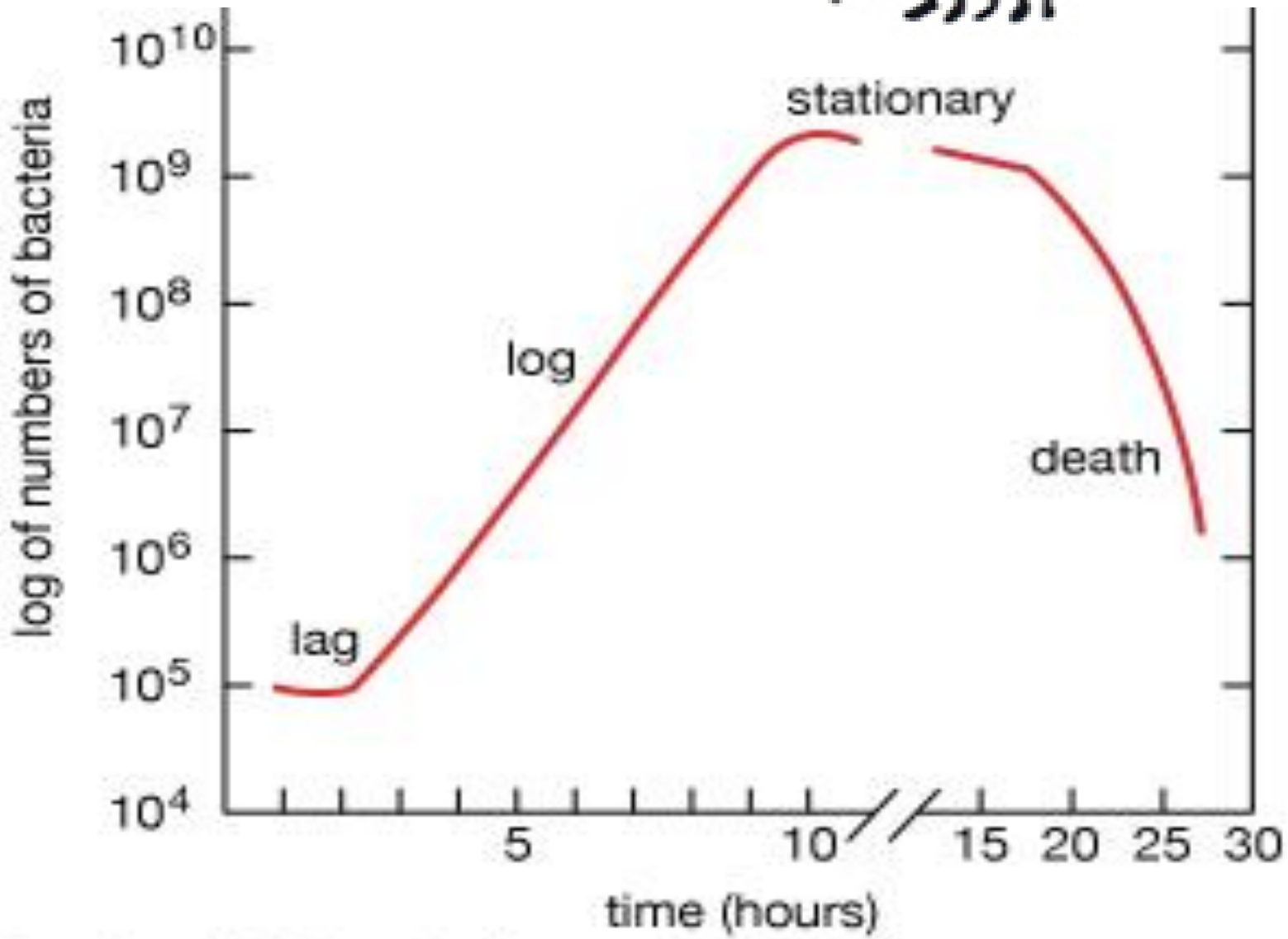
c) Vegetative vs Spore forming bacteria

C.1) ATP AND AMP definition

d) How to measure bacterial growth

- d.1) RLU vs ATP
- d.1.1) ATP vs ATP + AMP
- d.2) ATP + AMP vs Protein detection
- d.3) ATP vs CFU

a) What is bacterial life cycle



b) How do we control bacterial growth

F = Food

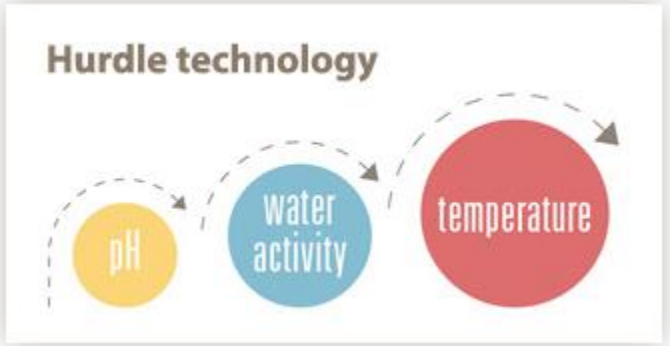
A = Acidity

T = Time

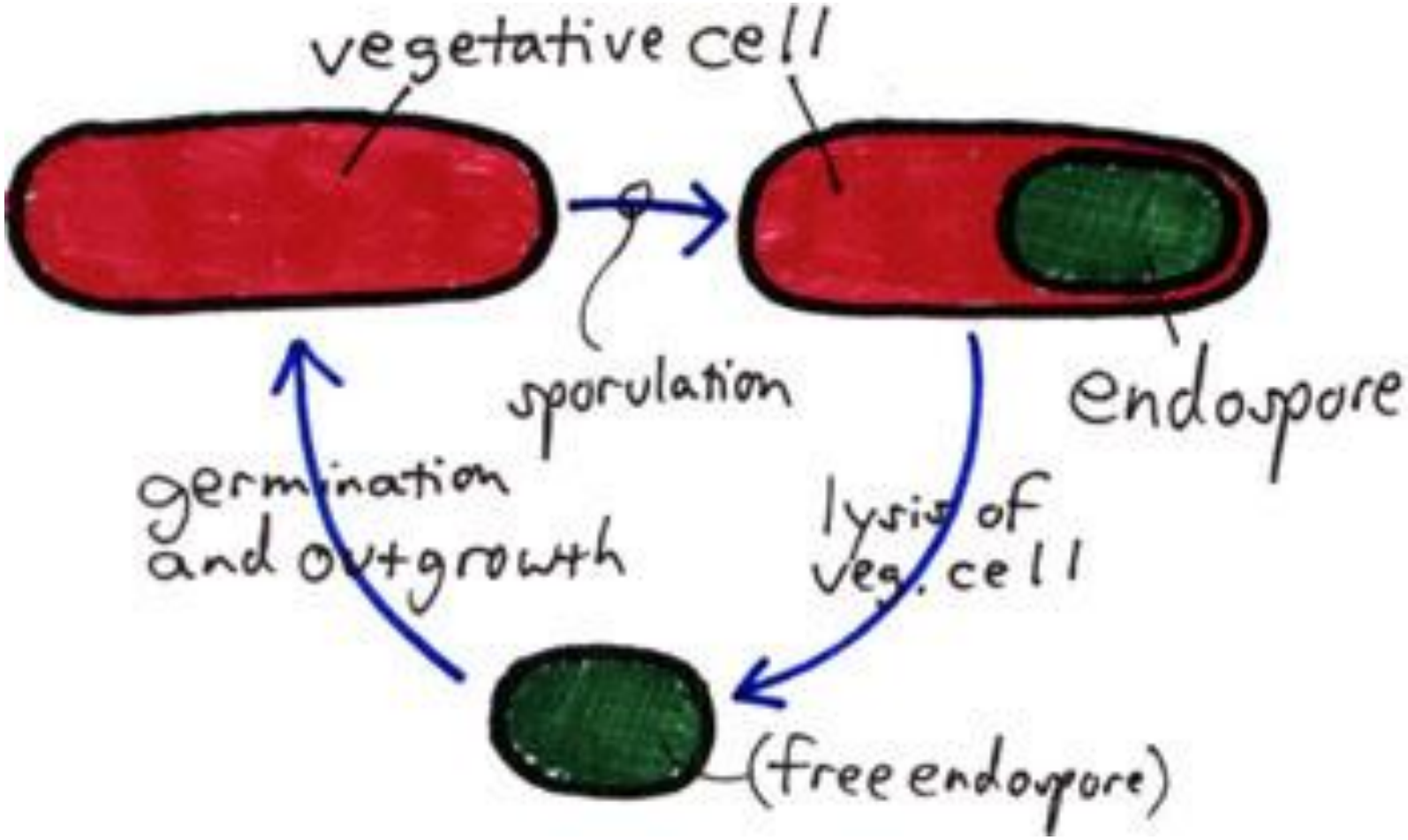
T = Temperature

O = Oxygen

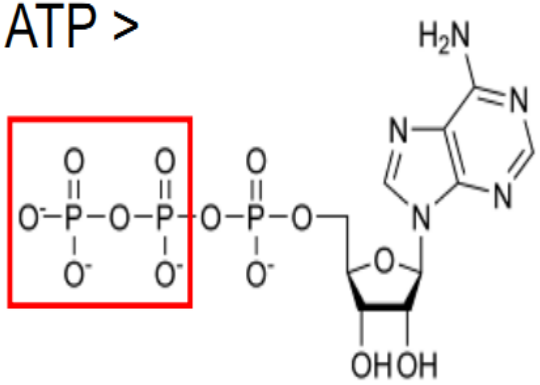
M = Moisture

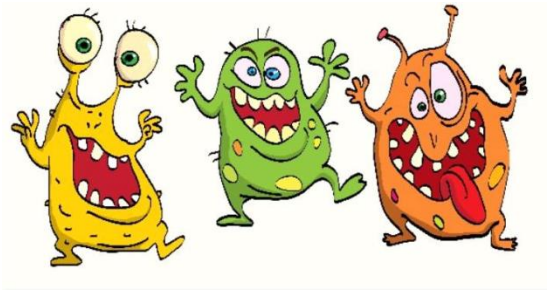


c) Spore Forming vs Vegetative Bacteria



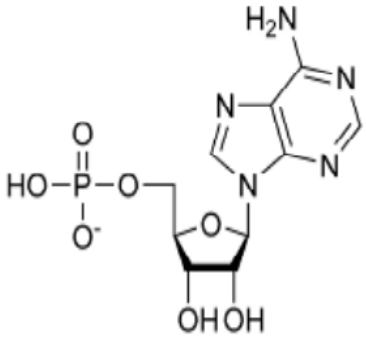
< ATP >





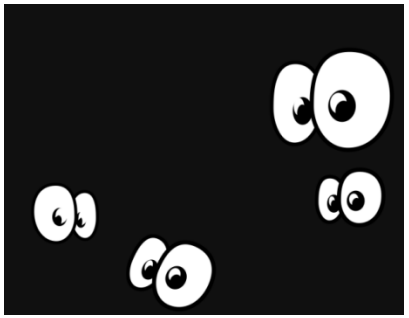
ATP is essential for all organisms to live

< AMP >

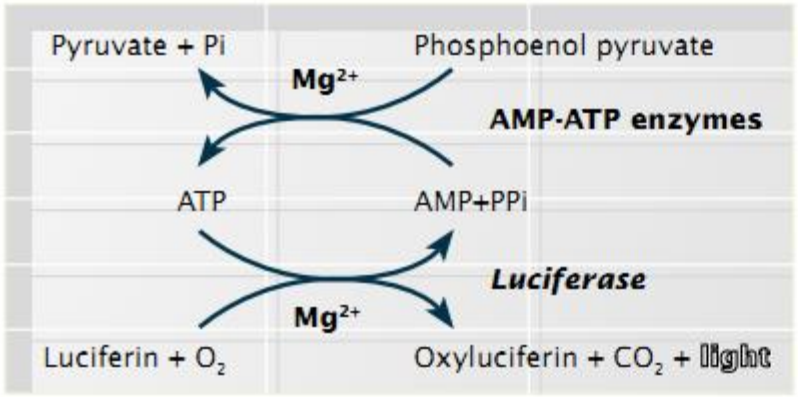


AMP exists in organisms universally too.

ATP degrades to AMP during heat Treatment, fermentation and so on.



Bioluminescent reaction

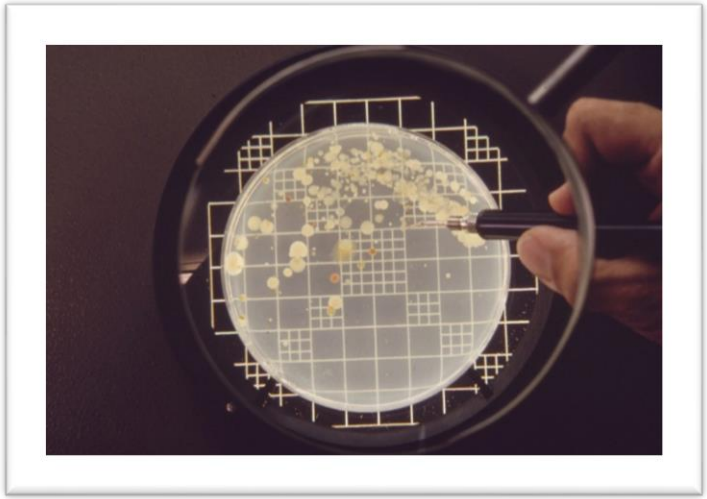


Patented AMP-ATP cycle technology

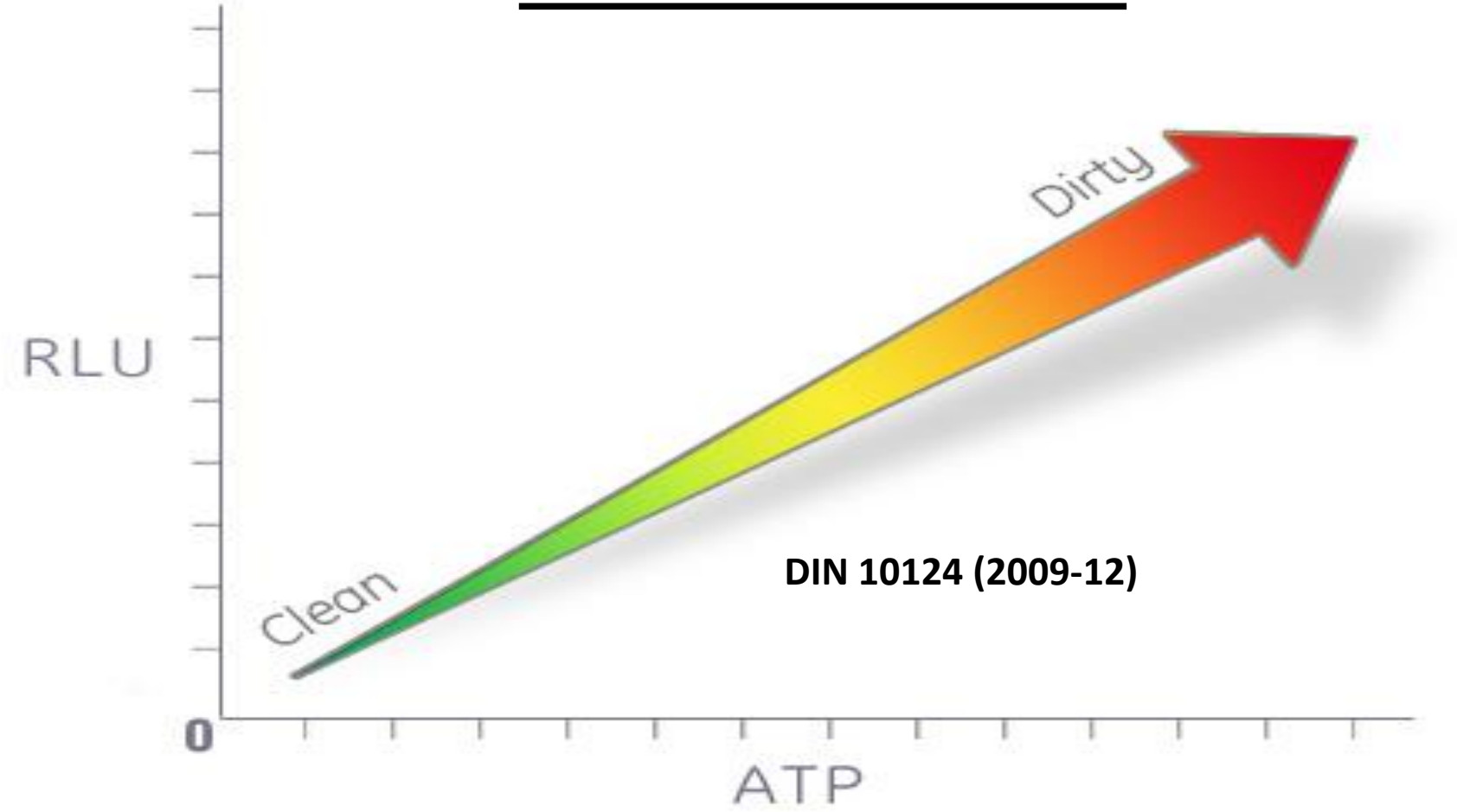
Lumitester PD-30 lets you measure ATP and AMP as part of the ATP cycle. This offers users increased security.

d) What equipment do you need to measure bacteria levels in food?

CFU vs RLU vs Protein detection



D1. RLU vs ATP



DIN 10124 (2009-12)

http://www.hyserve.com/files/Flyer_PD30-English.pdf

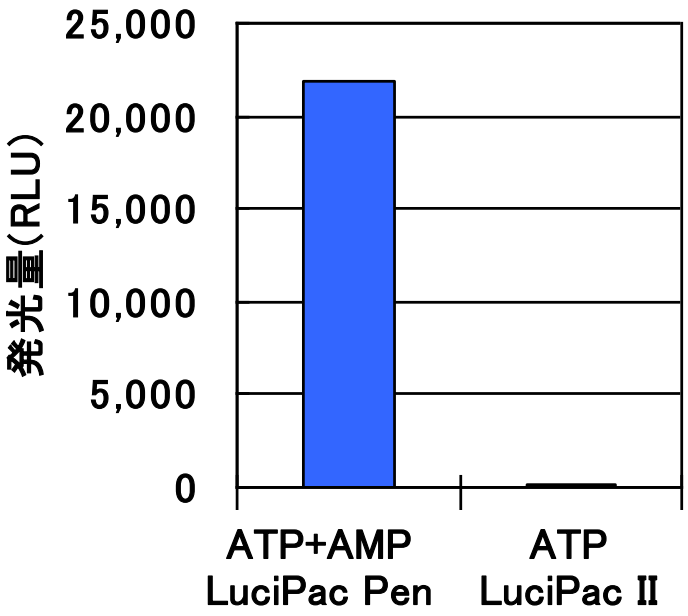
d.2 ATP + AMP detection

- Insufficient sensitivity of ATP detection -

Method: Stamped meat on a cutting board and swabbed its surface.

Poultry

Luminescence

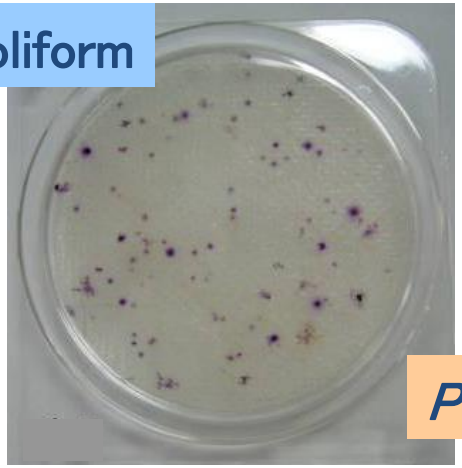


Measuring substances	ATP+AMP	ATP
Luminescence (RLU)	13,696	100

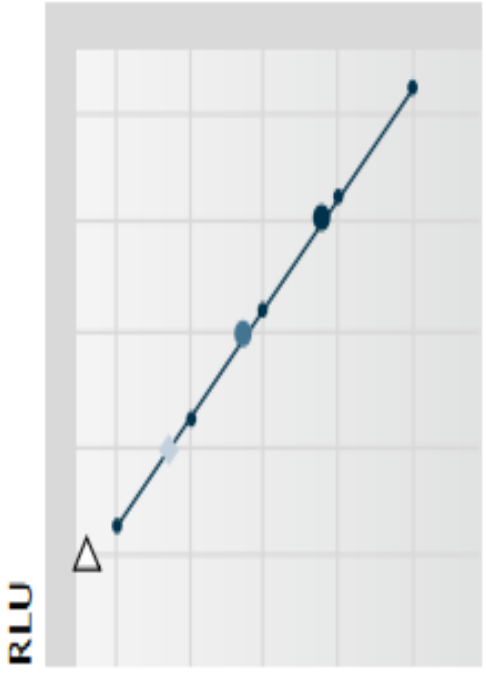
Aerobic plate counts

5,100 CFU/swab

Coliform

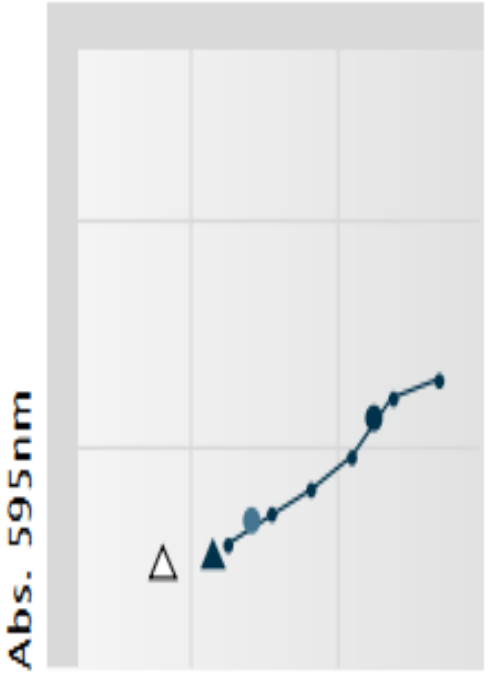


Positive



ATP + AMP (Mol)

- Standard ATP curve
- x 10,000 dilutions
- x 100,000 dilutions
- Sample
- △ Detection limit (blank value + 3SD)



Protein conc. (µg/mL)

- Standard ATP curve
- x 10,000 dilutions
- x 100,000 dilutions
- ▲ Detection limit (theoretical)
- △ Detection limit (blank value + 3SD)

d.3 **ATP-AMP detection v. protein detection**

The detection of ATP and AMP is much more sensitive compared to protein detection using staining. ATP-AMP measurement is characterised by outstanding linearity over the entire measurement range.

http://www.hyserve.com/files/Flyer_PD30-English.pdf

d.4 ATP vs CFU

Table 1. Proportionate samples according to four chosen relative light unit (RLU) values for each microbial growth category

Microbial growth category	Total number of samples	ATP values within each microbial growth category that exceeded the ATP values			
		1,000 RLU	5,000 RLU	10,000 RLU	50,000 RLU
NG	21	17(81%)	4(19%)	1(5%)	0(0%)
SG(<2.5 cfu/cm²)	52	51(98%)	32(62%)	32(62%)	4(8%)
LG(2.5–12 cfu/cm²)	25	25(100%)	17(68%)	11(44%)	4(16%)
MG(12–40 cfu/cm²)	7	7(100%)	7(100%)	4(57%)	1(14%)
HG(>40 cfu/cm²)	1	1(100%)	1(100%)	0(0%)	0(0%)

All microbial growth values are presented as aerobic colony counts per cm². NG, no growth; SG, scanty growth; LG, light growth; MG, moderate growth; HG, heavy growth.

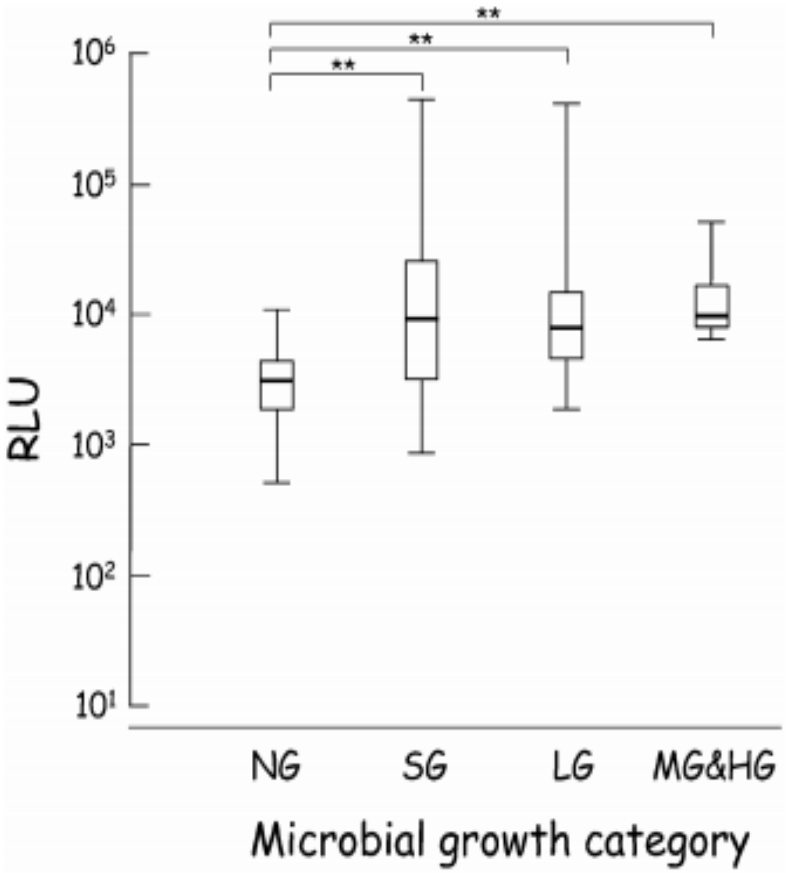


Figure 4. ATP values (expressed as relative light units: RLU) for microbial growth categories

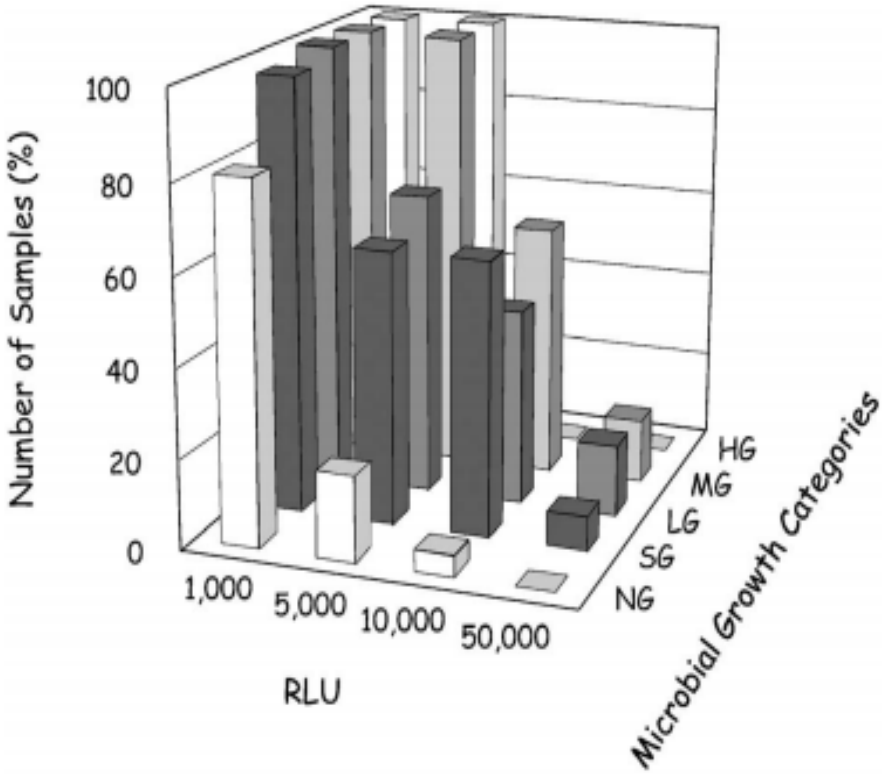


Figure 5. Percentage of positive samples for four ATP levels according to microbial growth categories

RLU, relative light units; NG, no growth; SG, scanty growth; LG, light growth; MG, moderate

https://www.jstage.jst.go.jp/article/jsei/29/6/29_14-004/_article

https://www.beuth.de/en/standard/din-10124/120047679

Specificity and Sensitivity

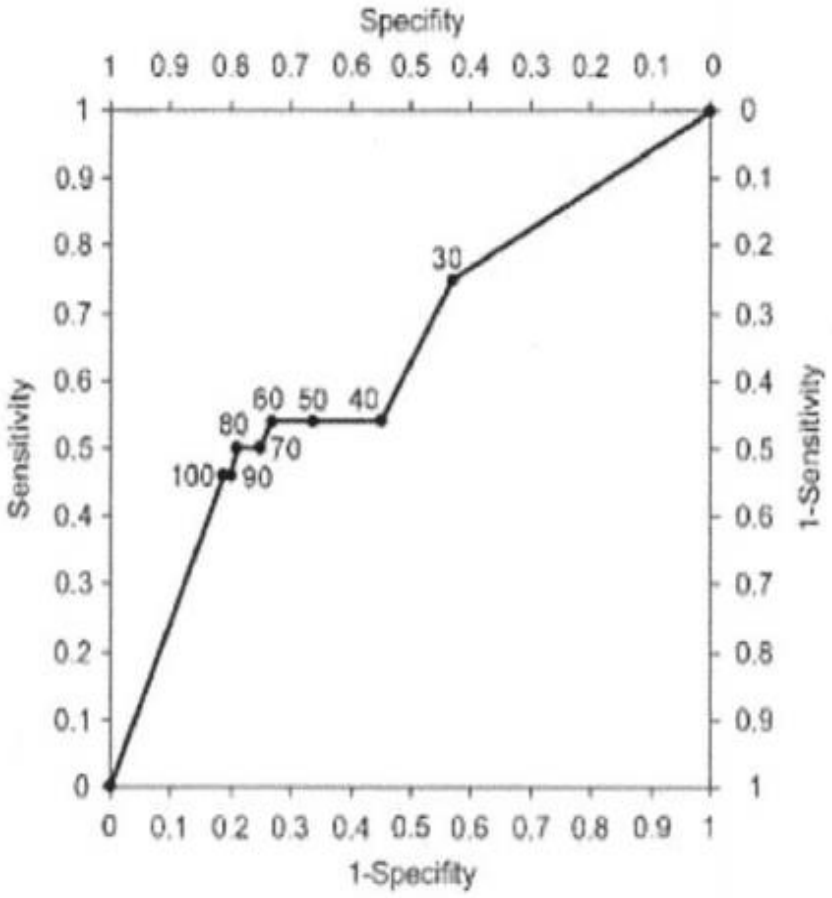


Figure 1: ROC curve of bioluminescence compared with microbiological culture as gold standard for thresholds between 30 and 100 RLU

II. Importance of Quantifying sanitation

a) Internationally
   - Importance and usage of Culture independent diagnostic tests comparative study from 2013 to 2016 in the health care industry

b) Nationally
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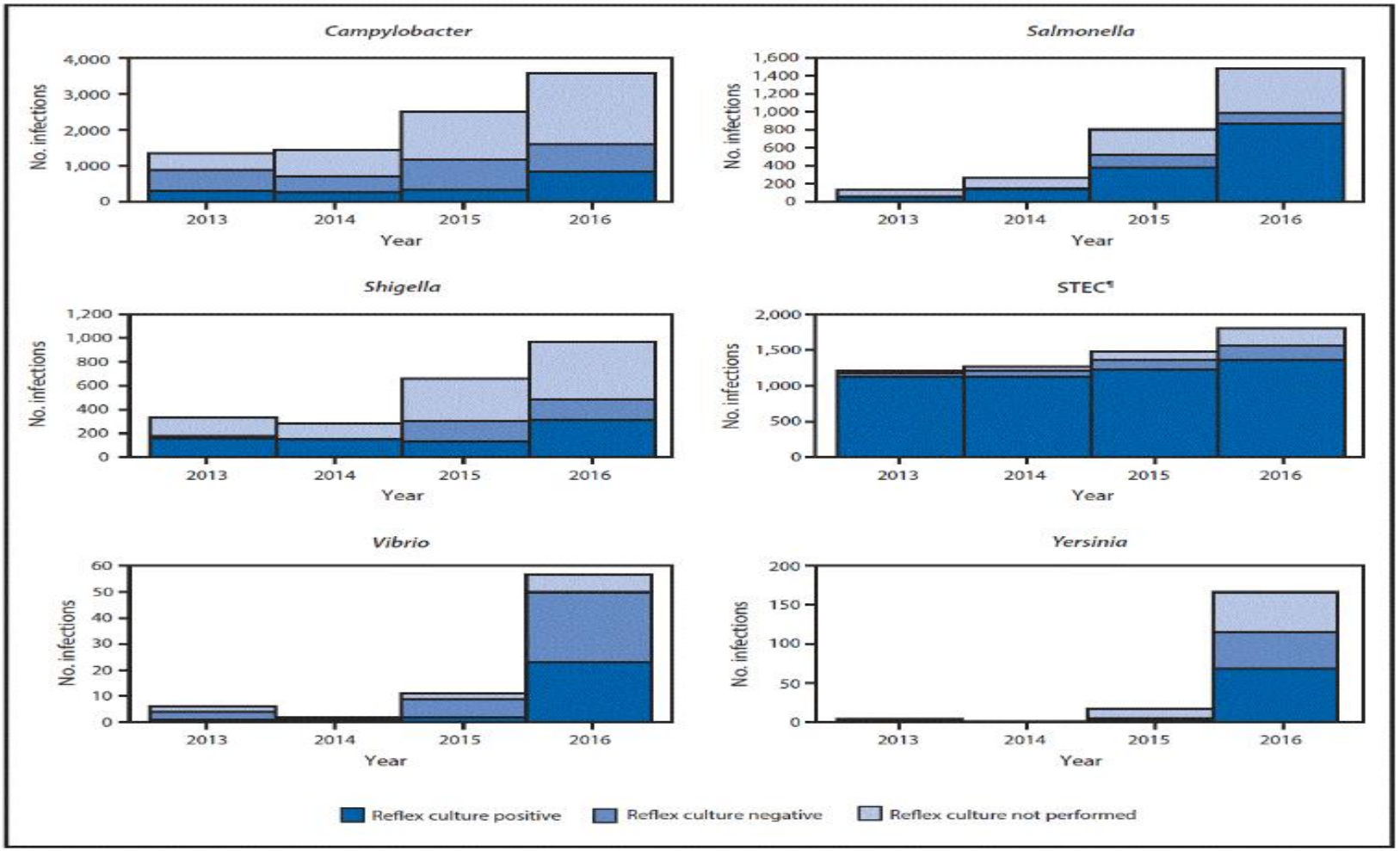
CDC Comparative study :

Incidence and Trends of Infections **with** Pathogens Transmitted Commonly Through Food **and** the Effect of Increasing Use of Culture-Independent Diagnostic Tests on Surveillance



https://www.cdc.gov/mmwr/volumes/66/wr/mm6615a1.htm

TABLE 2. Percentage change in incidence of confirmed and CIDT positive-only* bacterial and confirmed parasitic infections in 2016† compared with 2013–2015 average annual incidence, by pathogen – FoodNet, 10 U.S. sites,§ 2013–2016

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	Confirmed			Confirmed or CIDT positive-only		
Pathogen	2016 IR¶	% Change**	95% CI	2016 IR¶	% Change**	95% CI
Campylobacter	11.79	-11	-18 to -3	17.43	+3	-4 to +10
Listeria††	0.26	+4	-18 to +30	–§§	–§§	–§§
Salmonella	15.40	+2	-4 to +8	16.66	+6	-1 to +12
Shigella	4.60	+7	-17 to +38	5.94	+25	-3 to +62
STEC¶¶	2.84	+21	+3 to +42	3.76	+43	+22 to +67
Vibrio	0.45	+2	-18 to +26	0.51	+16	-6 to +42
Yersinia	0.42	+29	+2 to +64	0.62	+91	+52 to +140
Cryptosporidium	3.70	+45	+11 to +89	–§§	–§§	–§§

https://www.cdc.gov/mmwr/volumes/66/wr/mm6615a1.htm

- Nationally
  - Use of rapid test technologies in SME’s for early detection in Lebanon

III. Recommendations

- Use of CIDT is important for rapid and early detection
- ATP+ AMP detection is more superior that ATP alone in detecting spore forming bacteria
- ATP and AMP detection cannot substitute microbial count but it is mandatory for early detection

IV. Acknowledgment

We would like to thank IAFP – PDG Sanitation for giving us the opportunity to discuss this matter with you, especialy Mr. Lari Yale, Ms. Dina Siedenburg and Ms. Nadia Narine

We would like also to thank Dr.Stephan Speidel from Hyserve for his continuous help and support with the materials and

We would like to thank Food scientists believers and those who believe that small actions can make a change.

V. Other references

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3. Rapid detection of staphylococcus aureus using bioluminescent enzyme immunoassay.
Fukuda S, Tatsumi H, Igarashi H, Igimi S
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