NEMIS Special Webinar 27. Nov. 2024

Sampling of large surfaces with the N-Light^{TM} MaxiSampler

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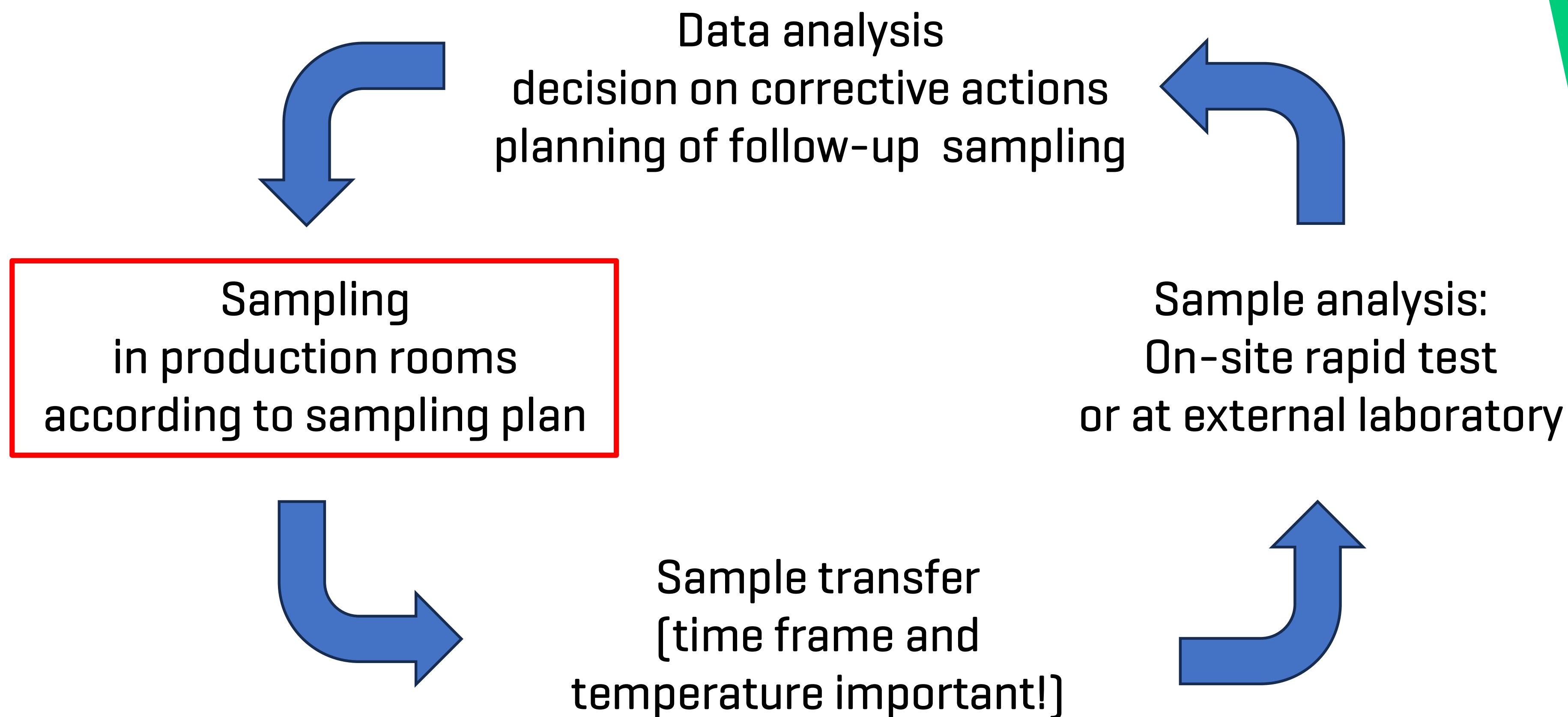
Sampling: A crucial part of environmental monitoring in food factories



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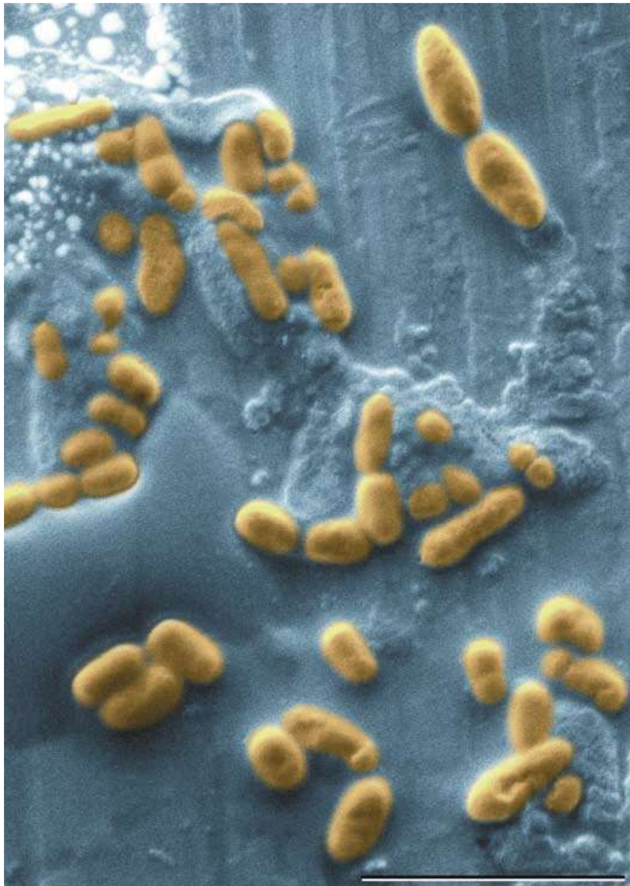
Microorganisms on surfaces: an incredibly complex topic

- Strength of adhesion influenced by properties of surface (material, morphology) and cells (outer polysaccharides, protein fimbriae)
- Drying will increase adhesion
- Cells can be in resting state or injured (hard to recover)
- Any additional matrix (food residues) will affect survival rate and strength of adhesion
- Microorganisms can form their own «sticky matrix» for highest persistence and protection = biofilm



Bacteria on stainless steel, scanning electron micrograph of "negative imprint" .
Kalab, M. *et al.* (2008). *infocus Magazine* 10.
DOI: 10.22443/rms.inf.1.33

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Why is the choice of the sampling device (and the moistening solution) important?

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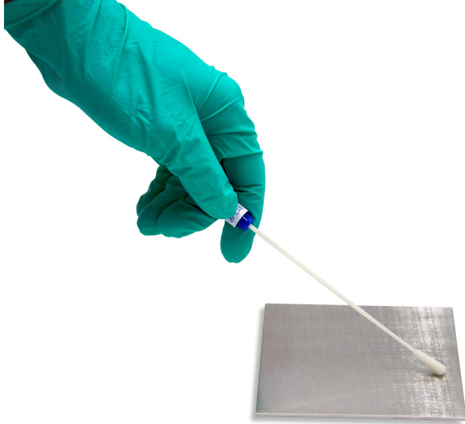
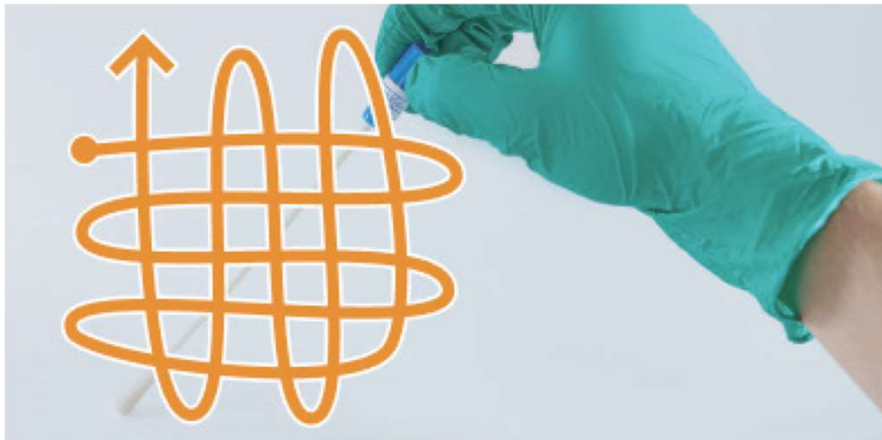
- Force that can be applied for removal of attached cells
- Possibility for sampling of hard-to-reach places (edges, grooves, valves, tubes, crevices, holes etc.)



- Surface area covered by device
- Effectiveness of and capacity for pick-up of cells
- Risk of cross-contamination during sampling
- Survival and/or growth of microorganisms on device during transport
- Release of cells into resuspension buffer/enrichment medium

Why is the sampling procedure important?

- Optimal moistening solution -> best recovery rate
- Sampled spot/area should be precisely defined
  -> best reproducibility in consecutive samplings (trend analysis)
- **Sampled area/spot should be covered multiple times** with one device
  (going back and forth in two to three different directions)



- **Sampling device should be turned** during sampling to take advantage of its full capacity
- **Sufficient pressure** should be applied without breaking the device
  -> optimal pick-up of adherent cells and biofilm

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Sampling devices available today

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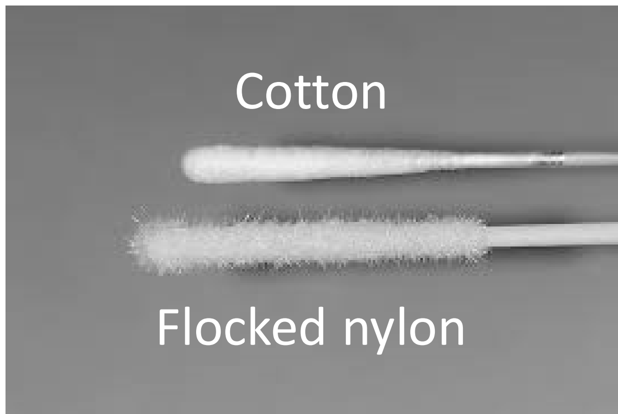
Swabs



Sponge sticks

Sponges and wipes

How do different sampling devices perform?

Recovery rate (% cfu recovered of cfu inoculated):

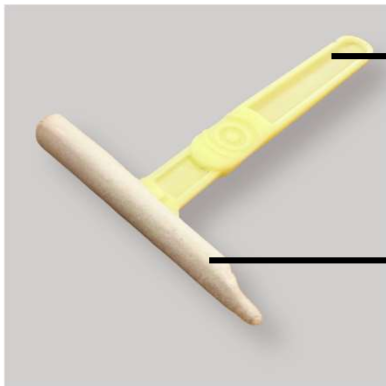
Device			*L. monocytogenes* strain 935 (human origin)	*L. monocytogenes* strain 437/07 (food origin)
Sponge (4 x 8 cm, with 10 ml BPW)			2.81 ± 0.63 BCDEF	0.97 ± 0.36 BCDEF
Premoistened towel (20 x 20 cm)			0.28 ± 0.18 G	0.06 ± 0.03 F
Cotton swab			1.01 ± 0.47 EFG	0.09 ± 0.07 F
Alginate swab			0.22 ± 0.15 G	0.14 ± 0.11 F
Metallic swab			0.24 ± 0.29 G	0.03 ± 0.03 F
Cotton disk			2.53 ± 0.96 CDEFG	1.22 ± 0.67 BCDE
Cotton g	Miniroller 1	White microfiber	0.40 ± 0.15 G	0.35 ± 0.20 DEF
MR1S			3.53 ± 1.17 BCD	1.30 ± 0.72 BCD
MR2S	Miniroller 2	100% Wool fiber–velour	6.27 ± 1.62 A	1.52 ± 0.37 B
MR3S			3.31 ± 0.10 BCDE	1.41 ± 0.55 BC
MR4S	Miniroller 3	100% White polyamide fiber	2.32 ± 0.48 CDEFG	0.43 ± 0.17 CDEF
MR5S			3.69 ± 0.64 BCD	0.59 ± 0.24 BCDEF
MR1V	Miniroller 4	White high-density foam	1.79 ± 0.36 DEFG	0.31 ± 0.12 DEF
MR2V			5.05 ± 2.19 AB	1.49 ± 0.65 B
MR3V	Miniroller 5	High-density foam flocked with polyamide fiber of 3 mm	4.23 ± 1.53 ABC	0.21 ± 0.18 EF
MR4V			1.90 ± 0.58 CDEFG	0.61 ± 0.33 BCDEF
MR5V			1.33 ± 0.66 DEFG	0.61 ± 0.46 BCDEF
Steel wool pad			0.75 ± 0.44 FG	0.31 ± 0.15 DEF
Petrifilm			0.25 ± 0.29 G	1.20 ± 0.36 BCDE
RODAC ALOA			2.50 ± 1.29 CDEFG	4.15 ± 1.03 A

Gomez *et al.* (2012). *J. Food Protection* 75: 1077-1082

Used stainless steel table, 10 x 10 cm squares (100 cm^2), *L. mono.* diluted in peptone water/minced meat suspension, 0.1 ml of 10^6 CFU suspension spread over square, dried for 15 min.

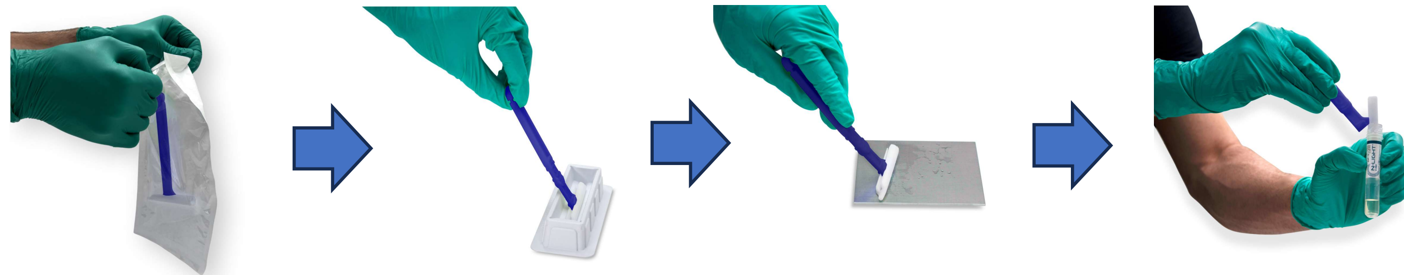
A new approach to sampling of large surfaces: The N-Light^{TM} MaxiSampler

The first large surface sampling device
compatible with rapid, on-site tube tests
Patent pending



Firmly attached, robust handle for forceful swabbing, removable by simple click mechanism

Giant swab barrel with fine tip, flocked fiber coating (similar to standard NEMIS swabs) on outside, hollow inside for dropping of N-Light^{TM} tablets to enrichment broth at bottom of N-Light^{TM} test tubes



How to use the N-Light^{TM} MaxiSampler

1. PREPARATION Repeat for 5-6 samples per reservoir and buffer tube

2. SAMPLING

Add the N-Light™ buffer to the sterile reservoir.

Mark/label the pouch according to your test plan. Open the sealed pouch.

Remove the N-Light ™ Maxi-Sampler from the pouch and wet it in the buffer.

Store the wetted MaxiSampler again in its pouch and use it within 4 hours.

Sample surface areas of up to 0.3 m^2 by scraping the area in 3 directions with some force. Place the N-Light™ Maxi-Sampler back into the pouch after sampling and proceed with step 3 within 4 hours.

Tips:

- For sampling wet surfaces, using dry MaxiSamplers is acceptable.
- Use the tip of the MaxiSampler swab to sample grooves and holes in the sampling area.
- Change the inclination of the handle during swabbing.

See also Instructions for Use (inlay in each box of MaxiSamplers, available as PDF)

How to use the N-Light^{TM} MaxiSampler

3. TRANSFER Procedure depends on the N-Light™ test

N-Light ™ *Listeria monocytogenes*
N-Light ™ *E. coli*

REF 00009 N-LIGHT™ LISTERIA MONOCYTOGENES
REF 00029 N-LIGHT™ E. COLI

Open the N-Light™ test tube. Insert the MaxiSampler swab and unclip the swab by turning the handle upwards.

Put the cap back on the test tube and lock the tube (press firmly until you hear a "click").

Rinsing: Hold tube between thumb and forefinger in horizontal orientation with fiber coating facing down. then incline to one side, then to the other, repeat 10 times

The MaxiSampler swab remains in the tube for the subsequent steps:
4. Incubate
5. Activate
6. Measure

N-Light ™ *Salmonella Risk*
N- Light ™ *Listeria spp.*

REF 00014 N-LIGHT™ SALMONELLA RISK
REF 00048 N-LIGHT™ LISTERIA spp.

Release the MaxiSampler swab into the N-Light™ test tube and carefully screw the cap back onto the tube. DO NOT LOCK THE TUBE.

Rinsing: Hold tube between thumb and forefinger in horizontal orientation with fiber coating facing down. then incline to one side, then to the other, repeat 10 times

Open the test tube again and insert the back of the handle into the MaxiSampler swab. REMOVE THE SWAB FROM THE TUBE. Discard the swab.

Salmonella Risk only: Add one starter tablet to the test tube and briefly shake the tube.

All: NOW LOCK THE TUBE.

(See also IFU)

***L. mono., E. coli*: MaxiSampler IN**

Important:
Rinse well with coating facing down before incubation

***Salmonella* Risk, *L.* spp.: MaxiSampler OUT**

Important:
Rinse well with coating facing down before removal and incubation

Recovery rate of N-Light™ MaxiSampler

- Bacterial cells diluted in sterile broth, 10x 10 µl «spotted» on 121 cm^2 stainless steel plates
- 1.5 h drying at room temperature
- MaxiSamplers moistened with N-Light™ Neutralizer, scrub samplers used as received.
- Surface swabbed in 3 directions while turning devices
- Devices resuspended in equal volumes of buffered peptone water (BPW)
- Inoculated and recovered (resuspended) cells (colony forming units) counted on plate count agar

Sampling Device	Test strain	CFU inoculated	CFU recovered after sampling	Recovery rate
N-Light™ MaxiSampler	*Listeria monocytogenes* ATCC 19111	39'000 ± 3'606	5'262 ± 127	14% ± 1%
	Salmonella Typhimurium ATCC 14028	17'000 ± 3'754	5'201 ± 585	30% ± 8%
3M Scrub Sampler	*Listeria monocytogenes* ATCC 19111	39'000 ± 3'606	6'319 ± 2'237	16% ± 6%
	Salmonella Typhimurium ATCC 14028	17'000 ± 3'754	3'606 ± 321	21% ± 5%

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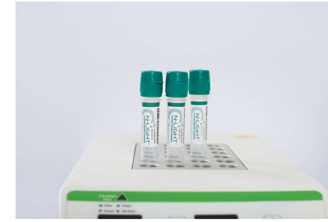
Use of N-Light™ MaxiSampler with the N-Light™ *Listeria monocytogenes* Test



- Bacterial cells diluted in BHI broth, 10x 10 µl «spotted» on stainless steel (121 cm^2)
- 1 h drying at room temperature
- MaxiSamplers moistened with N-Light™ Neutralizer, stainless steel plates swabbed in 3 directions
- MaxiSamplers left IN, incubation and read-out according to IFU for N-Light™ *Listeria monocytogenes*

Inoculation level (cfu/ 100 cm^2 steel plate)	Positive N-Light™ *Listeria monocytogenes* tests	
	L. mono. ATCC 13932	*L. mono.* ATCC 19111
1000-2000	5 / 5	5 / 5
100-200	5 / 5	5 / 5
10-20	4 / 5	5 / 5

N-Light™ MaxiSampler compared to N-Light™ swabs BP

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N-Light *L. mono.* tests

Moistening solution: N-Light™ Neutralizer

MaxiSampler/swabs left IN

Threshold for positive tests: 10'000 RLU

MaxiSampler	**RLU**	**RLU**	**Positives**
Stainless steel, 11 x 11 cm	average	st. dev.	
L. mono. ATCC 13932, 12'000 cfu	391'263	81'293	**5/5**
L. mono. ATCC 13932, 1'200 cfu	612'271	329'705	**5/5**
L. mono. ATCC 13932, 120 cfu	252'008	237'596	**4/5**
L. mono. ATCC 13932, 12 cfu	2'775	1'167	**0/5**
Negative control (Sterile BHI on steel)	1'878	NA	**0/1**

Swabs BP	**RLU**	**RLU**	**Positives**
Stainless steel, 2.5 x 2.5 cm	average	st. dev.	
L. mono. ATCC 13932, 12'000 cfu	3'026'692	479'233	**5/5**
L. mono. ATCC 13932, 1'200 cfu	3'151'930	962'436	**5/5**
L. mono. ATCC 13932, 120 cfu	3'029'002	1'152'811	**5/5**
L. mono. ATCC 13932, 12 cfu	2'100'827	1'922'773	**3/5**
Negative control (Sterile BHI on steel)	2'759	NA	**0/1**

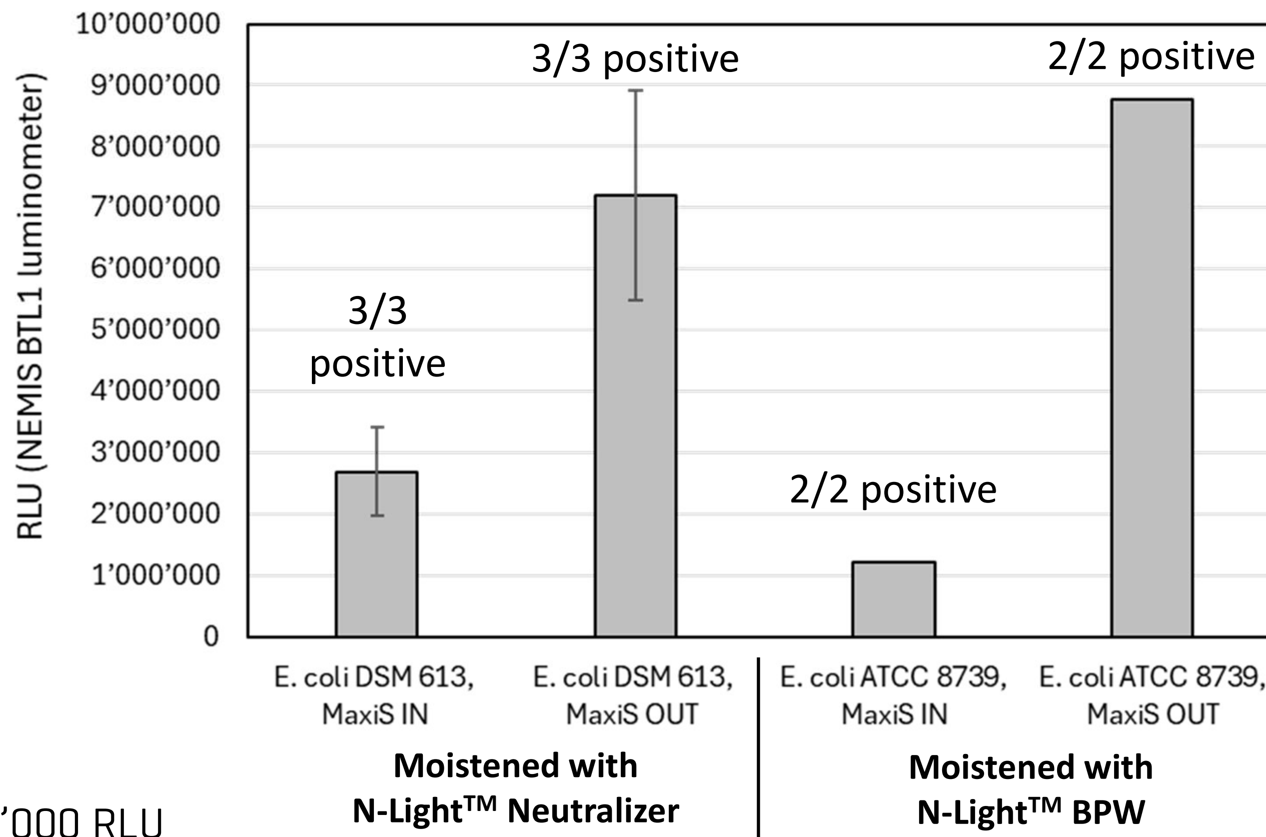
-> N-Light™ swabs BP peform better than MaxiSamplers when used for small areas (≤ 10 cm^2)

Use of N-Light™ MaxiSampler with the N-Light™ *Escherichia coli* Test

- Bacterial cells diluted in TSB, 10x 10 µl «spotted»
- 2 h drying
- MaxiSamplers with cells kept 2 h at room temp.
- Incubation and read-out according to IFU for N-Light™ *E. coli*

Negative control:
(sterile broth on steel)
MaxiS IN 1'008 RLU
MaxiS OUT 1'465 RLU

Threshold for positives: 10'000 RLU



Use of N-Light™ MaxiSampler with the N-Light™ *Salmonella* Risk Test

Similar experimental procedure as for the N-Light *E. coli* test, except for removal of MaxiSampler after rinsing, moistening with Neutralizer

Inoculation level *Salmonella* Typhimurium ATCC 14028 (cfu/ 121 cm^2 steel plate)	Result N-Light™ *Salmonella* Risk Test	
	Positives	RLU, average ± SD
790	3 / 3	538'738 ± 202'228
70	3 / 3	206'659 ± 38'642
0 (sterile TSB broth)	0 / 2	1'106

Threshold for positives: 20'000 RLU

Use of N-Light™ MaxiSampler with the N-Light™ *Listeria* spp. Test

Cells diluted in BHI broth, otherwise similar experimental procedure as for the *E. coli* and *Salmonella* Risk tests, MaxiSamplers removed after rinsing (before incubation)

Inoculation level and strain (cfu/ 121 cm² steel plate)	Result N-Light™ *Listeria* spp. tests	
	Positives	RLU
98 cfu *L. mono.* ATCC 13923	2 / 2	11'173 / 15'534
230 cfu *L. innocua* ATCC 13923	2 / 2	24'097 / 17'703
0 (sterile BHI)	0 / 5	1'147 / 1'094

Threshold for positives: 4'000 RLU

Field tests with N-Light™ MaxiSampler

Ready-to-eat food factory 1

Moistening solution	Positives N-Light™ *Salmonella* Risk	PCR analysis of N-Light tests, *Salmonella* positive
N-Light™ BPW	4 / 20 (20%)	0 / 20
N-Light™ Neutralizer	5 / 20 (25%)	0 / 20

Ready-to-eat food factory 2

Positives N-Light™ *L. mono.*	ISO analysis of developed N-Light tests, *L. mono.* positive
2 / 23 (9%)	0 / 23

Summary

- **Sampling** is determines to a large part the **quality of environmental monitoring** data
- **Microorganisms** can be **firmly attached to surfaces** (and hard to pick-up), particularly in biofilms
- There is **no one-size fits all sampling device**
- Large (MaxiSampler, sponges, sponge sticks) and small (swabs) sampling devices each have distint advantages and disantvantages
- The surface contact **material of sampling devices matters**, but more research is needed
- The novel NEMIS **N-Light™ MaxiSampler** allows efficient **sampling of large surfaces**
- The MaxiSampler is **compatible with all N-Light™ pathogen/indicator tests**
- Overloading of MaxiSamplers with food matrix/dirt should be avoided

Thanks for your participation !

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