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1. Scope

The **SENSIStrip Crustaceans LFD** is designed for the determination of crustaceans in food, contaminated surfaces or CIP water. It is based on an antibody against crustacean tropomyosin. The present report describes the validation process and summarizes its results.

2. General Considerations

Allergen lateral flow devices are mainly intended as qualitative analytical method. Anyhow, for the sake of definition of specifications, profound evaluation and semi-quantitative result comparison a color card was used. This graduates results into 10 incremental classes where “1” indicates absence of any visible signal and “10” the most intense line. “Classes” as stated in the tables below refer to the increments provided by the color card.

Applying semi-quantitative evaluation, calculating a Coefficient of Variation is deemed to be mere mathematical exercise without practical relevance. Thus, only a qualitative assessment is given wherever appropriate.

3. Precision

3.1. Repeatability

Repeatability was determined by testing one negative and two positive samples at different concentration levels in 20fold replicates.

Table 1: Repeatability of the SENSIStrip Crustaceans LFD

Replicate	Level 1 [0 ppb]	Level 2 [75 ppb]	Level 3 [1250 ppb]
1	1	3	8
2	1	3	8
3	1	3	8
4	1	3	8
5	1	3	8
6	1	3	8
7	1	3	8
8	1	3	8
9	1	3	8
10	1	3	8
11	1	3	8
12	1	3	8
13	1	3	8
14	1	3	8

Replicate	Level 1 [0 ppb]	Level 2 [75 ppb]	Level 3 [1250 ppb]
15	1	3	8
16	1	3	8
17	1	3	8
18	1	3	8
19	1	3	8
20	1	3	8
Mean	1.0	3.0	8.0

Data show good repeatability of results, including 0 ppb standard assessed as “negative” in all cases.

3.2. Reproducibility

3.2.1. Inter-lot Variability

Inter-lot variability was determined by testing one negative and two positive samples at different concentration levels in 3 different kit lots as duplicates.

Table 2: Inter-lot reproducibility of the SENSIStrip Crustaceans LFD

Lot	Level 1 [0 ppb]	Level 2 [75 ppb]	Level 3 [1250 ppb]
1	1/1	3/3	7/7
2	1/1	3/3	8/8
3	1/1	3/3	7/7
Mean	1.0	3.0	7.3

Data show good inter-lot reproducibility of results, including 0 ppb standard assessed as “negative” in all cases.

3.2.2. Inter-assay Variability

Inter-assay variability was determined by testing one negative and two positive samples at 4 different days as duplicates in the same kit lot.

Table 3: Inter-assay reproducibility of the SENSIStrip Crustaceans LFD

Day	Level 1 [0 ppb]	Level 2 [75 ppb]	Level 3 [1250 ppb]
1	1/1	3/3	7/7
2	1/1	3/3	7/7
3	1/1	4/3	7/7
4	1/1	3/3	7/7
Mean	1.0	3.1	7.0

Data show good inter-assay reproducibility of results, including 0 ppb standard assessed as “negative” in all cases.

3.2.3. Personal Factors

To assess usability of the device and the robustness of the method with respect to individual handling one negative and two positive samples at different concentration levels were tested and evaluated as duplicates by three different persons. All persons were trained lab technicians, but not necessarily experienced with this peculiar design of lateral flow devices.

Table 4: Usability of the SENSIStrip Crustaceans LFD

User	Level 1 [0 ppb]	Level 2 [75 ppb]	Level 3 [1250 ppb]
1	1/1	4/4	7/7
2	1/1	3/3	7/7
3	1/1	3/3	7/7
Mean	1.0	3,3	7.0

Data show reproducible performance of the test by various persons, including 0 ppb standard assessed as “negative” in all cases.

3.2.4. Robustness

Robustness experiments shall characterize limitations of the tests with respect to variances of various parameters while performing the test. For this, incubation times, temperature, sample volume and time to evaluation were varied. All other conditions were kept constant according the Instruction for Use. One negative and two positive samples at different concentration levels were tested in duplicates.

Table 5: Robustness of the SENSIS*Strip* Crustaceans LFD with respect to 1. incubation time

1. Incubation time [min]	Level 1 [0 ppb]	Level 2 [75 ppb]	Level 3 [1250 ppb]
1	1/1	3/3	7/7
2	1/1	3/3	7/7
3	1/1	3/3	7/7
4	1/1	3/3	7/7
5	1/1	3/3	7/7
6	1/1	3/3	7/7
7	1/1	3/3	7/7
10	1/1	3/3	7/7
15	1/1	3/3	8/8
20	1/1	3/3	7/7

1. Incubation time does not affect the accuracy of negative samples up to 20 minutes.

After 1 minutes 1. incubation time signals of positive samples reach equilibrium and do not change up to 20 minutes.

Thus, 1. incubation time yields constant results for negative and positive samples ranging from 1 to 20 minutes.

Table 6: Robustness of the SENSIS*Strip* Crustaceans LFD with respect to 2. incubation time

2. Incubation time [min]	Level 1 [0 ppb]	Level 2 [75 ppb]	Level 3 [1250 ppb]
1	1/1	2/2	6/6
2	1/1	2/2	7/7
3	1/1	3/3	7/7
4	1/1	3/3	7/7
5	1/1	3/3	7/7
6	1/1	3/4	7/8
7	1/1	4/4	8/8
10	1/1	5/5	8/8
15	2/2	6/6	8/8
20	2/2	6/6	8/8

2. Incubation time does not affect the accuracy of negative samples up to 10 minutes.

After 3 minutes 2. incubation time signals of positive samples reach equilibrium and do not change up to 7 minutes.

Thus, 2. incubation time yields constant results for negative and positive samples ranging from 3 to 7 minutes.

Table 7: Robustness of the SENSIS*Strip* Crustaceans LFD with respect to incubation temperature

Incubation temperature [°C]	Level 1 [0 ppb]	Level 2 [75 ppb]	Level 3 [1250 ppb]
4	1/1	3/3	7/7
15	1/1	3/3	7/7
20	1/1	3/3	7/8
25	1/1	3/3	7/7
30	1/1	3/3	7/7
37	1/1	3/3	7/8

Incubation temperature does not affect the accuracy of negative samples between 4 and 37°C.

Signals for positive samples remain constant between 4-37°C incubation temperature.

Thus, incubation temperature yields constant results for negative and positive samples ranging from 4 to 37°C.

Table 8: Robustness of the SENSIS*Strip* Crustaceans LFD with respect to sample volume

Sample volume [µL]	Level 1 [0 ppb]	Level 2 [75 ppb]	Level 3 [1250 ppb]
100	1/1	4/4	7/7
200	1/1	4/4	7/7
300	1/1	4/4	7/7
400	1/1	4/4	7/7
500	1/1	3/3	7/7
600	1/1	3/3	6/6
700	1/1	2/2	5/5
800	1/1	2/2	4/4
1000	1/1	1/1	1/1

Sample volume does not affect the accuracy of negative samples between 100 and 1000 µL.

Signals for positive samples remain constant between 100-600 µL sample volume.

Thus, sample volume yields constant results for negative and positive samples ranging from 100 to 600 µL.

Table 9: Robustness of the SENSIS*Strip* Crustaceans LFD with respect to time to evaluation

Time to evaluation [min]	Level 1 [0 ppb]	Level 2 [75 ppb]	Level 3 [1250 ppb]
0	1/1	4/3	7/7
2	1/1	3/4	7/7
5	1/1	3/4	7/7
10	1/1	3/3	7/7
15	1/1	4/4	7/7
30	2/2	4/4	7/7
60	2/2	4/4	7/7
120	2/2	4/4	7/7
1200	2/2	4/4	7/7

Time to evaluation does not affect the accuracy of negative samples up to 15 minutes. Signals for positive samples remain constant between 0-1200 minutes time to evaluation. Thus, time to evaluation yields constant results for negative and positive samples ranging from 0 to 15 minutes.

Although the above results concerning robustness show some degrees of freedom, real samples could behave in a different manner. Thus, test execution and evaluation should always be done according the IFU.

3.3. Analytical Sensitivity

For determination of the analytical sensitivity negative as well as multiple positive samples with increasing concentrations were tested in triplicates. The test was performed and evaluated by three different users.

Table 10: Limit of detection (LOD) of the SENSIS*Strip* Crustaceans LFD

User	Level 1 [0 ppb]	Level 2 [25 ppb]	Level 3 [50 ppb]	Level 4 [100 ppb]	Level 5 [250 ppb]	Level 6 [500 ppb]	Level 7 [1000 ppb]
1	1/1/1	3/3/3	4/4/4	5/5/4	6/6/7	7/7/7	8/8/8
2	1/1/1	3/4/3	4/4/3	5/5/5	7/7/7	7/7/7	8/8/8
3	1/1/1	2/3/3	3/4/3	4/4/5	7/6/6	7/7/7	8/8/8

LOD was defined as the lowest concentration which was correctly assessed as a value of ≥ 3 . Thus, LOD is 25 ppb.

3.4. Dynamic Range

Dynamic range is defined as the interval between LOD and beginning High-Dose-Hook Effect. For assessing the latter multiple positive samples with increasing concentrations were tested in duplicates.

Table 11: High-Dose-Hook Effect of the SENSIStrip Crustaceans LFD

Level [ppb]	Intensity
0	1/1
25	3/3
50	3/3
100	4/4
250	5/5
500	6/6
1000	7/7
2500	8/8
5000	8/8
10000	8/8
20000	7/7
40000	6/6
50000	5/6
100000	5/5

Signals are constant up to 20000 ppb indicating no High-Dose-Hook Effect. Thus, with LOD as determined according to 3.3. dynamic range is defined as 25-20000 ppb.

3.5. Accuracy

3.5.1. Spiked Matrices

Relevant matrices were spiked at various levels and extracted according the Instruction for Use. Extracts were tested undiluted as well as in various dilutions. Additionally, non-spiked zero matrix was tested. All tests were performed in duplicates.

Table 12: Accuracy (spiked matrices) of the SENSIStrip Crustaceans LFD

Matrix	Level 1 [0 ppb]	Level 2 [25 ppb]	Level 3 [50 ppb]	Level 4 [100 ppb]	Level 5 [200 ppb]	Level 6 [400 ppb]
Vegetable soup	1/1	3/3	4/4	5/5	6/6	7/7
Cracker	2/2	3/3	4/4	5/5	6/6	7/7
Sausage	1/1	3/3	4/4	5/5	6/6	7/7
Fish(cod)	1/1	2/2	3/3	4/4	5/5	6/6
Soy sauce	1/1	3/3	4/4	5/5	6/6	6/6

Matrix-dependent analytical sensitivity is ranging from 25 to 50 ppb.

3.5.2. Heat-treated Matrices

To exemplarily assess changes in analytical sensitivity for processed food samples, a soup matrix was spiked with 160 ppm of shrimps and divided in two parts. One part was cooked at 100 °C for 15 min. Processed as well as unprocessed extracts were tested undiluted as well as in various dilutions. Additionally, non-spiked zero matrix was tested. All tests were performed in duplicates.

Table 13: Accuracy (heat-treated matrices) of the SENSIStrip Crustaceans LFD

Matrix	Level 1 [0 ppm]	Level 2 [5 ppm]	Level 3 [10 ppm]	Level 4 [20 ppm]	Level 5 [40 ppm]	Level 6 [80 ppm]	Level 7 [160 ppm]
Soup / unprocessed	1/1	3/3	3/3	5/5	6/7	7/7	8/7
Soup / processed	1/1	2/2	2/3	4/3	4/4	5/5	6/6

Analytical sensitivity for the heat-treated matrix decreases from 5 to 10 ppm (Shrimp).

3.5.3. Routine Samples from Labs

Other than spiked samples routine samples give more profound information about realistic conditions as they occur while food manufacturing. Expectancy values might not be too well established, but the analytes have undergone all processing steps with full impact of the sample matrix.

Thus, routine samples from labs were acquired and tested according to the Instruction for Use.

Table 14: Accuracy (routine samples) of the SENSIStrip Crustaceans LFD

Sample description	Expectancy evaluated by	Expectancy [Tropomyosin ppb]	Intensity
Feed, flour/groats, seaweed	ELISA	< 20	1/1
Cheese, Soft cheese	ELISA	> 300	7/7
Curry	ELISA	< 20	1/1
Seaweed, dried	ELISA	> 300	3/3
Seaweed, dried	ELISA	110	5/5
Seaweed, dried	ELISA	82	2/3
Tea, dried	ELISA	47	3/2
Seaweed, dried	ELISA	45	3/4
Pizza	ELISA	< 20	1/1
Lecithin	ELISA	< 20	1/1
Dietary supplement	ELISA	< 20	1/1

Sample description	Expectancy evaluated by	Expectancy [Tropomyosin ppb]	Intensity
Lettuce	ELISA	< 20	1/1
Flour, fish flour	ELISA	< 20	1/1
Dietary supplement	ELISA	< 20	1/1
Cookie	ELISA	< 20	1/1
Tomato powder	ELISA	< 20	1/1

3.5.4. Proficiency Test material

In addition to routine samples proficiency test material serves as valuable information concerning the performance of a test system

Different proficiency test materials from DLA were applied and tested according the Instruction for use.

Table 15: Results of the SENSIStrip Crustaceans LFD applied on Proficiency Test material

Source	Sample description	Concentration, according provider [ppm]	Intensity
DLA 07/2014	Instant product, Sample A	0	1
DLA 07/2014	Instant product, Sample B	312 (crab protein)	5
DLA 07/2015	Instant soup powder, Sample A	0	1
DLA 07/2015	Instant soup powder, Sample B	113 (shrimp powder)	3
DLA 07/2016	Instant soup powder, Sample A	0	1
DLA 07/2016	Instant soup powder, Sample B	45 (dried prawn)	3
DLA 07/2018	Instant soup powder, Sample A	73 (dried river crayfish)	3
DLA 07/2018	Instant soup powder, Sample B	0	1

All negative samples were identified as negative. All of the positive samples with expectancy values > LOD are identified as positive with reasonable intensity.

3.5.5. Biological or processed variants

Crustaceans might occur in various variants due to biological diversity. Anyhow, in creating the immunological components of the test device only one of those can be considered. In the peculiar case antibodies are derived against shrimp tropomyosin. To assess the reactivity towards different crustacean species, those were extracted and diluted to various reasonable levels going down to 1 ppm as stated in chapter 3.3.

Table 16: Accuracy (relevant analyte variants) of the SENSIStrip Crustaceans LFD

Variant	Level 1 [1 ppm]	Level 4 [10 ppm]	Level 5 [100 ppm]	Level 6 [1000 ppm]	Level 6 [10000 ppm]
Spiny lobster, raw	1/1	1/1	3/2	5/5	8/8
Spiny lobster, cooked	1/1	2/2	7/7	8/8	8/8
Crab, cooked	1/1	2/2	5/5	8/8	8/8
River crayfish, raw	1/1	2/3	5/5	8/8	9/9
River crayfish, cooked	1/1	2/1	3/3	6/6	8/8
Shrimps, raw	1/1	3/3	5/6	7/8	8/8
Shrimps, cooked	1/1	2/3	7/6	7/7	8/7
Lobster, raw	1/1	3/3	6/6	8/8	8/8
Lobster, cooked	1/1	2/2	5/5	8/8	8/8
Black tiger prawn, raw	1/2	4/4	8/8	9/9	9/9
Black tiger prawn, cooked	1/1	3/3	6/6	8/8	8/8

Variant-dependent analytical sensitivity is ranging between 10 to 100 ppm depending on individual species and processing.

3.6. Swabbing Application

Major application for lateral-flow devices is the test of swab samples. SENSIStrip product line is harmonized with the SENSISwab swabbing kits. To assess its applicability, surfaces were contaminated with spiked sample matrices at various levels. Those were dispersed in assay buffer and dispensed on a 5x5 cm surface area in duplicates. Each swabbing sample was tested in duplicates as well, resulting in a total of 4 determinations. Concentrations below refer to the spiked samples.

Table 17: Accuracy (relevant analyte variants) of the SENSIStrip Crustaceans LFD

Sample description	Level 1 [0 ng/cm ²]	Level 2 [0.67 ng/cm ²]	Level 3 [2.00 ng/cm ²]	Level 4 [6.67 ng/cm ²]
Buffer	1/1	2/2	4/4	6/6
	1/1	2/2	4/4	6/6
Cookie (TUC)	1/1	2/2	3/3	6/6
	1/1	2/2	3/3	6/6

Sample description	Level 1 [0 ng/cm ²]	Level 2 [0.67 ng/cm ²]	Level 3 [2.00 ng/cm ²]	Level 4 [6.67 ng/cm ²]
Soy sauce	1/1	2/2	4/4	6/6
	1/1	2/2	4/4	6/6
Vegetable soup	1/1	1/1	2/2	4/4
	1/1	1/1	2/2	4/4

Dependent on the sample matrix, swab samples can be detected as low as 2 ng/cm².

Repeated swabbing of the same surface indicates that varying recoveries are an intrinsic feature of the method which can only be improved by multiple repetitions. Recoveries are affected by solubility of proteins, residual liquid and complete swabbing of the surface area.

Note that surfaces were contaminated by protein extracts and not complete commodities. As the latter are more particulate by nature they might be swabbed more easily. Thus, the approach chosen here reflects a worst-case scenario.

3.7. Rinse water / CIP

The sensitivity for rinse water / CIP was determined by diluting crustacean extract in different commonly applied water-based rinse solutions to various concentrations. The pH was adjusted to 7.0 ± 0.5 before running the test. All tests were performed in duplicate.

Table 18: Sensitivity of the SENSIStrip Crustaceans LFD for Rinse Solutions

Rinse solution	Level 1 [0 µg/L]	Level 2 [1.7 µg/L]	Level 3 [3.3 µg/L]	Level 4 [6.7 µg/L]	Level 5 [13 µg/L]	Level 6 [27 µg/L]
Pure water	1/1	3/3	3/3	4/4	5/5	7/6
2% NaOH	1/1	1/2	2/2	2/2	4/4	5/5
0.5% Citric acid	1/1	2/2	3/3	4/4	5/5	6/6
0.6% nitric acid	1/1	1/1	1/1	2/2	3/4	5/5
0.5% sodium tripolyphosphate	1/1	1/1	2/3	4/4	4/5	5/6

LOD for rinse solutions was defined as the lowest concentration which was correctly assessed as a value of ≥ 3 . Thus, LOD for pure water is 1.7 µg/L, LOD for 0.5% citric acid and 0.5% sodium tripolyphosphate solution is 3.3 µg/L and LOD for 2% NaOH and 0.6% nitric acid is 13 µg/L.

3.8. Specificity

Specificity was assessed by extracting and testing multiple commodities in pure form. Tests were performed in duplicates.

Table 19: Specificity (cross-reactivity) of the SENSIStrip Crustaceans LFD

Commodity	Intensity	Commodity	Intensity	Commodity	Intensity
Almond	1/1	Curcuma	1/1	Paprika	1/1
Adzuki bean	1/1	Dill	1/1	Pea	1/1
Barley	2/2	Duck	1/1	Peach	1/1
Bean, white	1/1	Egg	1/1	Peanut	1/1
Beef	1/1	Ewe's milk	1/1	Pecan	1/1
Blue mussel	7/7	Fennel	1/1	Peeper	1/1
Bovine gelatin	1/1	Fenugreek	1/1	Pine seed	1/1
Brazil nut	1/1	Fish(cod)	1/1	Pistachio	1/1
Buckwheat	1/1	Flaxseed	1/1	Poppy seed	1/1
Caraway	1/1	Garden cress	1/1	Pork	2/2
Cardamom	1/1	Garlic	1/1	Potato	1/1
Carob bean	2/2	Gliadin	1/1	Pumpkin seed	1/1
Carpet shell	8/8	Goat's milk	1/1	Radish	1/1
Carrot	1/1	Guar gum	1/1	Rice	1/1
Cashew	1/1	Hazelnut	1/1	Rye	2/2
Cayenne	1/1	Horseradish	1/1	Sesame	1/1
Celery	2/2	Kidney bean	1/1	Soy flour	1/1
Cherry	1/1	Kiwi	1/1	Soy lecithin	1/1
Chestnut	1/1	Lamb	1/1	Soy milk	1/1
Chia	1/1	Leek	1/1	Split peas	1/1
Chicken	1/1	Lentil	1/1	Sucrose	1/1
Chickpea	1/1	Lupin	1/1	Sunflower seed	1/1
Chili	1/1	Macadamia	1/1	Thyme	1/1
Cinnamon	1/1	Mustard	1/1	Tomato	1/1
Clove	1/1	Nutmeg	1/1	Turkey	1/1
Cocoa	1/1	Oats	2/2	Walnut	1/1
Coconut	1/1	Onion	1/1	Wheat	1/1
Corn	1/1	Oyster	7/7	White cabbage	1/1
Cow's milk	2/2				

With LOD = 25 ppb (see 3.3.) for most commodities no cross-reactivity could be determined.

For oyster, blue mussel and carpet shell additional dilutions of the extract were tested. Cross-reactivity was calculated considering concentration and LOD.

Table 20: Specificity (cross-reactivity) of the SENSIStrip Crustaceans LFD (2)

Commodity	Concentration [ppb]	Intensity	Cross-reactivity
Oyster	1 B	7/7	0.001%
	500 M	7/7	
	250 M	7/7	
	60 M	7/7	
	15 M	6/6	
	7 M	5/5	
	3,5 M	4/4	
	1.75 M	3/3	
	1.2 M	2/2	
Blue mussel	1 B M	7/7	0.002%
	500 M	7/7	
	250 M	7/7	
	60 M	7/7	
	15 M	6/6	
	3.5 M	5/5	
	1.75 M	4/4	
	1.2 M	3/3	
	800 K	2/2	
Carpet shell	1 B	8/8	0.001%
	500 M	8/8	
	250 M	7/7	
	60 M	7/7	
	15 M	6/6	
	3.5 M	5/5	
	2.5 M	4/3	
	1.75 M	3/3	
	1.2 M	2/2	