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1. Scope

The **SENSIStrip Mustard LFD** is designed for the determination of mustard in food, contaminated surfaces or CIP water. It is based on an antibody against mustard proteins. 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 semiquantitative 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 Mustard LFD

Replicate	Level 1 [0 ppm]	Level 2 [15 ppm]	Level 3 [250 ppm]
1	1	7	8
2	1	7	8
3	1	7	8
4	1	7	8
5	1	7	8
6	1	7	8
7	1	7	8
8	1	7	8
9	1	7	8
10	1	7	9
11	1	7	8
12	1	7	8
13	1	7	8
14	1	7	8
15	1	7	8

Replicate	Level 1 [0 ppm]	Level 2 [15 ppm]	Level 3 [250 ppm]
16	1	7	8
17	1	7	8
18	1	7	9
19	1	7	9
20	1	7	8
Mean	1.0	7.0	8.2

Data show good repeatability of results, including 0 ppm 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 Mustard LFD

Lot	Level 1 [0 ppm]	Level 2 [15 ppm]	Level 3 [250 ppm]
1	1/1	7/7	9/9
2	1/1	6/6	9/8
3	1/1	7/7	9/9
Mean	1.0	6.7	8.8

Data show good inter-lot reproducibility of results, including 0 ppm 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 Mustard LFD

Day	Level 1 [0 ppm]	Level 2 [15 ppm]	Level 3 [250 ppm]
1	1/1	6/6	9/8
2	1/1	6/6	8/8
3	1/1	6/6	8/8
4	1/1	6/7	8/9
Mean	1.0	6.1	8.3

Data show good inter-assay reproducibility of results, including 0 ppm 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 Mustard LFD

User	Level 1 [0 ppm]	Level 2 15 ppm]	Level 3 [250 ppm]
1	1/1	6/7	9/9
2	1/1	6/6	8/7
3	1/1	6/6	8/8
Mean	1.0	6.2	8.2

Data show reproducible performance of the test by various persons, including 0 ppm 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 SENSIStrip Mustard LFD with respect to 1. incubation time

1. Incubation time [min]	Level 1 [0 ppm]	Level 2 [15 ppm]	Level 3 [250 ppm]
1	1/1	4/4	7/7
2	1/1	6/6	8/8
3	1/1	6/6	8/8
4	1/1	6/6	8/8
5	1/1	6/6	8/8
6	1/1	6/6	8/8
7	1/1	6/6	8/8
10	1/1	7/7	8/8
15	1/1	7/7	8/8

1. Incubation time [min]	Level 1 [0 ppm]	Level 2 [15 ppm]	Level 3 [250 ppm]
20	1/1	4/4	8/8

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

After 2 minutes 1. incubation time signals of positive samples reach equilibrium and do not change up to 15 minutes.

Thus, 1. incubation time yields constant results for negative and positive samples ranging from 2 to 7 minutes.

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

2. Incubation time [min]	Level 1 [0 ppm]	Level 2 [15 ppm]	Level 3 [250 ppm]
1	1/1	3/3	7/7
2	1/1	4/4	7/7
3	1/1	6/6	8/8
4	1/1	6/6	8/8
5	1/1	6/6	8/9
6	1/1	6/6	9/9
7	1/1	6/6	9/9
10	1/1	7/7	9/9
15	1/1	7/7	9/9
20	1/1	7/7	9/9

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

After 3 minutes 2. incubation time signals of positive samples reach equilibrium and do not change up to 20 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* Mustard LFD with respect to incubation temperature

Incubation temperature [°C]	Level 1 [0 ppm]	Level 2 [15 ppm]	Level 3 [250 ppm]
4	1/1	6/6	8/8
15	1/1	6/6	8/8
20	1/1	6/6	8/8
25	1/1	6/6	8/8
30	1/1	6/6	8/8
37	1/1	6/5	8/8

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 SENSIStrip Mustard LFD with respect to sample volume

Sample volume [µL]	Level 1 [0 ppm]	Level 2 [15 ppm]	Level 3 [250 ppm]
100	1/1	6/6	8/8
200	1/1	6/6	8/8
300	1/1	6/6	8/8
400	1/1	6/6	8/8
500	1/1	6/6	8/8
600	1/1	6/6	8/8
700	1/1	6/6	8/8
800	1/1	6/6	8/8
1000	1/1	6/6	8/8

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

Signals for positive samples remain constant between 100-1000 µL sample volume.

Thus, sample volume yields constant results for negative and positive samples ranging from 100 to 1000 µL.

Table 9: Robustness of the SENSIStrip Mustard LFD with respect to time to evaluation

Time to evaluation [min]	Level 1 [0 ppm]	Level 2 [15 ppm]	Level 3 [250 ppm]
2	1/1	4/4	7/7
5	1/1	6/6	8/8
10	1/1	7/7	8/8
15	1/1	7/7	9/9
30	1/1	7/7	9/9
60	1/1	7/7	8/8
120	1/1	7/7	8/8
1200	1/1	7/7	8/8

Time to evaluation does not affect the accuracy of negative samples up to 1200 minutes.

After 5 minutes time to evaluation signals of positive samples reach equilibrium and do not change up to 1200 minutes.

Thus, time to evaluation yields constant results for negative and positive samples ranging from 5 to 1200 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 SENSIStrip Mustard LFD

User	Level 1 [0 ppm]	Level 2 [2.5 ppm]	Level 3 [5 ppm]	Level 4 [10 ppm]	Level 5 [20 ppm]	Level 6 [40 ppm]
1	1/1/1	3/3/3	5/5/5	6/6/6	6/6/6	7/7/7
2	1/1/1	3/3/3	6/6/6	7/7/7	8/8/8	8/8/8
3	1/1/1	4/4/3	6/6/6	7/6/7	8/8/8	9/9/8

LOD was defined as the lowest concentration which was correctly assessed as a value of ≥ 3 . Thus, LOD is 2.5 ppm.

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 Mustard LFD

Level [ppm]	Intensity
0	1/1
5	5/5
10	6/6
25	7/7
50	8/8
100	8/8
250	9/9
500	9/9
1000	9/9
2500	9/9

Level [ppm]	Intensity
5000	8/8
10000	7/7
25000	7/7
50000	6/6
100000	5/5
250000	2/2

Signals are constant up to 5000 ppm indicating no High-Dose-Hook Effect. Thus, with LOD as determined according to 3.3. dynamic range is defined as 2.5-5000 ppm.

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 Mustard LFD

Matrix	Level 1 [0 ppm]	Level 2 [2.5 ppm]	Level 3 [5 ppm]	Level 4 [10 ppm]	Level 5 [20 ppm]	Level 6 [40 ppm]	Level 7 [80 ppm]
Pea	1/1	3/3	4/4	5/5	6/5	6/6	8/8
Garlic	1/1	2/2	3/3	5/4	5/5	5/5	6/6
Sausage	1/1	2/2	3/3	4/4	5/5	6/6	6/6
Vegetable soup	1/1	2/2	3/3	4/4	5/5	6/6	7/7
Herb mix	1/1	3/3	5/5	6/5	6/6	7/7	7/7
Lentil	1/1	2/2	3/3	4/4	5/5	6/6	6/6
Soy sauce	1/1	2/2	3/3	4/4	5/6	6/6	6/6
Spice Mix	1/1	2/1	3/3	4/4	5/5	5/5	5/5
Wheat	1/1	2/2	3/3	4/4	5/5	6/6	7/7
Cheese	1/1	3/3	4/4	5/5	6/6	7/7	7/8
Herbs dressing	1/1	3/3	4/4	5/5	6/6	7/7	7/8
Yoghurt dressing	1/1	3/3	4/4	5/5	6/6	7/7	7/7

Matrix-dependent analytical sensitivity is ranging from 2.5 to 5 ppm.

3.5.2. Heat-treated Matrices

To exemplarily assess changes in analytical sensitivity for processed food samples, a soup matrix was spiked with 80 ppm of mustard 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 Mustard LFD

Matrix	Level 1 [0 ppm]	Level 2 [2.5 ppm]	Level 3 [5 ppm]	Level 4 [10 ppm]	Level 5 [20 ppm]	Level 6 [40 ppm]	Level 7 [80 ppm]
Soup / unprocessed	1/1	3/3	5/5	6/6	7/7	8/8	8/8
Soup / processed	1/1	1/2	2/2	2/3	3/3	4/4	5/5

Analytical sensitivity for the heat-treated matrix decreases from 2.5 to 10 ppm.

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 Mustard LFD

Sample description	Expectancy evaluated by	Expectancy [Mustard ppm]	Intensity
Cumin	ELISA	3.6	6/5
Psyllium husk	ELISA	92	6/5
Sauce, already made	ELISA	15	2/2
Sauce, for frying	ELISA	14	1/1
Psyllium husk	ELISA	>30	4/4
Liver meat loaf	PCR	positive	7/7
Wiener sausage	PCR	positive	7/7
Red curry paste	PCR	positive	5/4
Massaman vegan sauce	PCR	positive	1/1
Plant protein	ELISA	<2.5	1/1
Pea protein	ELISA	<2.5	1/1
Instant food	ELISA	<2.5	1/1
Cumin	ELISA	<2.5	1/1
Lentilles corail	ELISA	<2.5	1/1
Baking mix	ELISA	<2.5	1/1
Sauce, already made	ELISA	<2.5	1/1
Field bean	ELISA	<2.5	1/1

Sample description	Expectancy evaluated by	Expectancy [Mustard ppm]	Intensity
Pork steak	ELISA	<2.5	1/1
Pork barbecue bacon	ELISA	<2.5	1/1
CIP water	ELISA	<2.5	1/1
Yellow lentil	ELISA	<2.5	1/1
Mountain lentil	ELISA	<2.5	1/1
Minced meat	PCR	negative	1/1
Durum semolina	PCR	negative	1/1
Wheat bran	PCR	negative	2/1
Wheat whole-grain flour	PCR	negative	6/6 ¹⁾
Wheat flour	PCR	negative	7/6 ¹⁾
Semolina	PCR	negative	4/4 ¹⁾
Muffin mix	PCR	negative	3/3 ¹⁾

¹⁾The positive results could be assumed as a contamination of cross-reacting rape seed and/or charlock mustard due to agricultural cultivation according to 3.5.5 and 3.7

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 Mustard LFD applied on Proficiency Test material

Source	Sample description	Concentration, according provider [ppm]	Intensity
DLA 04/2016	Soup powder, Sample A	50	6
DLA 04/2016	Soup powder, Sample B	0	1
DLA 04/2017	Sausage meat, Sample A	372	6
DLA 04/2017	Sausage meat, Sample B	0	1
DLA 04/2018	Potato chips, Sample A	0	1
DLA 04/2018	Potato chips, Sample B	98	7
DLA 04/2019	Spice salt, Sample A	49	5
DLA 04/2019	Spice salt, 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

Mustard 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 yellow mustard. To assess the reactivity towards other variants, those were extracted and diluted to various levels going down to LOD as stated in chapter 3.3.

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

Variant	Level 1 [2.5 ppm]	Level 2 [5 ppm]	Level 3 [10 ppm]	Level 4 [20 ppm]	Level 5 [40 ppm]	Level 6 [80 ppm]
Yellow mustard	3/3	3/3	4/5	6/6	7/7	7/7
Black mustard	2/2	3/3	5/5	5/5	6/6	6/7
Brown mustard	2/2	3/3	4/3	5/5	6/6	7/6
Charlock mustard	2/2	4/3	4/4	6/6	7/7	7/7

Variant-dependent analytical sensitivity is ranging from 2.5 to 5 ppm.

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 Mustard LFD

Sample description	Level 1 [0 µg/cm ²]	Level 2 [0.07 µg/cm ²]	Level 3 [0.20 µg/cm ²]	Level 4 [0.67 µg/cm ²]
Buffer	1/1	3/3	5/5	8/8
	1/1	3/3	5/5	7/8
Herb mix	1/1	3/3	6/6	7/7
	1/1	3/3	6/6	7/7
Vegetable soup	1/1	4/4	6/6	8/8
	1/1	4/4	6/6	8/8
Sausage	1/1	3/3	6/6	8/8
	1/1	3/3	5/5	8/8

Dependent on the sample matrix, swab samples can be detected as low as 0.07 µg/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 mustard 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 Mustard LFD for Rinse Solutions

Rinse solution	Level 1 [0 mg/L]	Level 2 [0.17 mg/L]	Level 3 [0.33 mg/L]	Level 4 [0.67 mg/L]	Level 5 [1.33 mg/L]	Level 6 [2.67 mg/L]
Pure water	1/1	3/3	5/5	6/6	7/7	8/8
2% NaOH	1/1	3/3	3/3	4/4	5/5	7/7
0.5% Citric acid	1/1	3/4	3/3	4/5	6/7	7/7
0.6% nitric acid	1/1	2/2	4/4	5/5	6/6	7/7
0.5% sodium tripolyphosphate	1/1	2/2	4/4	5/5	6/6	7/7

LOD for rinse solutions was defined as the lowest concentration which was correctly assessed as a value of ≥ 3 . Thus, LOD for pure water, 2% NaOH and 0.5% citric acid is 0.17 mg/L and LOD for 0.6% nitric acid and 0.5% sodium tripolyphosphate solution is 0.33 mg/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 Mustard LFD

Commodity	Intensity	Commodity	Intensity	Commodity	Intensity
Adzuki bean	1/1	Curcuma	1/1	Peach	1/1
Almond	1/1	Dill	2/2	Peanut	1/1
Apricot	1/1	Duck	1/1	Pecan	1/1
Barley	1/1	Ewe's milk	1/1	Pepper	1/1
Bean, white	1/1	Fennel	1/1	Pine seed	1/1
Beef	1/1	Fenugreek	1/1	Pistachio	1/1
Bovine gelatin	1/1	Flaxseed	1/1	Poppy seed	1/1
Brazil nut	1/1	Garden cress	1/1	Pork	1/1
Buckwheat	1/1	Garlic	1/1	Potato	1/1
Caraway	1/1	Gliadin	1/1	Prawn, cooked	1/1
Cardamom	1/1	Goat's milk	1/1	Pumpkin seed	1/1

Commodity	Intensity	Commodity	Intensity	Commodity	Intensity
Carob bean	1/1	Guar gum	1/1	Radish	1/1
Carrot	2/1	Hazelnut	1/1	Rice	1/1
Cashew	1/1	Horseradish	1/1	Rye	1/1
Cayenne	1/1	Kidney bean	1/1	Sesame	1/1
Celery	1/1	Kiwi	1/1	Shrimp	2/2
Cherry	1/1	Lamb	1/1	Soy flour	2/1
Chia	1/1	Leek	1/1	Soy lecithin	1/1
Chicken	1/1	Lentil	1/1	Soy milk	1/1
Chickpea	1/1	Lupin	1/1	Split peas	1/1
Chili	1/1	Macadamia	1/1	Sucrose	1/1
Cinnamon	1/1	Marone	1/1	Sunflower seed	1/1
Clove	1/1	Nutmeg	1/1	Thyme	2/1
Cocoa	1/1	Oats	1/1	Tomato	1/1
Coconut	1/1	Onion	1/1	Turkey	1/1
Cod	1/1	Oyster	1/1	Walnut	1/1
Corn	1/1	Paprika	1/1	Wheat	1/1
Cow's milk	1/1	Pea	1/1	White cabbage	1/1
Cumin	1/1				

With LOD = 2.5 ppm (see 3.3.) for all commodities no cross-reactivity could be determined.

Since being biological related to mustard and representing a frequent source of contamination due to agricultural cultivation rape see was tested according the chapter 3.5.5.

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

Commodity	Concentration [ppm]	Intensity	Cross-reactivity
Rape seed	10000	5/5	3.1%
	1000	5/5	
	100	4/4	
	80	3/3	
	40	2/2	
	10	1/1	