

Validation Report

SENSIStrip Peanut LFD

(Cat. No. HU0030128)

Version 2

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1. Scope

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

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

Replicate	Level 1 [0 ppm]	Level 2 [15 ppm]	Level 3 [250 ppm]
16	1	6	8
17	1	6	8
18	1	6	8
19	1	6	8
20	1	7	8
Mean	1.0	6.3	8.0

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 Peanut LFD

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

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 Peanut LFD

Day	Level 1 [0 ppm]	Level 2 [15 ppm]	Level 3 [250 ppm]
1	1/1	6/6	8/8
2	1/1	6/6	9/9
3	1/1	7/7	9/9
4	1/1	7/7	9/9
Mean	1.0	6.5	8.8

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 Peanut LFD

User	Level 1 [0 ppm]	Level 2 [15 ppm]	Level 3 [250 ppm]
1	1/2	6/7	9/9
2	1/1	7/7	8/9
3	1/1	7/7	9/9
Mean	1.2	6.8	8.8

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 Peanut 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	8/8
2	1/1	6/6	9/9
3	1/1	7/7	9/9
4	1/1	6/7	9/9
5	1/1	7/7	9/9
6	1/1	7/7	9/9
7	1/1	7/7	9/9
10	1/2	7/7	9/9
15	1/1	7/7	9/9

1. Incubation time [min]	Level 1 [0 ppm]	Level 2 [15 ppm]	Level 3 [250 ppm]
20	2/3	8/8	9/9

With respect to the negative sample no visible lines are found up to 7 minutes 1. incubation time. False positive results cannot totally be excluded after 20 minutes.

After 3 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 3 to 7 minutes.

Table 6: Robustness of the SENSIS*Strip* Peanut 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	5/5	9/9
2	1/1	6/6	9/9
3	1/1	7/6	9/9
4	1/1	7/7	9/9
5	1/1	8/8	9/9
6	1/1	7/8	9/9
7	1/1	8/8	9/9
10	2/3	8/8	9/9
15	3/3	8/8	9/9
20	4/3	8/8	9/9

With respect to the negative sample no visible lines are found up to 7 minutes 2. incubation time. False positive results cannot totally be excluded after 10 minutes.

After 4 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 4 to 7 minutes.

Table 7: Robustness of the SENSIS*Strip* Peanut 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	7/6	8/8
25	1/1	7/7	9/9

Incubation temperature [°C]	Level 1 [0 ppm]	Level 2 [15 ppm]	Level 3 [250 ppm]
30	1/1	6/6	8/8
37	1/1	6/6	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 SENSIS*Strip* Peanut LFD with respect to sample volume

Sample volume [µL]	Level 1 [0 ppm]	Level 2 [15 ppm]	Level 3 [250 ppm]
100	1/1	6/7	8/8
200	1/1	7/7	9/9
300	1/1	7/6	9/9
400	2/1	7/8	9/9
500	1/1	7/7	9/9
600	1/1	6/7	9/9
700	1/1	5/5	8/9
800	1/1	5/5	8/8
1000	1/1	1/1	1/1

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

At 100 µL sample volume signals of positive samples reach equilibrium and do not change up to 600 µL.

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

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

Time to evaluation [min]	Level 1 [0 ppm]	Level 2 [15 ppm]	Level 3 [250 ppm]
0	1/1	5/6	9/9
2	1/1	5/5	9/9
5	1/1	6/6	9/9
10	1/1	7/6	9/9
15	1/1	7/7	9/9

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

With respect to the negative sample no visible lines are found up to 30 minutes time to evaluation. False positive results cannot totally be excluded after 60 minutes.

Signals for positive samples remain constant between 0-10 minutes time to evaluation.

Thus, time to evaluation yields constant results for negative and positive samples ranging from 0 to 10 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 Peanut 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	3/3/3	6/6/6	7/7/7	8/8/8
2	1/1/1	3/3/3	5/5/5	6/6/6	7/7/7	8/8/8
3	1/1/1	2/3/3	4/4/4	6/6/6	6/7/7	8/8/8

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 Peanut LFD

Level [ppm]	Intensity
0	1/1
5	6/6
10	7/7
25	8/8
50	8/8
100	8/8
250	8/8
500	8/9
1000	9/9
2500	9/9
5000	9/9
10000	9/9
25000	9/9
50000	8/8
100000	7/7
250000	7/7

Signals are constant up to 50000 ppm indicating no High-Dose-Hook Effect. Thus, with LOD as determined according to 3.3. dynamic range is defined as 2.5-50000 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 Peanut 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]
Cookie (TUC)	1/2	4/4	5/5	6/6	7/7	8/8	8/8
Biscuit	1/1	3/4	5/5	6/6	6/7	7/7	8/8
Shortbread	1/1	4/4	5/5	6/6	7/7	8/8	8/8
Ice cream	1/2	4/4	6/6	6/7	7/7	8/8	8/8
Chocolate ¹⁾	1/1	3/3	3/3	3/3	4/4	4/4	4/4
Garlic, grained	1/1	4/4	5/6	7/6	7/7	8/8	8/8
Garlic, fresh	1/1	3/4	5/5	7/7	7/7	8/8	8/8

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]
Spices (Paprika)	1/2	3/3	4/4	5/5	6/7	7/7	8/8
Milk	1/1	3/3	4/4	5/6	5/7	7/7	7/8
Muesli	1/1	3/3	4/5	6/6	7/7	7/7	7/8

¹⁾ addition of extraction additive for polyphenol containing samples

Irrespective of the peculiar matrix, LOD as determined according to chapter 3.3. was confirmed. Special care has to be taken for chocolate where extraction additive was added to improve the extraction.

3.5.2. Heat-treated Matrices

To exemplarily assess changes in analytical sensitivity for processed food samples, a cookie matrix was spiked with 80 ppm of peanut and divided in two parts. One part was baked at 150 °C for 20 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 Peanut 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]
Cookie / unprocessed	1/1	3/3	4/3	6/5	7/6	7/6	7/7
Cookie / processed	1/1	2/2	2/3	4/3	4/4	5/5	6/6

Analytical sensitivity for the heat-treated matrix decreases from 2.5 to 5 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 Peanut LFD

Sample description	Expectancy evaluated by	Expectancy [Peanut ppm]	Intensity
Garlic. dried	ELISA	<0.2	1/1
Chili/Cayenne	ELISA	<0.2	1/1
Sweets in chocolate	ELISA	<0.2	1/1
Coconut concentrate	ELISA	<0.2	2/2

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Sample description	Expectancy evaluated by	Expectancy [Peanut ppm]	Intensity
Food. not specified	ELISA	<0.2	2/2
Food. not specified	ELISA	<0.2	1/1
Curcuma	ELISA	<0.2	1/1
Paprika	ELISA	<0.2	1/1
Garlic. dried	ELISA	<0.2	1/1
Plant protein	ELISA	<0.2	1/1
Rose hip. dried	ELISA	<0.2	2/2
Peanut oil	ELISA	<0.2	1/1
Oil seed/Mustard seed	ELISA	<0.2	1/1
Cumin	ELISA	<0.2	1/1
Process water	ELISA	<0.2	1/1
Hazelnut Paste	ELISA	<0.2	1/1
Paprika	ELISA	<0.2	1/1
Chili/Cayenne	ELISA	<0.2	2/2
Gingseng. extract	ELISA	<0.2	1/1
Parsley. dried	ELISA	<0.2	2/2
Bread	ELISA	<0.2	1/1
Onion. dried	ELISA	<0.2	2/2
Oil seed/Mustard seed	ELISA	<0.2	1/1
Potato product	ELISA	<0.2	1/1
Cocoa compound	ELISA	<0.2	1/1
Cocoa creme	ELISA	7.3	5/5
Garlic. dried	ELISA	1.3	6/6
Cocoa creme	ELISA	21	5/5
Sweets in chocolate	ELISA	11	5/5
Garlic powder	ELISA	4.1	7/7
Garlic. dried	ELISA	0.94	4/4
Garlic. dried	ELISA	7.7	6/6
Cumin	ELISA	1.1	1/1
Chili/Cayenne	ELISA	0.53	1/1
Food. not specified	ELISA	2.9	2/3
Food. not specified	ELISA	1.2	2/3

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.

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

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

Source	Sample description	Concentration, according provider [ppm]	Intensity
DLA 05/2014	Bakery products, sample A	123	7/7
DLA 05/2014	Bakery products, sample B	0	2/1
DLA 05/2016	Butter cookie, sample A	0	1/2
DLA 05/2016	Butter cookie, sample B	10	7/7
DLA 05/2017	Potato powder, unroasted peanut	29	8/8
DLA 05/2017	Potato powder, roasted peanut	32.2	8/8
DLA 05/2017	Potato powder, peanut butter	26.3	7/7
DLA 05/2017	Potato powder, Peanut paste	32.7	7/7
DLA 05/2017	Potato powder, Peanut extrudate	32.5	7/7
DLA 05/2017	Potato powder, negative	0	1/1
DLA 16/2017	Peanut in cookie matrix	0	1/2
DLA 16/2017	Peanut in cookie matrix	0.51	2/2
DLA 16/2017	Peanut in cookie matrix	2.52	4/3
DLA 16/2017	Peanut in cookie matrix	5.05	5/5
DLA 16/2017	Peanut in cookie matrix	12.6	7/6
DLA 16/2017	Peanut in cookie matrix	25.3	7/7
DLA 07/2017	Soup powder, sample A	14.4	5/6
DLA 07/2017	Soup powder, sample B	0	2/2
DLA 05/2018	Butter cookie, sample A	0	1/1
DLA 05/2018	Butter cookie, sample B	32	7/7
DLA 06/2019	Cocoa cream, sample A	0	1/1
DLA 06/2019	Cocoa cream, sample B	37.5	7/7

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

Peanut might occur in various variants due to industrial processing. Anyhow, in creating the immunological components of the test device only one of those can be considered. In

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the peculiar case antibodies are derived against unroasted peanut. 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 Peanut LFD

Variant	Level 2 [2.5 ppm]	Level 3 [5 ppm]	Level 4 [12.5 ppm]	Level 5 [25 ppm]	Level 6 [50 ppm]	Level 7 [100 ppm]
Peanut, unroasted	3/3	4/4	5/5	6/6	7/7	7/7
Peanut, roasted at 150°C, 30 min	2/2	3/3	3/3	4/4	5/5	6/6

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 Peanut 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	4/4	7/7
	1/1	3/3	4/4	6/6
Cookie (TUC)	1/1	2/3	3/4	7/7
	1/1	3/3	4/4	7/7
Ice Cream	1/1	3/3	5/5	7/7
	1/1	3/3	5/5	6/7
Chocolate	1/1	3/3	4/4	6/6
	1/1	3/3	3/4	6/6

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 peanut 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 Peanut 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	6/6	7/7
2% NaOH	1/1	2/2	3/3	4/4	5/5	6/6
0.5% Citric acid	1/1	2/3	5/5	6/6	7/7	7/7
0.6% nitric acid	1/1	2/3	4/4	5/5	6/6	7/7
0.5% sodium tripolyphosphate	1/1	3/3	5/5	5/6	6/7	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, 0.5% citric acid, 0.6% nitric and 0.5% sodium tripolyphosphate solution is 0.17 mg/L and LOD for 2% NaOH 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 Peanut LFD

Commodity	Intensity	Commodity	Intensity	Commodity	Intensity
Almond	1/1	Cow's milk	1/1	Oyster	1/1
Adzuki bean	1/1	Cumin	1/1	Paprika	1/1
Apple	1/1	Curcuma	1/1	Pea	1/1
Apricot	1/1	Dill	1/1	Peach	1/1
Barley	1/1	Dried milk	1/1	Pecan nut	1/1
Bean, white	1/1	Duck	1/1	Pepper	1/1
Beef	1/1	Ewe's milk	1/1	Pine nut	1/1
Bell pepper	1/1	Fennel	1/1	Pistachio	1/1
Brazil nut	1/1	Flaxseed	2/2	Poppy seed	1/1
Buckwheat	1/1	Garden cress	1/1	Pork	1/1
Caraway	1/1	Garlic	1/1	Potato	1/1
Cardamom	1/1	Gelatin	1/1	Pumpkin seed	1/1
Carob bean	1/1	Gliadin	1/1	Radish	1/1
Carrot	1/1	Goat's milk	1/1	Rice	1/1
Cashew	5/5	Guar gum	2/2	Rye	1/1

Commodity	Intensity	Commodity	Intensity	Commodity	Intensity
Cayenne	1/1	Hazelnut	1/1	Sesame	1/1
Celery	1/1	Horseradish	1/1	Shrimp	1/1
Cherry	1/1	Kidney bean	2/2	Soy flour	1/1
Chestnut	1/1	Kiwi	1/1	Soy lecithin	1/1
Chia	1/1	Lamb	1/1	Soy milk	1/1
Chicken	1/1	Leek	1/1	Split peas	1/1
Chickpea	1/1	Lentil	1/1	Sucrose	1/1
Chili	1/1	Lupin	1/1	Sunflower seed	1/1
Cinnamon	1/1	Macadamia nut	1/1	Thyme	2/2
Clove	1/1	Milk powder	1/1	Tomato	1/1
Cocoa	1/1	Mustard, yellow	1/1	Turkey	1/1
Coconut	1/1	Nutmeg	1/1	Walnut	1/1
Cod	1/1	Oats	1/1	Wheat	1/1
Corn	1/1	Onion	1/1	White cabbage	1/1

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

For cashew additional dilutions of the extract were tested. Cross-reactivity was calculated considering concentration and LOD.

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

Commodity	Concentration [ppm]	Intensity	Cross-reactivity
Cashew	1 M	6/6	0.004%
	500 K	5/5	
	250 K	5/5	
	125 K	3/3	
	62.5 K	3/3	
	31.3 K	2/2	
	15.6 K	1/1	