NMKL - NordVal International
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NordVal International Certificate

Issued for:	LactoSens®R
NordVal No:	048
First approval date:	5 February 2018
Extension date:	7 March 2021
Valid until:	7 March 2023

LactoSens®R

Manufactured by:
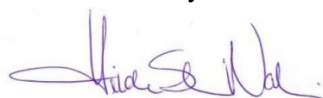**DirectSens GmbH**
Am Rosenbühel 38
3400 Klosterneuburg
Austria

fulfils the requirements of the NordVal Validation Protocol 2. The performance of the LactoSens®R assay was validated to demonstrate conformity with AOAC SMPR 2018.009 "Standard Method Performance Requirements (SMPR) for Lactose in Low-Lactose or Lactose-Free Milk, Milk Products, and Products Containing Dairy Ingredients". LactoSens®R was compared against the method "High-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD)". LactoSens®R is adopted as an AOAC method, AOAC 2020.01, and published in J AOAC Int. 2020;103(6):1534-1546. The method validation was carried out by the Austrian Agency for Health and Food Safety (AGES).

The results obtained are satisfactory and document that there is no statistical difference in the performances between the methods for lactose in milk and milk products (white mass and flavoured products) for levels at or above 0.008%.
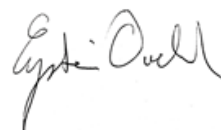
LactoSens® biosensor production is ISO 9001:2015 certified by TÜV NORD Austria.

Date: 3 February 2021

Yours sincerely,

Hilde Skår Norli
Chair of NordVal International

Eystein Oveland
NMKL Secretary General

PRINCIPLE OF THE METHOD:

DirectSens® has developed a biosensor for the determination of residual lactose in lactose-free milk products. An enzyme immobilized on a disposable test strip can detect lactose directly and accurately using the LactoSens® Reader with sensors from the LactoSens®R Biosensor Assay Kit (LK1225). The electrochemical measurement is based on a principle called amperometry. The highly specific enzyme on the biosensor oxidizes the lactose molecules in the sample. Resulting electrons are conducted to the LactoSens® Reader and the software transfers the analytical signal into a lactose concentration according to the calibration function. Samples with a fat content ≥ 20% must be centrifuged for 10 min. at 10,000 rpm, analysis performed on the aqueous phase, and the reading result multiplied with the dilution factor.

FIELD OF APPLICATION:

Milk and milk products. Validation only for cow/bovine milk.

HISTORY:

In 2019, the certificate for LactoSens® 0.01% method was extended to include milk samples with a fat content up to 36% and fermented milk products; yoghurt, cream, sour cream and curd. Further, it was evaluated that the sensor also is applicable to lactose-free flavoured yoghurts and milk drinks (coffee, cacao, fruits). Finally, it was evaluated that the sensors are stable for at least 3 days storage at 30ºC. By the time of extension, the name of the method was changed to LactoSens®R.

METHOD COMPARISON STUDY

The validation study was carried out by the expert laboratory AGES Linz. Unless otherwise stated, the measurement has been performed five times on the same sample.

RUGGEDNESS

The ruggedness of the method was tested by repeatedly analysing sample with lactose at various

- measurement temperatures (20ºC and 24ºC)
- sample temperatures (4ºC, 20ºC and 40ºC)
- fat content of the milk (0.8 – 36%)
- pH and texture

Table 1: The samples tested, their pH range and fat content

Sample	pH range	Fat content (%)
Milk/Cream		0.8 - 36
Yoghurt	4.4-4.6	0.1 - 3.5
Sour cream	4.3-4.4	15
Curd	3.8-3.9	20

The obtained relative standard deviations, RSD, of the measurements were no more than 9%

and the recovery were 93% and above. The results of the extension study, documenting that the LactoSens®R method is applicable to milk samples with a fat content up to 36% and fermented milk products; yoghurt, cream, sour cream and curd, are given in Table 2.

Table 2: The samples and results of yoghurt, cream, sour cream and curd

Sample	Fat content	pH	Alternative method				Reference method (% lactose)
			Mean (% lactose)	SD (%)	RSD (%)	Recovery (%)	
Cream	10	-	<0.008	-	-	-	<0.008
	10	-	0.066	0.002	4	99	0.066
	22	-	<0.008	-	-	-	<0.008
	22	-	0.060	0.001	2	93	0.064
	36	-	<0.008	-	-	-	<0.008
	36	-	0.068	0.002	3	95	0.071
Yoghurt	0.1	4.4	0.011	0.001	9	98	0.014
	0.1	4.5	0.033	0.001	3	108	0.031
	1	4.5	0.046	0.004	9	101	0.046
	1.8	4.6	0.013	0.001	9	98	0.014
	2.5	4.5	<0.008	-	-	-	<0.008
	3.5	4.6	0.092	0.003	3	108	0.085
Sour cream	15	4.3	<0.008	-	-	-	<0.008
	15	4.4	<0.008	-	-	-	<0.008
	15	4.3	<0.008	-	-	-	<0.008
Curd	20	3.9	<0.008	-	-	-	<0.008
	20	3.8	<0.008	-	-	-	<0.008
	20	3.9	<0.008	--	-	-	<0.008

The results showed that the method is rugged for the parameters tested.

SPECIFICTY
In order to check for interferences, samples with lactose content of 0.022% were spiked with different sugars; glucose, galactose, sucrose and fructose. The content of the spiked sugars were 1% and 2%, respectively. The study showed that there were no cross-reactivity interferences from the added sugars.

Further, the specificity was tested for vitamins. Milk samples, with lactose content of less than 0.010%, were fortified with Vitamin A (5 mg/L), Vitamin D (0.045 mg/L), Vitamin B6 (12 mg/L) and Vitamin E (60 mg/L). The results showed that the vitamins did not influence on the measurement of lactose content.

Interferences from flavours and added fruit preparations were also tested. The tested flavours were coffee, vanilla, cocoa at concentrations 0.3-1% and banana, peach/apricot, strawberry and cherry at concentrations 17 or 18%. No interferences were observed from flavours or fruit preparations.

STABILITY TESTING AND BATCH-TO-BATCH VARIATION

Sensors stored at 4ºC regularly measured with the samples containing various concentration of lactose showed that the sensors are stable for at least 12 months.

During production of LactoSens® biosensors every critical step is strictly monitored, and the final product is tested according to quality control requirements. Every batch of biosensors is factory calibrated to ensure absolutely repeatability of lactose determination between the batches.

Storage at 30°C for three days was tested and showed recoveries of 95-100%, indicating that sensors are stabile for at least 3 days at 30°C.

LIMIT OF QUANTIFICATION (LOQ)
The minimum level at which lactose can be quantified with acceptable precision and recovery was determined by using the reference method and the alternative method, analysing different levels of lactose. The results are given in Table 3.

Table 3: Determination of LOQ

Sample	Reference method		Alternative method		
	N	Mean ± SD (%)	N	Mean ± SD (%)	Recovery (%)
1	2	<0.005	5	<0.008	-
2	2	0.0077 ± 0.002	5	0.008 ± 0.000	104
3	2	0.0088 ± 0.001	5	0.008 ± 0.001	91
4	2	0.011 ± 0.002	20	0.012 ± 0.001	109
5	1	0.012	5	0.011 ± 0.001	92
6	2	0.0225 ± 0.002	5	0.022 ± 0.002	96
7	2	0.032 ± 0.005	5	0.031 ± 0.003	97
8	2	0.062 0.006	5	0.064 ± 0.003	103

N = number of replicates
The lowest level tested with satisfactory precision (SD) and accuracy (recovery) is 0.008% lactose content. Values below 0.0075% are displayed as <0.008% by the LactoSens® software. All other values are shown as in the table.

The lowest level tested with satisfactory precision (SD) and accuracy (recovery) is 0.008% lactose content.

RELIABILITY: PRECISION - INTERNAL REPRODUCIBILITY
The reliability of the method was tested by including milk samples at different concentration levels. Each sample was measured 5 times and repeated on 3 different days. The relative standard deviation of the repeatability, RSD, for each day and the relative standard deviation of the internal standard deviation RSDIR for the results obtained at different days along with the HorRat values were calculated. A HorRat value of no more than 2 is considered acceptable. Table 4 shows a summary of the results.

Table 4: Results of 3 different samples with different lactose concentrations levels over 3 days.

Sample (% lactose)	Day	Mean (%)	SD (%)	RSD (%)	Mean (%)	RSD$_{IR}$ (%)	HorRat	Recovery (%)
Milk (0.023)	1	0.022	0.001	5.93	0.022	6.95	0.97	98
	2	0.023	0.001	3.82				
	3	0.022	0.002	9.44				
Milk (0.062)	1	0.064	0.003	4.69	0.063	6.61	1.10	102
	2	0.061	0.005	8.99				
	3	0.064	0.003	4.37				
Milk (0.128)	1	0.132	0.007	5.10	0.128	4.85	0.88	103
	2	0.128	0.005	4.16				
	3	0.137	0.002	1.73				

The results show that the precision as well as the accuracy are satisfactory.

INTERMEDIATE STUDY

An intermediate study was performed at a second independent expert laboratory: Landwirtschaftliches Zentrum Baden-Wûrttemberg – Milchwirtschaft Wangen (Germany). Samples of milk, yoghurt, sour cream and curd at different levels were analysed. The results are given in Table 5.

Table 5: Results of the intermediate study

Sample	(%) lactose by reference method	N	Mean (%) lactose by LactoSens®	RSD (%)	Recovery (%)
Milk	0.005	5	<0.008	-	-
	0.011	5	0.012	5.9	109
	0.023	5	0.021	5.9	91
Yoghurt	0.085	3	0.083	3	97
	0.028	3	0.031	3	91
	<0.008	3	<0.008	-	-
Sour cream	<0.008	3x3	<0.008	-	-
Curd	<0.008	3x3	<0.008	-	-

CONCLUSION

The validation study shows that the LactoSens®R performs satisfactorily for lactose levels of 0.008% and above with equivalent results to the reference method.