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NordVal International Certificate

Issued for:	LactoSens® 0.01%
NordVal No:	048
First approval date:	15 February 2018
Valid until:	15 February 2020

LactoSens® 0.01%

Manufactured by:
DirectSens GmbH
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3400 Klosterneuburg
Austria

fulfils the requirements of the NordVal Validation Protocol 2. The performance of the LactoSens® 0.01% assay has been compared against the following reference method:

ISO 22662 IDF 198 Milk and milk products – Determination of lactose content by HPLC.

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) for levels at or above 0.01%.

Date: 15 February 2018

Yours sincerely

Hilde Skår Norli
Chair of NordVal International

Nina Skall Nielsen
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 (LR01) with sensors from the LactoSens® Test Kit (LK0225). 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.

FIELD OF APPLICATION:

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

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 content of 0.022% at various

- ambient temperatures (20ºC and 24ºC)
- sample temperatures (4ºC, 20ºC and 40ºC)
- fat content of the milk (0,8 – 3,5%)

The obtained relative standard deviations, RSD, of the measurements were no more than 8.6% and the recovery were 93% and above. 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.

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.

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 1.

Table 1: Determination of LOQ

	Reference method		**Alternative method**		
Sample	**N**	**Mean ± SD (%)**	**N**	**Mean ± SD (%)**	**Recovery (%)**
MIN28	2	<0.005	5	<0.001	-
MIN16	2	0.0077 ± 0.002	5	0.008 ± 0.000	104
M69	2	0.0088 ± 0.001	5	0.008 ± 0.001	91
M70	2	0.011 ± 0.002	20	0.012 ± 0.001	109
M74	1	0.012	5	0.011 ± 0.001	92
M37	2	0.0225 ± 0.002	5	0.022 ± 0.002	96
MH41S11	2	0.032 ± 0.005	5	0.031 ± 0.003	97
OH41S7	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 0.0095% are displayed as <0.01% by the LactoSens software. All other values are shown as in the table.

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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 RSD_{IR} 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 2 shows a summary of the results.

Table 2: Results of 3 different samples with different concentrations levels over 3 days.

Sample	**Day**	**Mean (%)**	**SD (%)**	**RSD (%)**	**Mean (%)**	**RSD_{IR} (%)**	**HorRat**	**Recovery (%)**
M37 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				
OH41S7 0.062%	1	0.064	0.003	4.69	0.063	6.61	1.1	102
	2	0.061	0.005	8.99				
	3	0.064	0.003	4.37				
MH41S8 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 another expert laboratory; LZBW Wangen (Germany). Three samples at different levels were analysed using five replicates. The results are given in Table 3.

Table 3: Results of the intermediate study

Sample	Ref. value (%)	N	Mean (%)	RSD (%)	Recovery (%)
MIN28	0.005	5	-	-	-
M70	0.011	5	0.012	5.9	109
M37	0.023	5	0.021	5.9	91

CONCLUSION

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