



<b>Guarantee/quality Control Milk and Foodstuffs Division</b>		<b>TITLE: CDR FoodLab Validation Test for the Determination of NH<sub>3</sub> in Whole Milk and Skim Milk</b>
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Whole milk

$$Y = 32.9200000 (\pm 0.0000002) X + 3.16700 (\pm 0.00001)$$

Skim milk

$$Y = 29.544 (\pm 0.001) X - 1.79 (\pm 0.06)$$

It is advisable to repeat the calibration each time the production lot of cuvette test and **R2** reagent are changed.

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## 5 – HOMOGENEITY OF THE VARIANCE

Before checking that the experimental points obtained are interpolated linearly, it is necessary to carry out a homogeneity test of the variance. One of the homogeneity of the variance that is not homogenous could, apart from being completely imprecise, create inaccuracy caused by the possible change of the inclination of the curve and therefore the sensitivity. Via the Hartley test it is possible to compare the relative variance at the points.

As can be observed in tables 1 and 2, the value of the  $F_{calc}$  is less than the value of the  $F_{tab}$ . For both straight lines, it is necessary to check that homogeneity of the variances is satisfactory.

**Table 1.** Homogeneity of the variance for whole milk

$F_{calc}$	$F_{tab}^a$
5.0625	161
<sup>a</sup> Significance = 5% degrees of freedom = 1.1	

**Table 2.** Homogeneity of the variance for skim milk

$F_{calc}$	$F_{tab}^a$
7.96594176	161
<sup>a</sup> Significance = 5% degrees of freedom = 1.1	

## 6 – LINEARITY

A preliminary study was also conducted on the linearity. Particular importance was attributed to the analysis of residues. By residues it is meant the difference between the true value and the estimated value via the model. If the model is adequate, the residues must derivate exclusively from the experimental error. Therefore it is necessary to wait for the said residues to be distributed normally around zero. Anomalous residues signify that there is probably a system error and therefore the model is not adequate. In particular, when the distribution of the residues assume a parabolic progression, the model is probably missing a quadratic term.

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Finally, the Mandel mathematic test was used for the statistical checks of the linearity. For this reason, calibration functions of the first order were used ( $y = b_0 + b_1x$ ) and the second order ( $y = b_0 + b_1x + b_2x^2$ ), including the corresponding residual variances ( $S_y$ ). This test foresees the use of an F-test where, if the value of F is less or equal to that tabulated, the calibration function of the second order do not supply a model significantly better than the linear model and therefore the calibration function is linear. In the case of levels superior to those tabulated, the function of the second order is that which best interpolates the points.

Comparing the calculated results ( $F_{calc}$ ) with those obtained from the table ( $F_{tab}$ ) for the data of the calibration obtained via the use of the CDR spectrophotometric technique (Table 3 and Table 4), it is possible to affirm that the calibration function of the second order does not supply a significantly better model of a linear function (with  $\alpha = 1\%$  for the whole milk and  $\alpha = 5\%$  for the skim milk).

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**Table 3.** Mandel test for the analysis conducted on samples of whole milk

F <sub>calc</sub>	12.779587
F <sub>tab</sub> <sup>á</sup>	10.130000
F <sub>tab</sub> <sup>á</sup>	34.1220000
<sup>á</sup> Significance = 5% degrees of freedom – 1.3	
<sup>á</sup> Significance = 1% degrees of freedom – 1.3	

**Table 4.** Mandel test for the analyses conducted on samples of skim milk

F <sub>calc</sub>	0.737716
F <sub>tab</sub> <sup>á</sup>	10.130000
<sup>á</sup> Significance = 5% degrees of freedom – 1.3	

## 7 – THE VARIANCE ANALYSIS (ANOVA)

The study conducted on the merits of adaptation of the linear model to the data relative to the sample of whole milk revealed how this is sufficiently adequate to describe the relationship between X and Y ( $\hat{\alpha} = 1\%$ ). This result is in line with the results obtained by the analysis of the residues and by the check on the linearity of the model (Mandel test).

The analysis on skim milk instead, revealed how the linear model is adequate to describe the relationship between X and Y with a confidence interval of 95%.

**Table 5.** Analysis of the variance (whole milk)

F <sub>lof</sub>	12.84
F <sub>tab</sub> <sup>á</sup>	10.13
F <sub>tab</sub> <sup>á</sup>	34.12
<sup>á</sup> Significance = 5% degrees of freedom – 1.3	
<sup>á</sup> Significance = 1% degrees of freedom – 1.3	

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**Table 6.** Analysis of the variance (skim milk)

Flof	0.81
Ftab <sup>á</sup>	10.13
Ftab <sup>á</sup>	34.12
<sup>á</sup> Significance = 5% degrees of freedom – 1.3	
<sup>á</sup> Significance = 1% degrees of freedom – 1.3	

## 8 – PRECISION

The precision was evaluated in terms of inter-day repeatability on five levels of concentration and carrying out three repeats for each level. A 2.5ppm – 8.93ppm interval of concentration was taken on whole milk, whilst for the skim milk, the range selected was between 2.16ppm – 12.29ppm inclusive. The parameters evaluated were: the variance of the repeatability, standard deviation of the repeatability, repeatability, the variation coefficient (VC%) and the confidence interval (CI). It is demonstrated in Table 8 how the variation coefficient is good only for high levels of concentration (5.95ppm, 7.94ppm and 8.93ppm) and as a consequence the high value of the repeatability in respect of the average measurements of the data. A possible explanation of this result could be connected to the lessening of sensitivity by the spectrophotometer in the reading of low concentrations of ammonia in samples of whole milk. Instead, in the case of skim milk, the variation coefficient is good at all levels of concentration.

**Table 7.** Inter-day repeatability (whole milk)

Level (ppm)	Variance of the repeatability	Standard deviation of repeatability	Repeatability (a = 5%)	Repeatability (a = 1%)
2.5	0.000004	0.002	0.012	0.028
4.19	5.63333E-05	0.008	0.046	0.105
5.95	3.23333E-05	0.006	0.035	0.080
7.74	7.03333E-05	0.008	0.051	0.118
8.93	1.63333E-05	0.004	0.025	0.057

**Table 8** Inter-day repeatability (whole milk)

Level (ppm)	VC% repeatability <sup>á</sup>	VC% standard deviation repeatability <sup>á</sup>	Maximum CI	Minimum CI
2.5	-121.7	-20.0	-0.005	-0.015
4.19	112.3	18.5	0.059	0.022
5.95	34.1	5.6	0.115	0.087
7.74	37.2	6.1	0.158	0.116
8.93	15.9	2.6	0.164	0.144

<sup>á</sup>The VC% was calculated with a  $\alpha = 5\%$ .

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**Table 9.** Inter-day repeatability (skim milk)

Level (ppm)	Variance of repeatability	Standard deviation of repeatability	Repeatability (a = 5%)	Repeatability (a = 1%)
2.16	0.000048	0.0069	0.0422	0.0972
3.95	0.000001	0.0010	0.0061	0.0140
5.95	0.000006	0.0025	0.0153	0.0353
8.93	0.000012	0.0035	0.0211	0.0486
12.29	0.000007	0.0026	0.0161	0.0371

**Table 10.** Inter-day repeatability (skim milk)

Level (ppm)	VC% repeatability <sup>a</sup>	VC% standard deviation of repeatability <sup>a</sup>	Maximum CI	Minimum CI
2.16	27.02	4.44	0.173211	0.138789
3.95	2.84	0.47	0.216484	0.211516
5.95	5.60	0.92	0.279918	0.267415
8.93	6.22	1.02	0.347605	0.330395
12.29	4.02	0.66	0.406572	0.393428

<sup>a</sup>The VC was calculated with a  $\alpha = 5\%$ .

A series of measurements was effectuated for the intermediate repeatability analysis on standard solutions with a known concentration of ammonia at different times and by different technicians but in the same laboratory. The range of concentration analysed was between 2.59 ppm and 14.76 ppm inclusive for the whole milk and between 2.61 ppm and 12.66 ppm inclusive for the skim milk. Eight levels of concentration and two repeats for each level were analysed within this interval.

**Table 11.** Intermediate repeatability (whole milk)

Level (ppm)	Variance of repeatability	Standard deviation repeatability	VC% standard deviation repeatability	Maximum CI	Minimum CI
2.59	0.000021	0.00459	6.885	0.100	0.033
3.05	0.000041	0.00636	8.107	0.125	0.031
4.56	0.000015	0.00389	3.600	0.138	0.080
5.77	0.000005	0.00300	1.685	0.153	0.119
6.76	0.000018	0.00424	2.431	0.206	0.143
8.3	0.000025	0.00495	2.340	0.247	0.175
11.29	0.000013	0.00353	1.198	0.321	0.269
14.76	0.000098	0.00990	2.484	0.471	0.326

<sup>a</sup>The VC% was calculated with a  $\alpha = 5\%$ .

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**Table 12.** Intermediate repeatability (skim milk)

Level (ppm)	Variance of repeatability	Standard deviation repeatability	VC% standard deviation repeatability <sup>a</sup>	Maximum CI	Minimum CI
2.61	0.000120	0.01096	4.965	0.301	0.140
3.39	0.000041	0.006364	2.619	0.289	0.196
4.08	0.000036	0.00601	2.266	0.309	0.221
5.20	0.000153	0.012374	4.041	0.397	0.215
6.35	0.000162	0.012728	3.700	0.437	0.250
7.09	0.000006	0.002475	0.686	0.379	0.342
9.69	0.001653	0.040659	8.600	0.771	0.174
12.66	0.000703	0.026517	4.737	0.754	0.365

<sup>a</sup>The VC% was calculated with a  $\alpha = 5\%$ .

The intermediate repeatability demonstrates how it is for the whole milk and the skim milk. The variation coefficient connected to the standard deviation of repeatability is lower at 9%.

## 9 – PROGRESSION OF THE CONCENTRATION IN TIME

The variation of the NH<sub>3</sub> concentration was evaluated in samples of milk over a period of time. With the scope of effectuating this analysis, the standards with known concentration of ammonia, previously frozen, were gently heated to obtain a delicate and homogenous defreezing.

In particular, measurements on the same sample of whole milk were repeated at a distance of 24 hours (T<sub>1</sub>) and 72 hours (T<sub>2</sub>) from the first measurement (T<sub>0</sub>): after 24 hours (T<sub>1</sub>) and 48 hours (T<sub>2</sub>) for the skim milk.

**Table 13.** Concentration over a period of time for samples of skim milk

Concentration (ppm)	Time		
	T <sub>0</sub>	T <sub>1</sub>	T <sub>2</sub>
2.5	2.5	2.11	2.925
4.19	4.19	4.79	4.08
5.95	5.95	6.39	6.77
7.74	7.74	7.36	7.81
8.93	8.93	8.59	8.575



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**Table 14.** Concentration over a period of time for samples of whole milk.

Concentration (ppm)	Time		
	T <sub>0</sub>	T <sub>1</sub>	T <sub>2</sub>
2.16	2.16	1.795	1.03
3.97	3.97	4.225	3.83
5.95	5.95	6.715	6.59
8.93	8.93	9.555	8.53
12.29	12.29	11.82	12.31

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## 10 – PROGRESSION OF CALIBRATION OVER A PERIOD OF TIME

A variation was observed in the lot of coefficients of the calibration straight line utilised for the analysis of ammonia on samples of milk. The straight lines to be considered are shown in Table 15 and were obtained by carrying out repeated measurements on standard solutions of a known concentration by using cuvette tests appertaining to diverse production lots.

**Table 15.** Variation of the calibration straight line in respect of the production lot.

	Equation of the straight line (whole milk)	Equation of the straight line (skim milk)
Lot 1	$Y = 32.92X + 3.16$	$Y = 29.54X - 1.79$
Lot 2	$Y = 35.46X + 0.61$	$Y = 30.62X - 3.90$

**Table 16.** Progression of the calibration straight line coefficients for whole milk.

	Straight line 1 <sup>a</sup>	Straight line 2 <sup>a</sup>	VC%
Inclination	35.46	32.92	5.25
Intercept	0.6126	3.167	95.57

<sup>a</sup>The straight line is a Y type = a + bX

**Table 17.** Progression of the calibration straight line coefficients for skim milk.

	Straight line 1 <sup>a</sup>	Straight line 2 <sup>a</sup>	VC%
Inclination	30.624	29.544	2.53
Intercept	3.9099	1.7975	52.34

<sup>a</sup>The straight line is a Y type = a + bX

It can be observed that a variation of calibration straight line coefficients is recorded when the production lot varies. The incidence of such a variation on the coefficients makes it advisable to recalibrate the instrument to the variation of the production lot of the cuvette test. However, it would seem that temporal variations within the same production lots do not bring about any movement of the calibration straight line.

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## 11 – NOTE

A comparison was effectuated between the specific electrode and the CDR spectrophotometer that is used for the analysis of ammonia in samples of milk. In particular, the costs of each analysis and the time necessary for the carrying out of the same were evaluated. From the following tables it can be observed how the CDR spectrophotometer requires an increased cost (Table 19) but less time for the analysis (Table 20) in respect of the analogous analysis effectuated by the specific electrode. The total cost of the analyses effectuated using the CDR instrument (Table 19) was reproduced and tested 7 times with the scope of making the cost comparable with the cost of the spectrophotometer, the membranes of which must be replaced every 7 tests.

In Table 19, the utilisation of glass and reagents required by the specific electrode was however omitted, because of the difficulty of quantifying these articles for each single test.

**Table 18.** Cost of the components necessary for the analysis of NH<sub>3</sub> in milk.

<b>Specific electrode</b>		<b>CDR FoodLab spectrophotometer</b>	
<b>Components</b>	<b>Cost (Euro)</b>	<b>Components</b>	<b>Cost (Euro)</b>
Membranes (packet of 20)	111	Cuvette test (packet of 10)	17,7
Internal solution (60 ml)	60	R2 reagent	Included in the price

**Table 19.** Cost of each single analysis of NH<sub>3</sub> in milk.

<b>Specific electrode</b>		<b>CDR FoodLab spectrophotometer</b>	
<b>Components</b>	<b>Cost of analysis (Euro)</b>	<b>Components</b>	<b>Cost of analysis (Euro)</b>
Membranes *	5,55	Cuvette test	1,70
Internal solution	2,50	R2 reagent	0
<b>Total (7 analyses)</b>	<b>8,05</b>	Single analysis	1,77
		<b>Total (7 analyses)</b>	<b>12,39</b>

\* The membrane can be utilised for 7 analyses.

**Table 20.** Time required for each single analysis of NH<sub>3</sub> in milk.

<b>Specific electrode</b>	<b>CDR FoodLab spectrophotometer</b>
<b>Time for analysis (minutes)</b>	<b>Time for analysis (minutes)</b>
15	10

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**Table 21.** Cost of one person for each single analysis of NH<sub>3</sub> in milk

<b>Specific electrode</b>		<b>CDR FoodLab spectrophotometer</b>	
<b>Time of analysis (minutes)</b>	<b>Cost of analysis (Euro)</b>	<b>Time of analysis (minutes)</b>	<b>Cost of analysis (Euro)</b>
15	6,41	10	4,27

The main aspect is the practicality in the overall aspects connected to the use of the two analytical instruments for the analysis of ammonia in samples of milk.

In fact, the CDR instrument requires less handling in respect of the addition of the R2 reagent. The analysis carried out via the specific electrode foresees a more consistent use of handling, glassware and reagents. In particular, with the specific electrode it is necessary to prepare a standard with a known concentration of ammonia chloride (1000 ppm) and a solution of soda 0.1N to add to the sample, with the consequent utilisation of the appropriate glassware (beaker, pipette, magnetic agitators....). Also the reading of the data is not immediate because it requires a concentrated evaluation within the scanning of the mV with the scope of correctly discovering the reaching of the plateau and the conversion of the result via the use of a suitable table.

The CDR instrument instead, supplies a direct result expressed in terms of concentration of ammonia present in the sample of milk and does not require preliminary treatments of the sample or the use of glassware and reagents different from the R2 reagent.