EVALUATION OF THE « *MELISA-TEK*^{TM} RUMINANT KIT» (ELISA TECHNOLOGIES. INC., GAINESVILLE, FL, USA) FOR THE DETECTION OF RUMINANT PROTEINS IN PROCESSED ANIMAL PROTEINS

SECOND PART BASED ON A LIMITED INTER-LABORATORY STUDY ON THE VARIABILITY OF THE BLANK CONTROL

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1. INTRODUCTION

In a first part of this report (Fumière *et al.*, 2009) on the evaluation of the "MELISA-TEK™ ruminant kit" (ELISA technologies, Inc., Gainesville, Florida, USA) for the detection of ruminant proteins in processed animal proteins, the CRL-AP came to the conclusion that when analysing the report made by the CCL (Vaessen *et al.*, 2009) the modified method (with an adapted decision criterion) worked well but when trying some limited experimental tests it was impossible for CRL-AP to reproduce the results of CCL. It looked as if the decision criterion defined by CCL was set too low. Parameters that might have influenced these results were systematically considered at CRL-AP and no handling error could be identified as the cause of the discrepancy in results with CCL. It might be possible nevertheless that a parameter not stressed within the protocol had a more crucial role than expected.

It was therefore considered during a meeting (September 9, 2009) between DG-SANCO, CCL and CRL-AP to organize a limited inter-laboratory study which could be launched easily because it did not even need PAP samples. Indeed, the CRL-AP came to the conclusion that only by looking at the variability of the results of the blanks, collected data could show if the kind of problem that in the hands of CRL-AP made it difficult to handle the decision criterion as set by CCL was encountered by others.

This report describes the inter-laboratory study that was carried out and the results that were obtained on the MELISA-TEK™ ruminant kit.

2. THE INTER-LABORATORY STUDY

The design of the inter-laboratory study was very simple. Each participant to the study had to analyze blank samples on plates consisting of three strips of eight wells (giving rise to 24 -well plates) and this analysis was repeated four times independently by each participant so as to use all the strips provided by one MELISA-TEK™ ruminant kit. For each 24-well plate, a participant had to measure results for blank samples within 15 wells. The remaining 9 wells were used to carry out the control tests that are required by the manufacturer of the kit (see Figure 1 in Annex 1). This means that, unless specific problems, each participant should have to end up with 60 results on the blank samples.

The protocol to be used was carefully described (see Annex 1) after several exchanges between CCL and CRL-AP to come up with a very precise protocol leaving no place to

different interpretations. As it is nevertheless impossible to have a completely identical way of working in each of the laboratories (e.g. not everybody uses MilliQ water), a questionnaire was set up to identify the exact way of working at each special step in each laboratory. These steps should normally not have been essential for the results but it was inferred that probably one of these parameters had been underestimated in its effects on the results.

The questionnaire can be found in Annex 2, while in the protocol it was stressed at which steps exactly a question of the questionnaire had to be answered by each participant.

3. THE PARTICIPANTS TO THE STUDY

During the discussions at DG-SANCO it was considered to try to organize the inter-laboratory trial with four laboratories:

- JRC - IRMM – Geel, Belgium
- RIKILT – Wageningen, The Netherlands
- CCL- nutricontrol, Veghel, The Netherlands
- CRA-W CRL-AP, Gembloux, Belgium (called hereafter CRL-AP)

JRC-IRMM and RIKILT were not present at the meeting of September 2009 but accepted to take part to the inter-laboratory study. During the preparation of the study in October 2009, two additional participants were included in the trial, one on demand of CCL and the other on demand of the CRL-AP:

- Rendac, Son, The Netherlands (a potential future user of the kit if it is positively evaluated),
- CRA-W, Department of Biological Control and Plant genetic resources (abbreviated to CRA-W D3 in the text) having more experience than the CRL-AP with immunological methods).

CRL-AP provided the kit to the four participants that where initially decided as well as to CRA-W D3. Rendac used kits they had. The batch numbers of each kit had been asked in the questionnaire that had to be filled by the participants. The kits were ordered from the Benelux representation for MELISA-TEK™ products (EuroProxima B.V. – Arnhem, The

Netherlands) and delivered directly to CCL, IRMM and CRL-AP. CRL-AP provided one kit to RIKILT (during the journey to Wageningen for the 3rd International Feed safety conference, 6th of October 2009) and another one to CRA-W D3.

4. THE RESULTS

Annex 3 gathers the results in terms of absorbance in optical density (OD) at 450 nm for each well of the four plates of the six participants to the study. All the control samples to be used gave results that met the criteria set by the manufacturer of the kit at each of the four experiments performed by all participants except for the plate of experiment 1 of JRC-IRMM which had too high optical densities for the negative samples. Therefore the OD measurements of the blank samples of experiment 1 of IRMM are not taken into consideration in the analysis that follows. Furthermore, on the plate of experiment 3 of CRL-AP, two OD measurements are missing because they were too high due to presence of air-bubbles in the wells. That is why these data were skipped too. Consequently the study gathers 343 OD measurements on blank samples spread over 23 runs. Annex 3 provides the answers to the several questions about some points that might differ between participants and therefore be a possible source of discrepancies.

Table 1 summarizes the responses that were collected to these several questions. It can be seen from that table that there might be some diversity but this corresponds also to the normal diversity to which in real life the kit might be confronted.

Table 1 – Overview of main responses to the questionnaire related to special items in the protocol

	Batch	Air condi-tioning	Room tempera-ture (°C)	Water used	Plate washer	Plate shaker	Multi-chanel pipette
CRA-W / D3	MRM 90126-48 7/79	No	21.1-21.8	Distilled	Yes	No	Yes
CRL-AP	MRM 90126-48 64/79	No	21.0-23.0	MilliQ	No	Yes	Yes
CCL	MRM 90910-55 14/78	Yes	19.5-20.0	MilliQ	Yes	No	Yes
JRC-IRMM	MRM 90910-55 12/78	Yes	21.6-23.6	MilliQ	Yes	Yes	Yes
RENDAC	MRM 90910-55 17/78, 20/78, 21/78	Yes	21	Reverse osmose	No	No	Yes
RIKILT	MRM 90910-55 13/78	No	21-23	Demineralised + MilliQ	Yes	No	No

Concerning the results obtained on the blank samples (raw data are gathered in Annex 4), an overall summary is provided in Table 2. Per well of each run, it takes into account if the

measure obtained for a well is positive or negative using to that purpose the criterion set by CCL. This latter criterion is as follows : a sample is considered as positive if its blank-subtracted OD is higher than 0.017 (Vaessen *et al.*, 2009). As we are dealing here only with blank samples to obtain a "blank-subtracted OD", the mean OD of the blanks of a run (*i.e.* a plate here with normally 15 blanks) is the figure to be subtracted from the OD of the well of a blank sample. Wells that provide a blank-subtracted OD value higher than the CCL criterion are highlighted in orange in the raw data of Annex 4.

Results of Table 2 clearly show that globally all participants met a certain amount of false positive results on the blank samples. This amount might be rather low but could reach up to a quarter or even a bit more than a third of the tested samples for some participants.

Table 2 – Overview of the results on the blank samples (with CCL cut-off value on blank-subtracted samples > 0.017 to have a positive sample) – SD : standard deviation.

		Parameters			
Laboratories	Number of valid blank measure-ments	% of blank samples giving positive results	% of runs with at least one positive blank sample (number of runs in brackets)	Overall mean OD of the blank samples	Overall SD of the blank samples
CRA-W-D3	60	1.7	25 (4)	0.100	0.010
CRL-AP	58 *	15.5	100 (4)	0.160	0.017
CCL	60	28.3	100 (4)	0.120	0.048
JRC-IRMM	45**	40.0	100 (3)	0.161	0.086
Rendac	60	3.3	50 (4)	0.049	0.010
RIKILT	60	16.7	100 (4)	0.070	0.021
Total of runs	343	16.6	78 (23)	0.107	0.057

* two samples not valid due to air bubbles in the well

** one plate (15 samples) not valid with the negative control

5. DISCUSSION ON THE RESULTS

Establishing any link between the handling details and the different measured parameters (especially the amount of false positive results) seems difficult. No clear pattern of relationship between some specific protocol variant and the quality of results do appear. Interestingly however, in this study the CCL obtained results that they considered among the worst they ever had and which are characterised by higher OD values and a much higher variability (second highest variability among participants). This however allows us to conclude that the hypothesis of a variability linked to badly mastered parameters of which the importance was not stressed in the protocol can be abandoned. Indeed, CCL worked in the same way as they did formerly. It therefore seems that there might be a great variability between the batches. This point was also considered when preparing the first part of the CRL-AP report (Fumière *et al.*, 2009) but it had not been retained as crucial as the two independent batches gave similar disappointing results.

Now the batch-dependency of results is clearly highlighted because participants themselves showed that they could obtain better results on an independent batch they had. RIKILT and CCL for instance did independent experiments on a kit of another batch for which they knew they had better results (raw results are given in the Annex 5 but Table 3 summarizes the main parameters).

Table 3 – Additional results of RIKILT and CCL with MELISA-TEK™ kits of other batches

Laboratories	Number of valid blank measure-ments	Parameters			
		% of blank samples giving positive results	% of runs with at least one positive blank sample (number of runs in brackets)	Overall mean OD of the blank samples	Overall SD of the blank samples
RIKILT	14	0	0 (1)	0.092	0.003
CCL	45	0	0 (3)	0.075	0.012
Total of runs	59	0	0 (4)	0.079	0.012

Independently of this batch-effect, it must nevertheless be stressed that even when the measured optical densities seem fine as well in terms of intensity – that should ideally be low – as in terms of variability (for instance results of Rendac or CRA-W D3 in Table2), there

still exists a small number of blank samples that will come out as positive. This latter point clearly shows that the decision criterion set by CCL to assess if a blank-subtracted measurement is positive or negative corresponds to a too low threshold. It should thus be higher than 0.017 with of course the risk that this might affect the sensitivity of the method. Taking as criterion that the results on the plates of Rendac should all be negative, the threshold should then be set at least at 0.046 (see raw data in Annex 4, §12.5). The reason to take results of Rendac is that it is the lab with the lowest mean OD and the lowest variability on the OD (together with CRA-D3 for the standard deviation) as can be seen from Table 2. Using a cut-off of 0.046 (lowest threshold required to get all Rendac measurements negative) makes it possible to recalculate in Table 4 a part of the parameters of Table 2 but with this new decision criterion. It can be seen that indeed the specificity is now increased. The fact that for some laboratories there are still bad results can be linked to the already mentioned batch effect.

Table 4 – New calculation of the parameters of Table 2 but with a cut-off at 0.046

	Parameters	
Laboratories	% of blank samples giving positive results	% of runs with at least one positive blank sample
CRA-W-D3	0	0
CRL-AP	0	0
CCL	11.7	100
JRC-IRMM	33.3	100
Rendac	0	0
RIKILT	5.0	75
Total of runs	16.8	46

As can be seen from Table 4, when using an increased decision threshold (0.046), three laboratories (CRA-W-D3, Rendac and CRL-AP) show no longer false positive results. If we recalculate a cut-off based on the results of these three labs using three times the variability on the blank-subtracted figures expressed as standard deviation we get a cut-off value of 0.034 (i.e. 3 X 0.0113) which would however not result in 0% false positive results for the data collected by these laboratories.

If however the manufacturer does not improve the kits, it means that the cut-off value should be three times the variability on the blank-subtracted figures of all 343 results (*i.e.*

here : the SD of 0.037 x 3) which corresponds to a value of 0.111. This is a figure close to what the manufacturer of the kit advised to use as criterion : a blank-subtracted OD of less than 0.100 is considered as negative – however strictly it is to be applied on the mean blank-subtracted OD of triplicates (MELISA-TEK™, 2009). Rates of false positive results with several cut-off values are given in Table 5 but even with 0.111 as threshold there are still false negative results. These ones drop considerably when 5 times the standard deviation on the blank-subtracted figures is used (Table 5).

Table 5 – Analysis of the results at several set cut-off values : 0.111 (3 x SD of blank-subtracted figures of the 6 labs), 0.148 (4 x SD) and 0.185 (5 x SD)

	Parameters with a cut-off set at 0.111		Parameters with a cut-off set at 0.148		Parameters with a cut-off set at 0.185	
Laboratories	% of blank samples giving positive results	% of runs with at least one positive blank sample	% of blank samples giving positive results	% of runs with at least one positive blank sample	% of blank samples giving positive results	% of runs with at least one positive blank sample
CRA-W-D3	0	0	0	0	0	0
CRL-AP	0	0	0	0	0	0
CCL	1.7	25	0	0	0	0
JRC-IRMM	8.9	100	8.9	100	2.2	33
Rendac	0	0	0	0	0	0
RIKILT	0	0	0	0	0	0
Total of runs	1.5	17	1.2	13	0.3	4

However it seems difficult to determine if a batch is good or wrong because even within a same batch it is not that evident that one can say that all the strips are good or wrong as resulted from another comparison made at CRL-AP by performing tests in parallel by a technician of CCL and by a technician of CRL-AP together on two different batches (Annex 6). According to the % of false positive results, one batch is good with the results of the technician from one institute while it is the other one with the result of the technician of the other institute, at least if the cut-off is set at 0.017. This once again points out that the threshold at 0.017 is a too low figure as cut-off. When calculating the cut-off values by taking three times the standard deviation of the blank-subtracted OD of both labs on a batch, one batch requires a much lower cut-off (0.038) than the other one (0.105) and this then confirms that one kit was globally less efficient but this does not necessarily lead to have always bad results.

The calculation of the cut-off value based on ratio's instead of on blank-subtracted values was also considered like in a study of the IRMM (Boix *et al*., 2009) but this approach did not deliver good results (too much false positive results with the obtained cut-off values) and was not considered furthermore. However, this is another evidence of the insufficient quality of the kits.

7. FINAL CONCLUSIONS

The inter-laboratory study pointed out that the MELISA-TEK kits seem to be **very variable from one batch to another one** leading to considerable differences in terms of variability of results. If the commercialized kits correspond to the quality of what was tested, it is impossible to use these kits with a decision criterion on blank-subtracted figures corresponding to a threshold value set at 0.017 by CCL. This cut-off is generally too low because as stressed by the collected results of this study, background noises may appear as significantly higher. All points out that the value of 0.100 that was set by the manufacturer is much more realistic and should in fact even be somewhat higher with the collected results of this study (but for the manufacturer it has to be applied on a mean of triplicates which results in smoothing possible peak values). This raises the question whether in these conditions the kit is still able to detect 1 or 2 % of ruminant meat and bone meal within processed animal proteins of other animal species. If the manufacturer would be able to improve the drawback that was observed (which may be a production problem or a stability problem linked to transport, this cannot be determined from the gathered data), and come with kits comparable to the one tested at Rendac, the **decision criterion to be applied to blank-subtracted figures should still be set higher than 0.017** and probably not less than 0.034 to avoid false positive results.

8. REFERENCES

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9. ANNEX 1 – PROTOCOL OF THE INTERLABORATORY STUDY

MELISA-TEK kit: study of the variability of the blank control
Design of the study

1. Introduction

The aim of this study is to determine whether a parameter not stressed by the kit protocol is crucial to obtain the same repeatability as the one reached experimentally by the CCL. We focus here only on the ELISA itself, not on the extraction protocol.

2. Recommendations

1. Read carefully the protocol before starting with the experiment.
2. Fill in the questionnaire provided with this document (page 4).

3. Design of the study

It is asked to the participants to test the variability of the blank control.

1. The participants have to perform the plates according to the protocol provided by the CCL (Detection of ruminant material (ELISA: MELISA-TEK^{TM} Ruminant) – ANAL-10443 – Version 3) and presented page 2.
2. The plate has to be performed four times independently as represented at the Figure 1. Two plates can be done in separate runs on the same day but the four repetitions of the plate have to be performed on more than one day.

STRIP	1	2	3	4	5	6	7	8	9	10	11	12
A	*LPC*	*LPC*	*LPC*									
B	*HPC*	*HPC*	*HPC*									
C	*Neg*	*Neg*	*Neg*									
D	*Blank*	*Blank*	*Blank*									
E	*Blank*	*Blank*	*Blank*									
F	*Blank*	*Blank*	*Blank*									
G	*Blank*	*Blank*	*Blank*									
H	*Blank*	*Blank*	*Blank*									
SPECIES	***Rum***	***Rum***	***Rum***	***Rum***	***Rum***	***Rum***	***Rum***	***Rum***	***Rum***	***Rum***	***Rum***	***Rum***

Legend :
Blank = Extraction Solution Only
LPC = Low Positive Control (0.05 %)
HPC = High Positive Control (1 %)
Neg = Negative Control (alternate species control)
Rum = Ruminant

Figure 1. Scheme of the plate to perform

4. Protocol

The present protocol describes only the ELISA step of the protocol provided by CCL (Detection of ruminant material (ELISA: MELISA-TEK™ Ruminant) – ANAL-10443 – Version 3). It is asked to the participants to conform to it as closely as possible.

4.1. Chemicals, reagents and standards

4.1.1. Chemicals

4.1.1.1. MELISA-TEK™ RUMINANT Species Kit; ELISA Technologies, Inc., cat.no. 510311 (store at 4-8°C). *Mention the batch number of the kit used – question 1 of the questionnaire)*

4.1.2. Reagents

4.1.2.1. Extraction solution: dissolve the content of one Extraction Solution packet completely in 1 liter of demineralised water *(or another type of water – question 4 of the questionnaire)* by stirring. This solution can be kept in the fridge for one week.

4.1.2.2. Wash solution: add one bottle of Wash Solution concentrate (100ml) to 900 ml of Millipore water *(or another type of water – question 4 of the questionnaire)* and mix gently by inverting several times. The solution can be kept in the fridge for one week.
To work all in the same conditions, we recommend to prepare freshly the wash solution before each run according the following:
Add 25 ml of Wash Solution concentrate to 225 ml of Millipore Water *(or another type of water – question 4 of the questionnaire)*.

4.1.3. Standards

4.1.3.1. Negative control; use a 10% solution of a species that does not match the species that should be detected. The 10% solution does not need to be diluted.

4.1.3.2. High positive control 1%; add 50 µl of the appropriate supplied 10% positive control to 450 µl Extraction Solution (6.2.1). Mix gently by pipetting. Diluted positive controls must be used the day they are prepared and then discarded.

4.1.3.3. Low positive control 0.05%; add 25 µl of the 1% high positive control (6.3.2) to 475 µl of the 10% negative control (6.3.1). Mix gently by pipetting. Diluted positive controls must be used the day they are prepared and then discarded.

4.1.3.4. Blank; extraction solution.

4.2. Equipment and resources

The equipment and resources written below may be substituted by other brands provided that the specifications of these brands are equal.

4.2.1. Equipment

4.2.1.1. Upper balance, Mettler Toledo

4.2.1.2. ELISA plate washer, Bio-Rad PW40 *(optional – question 6 of the questionnaire)*

4.2.1.3. Vortex, IKA MS2 mini shaker

4.2.1.4. Plate shaker *(optional – question 7 of the questionnaire)*

4.2.1.5. ELISA plate reader with 450 nm filter, Bio-Rad model 550 *(or another ELISA plate reader – question 8 of the questionnaire)*

4.2.1.6. Timer

4.2.1.7. Thermometer

4.2.2. Resources

4.2.2.1. Adjustable pipettes 20-200 µl and 200-1000 µl

4.2.2.2. Multichannel pipette 50-300 µl *(optional – question 10 of the questionnaire)*

4.2.2.3. Pipette tips

4.3. ELISA procedure

Please indicate the temperature of the room when you start the experiment and when you end it (questions 2 and 3 of the questionnaire)

- Pipette 100 µl of Negative control (4.1.3.1.), High positive control (4.1.3.2.), Low positive control (4.1.3.3.) and Blank (Extraction Solution – 4.1.3.4.) into the wells of the test strips according to the plate plan (see point 3. Design of the study).
- Incubate 20 minutes at room temperature.
- Wash the wells 3 times using 300 µl Wash Solution (4.1.2.2) and a plate washer *(manually if you have no plate washer – question 6 of the questionnaire)*.
- Pipette 50 µl Biotinylated Secondary Antibody into each well and incubate 20 minutes at room temperature.
- Wash the wells 3 times using 300 µl Wash Solution (4.1.2.2) and a plate washer *(manually if you have no plate washer – question 6 of the questionnaire)*.
- Pipette 50 µl Avidin-Peroxidase Solution into each well and incubate 20 minutes at room temperature.
- Wash the wells 6 times using 300 µl Wash Solution (4.1.2.2) and a plate washer *(manually if you have no plate washer – question 6 of the questionnaire)*.
- Pipette 50 µl TMB Substrate into each well. Incubate 20 minutes at room temperature **in the dark** (put the plate in a drawer).
- DO NOT WASH but finally pipette 50 µl Stop Solution into each well.
- Read the plate on a 96-well plate reader with a 450nm filter (4.2.1.5) within 10 minutes of adding Stop Solution *(please indicate the elapsed time between the adding of the stop solution and the reading of the OD – question 9 of the questionnaire)*.
- Fill in the Excel file for the reporting of the results.

10. ANNEX 2 – QUESTIONNAIRE LINKED TO THE INTER-LABORATORY STUDY

MELISA-TEK kit: study of the variability of the blank control
Questionnaire to fill in by the participants

Dear colleagues,
The aim of this study is to determine whether a parameter not stressed by the kit protocol is crucial to obtain the same repeatability as the one reached experimentally by the CCL.
Please, fill in carefully this questionnaire.

Name of the laboratory :

1. What is the batch number of your kit?
   ……
2. Is the temperature of the lab controlled with an air conditioning?
   ☐ Yes ☐ No
3. What is the temperature of the lab during the experiments? (ºC)

	Beginning of experiment	End of experiment
Experiment 1	.....	.....
Experiment 2	.....	.....
Experiment 3	.....	.....
Experiment 4	.....	.....

4. What type of water do you use? (e.g. *MilliQ*, *bidistilled*,…)
   ……
5. At what date is performed each experiment and mention through codes (e.g. numbers or initials) the analyst having done the test

	Date (e.g. *23/10/09 AM*)	Analyst (e.g. *Analyst #1*)
Experiment 1	.....	.....
Experiment 2	.....	.....
Experiment 3	.....	.....
Experiment 4	.....	.....

6. Do you use a plate washer?
   ☐ Yes ☐ No
7. Do you use a plate shaker?
   ☐ Yes ☐ No
8. What is your type of plate reader? (Brand + model. e.g. *BioRad Novapath™ Microplate Reader*)
   .....
9. Indicate the elapsed time between the adding of the stop solution and the reading of the OD

	Time (mm:ss)
Experiment 1	.....
Experiment 2	.....
Experiment 3	.....
Experiment 4	.....

10. Do you use a multichannel pipette?
    ☐ Yes ☐ No

11. ANNEX 3 – FILLED QUESTIONNAIRES

3.1. Filled form of CRA-W D3

Name of the laboratory : CRA-W D3 Mycologie

1. What is the batch number of your kit?
   MRM90126-48
2. Is the temperature of the lab controlled with an air conditioning?
   ☐ Yes ☑ No
3. What is the temperature of the lab during the experiments? (ºC)

	Beginning of experiment	End of experiment
Experiment 1	21.1°C	21.3°C
Experiment 2	21.7°C	21.8°C
Experiment 3	21.6°C	21.6°C
Experiment 4	21.1°C	21.3°C

4. What type of water do you use? (e.g. *MilliQ*, *bidistilled*,…)
   Distilled
5. At what date is performed each experiment and mention through codes (e.g. numbers or initials) the analyst having done the test

	Date (e.g. *23/10/09 AM*)	Analyst (e.g. *Analyst #1*)
Experiment 1	27/10/09 PM	N C
Experiment 2	28/10/09 PM	N C
Experiment 3	29/10/09 PM	N C
Experiment 4	30/10/09 PM	N C

6. Do you use a plate washer?
   ☑ Yes ☐ No
7. Do you use a plate shaker?
   ☐ Yes ☑ No
8. What is your type of plate reader? (Brand + model. e.g. *BioRad Novapath™ Microplate Reader*)
   THERMO – Multiscan Ascent
9. Indicate the elapsed time between the adding of the stop solution and the reading of the OD

	Time (mm:ss)
Experiment 1	2 min 30 sec
Experiment 2	2 min
Experiment 3	2 min
Experiment 4	2 min

10. Do you use a multichannel pipette?
    ☑ Yes ☐ No

3.2. Filled form of CRA-W

Name of the laboratory : CRA-W D7

1. What is the batch number of your kit?
   MRM90126-48
2. Is the temperature of the lab controlled with an air conditioning?
   ☐ Yes ☑ No
3. What is the temperature of the lab during the experiments? (ºC)

	Beginning of experiment	End of experiment
Experiment 1	22.5°C	23.0°C
Experiment 2	21.5°C	22.0°C
Experiment 3	21.0°C	22.0°C
Experiment 4	22.5°C	23.0°C

4. What type of water do you use? (e.g. *MilliQ*, *bidistilled*,…)
   MilliQ
5. At what date is performed each experiment and mention through codes (e.g. numbers or initials) the analyst having done the test

	Date (e.g. *23/10/09 AM*)	Analyst (e.g. *Analyst #1*)
Experiment 1	28/10/09 PM	J H
Experiment 2	29/10/09 AM	J H
Experiment 3	30/10/09 AM	J H
Experiment 4	30/10/09 PM	J H

6. Do you use a plate washer?
   ☐ Yes ☑ No
7. Do you use a plate shaker?
   ☑ Yes ☐ No
8. What is your type of plate reader? (Brand + model. e.g. *BioRad Novapath™ Microplate Reader*)
   BioRad Novapath™
9. Indicate the elapsed time between the adding of the stop solution and the reading of the OD

	Time (mm:ss)
Experiment 1	01:00
Experiment 2	01:00
Experiment 3	01:40
Experiment 4	01:00

10. Do you use a multichannel pipette?
    ☑ Yes ☐ No

3.3. Filled form of CCL

Name of the laboratory : CCL – Nutricontrol, Veghel, NL

1. What is the batch number of your kit?
   MRM90910-55 (14 of 78)
2. Is the temperature of the lab controlled with an air conditioning?
   ☑ Yes ☐ No
3. What is the temperature of the lab during the experiments? (ºC)

	Beginning of experiment	End of experiment
Experiment 1	20.0°C	20.0°C
Experiment 2	19.5°C	20.0°C
Experiment 3	20.0°C	20.0°C
Experiment 4	19.5°C	20.0°C

4. What type of water do you use? (e.g. *MilliQ*, *bidistilled*,…)
   MilliQ
5. At what date is performed each experiment and mention through codes (e.g. numbers or initials) the analyst having done the test

	Date (e.g. *23/10/09 AM*)	Analyst (e.g. *Analyst #1*)
Experiment 1	19/10/09 PM	AvD
Experiment 2	29/10/09 AM	AvD
Experiment 3	29/10/09 PM	AvD
Experiment 4	30/10/09 AM	AvD

6. Do you use a plate washer?
   ☑ Yes ☐ No
7. Do you use a plate shaker?
   ☐ Yes ☑ No
8. What is your type of plate reader? (Brand + model. e.g. *BioRad Novapath™ Microplate Reader*)
   BioRad model 550
9. Indicate the elapsed time between the adding of the stop solution and the reading of the OD

	Time (mm:ss)
Experiment 1	20:10
Experiment 2	20:03
Experiment 3	20:04
Experiment 4	20:03

10. Do you use a multichannel pipette?
    ☑ Yes ☐ No

3.4. Filled form of JRC-IRMM

Name of the laboratory : IRMM

1. What is the batch number of your kit?
   MRM90910-55
2. Is the temperature of the lab controlled with an air conditioning?
   ☑ Yes ☐ No
3. What is the temperature of the lab during the experiments? (ºC)

	Beginning of experiment	End of experiment
Experiment 1	22.0°C	22.1°C
Experiment 2	22.0°C	22.0°C
Experiment 3	21.6°C	23.3°C
Experiment 4	22.8°C	23.6°C

4. What type of water do you use? (e.g. *MilliQ*, *bidistilled*,…)
   MilliQ
5. At what date is performed each experiment and mention through codes (e.g. numbers or initials) the analyst having done the test

	Date (e.g. *23/10/09 AM*)	Analyst (e.g. *Analyst #1*)
Experiment 1	09/11/09 AM	*Analyst #1*
Experiment 2	09/11/09 PM	*Analyst #1*
Experiment 3	10/11/09 AM	*Analyst #1*
Experiment 4	10/11/09 PM	*Analyst #1*

6. Do you use a plate washer?
   ☑ Yes ☐ No
7. Do you use a plate shaker?
   ☑ Yes ☐ No
8. What is your type of plate reader? (Brand + model. e.g. *BioRad Novapath™ Microplate Reader*)
   Hybrizyme multilaber counter 1420 (Perkin Elmer)
9. Indicate the elapsed time between the adding of the stop solution and the reading of the OD

	Time (mm:ss)
Experiment 1	04:30
Experiment 2	04:30
Experiment 3	03:06
Experiment 4	03:00

10. Do you use a multichannel pipette?
    ☑ Yes ☐ No

3.5. Filled form of Rendac

Name of the laboratory : Rendac Son

1. What is the batch number of your kit?
   Exp 1 en 2 MRM90910-55 (17 of 78)
   Exp 3 MRM90910-55 (20 of 78)
   Exp 4 MRM90910-55 (21 of 78)
2. Is the temperature of the lab controlled with an air conditioning?
   ☑ Yes ☐ No
3. What is the temperature of the lab during the experiments? 21ºC

	Beginning of experiment	End of experiment
Experiment 1	09:00	11:00
Experiment 2	13:00	15:00
Experiment 3	09:00	11:00
Experiment 4	13:00	15:00

4. What type of water do you use? (e.g. *MilliQ*, *bidistilled*,…)
   Reverse osmoses
5. At what date is performed each experiment and mention through codes (e.g. numbers or initials) the analyst having done the test

	Date (e.g. *23/10/09 AM*)	Analyst (e.g. *Analyst #1*)
Experiment 1	28/10/09 AM	Marjo Vernie
Experiment 2	28/10/09 PM	Marjo Vernie
Experiment 3	03/11/09 AM	Marjo Vernie
Experiment 4	04/11/09 PM	Marjo Vernie

6. Do you use a plate washer?
   ☐ Yes ☑ No
7. Do you use a plate shaker?
   ☐ Yes ☑ No
8. What is your type of plate reader? (Brand + model. e.g. *BioRad Novapath™ Microplate Reader*)
   BIORAD 550 Microplate reader
9. Indicate the elapsed time between the adding of the stop solution and the reading of the OD

	Time (mm:ss)
Experiment 1	01:00
Experiment 2	01:00
Experiment 3	01:00
Experiment 4	02:00

10. Do you use a multichannel pipette?
    ☑ Yes ☐ No

3.6. Filled form of RIKILT

Name of the laboratory : RIKILT - WUR

1. What is the batch number of your kit?
   MRM90910-55 13/78
2. Is the temperature of the lab controlled with an air conditioning?
   ☐ Yes ☑ No no airconditionning
3. What is the temperature of the lab during the experiments? (ºC)

	Beginning of experiment	End of experiment
Experiment 1	22.0°C	23.0°C Humidity 34%
Experiment 2	21.0°C	21.0°C Humidity 33%
Experiment 3	22.0°C	22.0°C Humidity 34%
Experiment 4	21.0°C	22.0°C Humidity 39%

4. What type of water do you use? (e.g. *MilliQ*, *bidistilled*,…)
   Demi water for the extraction buffert and MilliQ for the washingbuffer
5. At what date is performed each experiment and mention through codes (e.g. numbers or initials) the analyst having done the test

	Date (e.g. *23/10/09 AM*)	Analyst (e.g. *Analyst #1*)
Experiment 1	23/10/09 PM	AKV
Experiment 2	28/10/09 AM	AKV
Experiment 3	28/10/09 PM	AKV
Experiment 4	30/10/09 AM	AKV

6. Do you use a plate washer?
   ☑ Yes ☐ No Wellwash 4MK2 from Thermo labsystems
7. Do you use a plate shaker?
   ☐ Yes ☑ No
8. What is your type of plate reader? (Brand + model. e.g. *BioRad Novapath™ Microplate Reader*)
   ELx 808 Ultra microplate reader Bio-Tek instruments
9. Indicate the elapsed time between the adding of the stop solution and the reading of the OD

	Time (mm:ss)
Experiment 1	1 mm
Experiment 2	1 mm
Experiment 3	1 mm
Experiment 4	1 mm

10. Do you use a multichannel pipette?
    ☐ Yes ☑ No I use a stepper, a multipette plus from Eppendrof

12. ANNEX 4 – RAW DATA

12.1. Results of CRA-W D3

Experiment 1

Date: 27/10/2009 pm Analyst x

	1	2	3				
OD LPC	0.452	0.45	0.416	Mean OD LPC :	0.43933	SD OD LPC :	0.02023
OD HPC	4.366	4.433	6.000	Mean OD HPC :	4.933	SD OD HPC :	0.92466
OD Neg	0.101	0.100	0.101	Mean OD Neg :	0.10067	SD OD Neg :	0.00058
OD Blank	0.092	0.095	0.096				
OD Blank	0.094	0.098	0.100				
OD Blank	0.094	0,103	0.116	Mean OD Blank :	0.1012	SD OD Blank :	0.00797
OD Blank	0.093	0.102	0.103	Max OD Blank :	0.116		
OD Blank	0.105	0.116	0.111	Min OD Blank :	0.092		

Experiment 2

Date: 28/10/2009 pm Analyst x

	1	2	3				
OD LPC	0.545	0,515	0,522	Mean OD LPC :	0.52733	SD OD LPC :	0.0157
OD HPC	5.210	4.470	5.512	Mean OD HPC :	5.064	SD OD HPC :	0.53612
OD Neg	0.108	0.103	0.122	Mean OD Neg :	0.111	SD OD Neg :	0.00985
OD Blank	0.104	0.101	0.116				
OD Blank	0.104	0.117	0.114				
OD Blank	0.11	0.112	0.125	Mean OD Blank :	0.11127	SD OD Blank :	0.00896
OD Blank	0.108	0.105	0.113	Max OD Blank :	0.132		
OD Blank	0.098	0.110	0.132	Min OD Blank :	0.098		

Experiment 3

Date: 29/10/2009 pm Analyst x

	1	2	3				
OD LPC	0.467	0.470	0.441	Mean OD LPC :	0.45933	SD OD LPC :	0.01595
OD HPC	4.281	4.909	4.211	Mean OD HPC :	4.467	SD OD HPC :	0.38438
OD Neg	0.101	0.101	0.095	Mean OD Neg :	0.099	SD OD Neg :	0.00346
OD Blank	0.101	0.097	0.092				
OD Blank	0.103	0.095	0.107				
OD Blank	0.094	0.093	0.093	Mean OD Blank :	0.0978	SD OD Blank :	0.00578
OD Blank	0.110	0.099	0.102	Max OD Blank :	0.11		
OD Blank	0.09	0.097	0.094	Min OD Blank :	0.09		

Experiment 4

Date: 30/10/2009 pm Analyst x

	1	2	3				
OD LPC	0.455	0.437	0.452	Mean OD LPC :	0.448	SD OD LPC :	0.00964
OD HPC	6.000	6.000	4.330	Mean OD HPC :	5.44333	SD OD HPC :	0.96417
OD Neg	0.108	0.088	0.089	Mean OD Neg :	0.095	SD OD Neg :	0.01127
OD Blank	0.086	0.088	0.09				
OD Blank	0.089	0.09	0.089				
OD Blank	0.088	0.089	0.089	Mean OD Blank :	0.08967	SD OD Blank :	0.00232
OD Blank	0.087	0.091	0.095	Max OD Blank :	0.095		
OD Blank	0.089	0.093	0.092	Min OD Blank :	0.086		

12.2. Results of CRL-AP

Experiment 1

Date: 28/10/2009 PM

Analyst JH

	1	2	3				
OD LPC	0.512	0.508	0.477	Mean OD LPC	0.499	SD OD LPC	0.01916
OD HPC	3.000	3.000	3.000	Mean OD HPC	3.000	SD OD HPC	0
OD Neg	0.142	0.144	0.152	Mean OD Neg	0.146	SD OD Neg	0.00529
OD Blank	0.169	0.155	0.154				
OD Blank	0.203	0.181	0.183				
OD Blank	0.174	0.161	0.170	Mean OD Blank	0.163	SD OD Blank	0.01748
OD Blank	0.160	0.155	0.145	Max OD Blank	0.203		
OD Blank	0.142	0.145	0.142	Min OD Blank	0.142		

Experiment 2

Date: 29/10/2009 AM

Analyst JH

	1	2	3				
OD LPC	0.575	0.570	0.571	Mean OD LPC	0.572	SD OD LPC	0.00265
OD HPC	3.000	3.000	3.000	Mean OD HPC	3.000	SD OD HPC	0
OD Neg	0.150	0.149	0.145	Mean OD Neg	0.148	SD OD Neg	0.00265
OD Blank	0.163	0.167	0.168				
OD Blank	0.179	0.173	0.187				
OD Blank	0.171	0.173	0.172	Mean OD Blank	0.166	SD OD Blank	0.01394
OD Blank	0.161	0.142	0.153	Max OD Blank	0.187		
OD Blank	0.141	0.183	0.150	Min OD Blank	0.141		

Experiment 3

Date: 30/10/2009 AM

Analyst JH

	1	2	3				
OD LPC	0.569	0.537	0.561	Mean OD LPC	0.556	SD OD LPC	0.01665
OD HPC	3.000	3.000	3.000	Mean OD HPC	3.000	SD OD HPC	0
OD Neg	0.145	0.158	0.135	Mean OD Neg	0.146	SD OD Neg	0.01153
OD Blank	0.161	0.140	0.186				
OD Blank	0.176	-*	-*	* Presence of air bubble (values not considered for statistics)			
OD Blank	0.159	0.157	0.156	Mean OD Blank	0.167	SD OD Blank	0.03215
OD Blank	0.159	0.147	0.157	Max OD Blank	0.242		
OD Blank	0.138	0.136	0.152	Min OD Blank	0.136		

Experiment 4

Date: 30/10/2009 PM

Analyst JH

	1	2	3				
OD LPC	0.512	0.479	0.477	Mean OD LPC	0.489	SD OD LPC	0.01966
OD HPC	3.000	3.000	3.000	Mean OD HPC	3.000	SD OD HPC	0
OD Neg	0.142	0.138	0.134	Mean OD Neg	0.138	SD OD Neg	0.004
OD Blank	0.155	0.150	0.142				
OD Blank	0.195	0.201	0.187				
OD Blank	0.148	0.150	0.153	Mean OD Blank	0.157	SD OD Blank	0.0204
OD Blank	0.146	0.155	0.141	Max OD Blank	0.201		
OD Blank	0.155	0.141	0.135	Min OD Blank	0.135		

12.3. Results of CCL

Experiment 1

Date: 22-10-2009 12.30 h
Analyst A

	1	2	3				
OD LPC	0.476	0.584	0.536	Mean OD LPC	0.532	SD OD LPC	0.05411
OD HPC	2.664	2.842	2.91	Mean OD HPC	2.80533	SD OD HPC	0.12703
OD Neg	0.072	0.082	0.06	Mean OD Neg	0.07133	SD OD Neg	0.01102
OD Blank	0.114	0.083	0.063				
OD Blank	0.095	0.143	0.09				
OD Blank	0.108	0.104	0.087	Mean OD Blank	0.1194	SD OD Blank	0.03764
OD Blank	0.157	0.195	0.088	Max OD Blank	0.195		
OD Blank	0.156	0.157	0.151	Min OD Blank	0.063		

Experiment 2

Date: 29-10-2009 8.30 h
Analyst A

	1	2	3				
OD LPC	0.662	0.658	0.708	Mean OD LPC	0.676	SD OD LPC	0.02778
OD HPC	2.948	2.990	3.088	Mean OD HPC	3.00867	SD OD HPC	0.07184
OD Neg	0.076	0.084	0.088	Mean OD Neg	0.08267	SD OD Neg	0.00611
OD Blank	0.068	0.089	0.082				
OD Blank	0.086	0.057	0.069				
OD Blank	0.098	0.078	0.198	Mean OD Blank	0.10093	SD OD Blank	0.04241
OD Blank	0.074	0.106	0.092	Max OD Blank	0.198		
OD Blank	0.115	0.104	0.198	Min OD Blank	0.057		

Experiment 3

Date: 29-10-2009 11.00 h
Analyst A

	1	2	3				
OD LPC	0.682	0.795	0.07	Mean OD LPC	0.51567	SD OD LPC	0.39007
OD HPC	2.785	2.949	2.876	Mean OD HPC	2.87	SD OD HPC	0.08216
OD Neg	0.151	0.074	0.068	Mean OD Neg	0.09767	SD OD Neg	0.04629
OD Blank	0.152	0.068	0.067				
OD Blank	0.129	0.103	0.062				
OD Blank	0.088	0.082	0.151	Mean OD Blank	0.11007	SD OD Blank	0.0318
OD Blank	0.122	0.116	0.162	Max OD Blank	0.162		
OD Blank	0.109	0.116	0.124	Min OD Blank	0.062		

Experiment 4

Date: 30-10-2009 8.30 h
Analyst A

	1	2	3				
OD LPC	0.677	0.670	0.593	Mean OD LPC	0.64667	SD OD LPC	0.04661
OD HPC	2.573	2.786	3.091	Mean OD HPC	2.81667	SD OD HPC	0.26036
OD Neg	0.082	0.099	0.079	Mean OD Neg	0.08667	SD OD Neg	0.01079
OD Blank	0.139	0.125	0.135				
OD Blank	0.121	0.068	0.084				
OD Blank	0.102	0.096	0.093	Mean OD Blank	0.14893	SD OD Blank	0.06301
OD Blank	0.22	0.138	0.211	Max OD Blank	0.271		
OD Blank	0.249	0.271	0.182	Min OD Blank	0.068		

12.4. Results of JRC-IRMM

Experiment 1

Date: 09/11/09 **am** or ~~pm~~

Analyst x

	1	2	3
OD LPC	3.228	3.187	3.147
OD HPC	3.189	3.147	3.112
OD Neg	3.193	3.156	3.113
OD Blank	0.206	0.179	0.125
OD Blank	0.261	0.184	0.061
OD Blank	0.298	0.085	0.151
OD Blank	0.11	0.116	0.143
OD Blank	0.235	0.292	0.117

Mean OD LPC	3.18733	SD OD LPC	0.0405
Mean OD HPC	3.14933	SD OD HPC	0.03855
Mean OD Neg	3.154	SD OD Neg	0.04004
Negative controls	are too	high	
Mean OD Blank	0.17087	SD OD Blank	0.07411
Max OD Blank	0.298		
Min OD Blank	0.061		

Experiment 2

Date: 09/11/09 ~~am~~ or **pm**

Analyst x

	1	2	3
OD LPC	0.752	0.883	0.858
OD HPC	3.182	3.151	3.106
OD Neg	0.075	0.152	0.197
OD Blank	0.249	0.173	0.073
OD Blank	0.077	0.152	0.057
OD Blank	0.098	0.183	0.221
OD Blank	0.148	0.097	0.092
OD Blank	0.09	0.368	0.215

Mean OD LPC	0.831	SD OD LPC	0.06955
Mean OD HPC	3.14633	SD OD HPC	0.03821
Mean OD Neg	0.14133	SD OD Neg	0.0617
Mean OD Blank	0.15287	SD OD Blank	0.08472
Max OD Blank	0.368		
Min OD Blank	0.057		

Experiment 3

Date: 10/11/09 **am** or ~~pm~~

Analyst x

	1	2	3
OD LPC	0.906	0.892	0.816
OD HPC	3.191	3.156	3.116
OD Neg	0.129	0.063	0.076
OD Blank	0.226	0.207	0.078
OD Blank	0.105	0.152	0.216
OD Blank	0.082	0.057	0.09
OD Blank	0.115	0.134	0.069
OD Blank	0.147	0.174	0.292

Mean OD LPC	0.87133	SD OD LPC	0.04843
Mean OD HPC	3.15433	SD OD HPC	0.03753
Mean OD Neg	0.08933	SD OD Neg	0.03496
Mean OD Blank	0.14293	SD OD Blank	0.0684
Max OD Blank	0.292		
Min OD Blank	0.057		

Experiment 4

Date: 10/11/09 ~~am~~ or **pm**

Analyst x

	1	2	3
OD LPC	0.839	0.742	0.786
OD HPC	3.16	3.139	3.088
OD Neg	0.121	0.109	0.216
OD Blank	0.088	0.073	0.109
OD Blank	0.156	0.100	0.266
OD Blank	0.152	0.068	0.359
OD Blank	0.09	0.248	0.259
OD Blank	0.247	0.209	0.371

Mean OD LPC	0.789	SD OD LPC	0.04857
Mean OD HPC	3.129	SD OD HPC	0.03703
Mean OD Neg	0.14867	SD OD Neg	0.05862
Mean OD Blank	0.18633	SD OD Blank	0.10172
Max OD Blank	0.371		
Min OD Blank	0.068		

12.5. Results of Rendac

Experiment 1

Date : 28-10-09 AM

Analyst Marjo

	1	2	3				
OD LPC	0.591	0.639	0.690	Mean OD LPC	0.640	SD OD LPC	0.04951
OD HPC	2.897	2.934	2.953	Mean OD HPC	2.928	SD OD HPC	0.02848
OD Neg	0.042	0.039	0.047	Mean OD Neg	0.04267	SD OD Neg	0.00404
OD Blank	0.053	0.048	0.047				
OD Blank	0.043	0.046	0.044				
OD Blank	0.070	0.042	0.050	Mean OD Blank	0.04773	SD OD Blank	0.0073
OD Blank	0.044	0.049	0.038	Max OD Blank	0.070		
OD Blank	0.046	0.044	0.052	Min OD Blank	0.038		

Experiment 2

Date : 28-10-09 PM

Analyst Marjo

	1	2	3				
OD LPC	0.575	0.669	0.653	Mean OD LPC	0.63233	SD OD LPC	0.05029
OD HPC	2.944	2.986	2.943	Mean OD HPC	2.95767	SD OD HPC	0.02454
OD Neg	0.051	0.047	0.048	Mean OD Neg	0.04867	SD OD Neg	0.00208
OD Blank	0.054	0.045	0.049				
OD Blank	0.070	0.046	0.046				
OD Blank	0.069	0.051	0.057	Mean OD Blank	0.0544	SD OD Blank	0.00915
OD Blank	0.051	0.049	0.065	Max OD Blank	0.070		
OD Blank	0.045	0.068	0.051	Min OD Blank	0.045		

Experiment 3

Date : 3-11-09 AM

Analyst Marjo

	1	2	3				
OD LPC	0.611	0.603	0.551	Mean OD LPC	0.58833	SD OD LPC	0.03258
OD HPC	2.991	2.990	2.929	Mean OD HPC	2.970	SD OD HPC	0.03551
OD Neg	0.050	0.058	0.043	Mean OD Neg	0.05033	SD OD Neg	0.00751
OD Blank	0.042	0.095	0.044				
OD Blank	0.043	0.042	0.045				
OD Blank	0.041	0.044	0.045	Mean OD Blank	0.04927	SD OD Blank	0.01407
OD Blank	0.045	0.044	0.063	Max OD Blank	0.095		
OD Blank	0.042	0.046	0.058	Min OD Blank	0.041		

Experiment 4

Date : 4-11-09 PM

Analyst Marjo

	1	2	3				
OD LPC	0.710	0.722	0.635	Mean OD LPC	0.689	SD OD LPC	0.04715
OD HPC	2.924	2.944	2.964	Mean OD HPC	2.944	SD OD HPC	0.020
OD Neg	0.047	0.041	0.041	Mean OD Neg	0.043	SD OD Neg	0.00346
OD Blank	0.041	0.045	0.042				
OD Blank	0.040	0.041	0.041				
OD Blank	0.041	0.041	0.042	Mean OD Blank	0.0432	SD OD Blank	0.003
OD Blank	0.045	0.044	0.045	Max OD Blank	0.052		
OD Blank	0.052	0.044	0.044	Min OD Blank	0.040		

12.6. Results of RIKILT

Experiment 1

Date: 23/10/2009 pm
Analyst AK

	1	2	3				
OD LPC	0.662	0.682	0.674	Mean OD LPC	0.67267	SD OD LPC	0.01007
OD HPC	4.373	4.482	4.543	Mean OD HPC	4.466	SD OD HPC	0.08612
OD Neg	0.089	0.053	0.058	Mean OD Neg	0.06667	SD OD Neg	0.0195
OD Blank	0.092	0.050	0.088				
OD Blank	0.054	0.055	0.077				
OD Blank	0.052	0.06	0.051	Mean OD Blank	0.06733	SD OD Blank	0.02017
OD Blank	0.055	0.058	0.056	Max OD Blank	0.122		
OD Blank	0.068	0.122	0.072	Min OD Blank	0.05		

Experiment 2

Date: 28/10/2009 am
Analyst AK

	1	2	3				
OD LPC	0.578	0.577	0.582	Mean OD LPC	0.579	SD OD LPC	0.00265
OD HPC	4.261	4.312	4.329	Mean OD HPC	4.30067	SD OD HPC	0.03539
OD Neg	0.048	0.047	0.049	Mean OD Neg	0.048	SD OD Neg	0.001
OD Blank	0.044	0.044	0.051				
OD Blank	0.047	0.061	0.045				
OD Blank	0.049	0.045	0.05	Mean OD Blank	0.0582	SD OD Blank	0.01835
OD Blank	0.06	0.053	0.069	Max OD Blank	0.114		
OD Blank	0.114	0.065	0.076	Min OD Blank	0.044		

Experiment 3

Date: 28/10/2009 pm
Analyst AK

	1	2	3				
OD LPC	0.626	0.645	0.651	Mean OD LPC	0.64067	SD OD LPC	0.01305
OD HPC	4.228	4.283	4.302	Mean OD HPC	4.271	SD OD HPC	0.03843
OD Neg	0.049	0.052	0.054	Mean OD Neg	0.05167	SD OD Neg	0.00252
OD Blank	0.049	0.081	0.088				
OD Blank	0.066	0.048	0.064				
OD Blank	0.061	0.068	0.071	Mean OD Blank	0.07113	SD OD Blank	0.01709
OD Blank	0.07	0.072	0.053	Max OD Blank	0.114		
OD Blank	0.114	0.089	0.073	Min OD Blank	0.048		

Experiment 4

Date: 30/10/2009 AM
Analyst AK

	1	2	3				
OD LPC	0.708	0.654	0.648	Mean OD LPC	0.67	SD OD LPC	0.03305
OD HPC	4.276	4.365	4.416	Mean OD HPC	4.35233	SD OD HPC	0.07085
OD Neg	0.103	0.076	0.052	Mean OD Neg	0.077	SD OD Neg	0.02551
OD Blank	0.081	0.079	0.046				
OD Blank	0.061	0.074	0.058				
OD Blank	0.102	0.095	0.088	Mean OD Blank	0.0822	SD OD Blank	0.0242
OD Blank	0.09	0.058	0.067	Max OD Blank	0.139		
OD Blank	0.115	0.080	0.139	Min OD Blank	0.046		

13. ANNEX 5 – ADDITIONAL RAW DATA

13.1. Additional data of RIKILT

Experiment 1

Date: 30-10-2009 AM

Analyst AK Kit nr MRM 90126-48

	1	2	3				
OD LPC	0.633	0.645	0.647	Mean OD LPC	0.64167	SD OD LPC	0.00757
OD HPC	4.218	4.339	4.332	Mean OD HPC	4.29633	SD OD HPC	0.06793
OD Neg	0.092	0.094	0.093	Mean OD Neg	0.093	SD OD Neg	0.001
OD Blank	0.089	0.089	0.089				
OD Blank		0.091	0.092				
OD Blank	0.09	0.09	0.095	Mean OD Blank	0.09186	SD OD Blank	0.00257
OD Blank	0.091	0.093	0.091	Max OD Blank	0.097		
OD Blank	0.095	0.097	0.094	Min OD Blank	0.089		

13.2. Additional data of CCL

Experiment 1

Date: 22-10-2009 12.30 h

Analyst Anniek lotnr MRM90513-52

	1	2	3				
OD LPC	0.504	0.494	0.482	Mean OD LPC	0.49333333	SD OD LPC	0.01101514
OD HPC	2.843	2.948	3.294	Mean OD HPC	3.02833333	SD OD HPC	0.23598799
OD Neg	0.099	0.085	0.088	Mean OD Neg	0.09066667	SD OD Neg	0.00737111
OD Blank	0.088	0.096	0.087				
OD Blank	0.091	0.082	0.079				
OD Blank	0.084	0.092	0.088	Mean OD Blank	0.0858	SD OD Blank	0.00487266
OD Blank	0.087	0.086	0.083	Max OD Blank	0.096		
OD Blank	0.078	0.084	0.082	Min OD Blank	0.078		

Experiment 2

Date: 29-10-2009 11.00 h

Analyst Anke lotnr MRM81126-47

	1	2	3				
OD LPC	0.352	0.347	0.279	Mean OD LPC	0.326	SD OD LPC	0.0407799
OD HPC	2.876	2.991	2.949	Mean OD HPC	2.93866667	SD OD HPC	0.05819221
OD Neg	0.06	0.06	0.071	Mean OD Neg	0.06366667	SD OD Neg	0.00635085
OD Blank	0.061	0.064	0.068				
OD Blank	0.058	0.061	0.06				
OD Blank	0.058	0.058	0.059	Mean OD Blank	0.06026667	SD OD Blank	0.00276371
OD Blank	0.06	0.061	0.061	Max OD Blank	0.068		
OD Blank	0.059	0.059	0.057	Min OD Blank	0.057		

Experiment 3

Date: 30-10-2009 8.30 h

Analyst Anke lotnr MRM90513-52

	1	2	3				
OD LPC	0.435	0.456	0.441	Mean OD LPC	0.444	SD OD LPC	0.01081665
OD HPC	2.951	2.844	2.95	Mean OD HPC	2.915	SD OD HPC	0.06148984
OD Neg	0.086	0.083	0.08	Mean OD Neg	0.083	SD OD Neg	0.003
OD Blank	0.082	0.085	0.081				
OD Blank	0.082	0.078	0.078				
OD Blank	0.075	0.076	0.079	Mean OD Blank	0.0794	SD OD Blank	0.00261315
OD Blank	0.078	0.078	0.082	Max OD Blank	0.085		
OD Blank	0.078	0.08	0.079	Min OD Blank	0.075		

14. ANNEX 6 – RAW DATA – COMPARISON TESTS CRL-AP/CCL

Experiment 1

Date: 25/11/2009 AM

Analyst JH Batch du CCL

	1	2	3				
OD LPC	0.613	0.640	0.614	Mean OD LPC	0.622	SD OD LPC	0.01531
OD HPC	3.000	3.000	3.000	Mean OD HPC	3.000	SD OD HPC	0
OD Neg	0.078	0.094	0.086	Mean OD Neg	0.086	SD OD Neg	0.008
OD Blank	0.120	0.094	0.236				
OD Blank				Figures suppressed – line systematically overestimated			
OD Blank	0.099	0.119	0.091	Mean OD Blank	0.110	SD OD Blank	0.04128715
OD Blank	0.093	0.089	0.103	Max OD Blank	0.236		
OD Blank	0.087	0.091	0.093	Min OD Blank	0.087		

Experiment 2

Date: 25/11/2009 AM

Analyst Anke Batch du CCL

	1	2	3				
OD LPC	0.675	0.609	0.633	Mean OD LPC	0.639	SD OD LPC	0.0334
OD HPC	3.000	3.000	3.000	Mean OD HPC	3.000	SD OD HPC	0
OD Neg	0.079	0.084	0.082	Mean OD Neg	0.082	SD OD Neg	0.00252
OD Blank	0.096	0.086	0.099				
OD Blank							
OD Blank	0.092	0.089	0.094	Mean OD Blank	0.090	SD OD Blank	0.00650117
OD Blank	0.085	0.085	0.094	Max OD Blank	0.099		
OD Blank	0.080	0.082	0.099	Min OD Blank	0.080		
			0.087				

Experiment 3

Date: 25/11/2009 AM

Analyst JH Batch du CRA-W

	1	2	3				
OD LPC	0.617	0.587	0.593	Mean OD LPC	0.599	SD OD LPC	0.01587
OD HPC	3.000	3.000	3.000	Mean OD HPC	3.000	SD OD HPC	0
OD Neg	0.157	0.127	0.144	Mean OD Neg	0.143	SD OD Neg	0.01504
OD Blank	0.143	0.144	0.143				
OD Blank							
OD Blank	0.153	0.152	0.140	Mean OD Blank	0.139	SD OD Blank	0.00939495
OD Blank	0.130	0.140	0.126	Max OD Blank	0.153		
OD Blank	0.142	0.138	0.122	Min OD Blank	0.122		

Experiment 4

Date: 25/11/2009 AM

Analyst Anke Batch du CRA-W

	1	2	3				
OD LPC	0.610	0.580	0.658	Mean OD LPC	0.616	SD OD LPC	0.03934
OD HPC	3.000	3.000	3.000	Mean OD HPC	3.000	SD OD HPC	0
OD Neg	0.111	0.110	0.114	Mean OD Neg	0.112	SD OD Neg	0.00208
OD Blank	0.120	0.120	0.123				
OD Blank							
OD Blank	0.126	0.126	0.120	Mean OD Blank	0.127	SD OD Blank	0.01564279
OD Blank	0.124	0.124	0.161	Max OD Blank	0.161		
OD Blank	0.111	0.111	0.156	Min OD Blank	0.111		

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