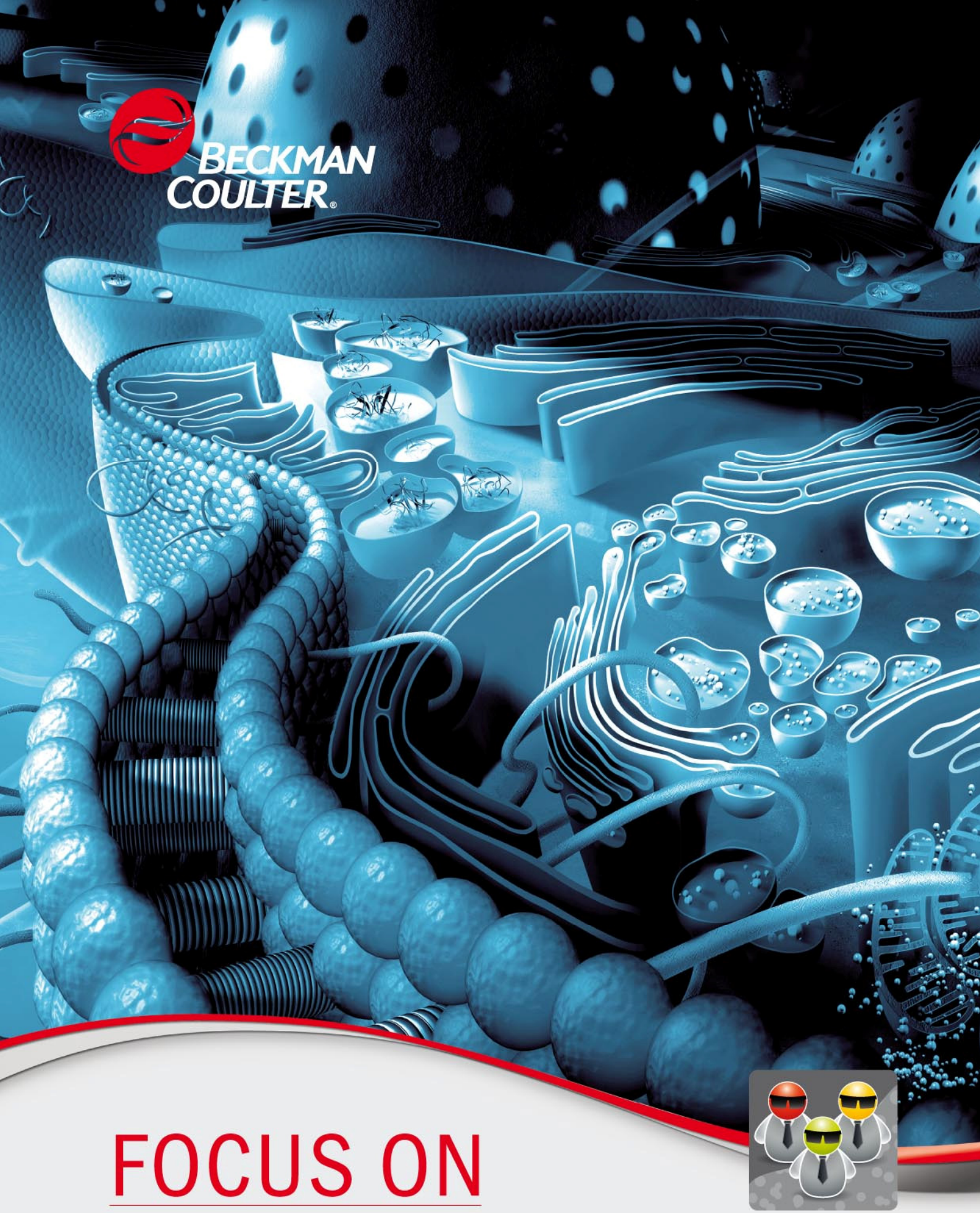


FOCUS ON

CEA IRMA, cat. No. IM2204, IM2274
new version

2012-01-03

CEA IRMA kit
Cat. No. IM2204, IM2274

We would like to inform you regarding a new version of CEA IRMA kit, cat. No. IM2204 and IM2274.

This assay will replace the previous version which was discontinued in autumn 2011.

The different monoclonal anti-CEA antibody is currently used for tube´s production.

Consequently, the assay protocol was changed to reach good continuity with the old CEA IRMA assay.

The new version will be launched with the January 9, 2012 availability date.

What will remain the same

Remaining	**Old version**	**New version**
Cat. No. of the kits	IM2204, IM2274	
Shelf life	10 weeks	
Incubation time	2 hours	
Analytical sensitivity	0.2 ng/mL	
Specificity	no cross-reaction with NCA antigens related to CEA	
Measurement range	0.2 – 325 ng/mL	
Hook effect	up to 10 000 ng/mL	

Summary of changes

Change	**Old version**	**New version**
Sample type	serum, plasma	serum
Sample volume	50 µL	30 µL
Tracer volume	200 µL	300 µL
Expected values	See detailed explanation in section 3. Expected values	
Functional sensitivity	0.65 ng/mL	0.38 ng/mL
Intra-assay	4.9 ng/mL	4.3 ng/mL
Inter-assay	12.3 ng/mL	6.2 ng/mL
Dilution test	97.3 – 112%	95.42 – 115.7%
Recovery test	89.8 – 110%	91.51 – 101.7%
Possible reduction of incubation time to 1 hour when the test is performed automatically	NO	YES

What was tested and compared?

Extensive studies were conducted to validate the new assay including the evaluation of all assay parameters. The clinical study using more than 1700 true patient samples was performed.

Please find important information below.

1. Sample Type: Serum is the only recommended sample type.

2. New assay protocol:

The volume of patient sample was decreased from 50 µL to 30 µL. The tracer volume was changed from 200 µL to 300 µL.

Step 1 Additions *	Step 2 Incubation	Step 3 Counting
To coated tubes, add successively: - **30** µL of calibrator, control or sample and - **300** µL of tracer. Mix.	Incubate 2 hours at 18-25°C with shaking (>280 rpm).	Aspirate carefully the contents of tubes (except the 2 tubes «total cpm»). Wash twice with 2 mL of wash solution. Count bound cpm (B) and total cpm (T) for 1 min.

3. Expected values

500 samples from ostensibly healthy Czech subjects were analysed and evaluated in new and old assays in Dec-2011. Very good conformity between both assays was found.

The evaluation for **new assay Dec-2011:**

Number of samples	Average	Standard deviation	**Probability 95 %**	**Probability 99 %**
500	0.98 ng/mL	0.852 ng/mL	< 2.5 ng/mL	< 4.6 ng/mL

The evaluation for **old assay Dec-2011:**

Number of samples	Average	Standard deviation	**Probability 95 %**	**Probability 99 %**
500	1.00 ng/mL	0.842 ng/mL	< 2.7 ng/mL	< 4.1 ng/mL

Newly obtained values for old assay are lower than those stated in Direction for use for old assay. An explanation of this difference may be in healthier life-style. The number of smokers has decreased and the quality of nutrition has improved in the Czech Republic since 1996.

The evaluation stated in Direction for use for old assay (measured in 1996):

Number of samples	Average	Standard deviation	**Probability 95 %**	**Probability 99 %**
515	1.58 ng/mL	1.025 ng/mL	< 3.7 ng/mL	< 5.1 ng/mL

3.1. Clinical study:

To evaluate the clinical utility, the serum samples of patients with three types of carcinoma were assayed, for both primary and secondary diagnosis:

- **Colorectal carcinoma** (n = 112 for primary diagnosis and n = 109 for secondary diagnosis, malignant tumour of colon, rectosignoidal junction and rectum).
- **Malignant tumour of breast** (n = 121 for primary diagnosis and n = 107 for secondary diagnosis).
- **Malignant tumour of lungs** (n = 48 for primary diagnosis and n = 102 for secondary diagnosis).

Results obtained with CEA IRMA kit were statistically evaluated. Cut off values, as well as clinical sensitivities at **95% clinical specificity** were established (current clinical consensus prefers to use 95% specificity to 90% specificity):

The same evaluation was done for both assays new and old. There is again very good conformity between both assays.

The evaluation for **new assay Dec-2011:**

	Cut off (ng/mL)		Sensitivity (%)	
	prim. diag.	sec. diag	prim. diag.	sec. diag
Colorectal carcinoma	2.50	3.91	64.3	93.6
Brest carcinoma	2.49	2.50	29.8	80.2
Lung carcinoma	2.50	3.74	56.3	54.9

The evaluation for **old assay Dec-2011:**

	Cut off (ng/mL)		Sensitivity (%)	
	prim. diag.	sec. diag	prim. diag.	sec. diag
Colorectal carcinoma	2.69	3.62	58.0	92.6
Brest carcinoma	2.73	2.62	23.1	71.7
Lung carcinoma	2.69	3.71	47.9	57.8

Newly obtained values for old assay are a little different than those stated in Direction for use for old assay. In primary diagnosis, the difference is again mainly caused by healthier life-style. The number of smokers has decreased and the quality of nutrition has improved in the Czech Republic since 1996. In secondary diagnosis, the difference is mainly caused by improved efficiency of treatment between years 1996 and 2011.

The cut off values and clinical sensitivity at **90% clinical specificity** for old assay stated in Direction for use for old assay (measured in 1996):

	Cut off (ng/mL)		Sensitivity (%)	
	prim. diag.	sec. diag	prim. diag.	sec. diag
Colorectal carcinoma	3.2	3.9	57.7	91.8
Brest carcinoma	3.3	3.7	55.9	75.7
Lung carcinoma	-	4.5	-	73.0

Because of very good correlation between the old and new versions (see chap. 13), it is possible to continue using the cut-off values that the labs established with the old version.

Also, there should not be any general shift in values during follow-up, though some individual differences cannot be excluded.

4. Analytical sensitivity

The analytical sensitivity of the new version is identical with old version: 0.2 ng/mL.

5. Functional sensitivity

Measured functional sensitivity of the new version is 0.38 ng/mL, functional sensitivity of old version was 0.65 ng/mL.

6. Specificity

The antibodies used in this kit exhibit no cross-reaction with NCA antigens related to CEA.

7. Intra-assay

The following information is presented in the new version of the Direction for use: ≤ 4.3 %, which is very well comparable with old kit version: ≤ 4.9 %.

Sample	**1**	**2**	**3**
Mean (ng/mL)	19.2	88.8	110
Median (ng/mL)	19.1	89.9	110
Min (ng/mL)	17.8	82.3	101
Max (ng/mL)	21.1	95.2	116
Number of determination	25	25	25
c.v. (%)	3.9	**4.3**	3.2

8. Inter-assay

The following information is presented in the new version of the Direction for use: ≤ 6.2 %, whilst ≤ 12.3 % was measured in the old kit version.

Sample	**1**	**2**	**3**
Mean (ng/mL)	2.31	12.5	205
Median (ng/mL)	2.36	12.6	205
Min (ng/mL)	2.00	11.8	197
Max (ng/mL)	2.45	13.7	214
Number of determination	10	10	10
c.v. (%)	**6.2**	4.8	2.5

9. Dilution test

Serum samples were serially diluted with the zero calibrator.

Following values are presented in Direction for use: 95.4 – 115.7 %.

Sample	Dilution factor	Measured (ng/mL)	Expected (ng/mL)	Recovery (%)
1	undiluted	24.17	-	**-**
	1:2	13.92	12.09	115.2
	1:4	6.99	6.04	**115.7**
	1:8	3.44	3.02	113.9
	1:16	1.73	1.51	114.5
	1:32	0.84	0.76	111.2
2	undiluted	74.50	-	**-**
	1:2	38.57	37.25	103.5
	1:4	18.89	18.63	101.4
	1:8	9.75	9.31	104.7
	1:16	4.49	4.66	96.43
	1:32	2.39	2.33	102.7
3	undiluted	99.08	-	**-**
	1:2	52.91	49.54	106.8
	1:4	26.43	24.77	106.7
	1:8	12.68	12.39	102.4
	1:16	6.08	6.19	98.18
	1:16	3.15	3.10	101.7
4	undiluted	>389.0	-	-
	1:2	254.19	-	-
	1:4	129.53	127.10	101.9
	1:8	63.12	63.55	99.33
	1:16	31.57	31.77	99.36
	1:16	15.16	15.89	**95.42**
5	undiluted	18.29	-	**-**
	1:2	9.12	9.15	99.73
	1:4	4.73	4.57	103.4
	1:8	2.36	2.29	103.2
	1:16	1.25	1.14	109.3
	1:32	0.66	0.57	115.5

10. Recovery test

Following values is presented in Direction for use: the recovery percentages ranged from 91.51 – 101.7 %.

Sample	Endogenous (ng/mL)	Added (ng/mL)	Expected (ng/mL)	Measured (ng/mL)	Recovery (%)
1	11.53	9.90	21.42	21.00	98.02
	11.17	19.18	30.35	30.86	**101.7**
	10.83	27.90	38.72	37.72	97.41
2	12.19	9.90	22.09	22.29	100.9
	11.81	19.18	30.99	28.87	93.15
	11.45	27.90	39.35	36.01	**91.51**
3	64.05	9.90	73.94	72.65	98.25
	62.04	19.18	81.22	80.54	99.16
	60.16	27.90	88.06	89.06	101.1
4	42.76	9.90	52.66	53.47	101.5
	41.43	19.18	60.61	61.36	101.2
	40.17	27.90	68.07	68.84	101.1
5	15.22	9.90	25.12	24.63	98.04
	14.75	19.18	33.93	31.63	93.23
	14.30	27.90	42.20	40.31	95.53

11. Measurement range

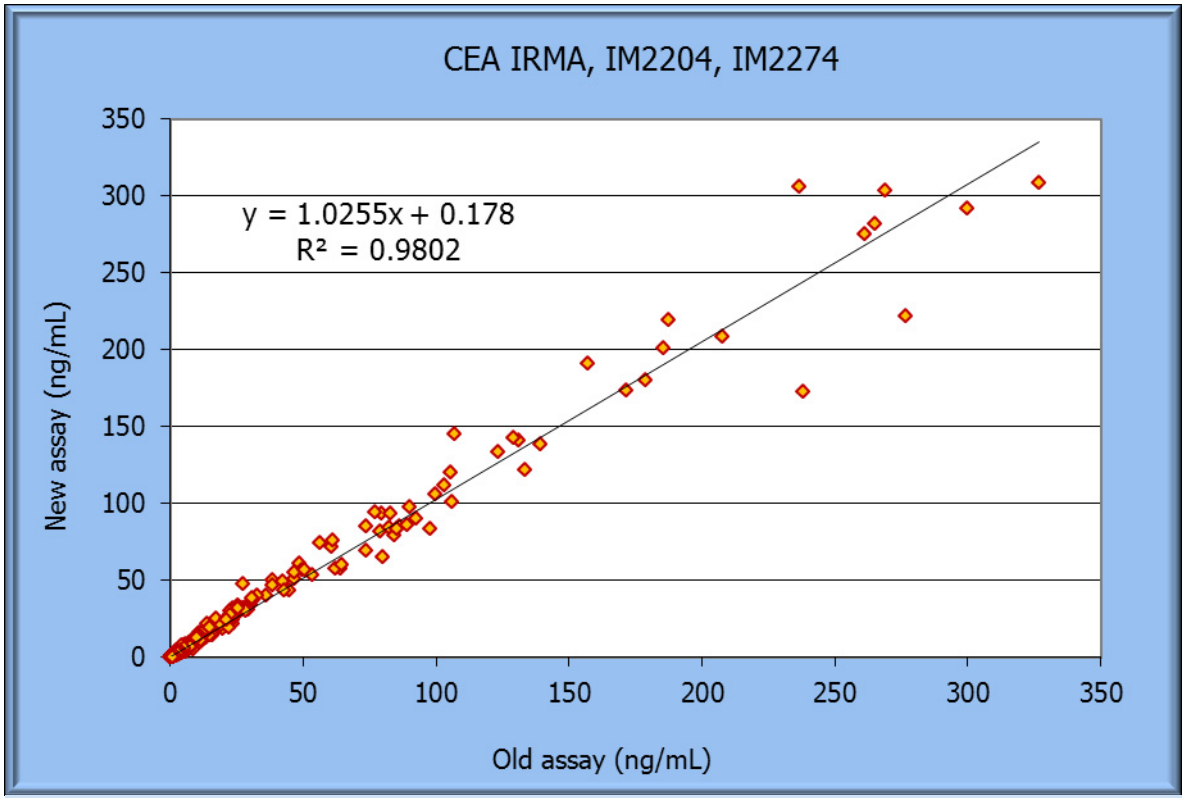
The measurement range of new method is the same with old version, approximately:
0.2 – 325 ng/mL.

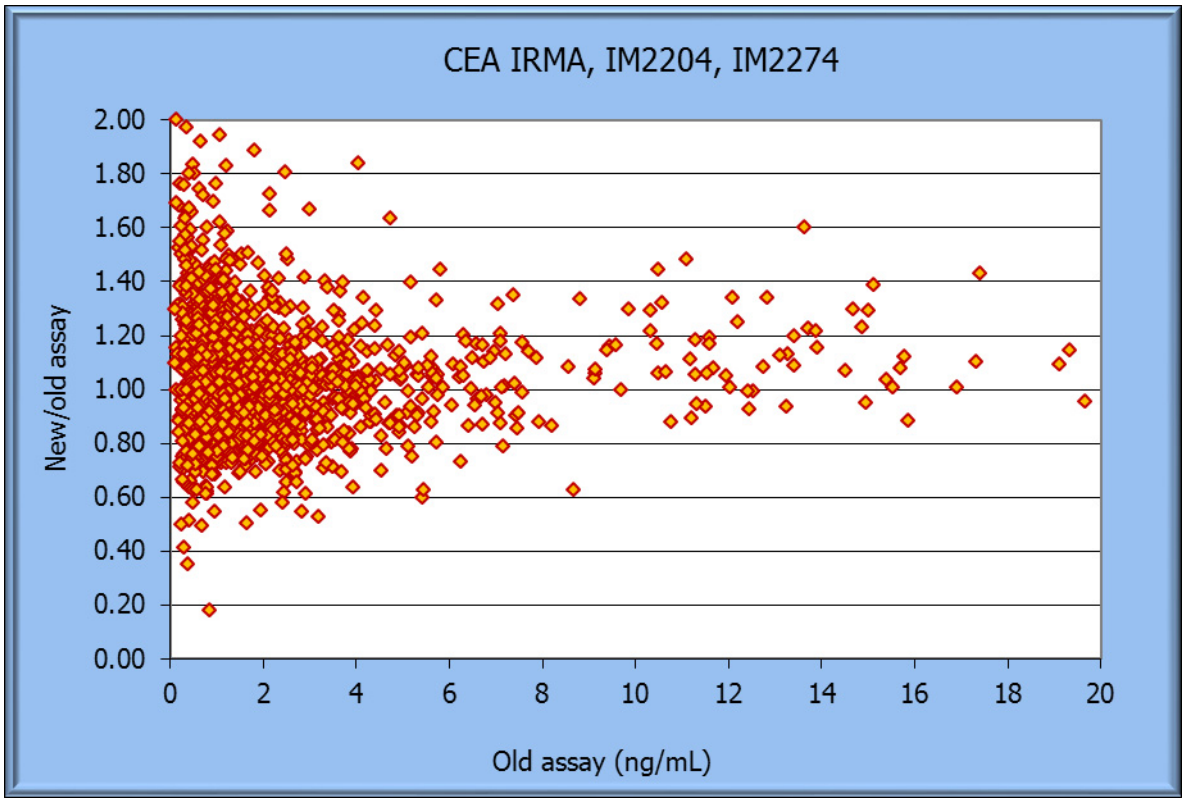
12. Hook effect

No hook effect was observed until 10 000 ng/mL. So, the same concentration level as for old assay is guaranteed for new assay.

13. Correlation between old and new version

Patient samples (n=1763) correlation between the old and new kit versions of IM2204, IM2274 is demonstrated in the following graphs:





14. Examples of old and new calibration curves, tracer 1 day old, performed manually:

	Old version (cpm)	New version (cpm)
Total	230 820	234 829
B0	178	43
B1	986	698
B2	4 539	3 493
B3	16 977	12 145
B4	76 305	51 578
B5	160 479	118 532

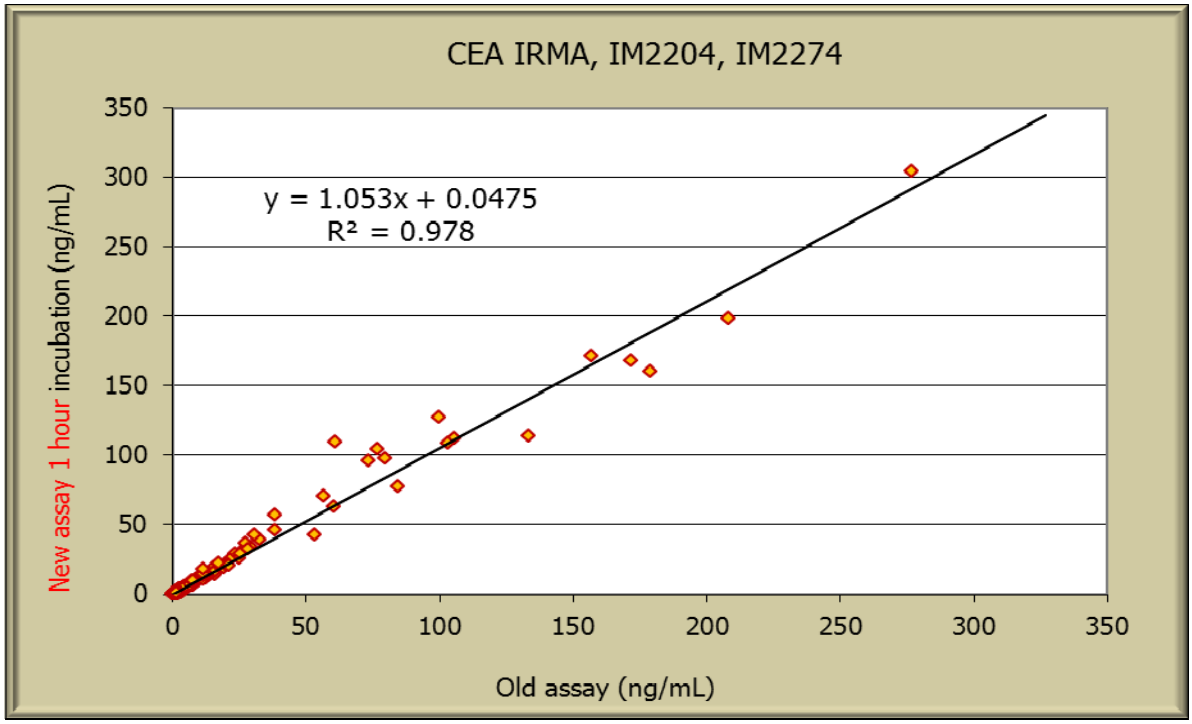
15. Examples of old and new calibration curves, tracer 21 days old, performed automatically:

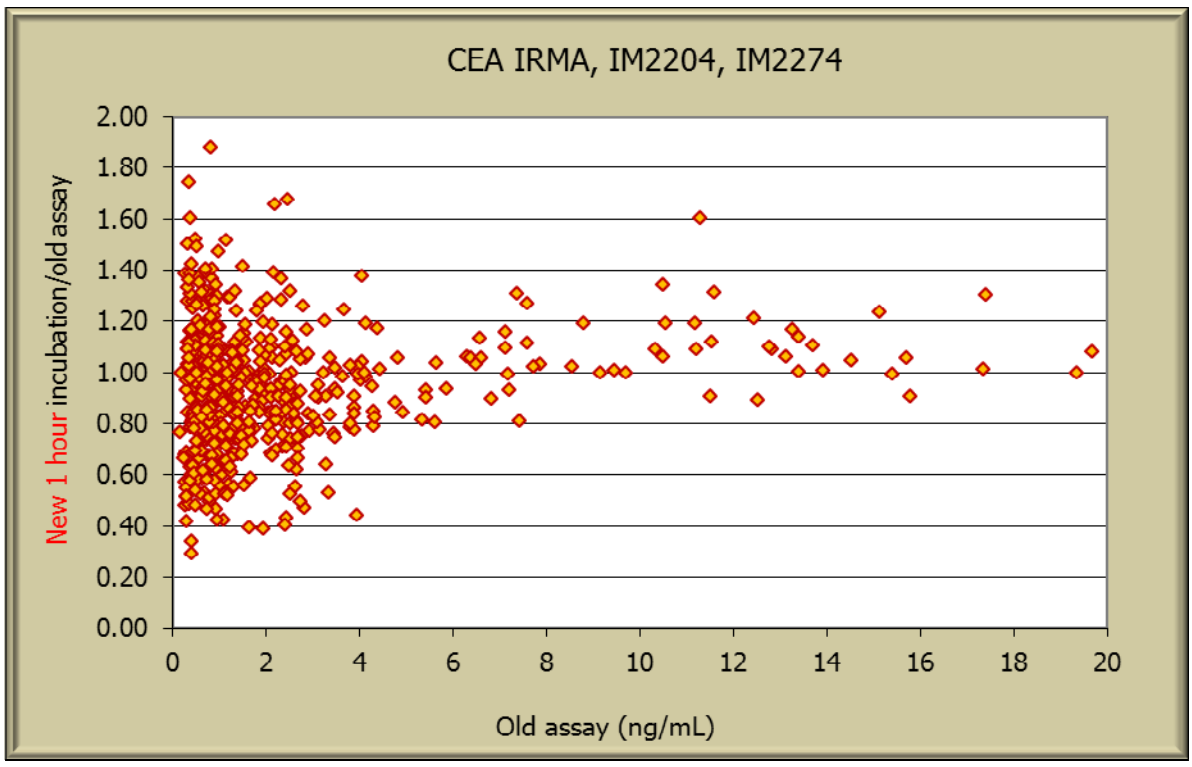
	Old version (cpm)	New version (cpm)
Total	182 255	175 787
B0	84	74
B1	509	562
B2	2 236	2 480
B3	8 896	9 170
B4	41 762	36 547
B5	87 407	81 432

16. Possible modification with 1 hour incubation time when the test is performed automatically at room temperature.

The reduction of incubation time from 2 hours to 1 hour was verified for automated determination.

16.1. The correlation of old assay and new 1 hour assay with 627 true samples was performed:





16.2. Expected values

The correlation between 2 and 1 hour incubation time was performed (automatically) with the satisfactory results (y=1.0365x-0.2437; R^2=0.97).

200 samples from ostensibly healthy Czech subjects were analysed and evaluated in new assay with 1 hour incubation in Dec-2011.

Number of samples	Average	Standard deviation	Probability 95 %	Probability 99 %
200	0.81 ng/mL	0.694 ng/mL	< 1.9 ng/mL	< 4.3 ng/mL

16.3. Examples of calibration curves with different incubation time, performed automatically, tracer 43 days old:

	New version 2 hrs (cpm)	**New version 1 hr (cpm)**
Total	150 475	150 475
B0	84	60
B1	400	264
B2	1 676	1 070
B3	6 176	4 010
B4	25 356	16 572
B5	59 086	42 134