Random Assay in Radioimmunoassay: Feasibility and Application Compared with Batch Assay

Jung Min Lee^{1,2} · Hwan Hee Lee^{1,2} · Sohyun Park^{1,2} · Tae Sung Kim¹ · Seok-Ki Kim¹

Received: 7 June 2016 / Revised: 27 June 2016 / Accepted: 5 July 2016 / Published online: 26 July 2016
© Korean Society of Nuclear Medicine 2016

Abstract

Purpose The batch assay has been conventionally used for radioimmunoassay (RIA) because of its technical robustness and practical convenience. However, it has limitations in terms of the relative lag of report time due to the necessity of multiple assays in a small number of samples compared with the random assay technique. In this study, we aimed to verify whether the random assay technique can be applied in RIA and is feasible in daily practice.

Methods The coefficients of variation (CVs) of eight standard curves within a single kit were calculated in a CA-125 immunoradiometric assay (IRMA) for the reference of the practically ideal CV of the CA-125 kit. Ten standard curves of 10 kits from 2 prospectively collected lots (pLot) and 85 standard curves of 85 kits from 3 retrospectively collected lots (Lot) were obtained. Additionally, the raw measurement data of both 170 control references and 1123 patients' sera were collected retrospectively between December 2015 and January 2016. A standard curve of the first kit of each lot was used as a master standard curve for a random assay. The CVs of inter-kits were analyzed in each lot, respectively. All raw measurements were normalized by decay and radioactivity. The CA-125 values from control samples and patients' sera were compared using the original batch assay and random assay.

Results In standard curve analysis, the CVs of inter-kits in pLots and Lots were comparable to those within a single kit.

The CVs from the random assay with normalization were similar to those from the batch assay in the control samples (CVs % of low/high concentration; Lot1 2.71/1.91, Lot2 2.35/1.83, Lot3 2.83/2.08 vs. Lot1 2.05/1.21, Lot2 1.66/1.48, Lot3 2.41/2.14). The ICCs between the batch assay and random assay using patients' sera were satisfactory (Lot1 1.00, Lot2 0.999, Lot3 1.00).

Conclusion The random assay technique could be successfully applied to the conventional CA-125 IRMA kits. The random assay showed strong agreement with the batch assay. The random assay procedure could increase the flexibility and decrease the turnaround time of the radioimmunoassay technique.

Keywords Radioimmunoassay · Immunoradiometric assay · Batch assay · Random assay · Standard curve · CA-125

Introduction

The radioimmunoassay (RIA) is an in-vitro assay that measures the concentration of a substance using radioisotopes and immunological principles [1, 2]. An immunoradiometric assay (IRMA) is the most common RIA technique, which generally uses a sandwich method comprised of the first and second antibodies labeled with radioisotopes and binding to the substance of concern [3, 4]. These methods have high sensitivity and specificity inherited by the characteristics of radioisotopes; the serum concentration of a small amount of hormone or tumor marker can be measured very accurately [5, 6].

A standard curve is acquired using multiple samples with known concentrations as calibrators in most quantitative assays in order to determine the concentration of an unknown sample by interpolation on the graph. The standard curve can be used as long as the measurement conditions do not change. Conventionally, RIA uses the batch assay technique for the

✉ Seok-Ki Kim
skkim@ncc.re.kr

¹ Department of Nuclear Medicine, National Cancer Center, 323 Ilsan-ro, Ilsandong-gu, Gyeonggi-do 410-769, Republic of Korea

² Department of Nuclear Medicine, Seoul National University Hospital, Seoul, Republic of Korea

precise standard curve; standard curves are obtained in every batch of measurements. A batch assay is advantageous for control over measurement errors from differences between kits and batches such as the decay of the radiotracer, radioactivity of the tracer, incubation time, and laboratory conditions, whereas the random assay, which usually obtains a standard curve once, needs relatively more meticulous quality controls.

Although the RIA using the batch assay technique is technically robust and easy to develop and perform, it needs the additional step of acquiring the standard curve and usage of standard samples in every batch measurement. This requires a relatively longer turnaround time and more resources in the setting of frequent and small amount requests [7]. If a standard curve of the IRMA kit can be used during a longer time interval such as once a lot, the random assay technique could be applied to the conventional IRMA kit.

A previous study conducted by Moon et al. showed that the first standard curve of a day could be used throughout the whole a day for an IRMA item. For the next step, we intended to verify whether a single master standard curve for all kits within the same lot number could be obtained and thereby whether a random assay using it is possible in the IRMA kit.

CA-125 is the most frequently used biomarker for ovarian cancer. The serum concentration of CA 125 is measured to detect a possible recurrence or response to therapy. The IRMA and RIA for CA 125 have high sensitivity and specificity [8–10]. CA-125 is the most commonly performed IRMA item at our institute. It was assumed to be the most appropriate item for this study. In this study, we analyzed standard curves from the 85 CA-125 IRMA kits of three lots. The raw measurement data of 170 control samples and 1123 patients' sera with decay and tracer radioactivity normalization were analyzed based on batch and random assays. The comparability between both methods was assessed in CA-125 IRMA.

Materials and Methods

Materials

Eight standard curves were obtained within one CA-125 immunoradiometric assay (IRMA) kit (Fujirebio Diagnostic Inc., Budapest, Hungary). Ten standard curves of ten kits were obtained from two prospectively collected lots (pLot) in CA-125 IRMA. Eighty-five standard curves of 85 kits were obtained from 3 retrospectively collected lots (Lot). Each standard curve was obtained from the first batch in each kit. Five kits of pLot1, 5 kits of pLot2, 37 kits of Lot1, 21 kits of Lot2, and 27 kits of Lot3 were analyzed, respectively. Additionally, the raw measurement data from both the 170 control references and 1123 patients' sera were retrospectively collected between December 2015 and January 2016 in Lots. The raw measurement data of patients' sample were composed of 461

patients' sera (Lot1), 288 patients' sera (Lot2), and 374 patients' sera (Lot3), respectively.

The kits from Lot1, Lot2, and Lot3 were used for 12, 10, and 12 days, respectively. Each kit was composed of standard reference samples with six different concentrations (0, 15, 30, 80, 200, and 500 U/ml) and control reference samples with low/high concentrations [49 (41–56) U/ml, 96 (83–113) U/ml].

All kits were stored in the refrigerator between 2 and 8 °C, and the laboratory room was kept at 15–25 °C during all measurements. Other conditions of procedures were followed according to the Korean Nuclear Medicine Recommendations. All measurements were performed complying with the assay procedure provided by the manufacturing company. All raw measurement data were retrieved from a gamma counter (Gamma 10, Shin Jin Medics MC, Goyang, Korea) and the quality control records. The gamma counter holds all the raw measurement data in counts per minute (CPM) and the actual value of the samples, controls, and standard references in U/ml for over 2 years. This study was exempted from approval by the institutional review board at our center.

Method

We evaluated the standard curves of the IRMA for the establishment of a master curve of a conventional IRMA kit and validated the efficacy of the random assay technique compared with the original batch assay results in practice. The processes of this study are shown schematically in Fig. 1.

Standard Curve Analysis

The coefficients of variation (CVs) of the raw measurement count in eight standard curves within a single kit were calculated for the reference of the practically ideal CV of the CA-125 IRMA kit. All raw measurement counts of standard samples from the second, third, fourth, and fifth kit in pLot1 were normalized based on the counts of the standard sample of the first kit. All raw measurement counts of pLot2 were normalized by the same method. The normalization was performed by two steps, decay correction and radiotracer correction. The decay correction was performed based on the manufactured day, and the ratio of tracer radioactivity of each kit based on that of the first kit was multiplied in all counts. The standard curves from pLot1 and pLot2 were drawn graphically and compared with each other before and after normalization (Fig. 2). Using these data, the CVs of inter-kits were evaluated in pLot1 and pLot2, respectively (Table 1). For analysis of Lots, the ratio of tracer radioactivity between kits was compared with the ratio of the value of standard reference measurements, which was calculated as the mean of the measurement count of the fourth and fifth standard reference sample in pLots.

Fig. 1 Schematic diagram of the study: For assessment of the standard curve as master curve, the CVs of standard curves within a single kit (intra-kit) were compared with each other (inter-kit; pLot1-2 and Lot1-3). For the next step, the master curve was applied to a conventional CA-125 IRMA kit and then batch and random assay techniques were compared in control samples and patients' sera after normalization

The standard curves from 85 kits (Lot1, 2, and 3) were normalized in terms of the decay time and radioactivity ratio by the same normalization method. The radioactivity concentration of Lots could not be obtained. In cases of Lots, we used the ratio (0.82–1.09) of the measurement mean count of the fourth and fifth standard reference sample instead of the ratio of radiotracer activity. The standard curves were compared in terms of mean and CV, respectively (Table 1).

The CVs of standard curves in each lot were compared with those within a single kit, respectively.

Control Reference Sample Analysis

The value of control samples was obtained twice by an original batch assay and random assay using the first standard curve of

the lot and normalized measurement counts. The 37 kits of Lot1, 21 kits of Lot2, and 27 kits of Lot3 were evaluated, respectively. For the batch assay, the value of the control samples was calculated based on each standard curve of each kit. In case of the random assay, the counts of control samples were normalized as per the method explained earlier in the “Standard curve analysis” section. The value of each control sample was calculated based on a master standard, i.e., the first standard curve of each lot. The values of the mean and CV were extracted. Comparison between the batch assay and random assay was performed using the paired *T* test or Wilcoxon sign-rank test, respectively (Table 2).

Patients' Sera Analysis

In order to evaluate the reliability of the batch assay and random assay using patients' sera data, values of patients' sera were calculated retrospectively by both methods. In the case of the batch assay, all the values were retrieved from the gamma counter. For the random assay, the values of the patients' sera were retrospectively calculated with the normalized measurement data using the previously explained normalization method.

The reliability between the batch assay and random assay within one lot was evaluated using the intra-class correlation coefficient (ICC) and Pearson's correlation [11] (Table 3). Bland-Altman plots were obtained (Fig. 3).

Statistical Analysis

The statistical analyses were performed using SPSS software (version 22.0; SPSS Inc., Chicago, IL, USA) and Matlab 7.0.4 (Mathwork Inc., Natick, MA, USA).

Results

Standard Curve Analysis

The CVs of eight standard curves within a single kit were 9.11, 10.81, 5.76, 6.42, 4.45, and 4.73 %, respectively, at each concentration (0, 15, 30, 80, 200, 500 U/ml, $n = 8$). The CVs of inter-kits were 1.29 %, 4.02 %, 3.04 %, 3.45 %, 1.71 %, 2.11 % in pLot1 and 5.04 %, 5.99 %, 6.82 %, 2.59 %, 1.06 %, 0.44 % in pLot2, respectively, at each concentration (0, 15, 30, 80, 200, 500 U/ml, $n = 5$). The CVs of inter-kits in pLot1 and 2 were comparable to those within a single kit after normalization. The CVs of inter-kits were 20.86 %, 6.15 %, 4.71 %, 2.20 %, 0.65 %, 0.64 % in Lot1, 15.60 %, 5.87 %, 5.09 %, 2.09 %, 0.74 %, 0.76 % in Lot2, and 17.76 %, 5.91 %, 5.11 %, 3.34 %, 2.24 %, 1.85 % in Lot3. The CVs of standard reference samples of Lot1, 2, and 3 were comparable to those within a single kit except the standard sample of 0 U/ml after normalization.

The means between pLot1 and pLot2 were different in all concentrations of standard reference samples ($p < 0.01$). The

Fig. 2 A significant difference was noted in the inter-kit precision between standard curves of different lot numbers as well as between the different kits. **a** After normalization of standard curves, no significant difference was noted between the standard curves in the same lot number **b**

means within the same lot number were not different after normalization ($p > 0.05$) (Table 1, Fig. 2). In the same context, the means between different lot numbers were significantly different in all standard samples of Lot1, 2, and 3 (one-way ANOVA $p = 0.00$). After normalization, no statistical difference was noted within the same lot number ($P > 0.05$).

The ratio of tracer radioactivity was very similar to the ratio of the value of standard reference measurements in pLot1 and 2 (pLot1 $R^2 = 0.944$, pLot2 $R^2 = 0.938$). The ratio of standard reference measurements was used for the normalization of Lot1, 2, and 3.

Control Reference Sample Analysis

The values of control samples were compared between the original batch assay and our random assay method. No

significant statistical difference was noted between the batch assay and random assay in Lot1, 2, and 3 (p -values of low/high concentration; Lot1 0.06/0.753, Lot2 0.054/0.05, Lot3 0.108/0.449). The CVs of the random assay (CVs % of low/high concentration; Lot1 2.05/1.21, Lot2 1.66/1.48, Lot3 2.41/2.14) were similar to those of the batch assay (CVs % of low/high concentration; Lot1 2.71/1.91, Lot2 2.35/1.83, Lot3 2.83/2.08) (Table 2).

Patients' Sera Analysis

Intra-class correlations of the values by batch assay and random assay of real patients' sera were satisfactory. The ICC between the batch assay and random assay were 0.999 (Lot1), 0.995 (Lot2), and 0.998 (Lot3). The coefficients of correlation between the batch assay and random assay were 0.999 (Lot1),

Table 1 Standard curve analysis

			Standard reference sample (U/ml)					
			STD1 (0)	STD2 (15)	STD3 (30)	STD4 (80)	STD5 (200)	STD6 (500)
Intra- kit	Within single kit ($n = 8$)	Mean (CPM)	310	1192	2217	6196	16307	38445
		CV	9.12	10.81	5.75	6.41	4.45	4.73
Inter-kits	pLot1 (5 kits)	Mean (CPM)	313	1323	2551	7416	19571	46322
		CV	1.29	4.02	3.04	3.54	1.71	2.11
	pLot2 (5 kits)	Mean (CPM)	246	1097	1920	5563	14676	34823
		CV	5.04	5.99	6.82	2.59	1.06	0.44
	Lot1 (37 kits)	Mean (CPM)	283	1351	2560	7242	18892	44845
		CV	20.86	6.15	4.71	2.20	0.65	0.64
	Lot2 (21 kits)	Mean (CPM)	220	1055	2042	5910	15622	36903
		CV	15.60	5.87	5.09	2.09	0.74	0.76
	Lot3 (27 kits)	Mean (CPM)	260	1224	2326	6517	17201	40575
		CV	17.76	5.91	5.11	3.34	2.24	1.85

CPM counts per minute, CV coefficient of variation (%)

Table 2 Comparison between the batch assay and random assay using control samples

	Assay	Low conc.			High conc.		
		Mean	C.V (%)	<i>p</i> -value*	Mean	C.V (%)	<i>p</i> -value*
Lot1 (37 kits)	Batch	55.12	2.71	0.06	103.29	1.91	0.753
	Random	54.42	2.05		103.21	1.21	
Lot2 (21 kits)	Batch	55.99	2.35	0.054 ^a	103.83	1.76	0.05 ^a
	Random	55.28	1.66		102.5	1.48	
Lot3 (27 kits)	Batch	55.9	3.13	0.108	104.34	2.46	0.449
	Random	56.45	2.41		104.74	2.14	

^a Wilcoxon signed-rank test*Paired *t*-test

0.996 (Lot2), and 0.998 (Lot 3) ($p = 0.00$) without normalization. After normalization, the ICCs between the batch assay and random assay became better. The ICCs between the batch assay and random assay were 1.00 (Lot1), 0.999 (Lot2), and 1.00 (Lot3). The coefficients of correlation between the batch assay and random assay were 1.000 (Lot1), 1.00 (Lot2), and 1.00 (Lot3) ($p = 0.00$). The ICC values and coefficient of correlation were more improved after normalization in all analyses (Table 3). The Bland-Altman plot showed the agreement of two measurements by both assays (Fig. 3). There were no differences between the two methods using patients' sera.

Discussion

In this study, we aimed to verify whether the obtained single master standard curve could be used and thereby the random assay could be used in daily practice. If an ideal master standard curve is obtained in the IRMA kit and can be used during longer time intervals such as once a lot, we can increase the flexibility of the RIA and decrease the turnaround time using the random assay technique.

We successfully validated the possibility of application of a master standard curve. The CVs of standard reference samples of inter-kits in each lot were comparable to those within a single kit. It was suggested that the standard curves within the same lot number are as homogeneous as the standard

curves of a single kit. Therefore, the single master curve can be applied to the measurements within the same lot number.

In this study, all counts of raw measurements were normalized by the decay and radioactivity concentration. The counts were affected by decay, considering the half-life of I-125, which is 60.14 days. The variation of standard curves was found not only in measurements done on different days but also those on the same day. It is suggested that there was a certain variation of standard curves other than the variation by the decay of tracer. We measured the tracer radioactivity of each kit, and different radioactivity values were observed between the kits of the same lot number. For these reasons, all raw measurement counts were normalized using tracer radioactivity as well as decay. The mean counts of standard samples within the same lot number were not statistically different after normalization.

The random assay can possibly obtain as precise a result as the batch assay. The analyses of pLots and Lots were consistent with each other. In the control sample analysis, the CVs from the random assay were similar to those from the batch assay, and the CVs of both techniques were below 10 %, which all were assumed to be acceptable [12–14]. The random assay showed strong agreement with the batch assay in patients' sera. Furthermore, the ICCs between the batch assay and random assay using patients' sera were improved after normalization. It is suggested that we can apply the random assay technique instead of the batch assay.

This study has several limitations. The radioactivity concentration could not be obtained in retrospectively collected

Table 3 Result of assessing agreement between the batch assay and random assay in patients' sera

		ICC	95 % Confidence interval	Pearson R ²
Lot1 (461 patients' sera)	Non-normalization	0.999	0.999 to 0.999	0.999 ($p = 0.00$)
	Normalization	1.000	1.000 to 1.000	1.000 ($p = 0.00$)
Lot2 (288 patients' sera)	Non-normalization	0.995	0.995 to 0.996	0.996 ($p = 0.00$)
	Normalization	0.999	0.999 to 1.000	1.000 ($p = 0.00$)
Lot3 (374 patients' sera)	Non-normalization	0.998	0.997 to 0.998	0.998 ($p = 0.00$)
	Normalization	1.000	1.000 to 1.000	1.000 ($p = 0.00$)

Fig. 3 The random assay showed strong agreement with the batch assay in the analysis of patients' sera

Lot1, 2, and 3. We used the ratio of the mean of measurement counts of the fourth and fifth standard reference sample instead of the ratio of radiotracer activity. The value of the range from between 0.82 and 1.09 was multiplied in all counts of Lot1, 2, and 3 after decay correction. However, the ratio of

radioactivity was very similar to the ratio of the value of the standard reference measurements in pLots. In this context, the ratio of radioactivity in Lots can be assumed from the value of the range from between 0.82 and 1.09. Therefore, the analysis of Lot1, 2, and 3 could be performed.

The CV value of standard reference samples of 0U/ml was high, above 10 % in Lot1, 2, and 3. It has low counts so that it can be influenced by the laboratory conditions. In our department, since the in-vivo imaging facility is close to the laboratory, the count value of the background might have been affected. Using additional shielding material can improve the CV of standard reference samples of 0U/ml.

Because we used one lot during only 10–12 days and only analyzed CA-125 IRMA, the strong agreement between the batch assay and random assay can be applied only to IRMA during 10–12 days. It could not be applied in the small-volume hospital using fewer kits. If additional research choosing other items that uses RIA items or a single lot for a long period is performed, it could be seen whether applying RIA items or using a single lot for a longer time is possible.

Conclusion

The random assay can be successfully applied to the conventional CA-125 IRMA kits. The random assay showed strong agreement with the batch assay in the analysis of control samples and patient sera. The random assay using a master standard curve was comparable to the original batch assay and could increase the flexibility and decrease the turnaround time of the radioimmunoassay technique.

Acknowledgments This study was supported by Woo-jae Won and Jina Kim at the Department of Nuclear Medicine, National Cancer Center. We are grateful to them for their excellent technical assistance.

Compliance with Ethical Standards

Conflict of Interest Jung Min Lee, Hwan Hee Lee, Sohyun Park, Tae Sung Kim, and Seok-ki Kim declare that they have no conflict of interest.

Ethical Statement All procedures followed were performed in accordance with the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration of 1975, as revised in 2013. This study was exempted from approval by the institutional review board at our center.

References

1. Goldsmith SJ. Radioimmunoassay: review of basic principles. *Semin Nucl Med.* 1975;5:125–52.
2. Hawker CD. Radioimmunoassay and related methods. *Anal Chem.* 1973;45:878A–90a.
3. Lindstedt G, Jacobsson A, Lundberg PA, Hedelin H, Pettersson S, Unsgaard B. Determination of prostate-specific antigen in serum by immunoradiometric assay. *Clin Chem.* 1990;36:53–8.
4. Peters J, Schmidt-Gayk H, Peters B, Armbruster FP, Quentmeier A, Mathias D. Immunoradiometric assay of carcinoembryonic antigen with use of avidin-biotin labeling. *Clin Chem.* 1989;35:573–6.
5. Sturgeon CM, Hussey AJ, Beynon L, Ritchie A, Chisholm GD, Micklem L, et al. Comparison of radioimmunoassay and immunoradiometric assay for serum prostatic acid phosphatase. *Clin Chim Acta.* 1986;161:47–57.
6. Woodhead JS, Addison GM, Hales CN. Radioimmunoassay and saturation analysis. The immunoradiometric assay and related techniques. *Br Med Bull.* 1974;30:44–9.
7. Moon SH, Lee HY, Shin SY, Min GS, Lee HJ, Jang SJ, et al. Comparison of batch assay and random assay using automatic dispenser in radioimmunoassay. *Nucl Med Mol Imaging.* 2009;43:323–9.
8. Niloff JM, Bast RC, Schaetzl EM, Knapp RC. Predictive value of CA 125 antigen levels in second-look procedures for ovarian cancer. *Am J Obstet Gynecol.* 1985;151:981–6.
9. Suh KS, Park SW, Castro A, Patel H, Blake P, Liang M, et al. Ovarian cancer biomarkers for molecular biosensors and translational medicine. *Expert Rev Mol Diagn.* 2010;10:1069–83.
10. Glenn J, Steinberg WM, Kurtzman SH, Steinberg SM, Sindelar WF. Evaluation of the utility of a radioimmunoassay for serum CA 19–9 levels in patients before and after treatment of carcinoma of the pancreas. *J Clin Oncol.* 1988;6:462–8.
11. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet.* 1986;1:307–10.
12. Won KS, Ryu JS, Moon DH, Lee HK. The evaluation of domestic immunoradiometric assay kit for alpha-fetoprotein. *Korean J Nucl Med.* 2000;34:353–9.
13. Sturgeon C, Dati F, Duffy M, Hasholzner U, Klapdor R, Lamerz R, et al. Quality requirements and control: EGTM recommendations. *Anticancer Res.* 1999;19:2791–4.
14. Zaki R, Bulgiba A, Ismail R, Ismail NA. Statistical methods used to test for agreement of medical instruments measuring continuous variables in method comparison studies: a systematic review. *PLoS One.* 2012;7:e37908.