

# Application of novel magnetizable cellulose particles in the development of immunoradiometric assay for C-peptide

R. R. Rasmi · K. Bhasker Shenoy · V. B. Kadwad · Jayula Sarnaik · H. M. Somashekarappa

Received: 9 October 2014 / Published online: 14 January 2015  
© Akadémiai Kiadó, Budapest, Hungary 2015

**Abstract** The present study was oriented to develop, optimize and validate immunoradiometric assay for the measurement of C-peptide in human serum by employing magnetizable cellulose particles prepared by a novel method of wet grinding. Six commercial monoclonal antibodies raised against the different epitopes of C-peptide were evaluated for their compatibility as sandwich partners by “cross matching”. The developed one step inclusive assay with best matched combination of sandwich complex, “magnetizable cellulose particles linked capture antibody–C-peptide–<sup>125</sup>I labelled detector antibody” had an analytical sensitivity of 0.16 ng/ml and standard range of 0.3–30 ng/ml covering required physiological and diagnostic range.

**Keywords** Magnetizable cellulose particles · C-peptide · Monoclonal antibodies · Immunoradiometric assay · Diabetes

## Introduction

Human C-peptide (3021 Daltons), composed of 31 amino acids, a by-product of insulin biosynthesis is produced by a

series of enzymatic cleavages of the precursor molecules preproinsulin and proinsulin [1]. Thereby, insulin and C-peptide are released in equimolar amounts (1:1 ratio) from  $\beta$ -cells of pancreas into the portal circulation [2]. Soon after their entry, both insulin and C-peptide are carried to the liver (prime target organ) wherein insulin is extracted and degraded [3]. Another vital property is that the half-life of C-peptide is two to five times higher as compared to insulin. As a result, C-peptide levels may be used as a stable indicator of insulin secretion and important marker of  $\beta$ -cell function [4, 5] and under conditions of beta cell culture (in vitro) [6]. Insulin measurement in diabetic patients treated with insulin may be complicated by anti-insulin antibodies and cross reactivity with partially degraded insulin, proinsulin and split forms of proinsulin [7]. Extensive research on C-peptide has been carried out over the past few decades and it was found that measurement of C-peptide alone is adequate to make accurate clinical predictions and also to change treatment modality. By measuring C-peptide levels, a wide gamut of beneficial properties has been reported include; differentiation of Type 1 and Type 2 diabetes. A study by Bakhtadze et al. [8] has shown that patients diagnosed with type 1 diabetes mellitus had significantly lower median fasting plasma C-peptide than patients with type 2 diabetes mellitus or diabetes mellitus that was unclassified. The median values were 0.73 ng/ml (0.24 nmol/l) for type 1 diabetes mellitus, 2.24 ng/ml (0.74 nmol/l) for type 2 diabetes mellitus, and 1.45 ng/ml (0.48 nmol/l) for unclassified diabetes mellitus. C-Peptide levels can also predict the occurrence of auto-antibodies. Torn et al. [9] have shown that C-peptide levels predict the presence of autoantibodies like islet cell antibodies, glutamic acid decarboxylase antibodies or tyrosine phosphatase antibodies. C-peptide levels also aid in diagnosis of various hypoglycemic disorders including islet cell

---

R. R. Rasmi · K. B. Shenoy (✉)  
Department of Applied Zoology, Mangalore University,  
Mangalagangothri 574 199, India  
e-mail: kshenoyb@gmail.com

V. B. Kadwad · J. Sarnaik  
Radiopharmaceuticals Programme, Board of Radiation and  
Isotope Technology (BRIT), Vashi Complex, Sector 20, Vashi,  
Navi Mumbai 400 703, India

H. M. Somashekarappa  
Centre for Application of Radioisotopes and Radiation  
Technology (CARRT), Mangalore University,  
Mangalagangothri 574 199, India

tumors and factitious hypoglycemia [10]. Levels of C-peptide were found to be elevated in some of the obese individuals with Type 2 diabetes mellitus indicating insulin resistance [11]. Immunoassays based on the solid phase separation are widely preferred due to their ease of separation despite of heavy antibody consumption. In sandwich assays, like immunoradiometric assay (IRMA) solid phase separation is essential.

IRMA usually makes use of two monoclonal antibodies raised against the same analyte, but directed towards two different epitopes. A polyclonal antibody can also replace one of these monoclonal antibodies. One of these antibody will be immobilized on the solid support extracts or picks up the antigen (analyte) molecule from the sample and hence called capture antibody. In order to detect and quantitate the amount of the analyte captured, the other antibody labelled with  $^{125}\text{I}$ , which is called as a detector antibody or tracer, is used. The development of IRMA further involves proper selection of capture and detector antibody pair, preparation of antibody coupled to solid support (immunosorbent), labelling of the antibody and assay optimization for desired sensitivity and range [12].

Among the available immunosorbents, single piece solid support like antibody coated plastic tubes are the most convenient, but large scale production of these coated tubes require sophisticated technology with automatic gadgets to ensure uniform coating of the antibody. Besides, this is expensive as large quantities of antibody along with good quality tubes are required. On the other hand, separation methods based on antibody coupled to magnetizable particles as solid phase (magnetizable immunosorbents) are relatively inexpensive and can serve as an attractive alternative to coated tubes, without compromising much upon simplicity of the assay system. Among the variety of magnetizable particles, magnetizable cellulose particles are often preferred as they are associated with low non specific binding (NSB) and higher stability of resulting immunosorbent besides being inexpensive [13]. The objective of the present study was to develop and validate a simple one step inclusive IRMA for C-peptide in human serum samples using magnetizable cellulose particles as solid support with special emphasis on cross matching of monoclonal antibody pairs and selection of a suitable pair for standardization of C-peptide IRMA.

## Experimental

### Materials

Bovine serum albumin (BSA; RIA Grade), C-peptide (Purity  $\geq 95\%$  (HPLC)), 1,1'-Carbonyldiimidazole (CDI) and Sephadex G-75 were from Sigma Chemical Company,

USA. Bovine serum was obtained from Himedia, India. A pair of monoclonal antibodies designated as A & B were obtained from Medix Biochemica, Finland, C & D from Fitzgerald Industries International, USA and E & F from Abcam, USA. Carrier free  $^{125}\text{I}$  as Sodium Iodide (Specific activity  $\sim 17\text{ mCi}/\mu\text{g}$ , radioactive concentration  $100\text{ mCi/ml}$ ) was obtained from BARC, India. Magnetizable cellulose particles were prepared in-house at BRIT Laboratories, Mumbai, India (Indian Patent No: 193445). Multiwell RIA gamma counter was from Stratec Biomedicals Systems, Germany and Single well gamma counter NaI(T) was from Electronic Corporation of India (ECIL), India. Paper electrophoresis system was from Biotech R&D Laboratories, India.

### Preparation of detector antibody

Radioiodination of monoclonal antibody was performed with  $^{125}\text{I}$ -NaI using Chloramine-T oxidation method as described by Greenwood et al. [14] with minor modifications. Radiolabeling procedures were performed with attention to good radiation safety practice. In particular, radioiodinations were performed in a well-ventilated fume hood in a glass test tube ( $75 \times 12\text{ mm}$ ) as detailed in Table 1. At the termination of the iodination reactions aliquots were immediately removed for trichloroacetic acid (TCA) precipitation/paper electrophoresis and the reaction mixture was immediately purified by gel filtration. Purification of radiolabelled anti-C-peptide monoclonal antibody from free radioiodide was carried out by using Sephadex G-75 column ( $30 \times 1\text{ cm}$ ) pre-equilibrated with  $0.05\text{ M}$  phosphate buffer and BSA.  $1\text{ ml}$  fractions were collected in  $1\text{ ml}$  of  $1\%$  BSA in pre-marked tubes at a flow rate of  $8\text{ drops/min}$ , and their radioactivity was monitored in a single well manual gamma scintillation counter calibrated for  $^{125}\text{I}$  with changed geometry. All the six monoclonal antibodies A–F were radioiodinated using the same protocol and radioiodinated antibodies are designated as A\*–F\*.  $^{125}\text{I}$ - anti-C-peptide monoclonal antibody fractions, were pooled and diluted in  $0.05\text{ M}$  phosphate buffer, pH 7.4

**Table 1** Radioiodination protocol for anti-C-peptide monoclonal antibody

Anti-C-peptide monoclonal antibody	20 $\mu\text{g}$ (20 $\mu\text{l}$ )
0.5 M Phosphate Buffer, pH 7.4	40 $\mu\text{l}$
$^{125}\text{I}$ -NaI	300 $\mu\text{Ci}$
Chloramine-T	10 $\mu\text{g}$ (10 $\mu\text{l}$ )
<i>Reaction time 60 s</i>	
0.05 M phosphate buffer, pH 7.4	500 $\mu\text{l}$
Sodium metabisulphite	20 $\mu\text{g}$ (20 $\mu\text{l}$ )
Potassium iodide	250 $\mu\text{g}$ (50 $\mu\text{l}$ )

containing 0.5 % BSA and stored in aliquots at  $-20\text{ }^{\circ}\text{C}$  for evaluation in the assay.

The radioiodination yield was estimated by paper electrophoresis and TCA precipitation of the reaction mixture prior to the purification. The radiochemical purity (RCP) of the purified and pooled fractions of radioiodinated anti-C-peptide monoclonal antibody was determined using paper electrophoresis and TCA precipitation. An aliquot of the reaction mixture and peak fractions obtained from the column were spotted on a 30 cm Whatman 3 mm chromatography paper. The electrophoresis was run for 1 h at 240 V using 0.025 M phosphate buffer, pH 7.4. At the end of the run, the paper was removed, dried and cut into 1 cm segments and measured for radioactivity. The electrophoresis pattern of the reaction mixture was used for calculating the percentage radioiodination yield while those of the purified fractions were used as a measure of the RCP. Radiochromatogram was plotted by counts against strip number. Radioiodination yield and RCP of tracer was evaluated. The free iodide moves towards the anode and labelled molecule remains at the point of application. TCA precipitation method involves addition of 50  $\mu\text{l}$  of the reaction mixture/peak fractions and 50  $\mu\text{l}$  of C-peptide free bovine serum followed by 1 ml of 10 % TCA. The contents were centrifuged and radioactivity associated with pellet is measured to calculate percentage radioiodination in the case of reaction mixture, whereas purified fraction for the RC purity [15].

#### Preparation of capture antibody

Anti-C-peptide monoclonal antibody was coupled to in-house prepared magnetizable cellulose particles using CDI activation method using 900  $\mu\text{g}$  of anti-C-peptide monoclonal antibody per g of magnetizable cellulose particles. 1 g of in-house prepared magnetizable cellulose particles were washed with 100 ml of double distilled water (DDW) followed by five washes with  $\sim 10$  ml of acetone. The washed particles were suspended in 10 ml acetone containing 500 mg of CDI for activation with gentle shaking for 1 h at room temperature. After activation, particles were washed with acetone followed by 0.1 M bicarbonate buffer, pH 8.6 (bicarbonate buffer) and suspended in 10 ml of bicarbonate buffer, pH 8.6 containing 900  $\mu\text{g}$  of anti-C-peptide monoclonal antibody. The total reaction volume was maintained at 20 ml. The reaction was allowed to proceed overnight with gentle shaking. Anti-C-peptide monoclonal antibody coupled magnetizable cellulose particles were washed with bicarbonate buffer, pH 8.6 and 0.1 M acetate buffer, pH 4 alternatively to block the residual free binding sites. Anti-C-peptide monoclonal antibody coupled particles was saturated with 0.05 M phosphate buffer, pH 7.4 containing 2 M glycine and 1 % BSA. Finally, the coupled magnetizable

cellulose particles were washed and suspended in 0.05 M phosphate buffer, pH 7.4 containing 0.5 % BSA and 0.1 % Tween-20 (assay buffer) and stored at  $4\text{ }^{\circ}\text{C}$  until further use. All the six monoclonal antibodies A-F were coupled to magnetizable cellulose particles using the same protocol and monoclonal antibodies coupled to antibodies magnetizable cellulose particles are designated as M-A, M-B, M-C, M-D, M-E and M-F.

#### Preparation of standards

A total of six C-peptide standards 0.3, 1.2, 6, 12, 18 and 30 ng/ml containing 0.1 % sodium azide as a preservative were prepared in C-peptide free bovine serum using highly purified synthetic C-peptide in aseptic conditions. The standards were later calibrated against WHO Ref IRP 84/510 and stored at  $4\text{ }^{\circ}\text{C}$  after lyophilization.

#### Compatibility studies for identification of capture and detector antibody

All the  $^{125}\text{I}$ -anti-C-peptide monoclonal antibodies (A\*-F\*: detector antibodies or tracer) and all the six monoclonal antibodies coupled to magnetizable cellulose particles (M-A to M-F: capture antibodies) were used for this study. The strategy applied for the selection of process was to identify a detector antibody to match capture antibody or vice versa.

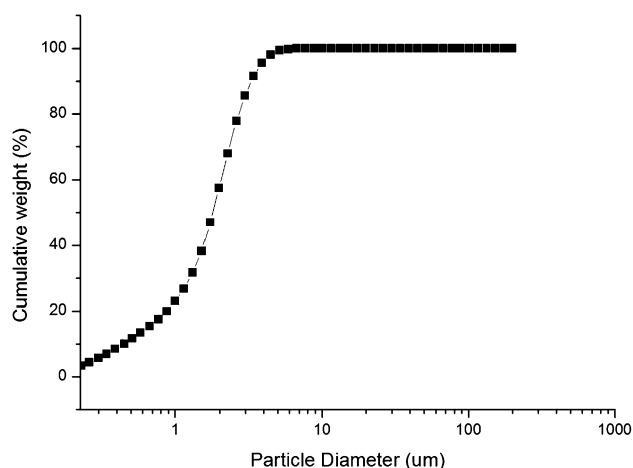
100  $\mu\text{l}$  of capture antibodies in the form of anti-C-peptide monoclonal antibodies coupled to magnetizable cellulose particles were incubated with 100  $\mu\text{l}$  C-peptide standards (0, 0.3 and 30 ng/ml) along with 100  $\mu\text{l}$  of detector antibodies ( $\sim 1.5 \times 10^5$  CPM) overnight with gentle stirring. At the end of the incubation, particles were washed with 1 ml of 0.05 M phosphate buffer, pH 7.4 containing 0.1 % Tween-20 (wash buffer) twice and measured for radioactivity in a multiwell automatic RIA gamma counter. Using best possible combination of antibodies in terms of NSB (binding with C-peptide standard of 0 ng/ml) and binding with C-peptide standard with highest concentration (30 ng/ml) IRMA procedure for C-peptide was developed and validated. Optimized assay procedure consists of simultaneous addition of all the reagents. 200  $\mu\text{l}$  of sample or standards (0.3–30 ng/ml) in bovine serum were incubated with 100  $\mu\text{l}$  of capture antibody coupled to magnetizable cellulose particles and 100  $\mu\text{l}$  of tracer on a shaker for overnight at room temperature. At the end of incubation, the particles were washed twice with wash buffer using a magnetic separator and counted for 1 min in a multiwell automatic RIA gamma counter. A standard curve was constructed by plotting %B/T (Y axis) against C-peptide standard concentration (X axis).

## Results and discussion

Radioiodination yield and RCP were determined by paper electrophoresis as well as by TCA precipitation. Radioiodination yield/specific activity observed in the case all monoclonal antibodies were almost similar with an average of specific activity of 10.65  $\mu\text{Ci}/\mu\text{g}$ . This corresponds to 0.71  $^{125}\text{I}$  atom per every antibody molecule. The average RCP of the fraction collected was found to be 92 % (Table 2). Specific activity and RC purity was also confirmed by TCA precipitation method with comparable results.

The paramagnetic magnetizable cellulose particles used in this work were prepared in-house by a novel method based on wet grinding of iron oxide and microcrystalline cellulose in a stirred ball [16]. Particle size distribution of the particles is depicted in Fig. 1. Average particle size was found to be 1.89  $\mu\text{m}$  with cutoff size of 7.7  $\mu\text{m}$ . Percentage on 2  $\mu\text{m}$  size particle was found to be 55.4 and the specific surface area as measured by the same equipment was found to be 57,128  $\text{cm}^2/\text{cm}^3$ . The process of wet grinding not only firmly binds magnetite and cellulose physically but also results in the formation of magnetizable cellulose with smaller particle size. High surface area of the particles resulted in uptake of antibody of >80 % which was determined during the preparation of magnetizable cellulose particles coupled antibody particles by measuring protein (antibody) concentration before and after coupling reaction. Antibody coupled to magnetizable cellulose particles stored at 4  $^{\circ}\text{C}$  retained immunoreactivity not less than 2 years. In-house prepared magnetizable particles also have been used for other radioimmunoassay (RIA) and IRMA procedures [17–20].

As the development of highly sensitive and specific sandwich IRMA systems can be hampered by the unpredictability and inflexibility of monoclonal antibodies as sandwich partners all the 30 possible combinations of capture—detector antibody pairs were tried (Table 3). Out of 30 possible combinations, the performance of the each



**Fig. 1** Particle size distribution of magnetizable cellulose particles

pair of the antibody in terms of NSB and maximum binding (binding with 30 ng/ml standard) differed considerably. Even though similar amount of antibodies were immobilized on magnetizable cellulose particles (900  $\mu\text{g}$  of antibody per 1 g of particles) and using radioiodinated monoclonal antibodies of specific activity of around 11  $\mu\text{Ci}/\mu\text{g}$ , yet there was a significant difference in the performance of these antibodies as a pair. In the case of the best possible combination (M–C and D\*), NSB and maximum binding observed were 0.2 and 34 % respectively. Results obtained in the case of rest of other combinations were poor with maximum binding as low as 0.26 % (M–B and C\*). NSB as high as 1.01 % and maximum binding as low as 0.46 % was also observed in one of the combinations (M–E and F\*). C-peptide standard of 0.3 ng/ml was also included in the study in order to assess the feasibility of sensitive assays. Results indicated that proper screening and careful selection of detector and capture antibody is necessary and the combination M–C and D\* was used for further optimization and validation of the assay system. Different dilutions (1:2, 1:3, 1:4 and 1:5) of the selected capture antibody (M–C) using assay buffer were tested

**Table 2** Specific activity and RCP of  $^{125}\text{I}$  labelled monoclonal antibodies

Monoclonal antibody	Iodination yield (%)	Specific activity ( $\mu\text{Ci}/\mu\text{g}$ )	$^{125}\text{I}$ Atom per antibody molecule	RCP (%)
A*	69	10.35	0.69	91
B*	67	10.05	0.67	94
C*	70	10.50	0.7	89
D*	78	11.70	0.78	98
E*	70	10.50	0.7	90
F*	72	10.80	0.72	92
Average	71	10.65	0.71	92

**Table 3** Cross matching of different capture antibody diluted (1:1) with detector antibody

Antibody pairs		Concentration of C-peptide %B/T		
Capture antibody	Detector antibody	0 ng/ml	0.3 ng/ml	30 ng/ml
M–A	B*	0.45	0.33	0.73
M–A	C*	0.26	0.26	0.27
M–A	D*	0.37	0.46	16.45
M–A	E*	0.39	0.36	0.75
M–A	F*	0.22	0.28	0.32
M–B	A*	0.47	0.45	0.52
M–B	C*	0.26	0.28	0.26
M–B	D*	0.3	0.24	0.62
M–B	E*	0.34	0.32	0.30
M–B	F*	0.27	0.35	0.46
M–C	A*	0.74	0.73	0.56
M–C	B*	0.41	0.44	0.31
<b>M–C<sup>a</sup></b>	<b>D*</b>	<b>0.20</b>	<b>0.55</b>	<b>34</b>
M–C	E*	0.36	0.41	0.42
M–C	F*	0.52	0.47	0.52
M–D	A*	0.61	0.59	12.4
M–D	B*	0.59	0.37	0.51
M–D	C*	0.4	0.45	11.08
M–D	E*	0.31	0.29	0.36
M–D	F*	0.26	0.30	0.32
M–E	A*	0.25	0.27	0.68
M–E	B*	0.43	0.34	0.59
M–E	C*	0.23	0.25	0.27
M–E	D*	0.27	0.33	0.35
M–E	F*	1.01	0.92	0.52
M–F	A*	0.40	0.44	0.62
M–F	B*	0.51	0.53	0.71
M–F	C*	0.21	0.20	0.37
M–F	D*	0.29	0.32	0.46
M–F	E*	0.36	0.99	0.72

<sup>a</sup> Pair selected for the final assay development

with its complementary detector antibody (D\*) with maximum binding of 33, 33, 29 and 20 % respectively. Further drastic reduction in binding was observed when the particles were diluted beyond 1:5.

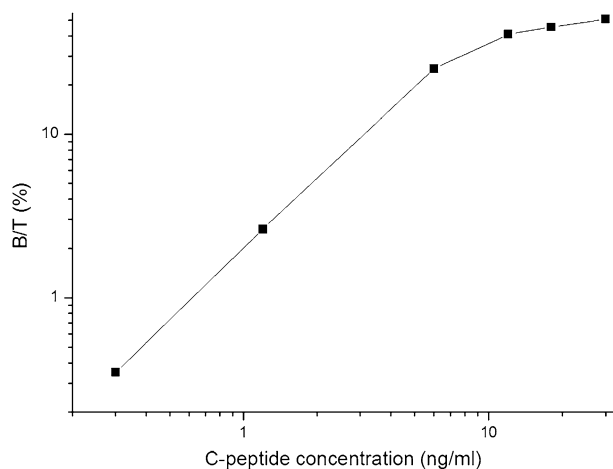
The other important requirement for developing any assay is the production of high quality tracer. Addition of a single iodine atom to the biological molecules does not alter the function and property of the labeled compounds. The substituted iodine does not cause any conformational change to the structure of biomolecules [21]. Tracer with specific activity of around 11  $\mu\text{Ci}/\mu\text{g}$  was used throughout the experiments and in order to find out the optimal concentration of the tracer required, effect of concentration of tracer in terms of CPM was studied. Total CPM were varied from  $5 \times 10^4$  to  $3 \times 10^5$  and NSB, % B/T for the highest standard were calculated. Total CPM of  $1.5 \times 10^5$  was found to be ideal with minimum NSB and high

maximum binding. Radioiodination of anti-C-peptide monoclonal antibody used in this technique is simple than performing radioiodination for tyrosylated-C-peptide as required in case of RIA as C-peptide is devoid of tyrosine or histidine residue which is essential for radioiodination. Typical standard curve using optimized assay parameters are depicted in Fig. 2.

In C-peptide immunoassays, limiting factors like interference of even minor cross reactivity with proinsulin may significantly interfere with the C-peptide immunoassay. Purification of C-peptide by HPLC [22] and precipitation by 25 % polyethylene glycol [23] prior to radioimmunoassay was also carried out by some research groups to remove cross reactivity with proinsulin. Treatment for diabetes by insulin therapy may produce antibodies against insulin which will lead to the retention of proinsulin resulting in concentrations high enough to interfere

**Table 4** Specificity of anti-C-peptide monoclonal antibody

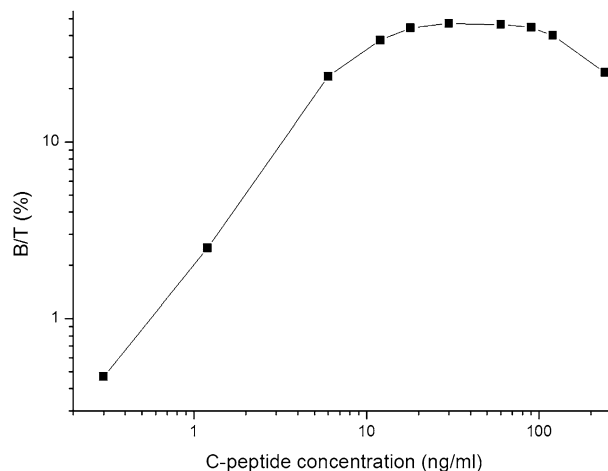
Hormone	Concentration added	Apparent concentration (%)
Human proinsulin	2 ng/ml	<0.05
Human insulin	200 $\mu$ IU/ml	<0.05
Glucagon	200 pg/ml	<0.01
Somatostatin	100 pg/ml	<0.01
Pancreatic polypeptide	1,000 pg/ml	<0.01

**Fig. 2** Typical standard curve

significantly in a C-peptide immunoassay [24]. To assess the extent of C-peptide specificity, we measured the cross reactivity with other related peptides/proteins like human Proinsulin, Insulin, Glucagon, Somatostatin and Pancreatic polypeptide in the assay at concentrations of 2 ng/ml, 200  $\mu$ IU/ml, 200, 100 and 1,000 pg/ml respectively. However, apparent concentration of added cross-reactants were less than 0.01 % indicating the absence of cross reactivity (Table 4). Such low levels of cross reactivity mainly with proinsulin are essential to ensure accurate and reproducible C-peptide measurements [25].

High dose hook effect is always associated with sandwich assays and usually observed when capture antibody sites are saturated with the antigen as in the case of samples with high antigen concentration. This problem can be tackled to some extent by increasing the concentration of capture antibody. Use of capture antibody prepared with magnetizable cellulose particles with high surface area facilitated the increase of capture antibody concentration in the assay system hook effect was not observed up to 50 ng/ml (Fig. 3). In addition, unlike antibody coated tubes or microliter plates, capture antibody prepared using magnetizable cellulose particles have the flexibility in varying their concentration as per the requirement.

Developed assay system was validated using standard assay parameters. Sensitivity of the assay determined by assaying 15 replicates of the zero standard and calculating

**Fig. 3** High dose hook effect

the concentration corresponding to the sum of the mean CPM and its two standard deviation was found to be 0.16 ng/ml. Sensitivity of the developed method is quite higher compared to radioimmunoassay reported in other studies [26, 27]. The intra-assay variation was determined by analyzing two serum samples 15 times in the same assay. Similarly, inter-assay variation was determined by analyzing the same two serum samples in ten different assays (each sample in duplicate) run over a period of a time (1 month) (Table 5). For estimation of recovery, serum sample containing C-peptide were spiked with a known amount of C-peptide standards, both the native sample and spiked sample were analyzed in the same assay. % Recovery ranged from 88 to 105 % which was well within acceptable limits of 80–110 % in immunoassays (Table 6). C-peptide samples were diluted twofold, fourfold and eightfold with zero standard. Both

**Table 5** Assay variation

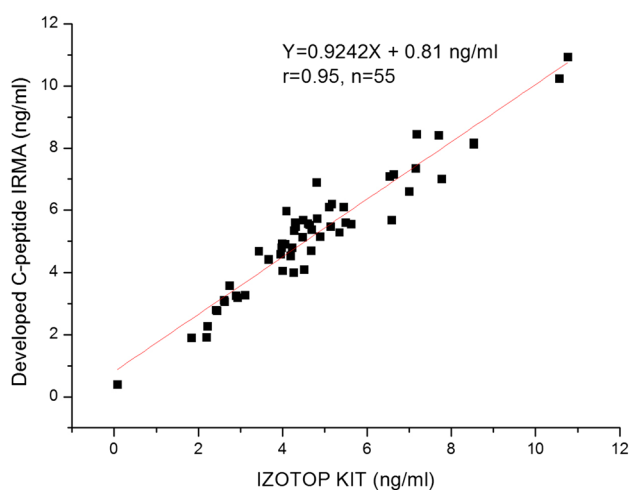
	Mean $\pm$ SD (ng/ml)	%CV
<i>Intra assay variation</i>		
Sample 1	0.97 $\pm$ 0.05	5.1
Sample 2	6.17 $\pm$ 0.45	7.3
<i>Inter assay variation</i>		
Sample 1	1.6 $\pm$ 0.07	4.4
Sample 2	7.3 $\pm$ 0.60	8.2

**Table 6** Recovery test

S. no.	Sample (ng/ml)	Standard (ng/ml)	Observed (ng/ml)	Expected (ng/ml)	% Recovery
1.	1.77	0.15	2.02	1.92	105
2.	0.57	0.6	1.09	1.17	93
3.	0.6	9	8.4	9.6	88
4.	6.39	3	9.2	9.4	98
5.	6.39	6	12.9	12.4	104

**Table 7** Linearity test

Sample	Dilution	Observed (ng/ml)	Expected (ng/ml)	% Recovery
Sample 1	Neat	18	–	–
	1:1	8.5	9	94
	1:4	4.2	4.5	93
	1:8	2.3	2.3	100
Sample 2	Neat	30	–	–
	1:1	17.4	15	116
	1:4	8	7.5	107
	1:8	3.8	3.75	101

**Fig. 4** Clinical sample analysis

native samples as well as diluted samples were analyzed in the assay. Percentage recovery for dilution parallelism ranged from 93 to 116 % (Table 7).

Around 55 clinical samples were analyzed using developed method. C-peptide values obtained were compared with those values obtained by an established commercial kit (IZOTOP, Hungary) with acceptable correlation of ( $Y = 0.9242X + 0.81$  ng/ml;  $r = 0.95$ ) (Fig. 4).

## Conclusion

Proper screening and careful selection of detector and capture antibody is necessary in development of an IRMA

system as shown in the present study as different antibody pairs behaved differently as sandwich partners affecting overall assay parameters. By using magnetizable immunosorbent prepared with in-house magnetizable cellulose particles an assay procedure with a sensitivity of 0.16 ng/ml can be used to assess the course, prognosis and management of diabetic disorders. Assay procedure can be compared with assays based on antibody coated tubes with an added advantage of economy and flexibility in the use of solid phase reagent.

**Acknowledgments** The authors are thankful to Dr. A. K. Kohli, Chief Executive, BRIT, Mr S. S. Sachdev, Senior General Manager, BRIT and Dr. N. Sivaprasad, Former Senior General Manager, BRIT for their support. We also thank Board of Research in Nuclear Sciences (BRNS) for the financial assistance.

## References

- Kley S, Caffall Z, Tittle E, Ferguson DC, Hoenig M (2008) *Domest Anim Endocrinol* 34:311–318
- Wahren J, Kallas A, Sima AA (2012) *Diabetes* 61:761–772
- Clark PM, Hales CN (1994) *Diabetes Metab Rev* 10:79–90
- Brandenburg D (2008) *Exp Diabetes Res*. doi:10.1155/2008/576862
- Ido Y, Vindigni A, Chang K, Stramm L, Chance R, Heath WF, Di Marchi RD, Di Cera E, Williamson JR (1997) *Science* 277:563–566
- Kayall AG, Flores LE, Lopez AD, Kutlu B, Baetge E, Kitamura R, Hao E, Beattie GM, Hayek A (2007) *Diabetes* 56(3):703–708
- Rendell M (1983) *Acta Diabetol* 20:105–113
- Bakhtadze E, Borg H, Stenstrom G, Fernlund P, Arnqvist HJ, Ekblom-Schnell A, Bolinder J, Eriksson JW, Gudbjornsdottir S, Nystrom L, Groop LC, Sundkvist G (2006) *Diabetologia* 49(8):1785–1794

9. Torn C, Landin-Olsson M, Scherstén B (2001) *Pract Diabetes Int* 18(3):83–88
10. Dimitriadis G, Raptis S, Zoupas C, Schizas N (1980) *Acta Diabetol Lat.* 17:67–71
11. Abdullah BB, Patil BS, Thaseen A (2010) *Al Ame en J Med Sci.* 3(1):6–78
12. Davies C (2005) In: Wild D (ed) *The immunoassay handbook.* Elsevier, Amsterdam, pp 14–16
13. Chapman RS, Sutherland RM, Ratcliffe G (1983) *Immunoassay for clinical chemistry*, 2nd edn. Churchill Livingstone, Edinburgh
14. Greenwood FC, Hunter WM, Klopper A (1964) *Br Med J* 1(5374):22–24
15. Hiemisch H, Gavrilyuk V, Atochina E, Slinkin M, Torchilin V, Muzykantov V, Danilov S (1993) *Nucl Med Biol* 20(4):435–441
16. Kadwad VB, Jyotsna N, Sivaprasad N, Sinha PK (1996) *J Radioanal Nucl Chem* 210(1):27–33
17. Rasmi RR, Shenoy KB, Sarnaik J, Kadwad VB, Somashekarappa HM, Sivaprasad N (2014) *J Radioanal Nucl Chem* 302: 1271–1275
18. Paradkar S, Vrindha C, Jyotsna N, Sivaprasad N (1999) *J Radioanal Nucl Chem* 241(3):561–567
19. Mohan RK, Kadwad V, Samuel G, Venkatesh M, Sivaprasad N (2006) *J Radioanal Nucl Chem* 268(3):461–466
20. Mehany NL, El-Kolaly MT, Ayyoub SM, Hassan SEM (2005) *J Radioanal Nucl Chem* 265(1):61–71
21. Eisenhut M, Mier W (2003) *Handbook of nuclear chemistry.* Kluwer Academic publishers, Dordrecht
22. Komjati M, Nowotny P, Waldhausl W (1987) *Anal Chem* 59(15):2010–2012
23. Heding LG, Ludvigsson J (1977) *Acta Paediatr Scand* 270(Suppl):48–52
24. Kuzuya H, Blix PM, Horwitz DL, Steiner DL, Rubenstein AH (1977) *Diabetes* 26(1):22–29
25. Palmer JP, Fleming GA, Greenbaum CJ, Herold KC, Jansa LD, Kolb H, Lachin JM, Polonsky KS, Pozzilli P, Skyler JS, Steffes MW (2004) *Diabetes* 53(1):250–264
26. Melani F, Rubenstein AH, Oyer PE, Steiner DF (1970) *Proc Nat Acad Sci USA* 67:148–155
27. Beischer W, Keller L, Maas M, Schiefer E, Pfeiffer EF (1976) *Klin Wochenschr* 54:709–715