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Evaluation of the correlation coefficient of polyethylene glycol treated and direct prolactin results and comparability with different assay system results

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ABSTRACT

The presence of Macro prolactin is a significant cause of elevated prolactin resulting in misdiagnosis in all automated systems. Poly ethylene glycol (PEG) pretreatment is the preventive process but such process includes the probability of loss of a fraction of bioactive prolactin.

Surprisingly, PEG treated EQAS & IQAS samples in Cobas e 411 are found out to be correlating with direct results of at least 3 immunoassay systems and treated and untreated Cobas e 411 results are comparable by a correlation coefficient. Comparison of EQAS, IQAS and patient samples were done to find out the trueness of such correlation factor. Study with patient's results have established the correlation coefficient is valid for very small concentration of prolactin also.

Materials and methods

EQAS, IQAS and 150 patient samples were treated with PEG and prolactin results of treated and untreated samples obtained from Roche Cobas e 411. 25 patient's results (treated) were compared with direct results in Advia Centaur, Architect I & Access2 systems.

Statistical calculations

Correlation coefficient was obtained from trend line of the treated and untreated results. Two tailed p-value obtained from regression coefficient(r) and sample size.

Results and discussion

The correlation coefficient is in the range (0.761-0.771). Reverse correlation range is (1.289-1.301). r value of two sets of calculated results were 0.995. Two tailed p- value is zero approving dismissal of null hypothesis.

Conclusion

- The z-score of EQAS does not always assure authenticity of results
- PEG precipitation is correlated by the factor 0.761 even in very small concentrations

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Abbreviations

***GFC**: gel filtration chromatography*
***PEG:** polyethylene glycol*
***EQAS:** external quality assurance system*
***M-PRL:** macro prolactin*
***PRL:** prolactin*
***ECLIA:** electro-chemiluminescence immunoassay*
***CLIA:** clinical laboratory improvement amendments*
***IQAS:** internal quality assurance system*
***r:** regression coefficient*

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INTRODUCTION

The presence of Macro prolactin (M-PRL) is a known cause of misdiagnosis, unnecessary investigation and inappropriate treatment. M-PRL in human blood consists of monomeric bioactive prolactin (PRL) of molecular mass 23kDa and a non reactive immunoglobulin G molecule with a molecular mass of approximately 150-170kDa causing a prolonged clearance rate. Though M-PRL is non reactive but it interferes with prolactin immunoassay and causes false elevation of prolactin (1, 2, 3).

The probable reasons for elevation may be:

- The assay antibodies are probably having affinity to different epitopes on PRL with which they react. The elevation of result is dependent on the availability of such epitopes on the M-PRL complex (4)
- The coupling of same pair of antibodies to different solid phase and signal generating system (5, 6)
- Incubation time has also been shown to be directly related to the reactivity with M-PRL (6). It was observed that Roche Elecsys System showed maximum elevated results (5, 7)

A study was done to examine the frequency of Macroprolactinemia in clinical practice and the ability of immunoassay systems to distinguish between M-PRL and PRL using 300 hyperprolactinemic serum samples. Overall, 71 results dropped to within the normal range following treatment of serum samples with PEG, indicating that 24% of hyperprolactinemia are approximately misdiagnosed due to interference by M-PRL. Ten out of these samples where elevation of results was due to interference of M-PRL were tested at 18 clinical laboratories. Two sets of PRL measurements of these serum samples were obtained from each of the nine most commonly used immunoassay systems. Across the nine assay systems, differences in the PRL estimates ranged from 2.3- to 7.8-fold. Elecsys users reported the highest PRL levels. Somewhat lower values were reported for DELFIA systems followed by Immuno-1, AxSYM, and Architect assay system.

The Immulite 2000 assay generated PRL levels equivalent to approximately 50% of those reported by the high-reading methods. The lowest PRL levels were reported by Access, ACS: 180, and Centaur systems (4, 8).

Two system of separation of M-PRL from bioactive PRL became popular, PEG precipitation, and Gel Filtration Chromatography (GFC)(9,10). A HPLC method has been developed using Agilent Zorbax GF-250 Column, tris buffer with saline at pH 7.2 which was found to have equal efficiency of GFC but still not very popular(11). GFC is time consuming and expensive, so not suitable for regular clinical laboratory performances. Therefore, PEG precipitation became common method of precipitation of M-PRL. Karolina et al (12) assessed elevation effect of M-PRL in 27 patients among which 19 with functional hyperprolactinemia and 8 with prolactinoma between PEG precipitation and ultracentrifugation. A high diagnostic agreement (95.9%) and positive correlation coefficient ($r=0.506$, $p<0.001$) was found out between two precipitation method. Both precipitation methods showed equal efficacy in functional hyperprolactinemia, and PEG precipitation was better method in prolactinoma.

Kit inserts of different systems (13, 14, 15, 16) mentioned that the PRL results may get affected due to the presence of M-PRL and PEG precipitation has been suggested where elevated result is obtained. It was also stated that a fraction (approximately 14%) of active prolactin may get co precipitated during PEG pre- treatment. The dilution effect also to be taken into consideration. No instruction on cutoff value above which PEG pretreatment to be done was mentioned in the inserts. Hence, a correlation study was felt to be necessary to get a guideline regarding such cutoff value.

In the current study, the author treated all samples twice, direct assay and after PEG precipitation. A reverse correlation of both results was done and regression coefficient (r) was calculated. The reverse correlation was necessary to substantiate the authenticity of correlation coefficient. The reason of finding out such correlation was to find out:

- Should PEG precipitation be done for all samples irrespective of normal/or elevated results? (i.e., whether there should be a lower cutoff?)
- Does the fraction of PRL being co precipitated with M-PRL affect patients' clinical status?
- Though M-PRL is still being mentioned as interfering molecule in all inserts but PEG pretreated results of Cobas e 411 are in agreement with untreated results of Access-2 system. Hence, the underlying problem of elevated prolactin results is still a matter of concern in Cobas e 411.
- Whether the correlation coefficients are within an acceptable uncertainty both in direct and reverse direction?

MATERIALS AND METHODS

Materials

A total of 150 patient samples were collected at random with direct prolactin results from 0.25-300 ng/ml. As the basic aim of the study was to check the dilution effect during PEG treatment and transferability of the expected values so clinical case history has not been considered to make the study a blind trial. Prolactin has been measured twice, direct measurement & after PEG precipitation in EQAS samples (BIORAD, Cycle 13), BIORAD immunoassay control levels 1, 2 & 3, lot 40330 and above mentioned patient samples.

Total 25 number samples were selected and tested in 4 different systems Roche Cobas e 411, Abbott Architect I, Advia Centaur and Access 2.

The laboratory performed tests in Cobas e 411 & Access 2 and outsourced in accredited laboratories having Advia Centaur & Abbott Architect I systems. It was informed not to treat the samples with PEG. In Access 2 and Cobas e 411 these 25 samples were measured twice ie, direct estimation and after PEG precipitation.

Methods

Cobas e 411

Electro Chemiluminescence Immunoassay (ECLIA) (13, 17).

Access 2

Chemiluminescence Immunoassay (CLIA) (14). The Access Prolactin assay is a simultaneous one-step immunoenzymatic ("sandwich") assay using Lumiphos* 530 as Chemiluminescent substrate (14).

Abbott, Architect i

Prolactin assay is a two-step immunoassay using Chemiluminescent Micro particle Immunoassay (CMIA) technology with flexible assay protocols, referred to as Chemiflex (15).

Advia Centaur

Chemiluminescence Immuno assay (CLIA) (16).

Precipitation using PEG

25% solution of poly ethylene glycol 6000(PEG) is prepared using deionized water. It is stable for 7 days. Sample and PEG solution was mixed in equal volume. Mixed for 10 seconds and centrifuged for 5-30 minutes between 1500-10000g (13).

Table 1 Comparison of EQAS values, BIORAD, Cycle-13

Sample no.	N#	Lab results* (ng/mL)	z-score	Peer mean of compared systems(ng/mL)				PEG treated results (Cobas-e 411) (ng/mL)	PEG X 1.289 (ng/mL)	Direct result X0.771 (ng/mL)
				Cobas e 411	Advia Centaur**	Access 2**	Architect**			
1	165	13.3	-0.67	13.9	9.01	9.9	10.6	10.6	10.57	10.25
2	217	19	-0.71	19.9	12.3	13.3	14.7	15.1	15.14	14.65
3	232	33.75	-0.56	35	22.2	23.3	25.7	26.6	26.63	26.02
4	236	47.16	-1.48	52.7	33.2	35.4	39.8	40.2	40.1	36.36
5	235	19.9	-0.25	20.2	11.9	13.4	14.3	15.4	15.37	15.34
6	243	54.63	0.40	53.3	33.6	35	39.3	40.34	40.56	42.12
7	238	14.96	0.75	14.2	8.84	9.91	10.5	11	10.8	11.53

8	232	19.84	-0.41	20.4	11.8	13.2	14.7	16.2	15.52	15.3
9	241	36.24	0.16	35.9	22.1	23.2	26.5	26.9	27.32	28
10	232	54.11	-0.03	54.2	34	34.6	40.6	41.85	41.24	41.71
11	246	14.56	0.01	14.6	8.91	9.67	10.5	11.55	11.11	11.45
12	225	37.03	0.43	36.1	22.1	23	26.6	28.2	27.47	28.55
r***	-	-	-	-	0.998	0.997	0.999	-	-	0.9995

p-value: <0.0001

**The lab has enrolled for Roche Cobas e 411 only.*

***Peer mean obtained from BIORAD, monthly EQAS assessment sheet.*
 # Participant laboratories of Roche Cobas e 411.

*r***- When compared with PEG pretreated results*

Figure 1 Correlation of EQAS results of Cycle 13

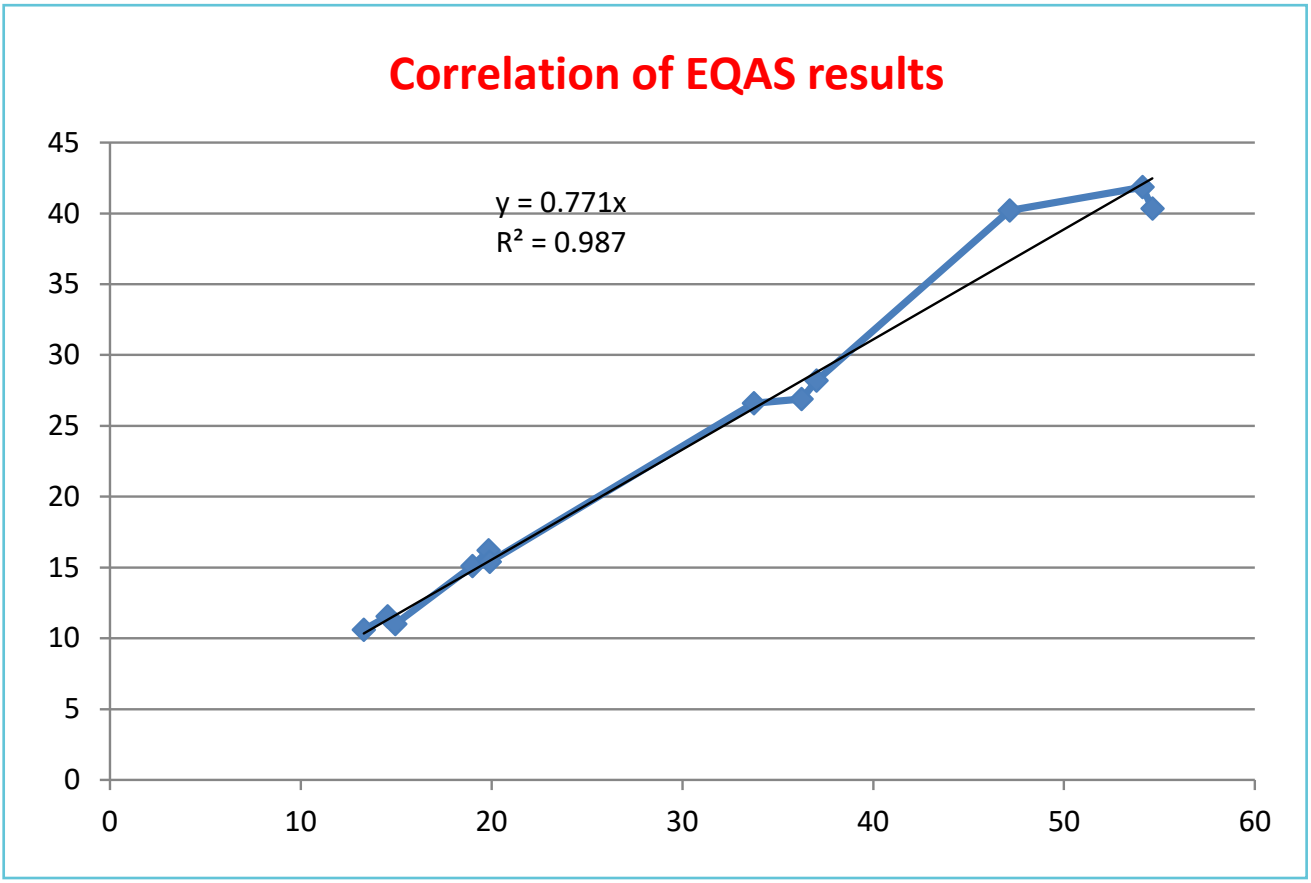
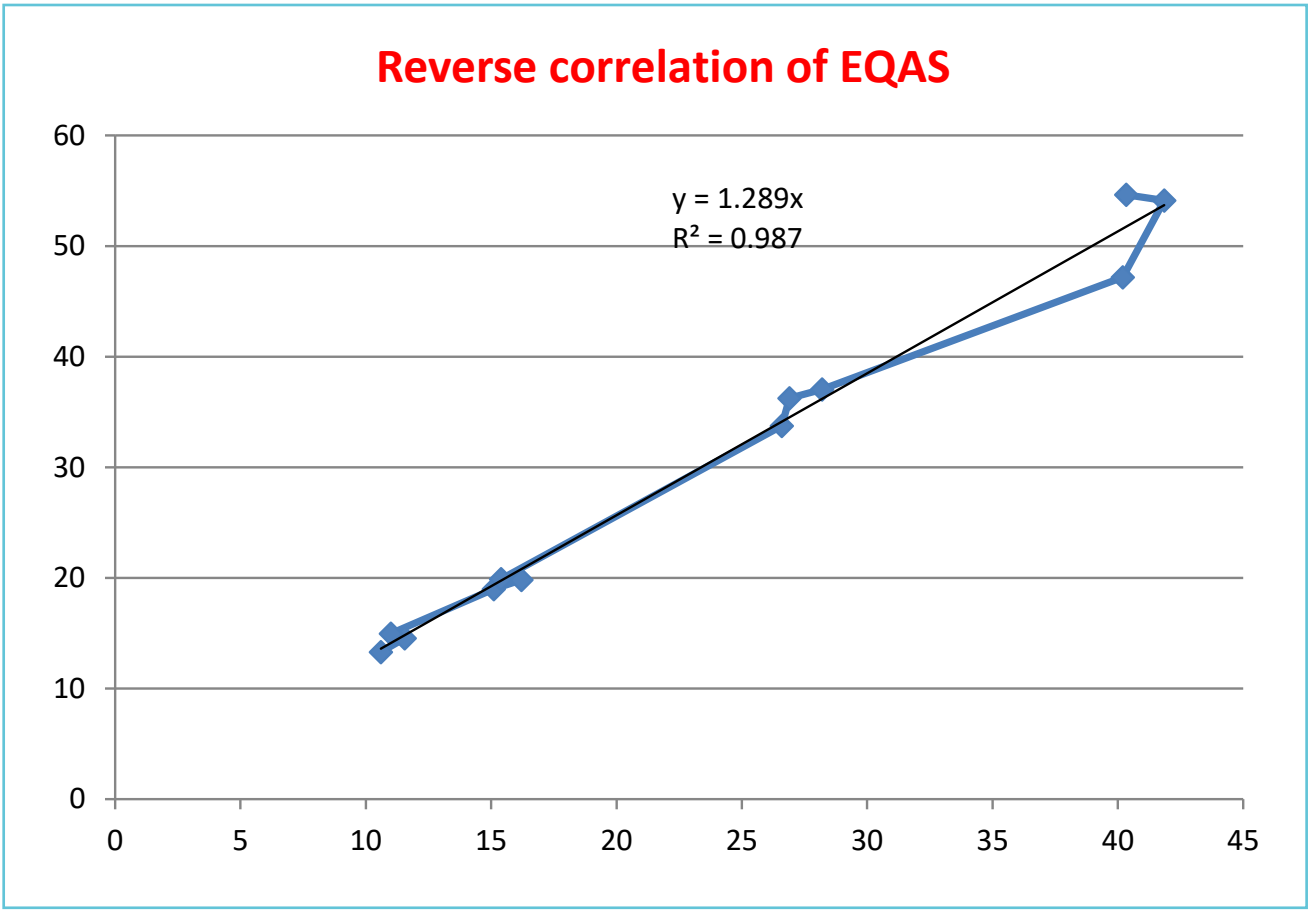


Figure 2 Reverse correlation of EQAS results Cycle 13



Calculations

PEG treated results were multiplied by 2(1+1ratio). EQAS results of direct and PEG treated values were compared twice, putting PEG results on Y-axis and direct result on X-axis and reversing the axis.

Correlation factors obtained from trend lines (Table 1, Figures 1 & 2). The factors were 0.761 & 1.306.

In a similar manner correlation factors obtained for Trilevel Immunoassay controls. Correlation factors were 0.762 & 1.307 (Table 2, Figures 3 & 4).

Such correlation was checked from 150 patient's data ranging from (0.298-355) ng/ml. Factors were 0.761 & 1.306 (Table 4, Figures 5 & 6)

Results and discussion

The z-scores of EQAS results (Immunoassay, BIORAD, Cycle 13) showed no outlying score in the complete cycle but remarkable discrepancy was observed with the peer mean of other immunoassay systems.

The laboratory observed recurrent complaints of elevated prolactin results from the patients though EQAS and IQAS results were appropriate to the peer mean values.

The patients obtained results mainly from Abbott Architect, Advia Centaur systems and laboratory started comparing results with Access 2 system.

The difference in results from Cobas e 411 and comparability of results of other systems were

Table 2 Correlation of IQC value (Lot 40330, BIORAD, immunoassay trilevel)

Control	Without PEG (ng/mL)	After pretreatment with PEG (ng/mL)	Access-2 (ng/mL)	Advia Centaur (ng/mL)	Architect-i (ng/mL)
L1	9.05	6.86	6.54	5.75	7.37
L2	27.3	21.1	17.9	15.6	21.5
L3	55.8	42.4	39.2	37.7	49.4

p-value: <0.01

Figure 3 Correlation of BIORAD trilevel immunoassay control (Lot 40330)

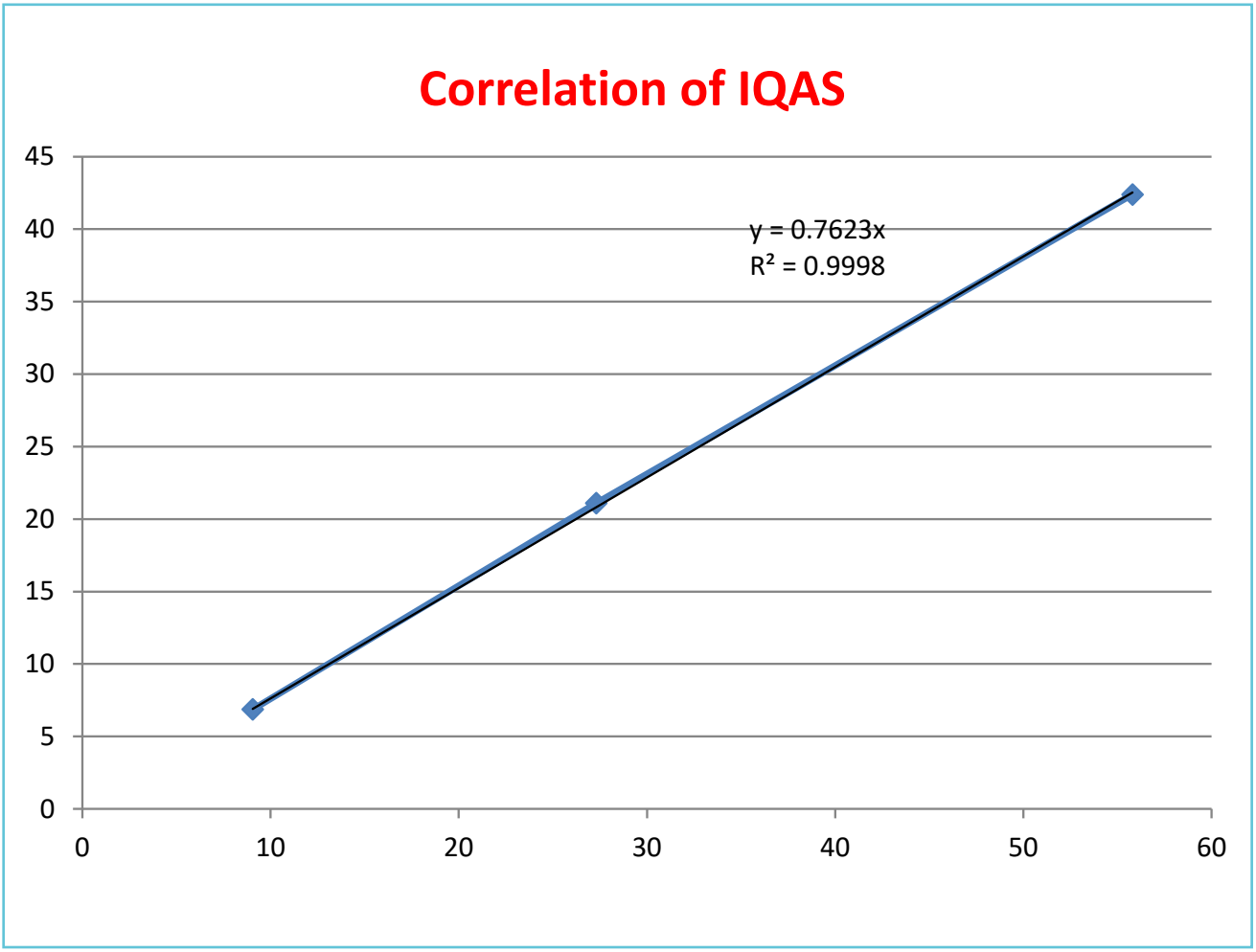


Figure 4 Reverse correlation of BIORAD trilevel immunoassay control (Lot 40330)

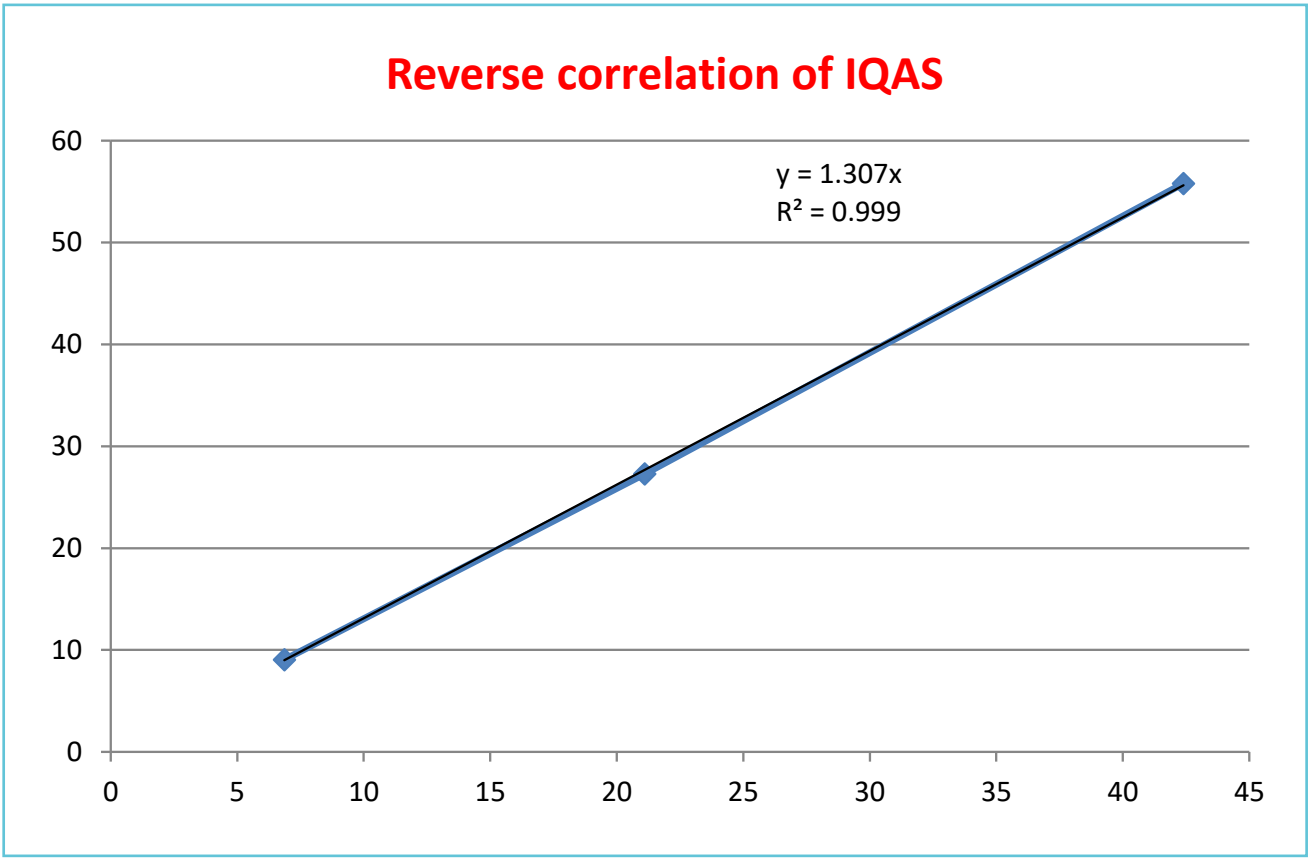


Table 3 Comparison of PEG treated PRL values of Roche Cobas e 411 with direct results of other Immunoassay systems
Sample: Patient sample chosen at random of concentration range 6ng/mL - 365ng/mL

Sl.no.	Roche Cobas e 411 (ng/mL)		Abbott Architect (direct) (ng/mL)	Access 2 Beckman (ng/mL)		Advia Centaur (direct) (ng/mL)
	Direct result	PEG treated result		Direct result	PEG treated result	
1	8.5	6.3	6.8	7.1	6.9	6.0
2	10.2	7.6	7.9	7.5	7.1	8.5
3	9.8	8	7.2	7.3	7.7	7.8
4	10.1	8	7.9	8.3	8.8	9.1

5	18.8	14.8	15.6	14.3	13.8	15.8
6	20.1	16.6	14.8	15.9	14.8	17.1
7	28	20.4	21.1	19.2	20.5	18.5
8	29	21.1	22.5	18.5	19	22.5
9	30.5	24	26.8	23.8	24	23.6
10	41	30	31.1	28.8	27.5	32.7
11	38.8	32	29.4	31.5	30.8	29
12	52	39.4	41.6	40.8	41.5	42.5
13	58.9	46.2	43.5	48.3	49	45.1
14	66.2	50.5	55	49.1	50.2	47
15	66.8	50.6	53.8	48.5	47.9	53
16	73	56	55	55.5	54.7	51
17	75	57	60.1	61.2	58.9	62.8
18	87	65	66	69	71.2	59
19	108	84.8	80	75	73.2	78
20	110	86	78	92	90	90
21	168	124	118	132	124	125
22	139	103	112	119	115	114
23	302	235	228	215	209	230
24	103	78	82	75	70	81

25	471	355	341	362	355	358
r*	-	-	0.9988	0.9967	0.997	0.9985

p-value: <0.001

r -- Each series of instrument results compared with Cobas e 411 after PEG treatment results*

Table 4 Correlation of prolactin direct & PEG treated results

Total no. of patients	Range of analysis (ng/mL)	Correlation factor of PEG to direct results	Correlation factor of direct to PEG results	Regr. of direct result & PEGX1.306	Regr. of Di rectx0.761 & PEG results
150	0.298-355	0.761	1.306	0.995	0.995

Figure 5 Comparison of data with PEG on Y-axis

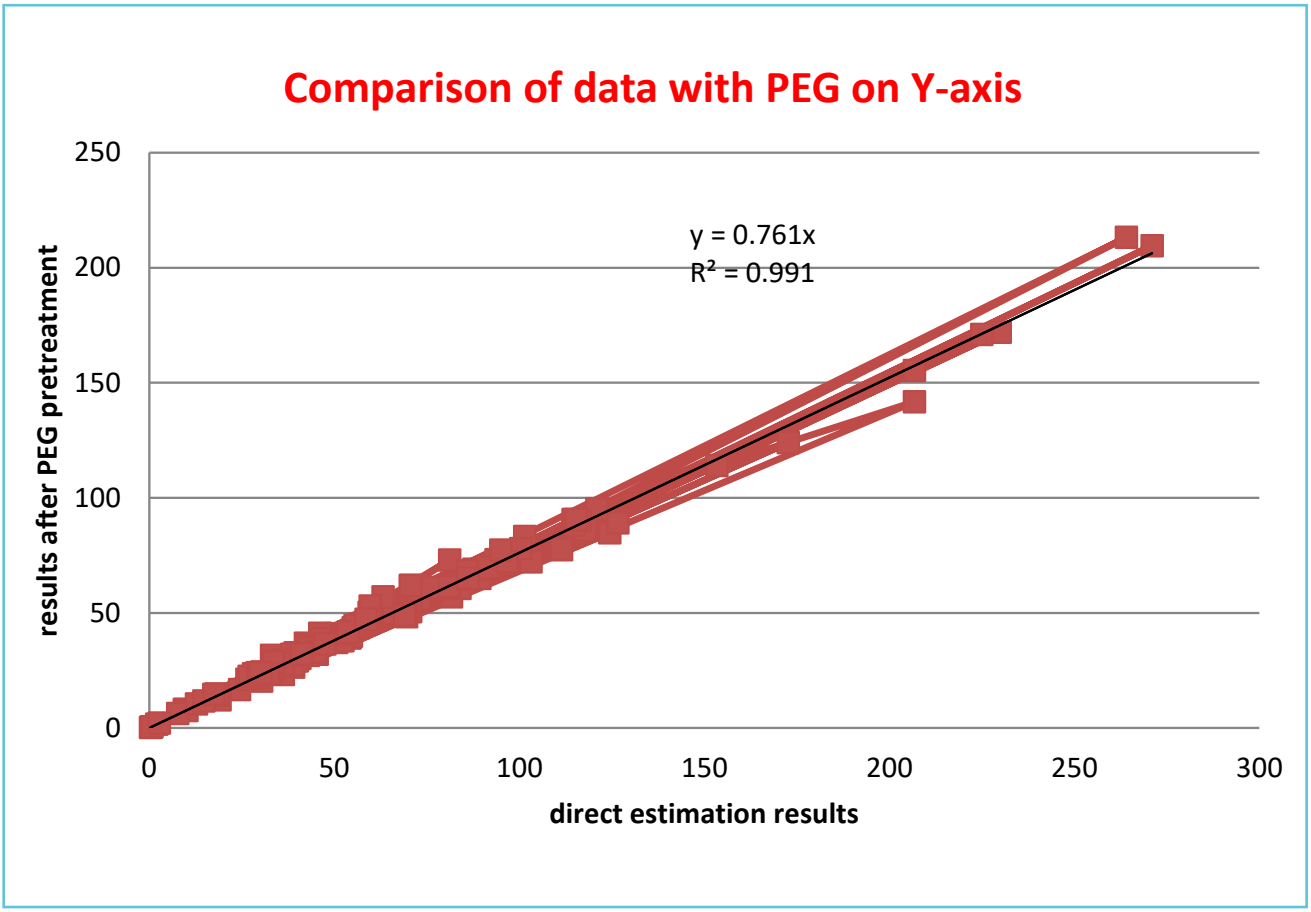
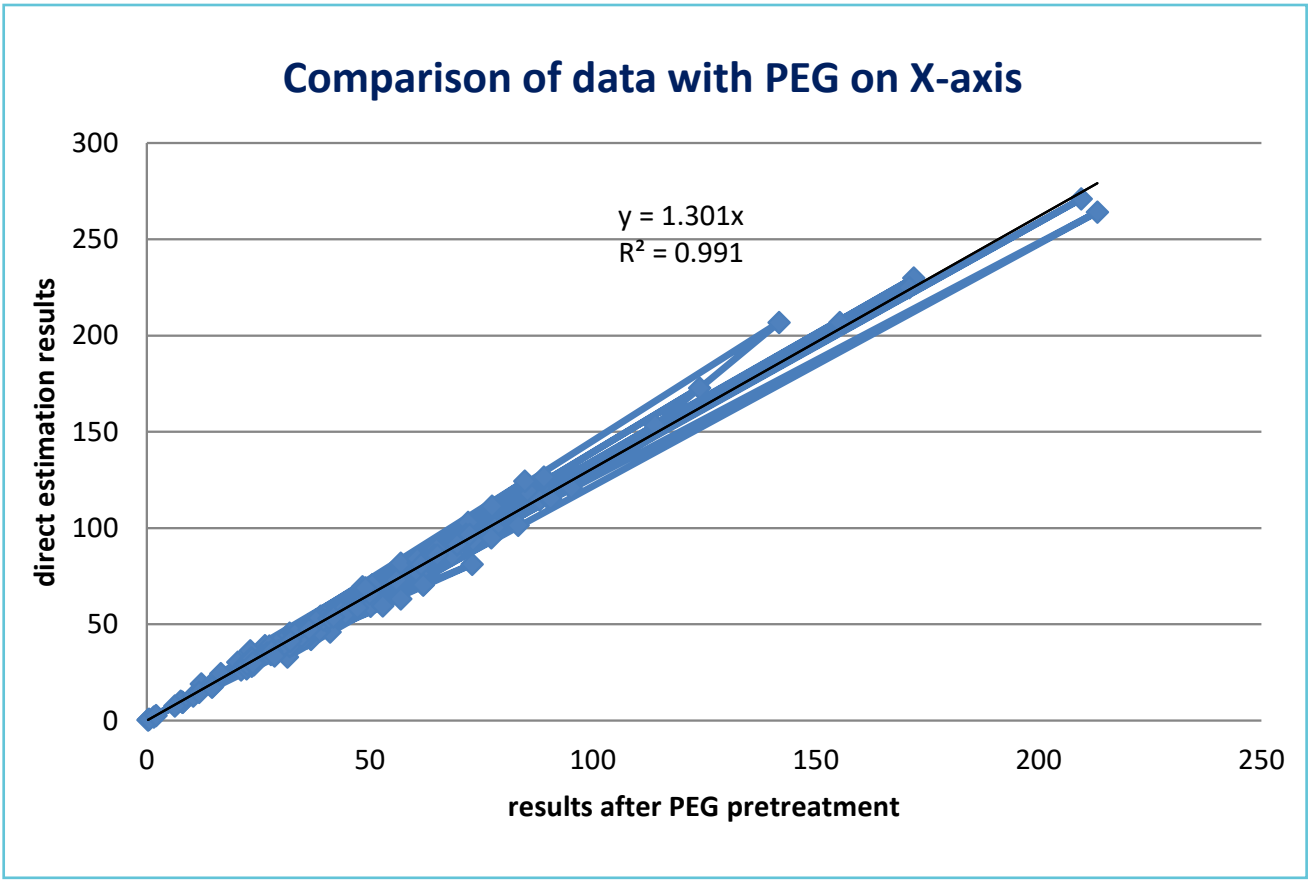


Figure 6 Comparison of data with PEG on X-axis (reverse comparison)



observed. Hence, the laboratory started comparing peer mean of EQAS and IQAS results in all chemiluminescent immunoassay systems. EQAS samples pretreated with PEG and compared the results with direct results. The correlation coefficients obtained from the slope twice.

i. ***Plotting PEG results on Y axis and direct results on X-axis:***
The correlation coefficient was 0.761 for EQAS results of Cycle 16 (Table 1, Fig. 1)

ii. ***Plotting PEG results on X axis and direct results on Y-axis:***
The correlation coefficient was 1.289 for EQAS results of Cycle 16 (Table 1, Fig. 2) i.e. reverse comparison.

The two step crosschecking was done and correlation coefficients were evaluated to confirm validity of the same within a limit of acceptable uncertainty.

It was also observed that PEG treated Cobas e 411 results are in accordance with Immunoassay systems Abbott Architect, Access 2 & Advia Centaur, r being 0.998, 0.997 & 0.999. The reason may be the EQAS samples either pretreated with PEG or the methods in other systems were modified so that involvement of M-PRL is not affecting the patient's results like Cobas e 411.

The insert of Lot 40330, BIORAD reflected similar consistency in results of above mentioned Systems and elevated prolactin results in Cobas e 411. So Trilevel immunoassay results were obtained and evaluated following the same procedure.

The correlation coefficients were found out to be 0.762 and 1.307 with R^2 0.999 (Table 2, Fig. 3, Fig. 4). The correlation coefficients of EQAS and IQAS both in direct and reverse direction are within the limit of acceptable uncertainty.

Now, to exclude PEG precipitation effect 25 samples were treated in the laboratory in Cobas e 411 and Access 2. In both systems direct and PEG treated sample results were recorded. The

Advia Centaur & Abbott Architect results obtained from accredited laboratories and it was instructed to send direct results only (Table 3). No remarkable deviation in results were noted between direct and after PEG treatment results in Access 2. The r value of Abbott Architect compared to PEG treated results of Cobas e 411 was 0.9988. The same for Access direct was 0.9967, for Access-PEG 0.997 and Advia Centaur was 0.9985. Though more than 25 samples could not be compared considering financial viability the range of prolactin results sent for comparison was extended from 6.0-365.0 ng/mL (Table 3).

The previous and current PRL insert of Roche Diagnostics were compared. No amendment in the procedure was observed (17). Though the inserts mentioned PEG treatment but no cutoff was instructed. To assess the validity of the laboratory defined correlation constant 150 samples were tested directly and after PEG precipitation. Correlation constants obtained are 0.761 & 1.301 with R^2 0.991 (Table 4, Figures 5, 6). Hence, the correlation constant is almost same for IQAS & human sample (0.761 & 0.762). Slightly different for EQAS samples (0.771) but such deviation is negligible and well within acceptable range. The EQAS cycle is a current one and difference in peers values confirm that Cobas e 411 values are still elevated and such elevation is measurable by a correlation coefficient.

CONCLUSION

- Correlation coefficient 0.761 is validated.
- When results are correlated in a wide range it can be concluded that PEG treatment to be done irrespective of concentration of prolactin within normal reference interval or above the biological reference interval. Within normal reference range result does not exclude the presence of M-PRL.
- As the PEG treated results are in correlation with other systems hence chance of inappropriate diagnosis is less. At least it may be concluded the precipitation of PRL is so small that it will not affect the results and interpretation.
- Possibility of systematic bias for the elevated results in Cobas e 411 cannot be excluded as it is not expected that presence M-PRL will be measurable by a correlation coefficient.
- Current peer mean results and range of IQAS and comparison data of EQAS, BIORAD is affirmative of the fact that the results of PRL in Cobas e 411 is yet elevated than other systems and needs modification/amendment of method. As peer mean of EQAS and IQAS are worldwide data hence the problem is a universal one and needs immediate resolution.

REFERENCES

1. Beda-Maluga K, Pisarek H, Komorowski J, Pawlikowski M, Swietostawaski J, Winczyk K. The detection of macroprolactin by precipitation and ultra filtration methods. Endocrynol Pol.62 (6); p.529-36. 2011.

2. Jurrige V. A possible cause of hyperprolactinemia, misdiagnosis and mistreatment. Pathology in Practice. Endoc rinology.26(7);p.26-30,2006.

3. Vaisya R, Gupta R, Arora S. Macroprolactin- A frequent cause of misdiagnosed hyperprolactinemia in clinical practice. J.Reprod. Infertil.11(3); p.161-7,2010.

4. Smith T P, Suliman A M, Fahie- Wilson M N, McKenne T J. Cross variability in the detection of prolactin in sera containing big-big prolactin(macroprolactin) by

commercial immunoassays. J Clin Endocrinol Metab.87; p.5410-5, 2001.

5. Fahie Wilson M N, Brunsden P, Surrey J, Everitt A. Macroprolactin and the Roche Elecsys Prolactin Assay: Characteristic of the reaction and detection by precipitation with poly ethylene glycol. Clin Chem. 46:p.1993-5,2000.

6. Hekim C, Alfyhan H, Leinonen J T, Stenman V H. Effect of incubation time on recognition of various forms of prolactin in serum by DELFIA assay. Clin Chem. 48, p.2253-6, 2002.

7. Schneider W, Marcovitz S, Al-Shammari S, Yago S, Chavalier S. Reactivity of Macroprolactin in common automated systems. Clin Biochem. 34,p.469-73,2001.

8. Leslie H, Courtney C H, Bell P M, Hadden D R,McCance D R, Ellis P K,Sheridan B, Atkinson A B. Laboratory and clinical experience in 55 patients with macroprolactinemia identified by a simple polyethylene glycol precipitation method. J Clin Endocrinol Metab. 86, p.2743-6, 2001.

9. Fahie-Wilson M N, John R, Ellis A R. Macroprolactin: high molecular mass forms of circulating prolactin. Ann Clin Biochem. 42,p.175-92,2005.

10. Gibney J, Smith T P, McKenna T J. The impact on clinical practice of routine screening for macroprolactin. J Clin Endocrinol Metab.90(7), p.3927-32,2005.

11. Bell D A, Hoad K, Leong L, Bakar J A, Sheehan P, Vasikaran S D. A high pressure liquid Chromatography method for separation of prolactin forms. Ann Clin Biochem. 49. p.285-8, 2012.

12. Karolina B M, Pisarek H, Romanowska I, Komorowski J, Swietoslawski J, Winczyk K. Ultrafiltration – an alternative method to poly ethylene glycol precipitation for macroprolactin detection. Arch Med Sci. 11(5),1001-7,2015.

13. Kit insert for Prolactin: Roche Diagnostics, p.1-5, 2010.

14. Kit insert for Prolactin: Access 2, Beckman Coulter, p.1-4,2010.

15. Kit insert for Prolactin: Abbott Diagnostics, p.1-6,2014.

16. Kit insert for Prolactin: Advia Centaur, p.1-5,2013.

17. Kit insert for Prolactin: Roche Diagnostics, p.1-4, 2016.