SIEMENS

IMMULITE®
Immunoassay System
IMMULITE® 1000
Immunoassay System

Siemens Healthcare Diagnostics Inc.

Customer Bulletin
CB2012-06-26
Supersedes CB2010-12-18
2012-06

IMMULITE / IMMULITE 1000 Insulin assay (U.S.)

Purpose:

The Purpose of this document is to announce the restoration of the IMMULITE / IMMULITE 1000 Insulin assay to historical performance. Siemens has addressed all of the following:

- Corrected low end negative bias identified in the December 2010 Customer Notification (CB2010-12-18)
- Restored Expected Values to the guidelines provided in the Instructions for Use (IFU)
- Improved alignment to WHO –IRP 66/304

Siemens strongly recommends that you re-establish your own medians and reference ranges based on your population.

Background Information

A low end negative bias was confirmed with the IMMULITE/IMMULITE 1000 Insulin (LKIN1, 10381429) assay kit lots 329 and above in late 2010. Customer Notification CB2010-12-18 was issued in December 2010, advising re-establishment of medians and reference ranges. Table 1 shows the revised 2010 reference range guidelines as well as the IFU Expected Values.

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Table 1. Reference range guidelines

	IFU Expected Values			December 2010 Revised Guidelines		
	Median (µIU/mL)	95th Percentile (µIU/mL)	n	Median (µIU/mL)	95th Percentile (µIU/mL)	n
IMMULITE / IMMULITE 1000	8.9	28.4	83	4.47	24.6	50

The root cause of the bias has been identified as a processing step used in manufacturing the reagent polyclonal conjugate. Siemens resolved the processing issue and the corrected conjugate will be available in kit lots 401 and above. Additional testing steps have been implemented to help prevent any future recurrences of this issue.

During the investigation into the root cause of the Insulin low end negative bias, Siemens also identified an opportunity to improve alignment to the WHO –IRP 66/304.

Low End Performance

The low end negative bias has been corrected with kit lots 401 and above. Table 2 shows the agreement between the IFU Expected Values and those obtained from a normal range study performed on 196 fasting, apparently healthy laboratory volunteers. The slight difference between the IFU and current normal range study may be attributed to the different demographics of the samples used. Please note the IFU values are to be used as guidelines only and each laboratory should establish their own reference ranges.

Table 2. IFU Expected Values Compared to Normal Range Study performed with Corrected Kit Lots 401 and above

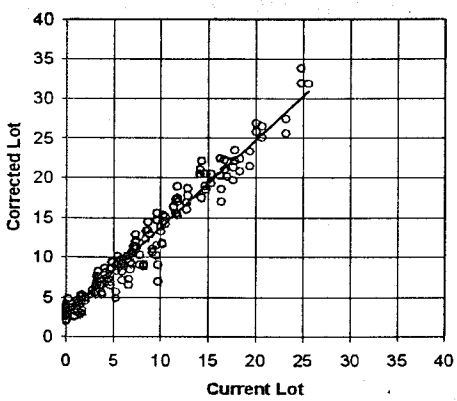
	Median (µIU/mL)	95th Percentile (µIU/mL)	n
IFU Expected Values	8.9	28.4	83
Lots 401 and above	8.0	33.3	196

The median difference between the current low biased and corrected kit lots is a combination of two effects:

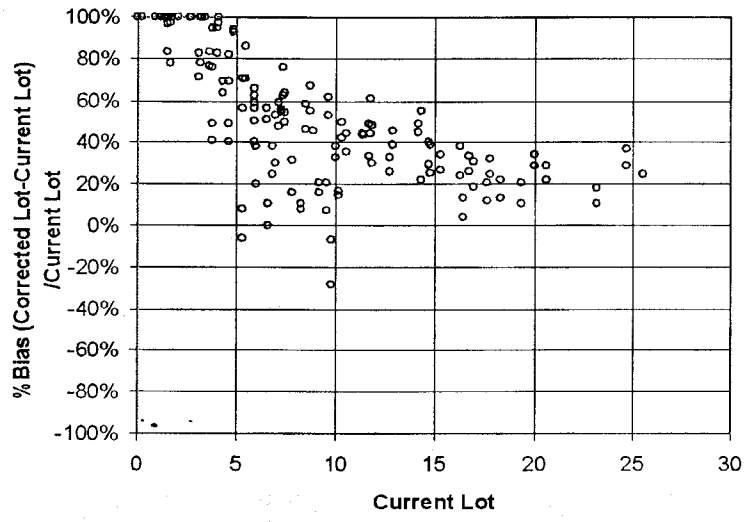
- The introduction of the corrected conjugate starting at kit lot 401 which impacts results at the low end of the assay range
- An approximate 20% positive shift across the entire assay curve to improve WHO alignment

The method comparison and bias plots for the low end of the assay, from 0 to 30 µIU/mL are shown in Figure 1.

Figure 1. IMMULITE / IMMULITE 1000 Insulin method comparison and bias plot between current lot and corrected lot up to 30 µIU/mL



Slope	1.10 95% CI: 1.07, 1.13
Intercept	2.73 95% CI: 2.43, 3.03
R (Corr. Coef.)	0.980
Mean of Corrected Lot	10.4
Mean of Current Lot	6.99
N	196



WHO Alignment

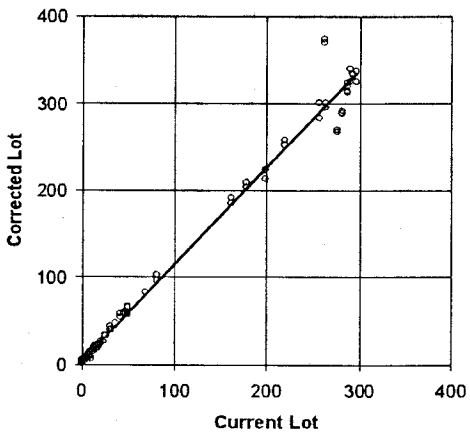
Siemens identified that the current Insulin assay recovers approximately 20% below the WHO –IRP 66/304. Kit lots 401 and above have been recalibrated to improve alignment. Table 3 shows the WHO alignment of the corrected kit lots 401 and above.

Table 3. WHO IRP Recovery for lots 401 and above (µIU/mL)

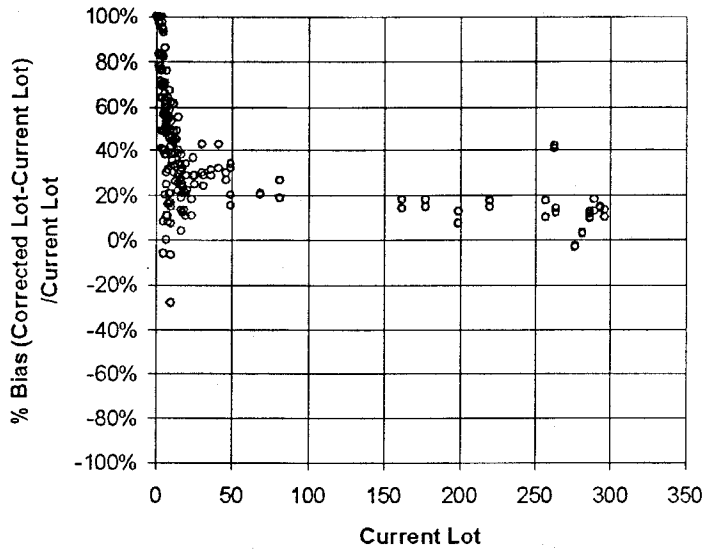
Expected	Observed	%O/E
6.0	5.9	98%
12.0	12.4	103%
25.0	26.3	105%
50.0	50.2	100%
100.0	98.1	98%
200.0	205.5	103%
300.0	298.3	99%

The approximate 20% positive shift across the assay range due to the WHO alignment is shown in the full method comparison between the current low biased and corrected kit lots below.

Figure 2. Figure 2: IMMULITE / IMMULITE 1000 Insulin method comparison and bias plot between current lot and corrected lot across the full assay range (µIU/mL)



Slope	1.12 95% CI: 1.11, 1.14
Intercept	2.99 95% CI: 1.76, 4.23
R (Corr. Coef.)	0.995
Mean of Corrected Lot	46.2
Mean of Current Lot	38.5
N	243



Next Steps

Slight differences in the IFU Expected Values and the normal range study (Table 2) may be attributed to the different demographics of the samples used. **Due to the demographic differences and the assay changes it is strongly recommended that you re-establish your median values based on your population.**

A positive shift in control values will be observed with the corrected kit lots 401 and above. Important Notices will be packed with the corrected kit lots to alert operators to the control revisions. Revised target values are also provided below:

Table 4. Revised Control Targets (µIU/mL)

Starting July 30, the corrected kit lots will ship routinely. If the older kit lots, 400 and below, are required, they will be available upon request through the end of 2012.

The following table shows the availability of the current and the corrected Insulin kit lots.

Table 5. Availability

Kit Lot	Up to July 27, 2012	Starting July 30, 2012
Current (LKIN kit lot 400 and below)	Will ship routinely	Specify kit lot number when ordering
Corrected (LKIN kit lot 401 and above)	Not available	Will ship routinely

Additional Data

There have been no changes to the assay's reportable range, analytical sensitivity, hook effect, linearity, recovery, specificity, tube type, or other parameters as stated in the IFU.

Precision

The conjugate correction which restores low end performance has no impact on assay precision. Precision data was generated and analyzed in line with CLSI EP15. Validation testing demonstrates that the precision of kit lots 401 and above is statistically equivalent to the IFU representative data as shown in Table 6.

Table 6. Precision (µIU/mL)

Current IFU Representative Data					Corrected Lot				
	Within Run		Total			Within Run		Total	
Mean	SD	CV	SD	CV	Mean	SD	CV	SD	CV
7.39	0.47	6.4%	0.59	8.0%	9.58	0.23	2.4%	0.23	2.4%
25.5	1.46	5.7%	1.50	5.9%	24.5	0.40	1.6%	0.60	2.5%
102	5.26	5.2%	6.20	6.1%	111	2.61	2.4%	2.50	2.3%

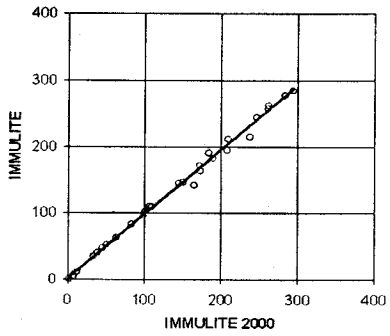
Commercial Insulin Controls

Siemens recommends using only the Insulin control LINC1/2 provided in the kit as stated in the IFU. Commercial control and survey materials may be problematic when tested with the IMMULITE /IMMULITE 1000 Insulin assay. This is due to the processed matrices used during manufacturing. We have frequently observed a shift in values with these types of samples when introducing a new raw material which is not reflected in patient results. The LINC 1/2 controls have been formulated to be less susceptible to raw material changes and better reflect patient performance.

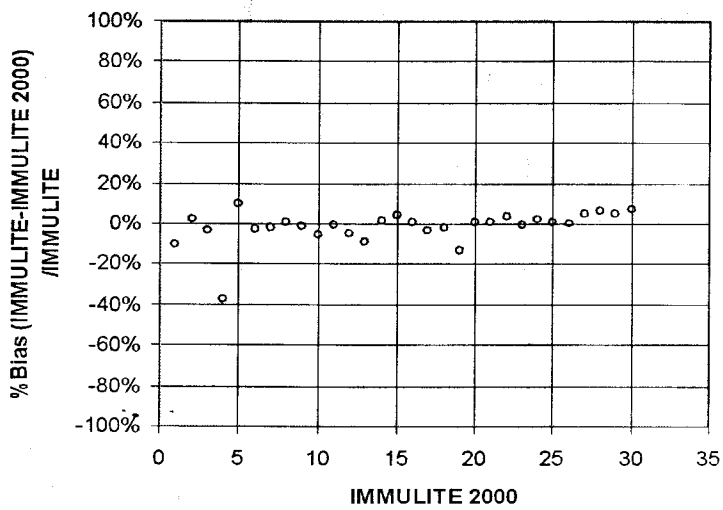
IMMULITE / IMMULITE 1000 against IMMULITE 2000 / IMMULITE 2000 XPi Insulin Method comparison

A cross platform comparison of corrected Insulin kit lots, 401 and above, is shown in Figure 3, which demonstrates good cross platform agreement.

Figure 3. Method Comparison IMMULITE 2000 / IMMULITE 2000 XPi against IMMULITE / IMMULITE 1000 Insulin kit lot 401(µIU/mL)



Slope	0.971 95% CI: 0.945, 0.996
Intercept	1.70 95% CI: -2.43, 5.83
R (Corr. Coef.)	0.998
Mean of IMMULITE	129
Mean of IMMULITE 2000	132
N	30



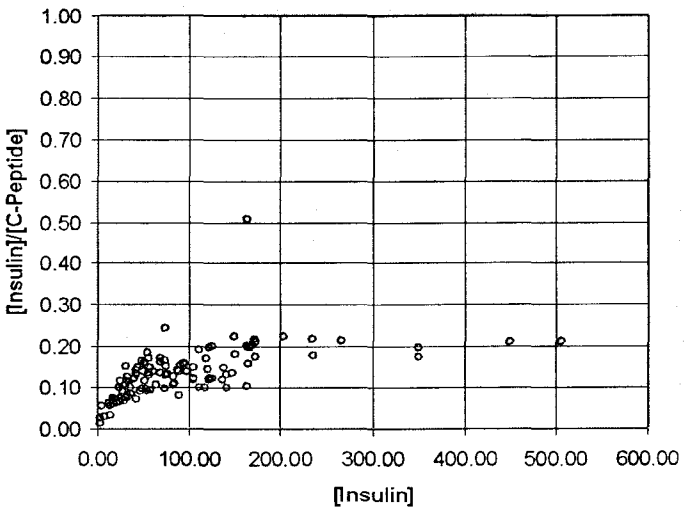
Insulin C-Peptide Molar Ratio

A study of IMMULITE 2000/IMMULITE 2000 XPi C-Peptide and the corrected Insulin assay results with 161 fasting volunteers is shown in Figure 4. After cleavage of pro-insulin into insulin and C-peptide in the pancreatic beta-cells, both molecules are secreted in the portal vein in a 1:1 molar ratio. The difference in metabolic action and clearance between insulin and C-peptide results in a much higher C-peptide half-life than insulin (30-35 minutes versus 5-10

minutes). As a result the observed ratio in fasting subjects and after food intake will be less than 1.0 despite the 1:1 molar secretion in the portal vein.

As would be expected, the IMMULITE 2000/IMMULITE 2000 XPi Insulin to C-Peptide ratios were all less than 1. Similar results would be expected with the IMMULITE/IMMULITE 1000.

Figure 4. Insulin:C-Peptide Molar Ratio (pmol/L)



FAQs

Questions	Answers
Will there be changes in Quality Control Material target values?	Yes. Revised Control values and ranges are shown in Table 4. Commercial controls can be problematic when used with the IMMULITE Insulin, and therefore Siemens recommends using only the LINC 1/2 provided in the kit as stated in the IFU.
Will there be any impact to survey samples?	Yes, survey samples will be affected. An upward shift is expected. Please refer to the method comparison and bias plots in Figures 1 and 2 for more information.

Questions	Answers
Is it necessary to carry out method comparison studies between current and corrected kit lots?	Siemens performed the method comparison study shown in Figure 2. If additional method comparisons are desired, both current and corrected kits will be available for a limited period of time. Corrected kits will begin routine shipment on July 30, 2012. The older kit lots will be available upon request through the end of 2012 – please state LKIN kit lot 400 and below when placing an order for the older kit lots.
Why is Siemens correcting the IMMULITE /IMMULITE 1000 Insulin assay?	As stated in the December 2010 bulletin, Siemens has been committed to restoring the low end performance of the Insulin assay. This has been achieved with kit lots 401 and above. In addition, the new lots have improved alignment to the WHO -IRP 66/304.
Will reference ranges need to be re-established?	Yes. As stated in the IFU, the Expected Values are guidelines only. Each laboratory should establish their own reference ranges.
Has the kit been reformulated?	No. The formulation of the assay has not been changed.
Why is there a greater positive shift at the low end?	The low end shift observed between the current kit lots and the corrected ones is the combination of two effects. The first is the change in the polyclonal conjugate processing that only affects the low end. The second is an approximate 20% positive shift based on the WHO recalibration. The WHO calibration impacts the entire range of the assay.
What has been done to prevent recurrence of the low end bias issue?	The root cause of the problem has been identified and corrected. Also, additional testing within the Expected Value dose range of the assay has been introduced to help identify any future issues.

Trademark Information

Additional Assistance

If you need additional assistance, please contact your Siemens Customer Service Center.