

ARCHITECT® Insulin: A Chemiluminescent Microparticle Assay

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Abstract

Objective

Immunoassays for insulin have been widely used to provide supplementary information for the diagnosis of diabetes mellitus and for differential diagnosis of fasting hypoglycemia to discriminate between insulinoma and factitious hypoglycemia. The objective of this study is to demonstrate the preliminary performance of the insulin assay run on the ARCHITECT instrument system to provide a more precise and convenient method of testing of insulin.

Methods

ARCHITECT Insulin is an automated assay for the quantitative determination of insulin in serum or plasma and is based on paramagnetic microparticle chemiluminescent technology. It is a one-step assay utilizing microparticles coated with the anti-insulin monoclonal antibody (MAb) and an Acridinium labeled anti-insulin MAb. Sample, microparticles and conjugate are combined in the first step, incubated and washed. After washing, Pre-trigger and Trigger are added to produce chemiluminescence, which is measured as relative light units (RLUs). The RLUs correspond to the concentrations of the insulin in the sample.

Results

The performance of ARCHITECT Insulin was confirmed at two sites as shown in the table. This assay has a dynamic range of 1 – 300 µU/mL with ability to run up to 200 tests/hour. The calibrators of this assay were standardized to the WHO reference (NIBSC 66/304). This assay was able to test both serum (including SST) and plasma (EDTA, heparin and NaF) samples. High dose hook was not observed for samples less than 15,000 µU/mL and a 1:2 auto-dilution function performed well. The comparison to Tosoh AIA-PACK IRI gave a slope of 0.832 and a correlation coefficient (r) of 0.995.

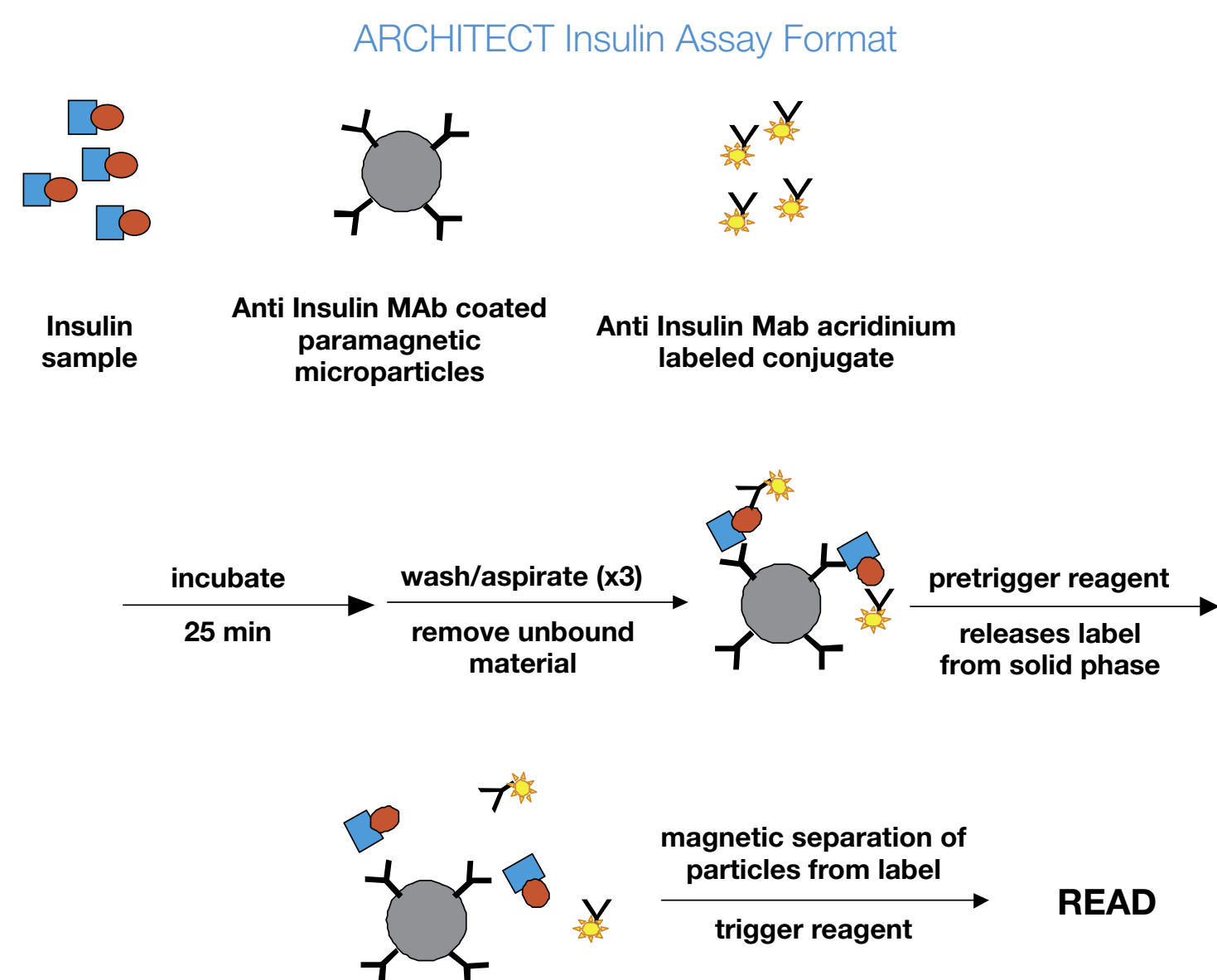
Conclusions

These preliminary results demonstrate the ARCHITECT Insulin assay has excellent sensitivity and precision, and correlates well with a reference method. The ARCHITECT Insulin assay provides a convenient method for measurement of insulin in serum or plasma in hospitals and clinical laboratories.

Assay Performance Summary

Character	Performance/ Results
Within-laboratory precision	Total precision (EP5-A2, CV): 1.9 – 5.2% (3 lot, 7 samples)
Analytical sensitivity	0.18 uU/mL (0 cal + 2 SD, mean of 12 runs)
Spike recovery	Serum sample (n=5): 93 – 95% Plasma sample (n=5): 93 – 100%
Dilution linearity	Serum sample (n=5): 103 – 106% Plasma sample (n=5): 98 – 102%
Analytical interference	Triglyceride (3840 mg/dL), Bilirubin (22 mg/dL), Protein (12 g/dL), Hemoglobin (1170 mg/dL), HAMA or RF affected less than 10%
Cross-reactivity	Very low cross-reactivity for proinsulin (≤ 0.1%), C-peptide (≤ 0.001%) or glucagon (≤ 0.001%)
Correlation (0 - 250 µU/mL)	y = 0.832 x + 0.071, r = 0.995, n = 100 (y: ARCHITECT, x: AIA-PACK IRI)
Dynamic range	1 – 300 µU/mL (2 – 600 µU/mL with auto-dilution)

Assay Format



Methods

Studies were conducted to characterize the performance of the ARCHITECT Insulin assay (available outside of the U.S.).

Precision: Panels and controls were tested following NCCLS protocol EP5-A2. The Repeatability and Within-laboratory precision are expressed as %CV.

Analytical Sensitivity: The upper limit of the 95% confidence interval representing the lowest measurable concentration that can be distinguished from zero (rep=10) was calculated.

Spike Recovery: Insulin antigen was spiked in serum and plasma samples. Recovery (%) was calculated using the Calibrator A as the control.

Dilution Linearity: Serum and plasma samples with elevated insulin concentrations were manually diluted using the Calibrator A and then compared with the undiluted assay value.

Analytical Interference: Serum samples were spiked with Triglyceride, Bilirubin, Protein and Hemoglobin and the values were compared with the control. For Human Anti-Mouse Antibodies (HAMA) and Rheumatoid Factor (RF), recovery of the spiked insulin was tested and the recovery % was compared with the normal sera.

Cross-reactivity: Serum samples spiked with Proinsulin, C-peptide and Glucagon were tested and the cross-reactivity was calculated.

Correlation: Serum samples were used in a correlation study versus the AIA-PACK IRI (Tosoh) assay. Correlation was assessed by least-squares regression analysis.

Results

Precision

Panels and controls were tested following NCCLS protocol EP5-A2 (20 days, 2 runs/day, 2 replicates). 3 lots of reagent were used.

Sample	Reagent Lot-1			Reagent Lot-2			Reagent Lot-3		
	Mean (µU/mL)	Repeatability CV%	Within-lab CV%	Repeatability CV%	Within-lab CV%	Repeatability CV%	Within-lab CV%		
Control-L	7.6	3.6%	4.5%	4.2%	5.2%	3.5%	4.7%		
Control-M	38.2	2.0%	2.3%	1.7%	2.8%	2.1%	2.4%		
Control-H	119.7	1.8%	2.1%	1.9%	2.1%	2.0%	2.3%		
Panel-1	8.6	3.1%	4.2%	3.0%	4.6%	3.4%	3.6%		
Panel-2	18.4	4.0%	4.5%	2.1%	3.6%	2.1%	3.0%		
Panel-3	54.0	1.9%	2.5%	2.2%	2.9%	2.3%	2.5%		
Panel-4	165.7	1.7%	1.9%	2.0%	2.2%	2.3%	2.3%		

Controls are buffer-based; Panels are serum-based

Analytical Sensitivity

The analytical sensitivity, as defined by the upper limit of the 95% confidence interval representing the lowest measurable concentration that can be distinguished from zero, was tested with 12 determinations (combination of 3 lots of reagent, 3 instruments and 2 runs). 10 replicates of A calibrator and 4 replicates of B calibrator were used per determination.

Instrument	Reagent Lot	Run	Analytical Sensitivity (µU/mL)
I201468	Lot 1	Run-1	0.19
		Run-2	0.15
	Lot 2	Run-1	0.13
		Run-2	0.20
I201253	Lot 1	Run-1	0.18
		Run-2	0.14
	Lot 3	Run-1	0.13
		Run-2	0.20
I202177	Lot 2	Run-1	0.15
		Run-2	0.16
	Lot 3	Run-1	0.21
		Run-2	0.28

In 12 determinations, Mean: 0.18 µU/mL and Mean+2SD: 0.26 µU/mL.

Spike Recovery

Three levels (20, 60 and 200 µU/mL) of insulin was spiked in 5 serum and 5 plasma samples. Recovery (%) was calculated using the Calibrator A as the control.

Sample Type	Endogenous Level (µU/mL)	Insulin Added (µU/mL)	Insulin Observed (µU/mL)	Percent Recovery	Mean in Sample
Serum 1	4.8	19.7	23.2	93.6%	92.5%
	4.8	60.6	60.3	91.6%	
	4.8	199.2	188.4	92.1%	
Serum 2	13.7	19.7	32.4	95.1%	95.3%
	13.7	60.6	72.6	97.2%	
	13.7	199.2	200.2	93.6%	
Serum 3	11.5	19.7	30.2	95.1%	93.2%
	11.5	60.6	68.1	93.4%	
	11.5	199.2	193.0	91.1%	
Serum 4	5.0	19.7	23.9	95.8%	94.5%
	5.0	60.6	62.3	94.6%	
	5.0	199.2	190.6	93.2%	
Serum 5	2.0	19.7	20.5	93.9%	93.6%
	2.0	60.6	59.1	94.2%	
	2.0	199.2	186.7	92.7%	
Plasma 1	6.0	19.7	25.3	97.9%	96.4%
	6.0	60.6	64.8	97.1%	
	6.0	199.2	193.5	94.1%	
Plasma 2	6.2	19.7	25.8	99.3%	99.7%
	6.2	60.6	67.8	101.6%	
	6.2	199.2	201.7	98.1%	
Plasma 3	8.8	19.7	27.6	95.7%	94.6%
	8.8	60.6	66.3	95.0%	
	8.8	199.2	194.4	93.2%	
Plasma 4	8.2	19.7	26.9	95.4%	93.6%
	8.2	60.6	63.6	91.5%	
	8.2	199.2	195.5	94.0%	
Plasma 5	6.1	19.7	24.5	93.7%	93.4%
	6.1	60.6	62.9	93.8%	
	6.1	199.2	190.7	92.7%	

Dilution Linearity

Dilution linearity was assessed by serial manual dilution using Calibrator A with five serum and five plasma samples of elevated insulin.

Sample	Dilution Factor	(µU/mL)			
		Avg Conc.	Corrected	Recovery	Mean
Serum 1	1:1	47.7			
	1:2	24.3	48.5	101.8%	
	1:4	12.3	49.1	103.0%	106.2%
	1:8	6.4	51.1	107.1%	
	1:16	3.4	53.7	112.7%	
Serum 2	1:1	56.9			
	1:2	29.8	59.7	103.1%	
	1:4	14.9	59.6	103.0%	105.2%
	1:8	7.5	60.0	103.7%	
	1:16	4.0	64.3	111.1%	
Serum 3	1:1	66.5			
	1:2	34.1	68.1	102.5%	
	1:4	16.8	67.0	100.8%	103.2%
	1:8	8.7	69.9	105.1%	
	1:16	4.3	69.3	104.2%	
Serum 4	1:1	42.8	dfddf		103.1%
	1:2 – 1:8				
Serum 5	1:1	87.6			103.4%
	1:2 – 1:16				
Plasma 1	1:1	165.3			
	1:2	84.3	168.6	102.0%	
	1:4	41.8	167.2	101.1%	101.8%
	1:8	20.9	167.4	101.2%	
	1:16	10.6	169.8	102.7%	
Plasma 2	1:1	43.8			
	1:2	22.3	44.5	101.7%	
	1:4	11.1	44.5	101.6%	102.1%
	1:8	5.6	45.0	102.8%	
	1:16	NA (observed value was <3.0)			
Plasma 3	1:1	58.3			
	1:2	29.0	58.1	99.5%	
	1:4	14.2	56.9	97.5%	100.6%
	1:8	7.4	58.8	100.8%	
	1:16	3.8	60.9	104.4%	
Plasma 4	1:1	67.1			99.4%
	1:2 – 1:16				
Plasma 5	1:1	99.5			98.3%
	1:2 – 1:16				

Analytical Interference

Four serum samples were spiked with Triglyceride (3840 mg/dL), Bilirubin (22 mg/dL), Protein (12 g/dL) and Hemoglobin (1170 mg/dL) and the values were compared with the control. For HAMA and RF, recovery of the spiked insulin was tested and the recovery % was compared with the normal sera.

Substance	Concentration	Insulin Conc of the Control (µU/mL)	Interference of 4 Samples
Triglyceride	3840 mg/dL	7.0 – 135.9	-0.2 – 2.8%
Bilirubin	22 mg/dL	8.3 – 163.3	-0.8 – 2.5%
Total Protein	12 g/dL	4.6 – 84.2	-7.7 – -4.3%
Hemoglobin	1170 mg/dL	8.0 – 149.6	-6.5 – -4.2%

Sample	Spiked Insulin Concentration	Average of % Recovery
Normal serum 5 samples	20 & 120 µU/mL	96.1%
HAMA specimen 5 samples	20 & 120 µU/mL	98.7%
RF specimen 5 samples	20 & 120 µU/mL	95.7%

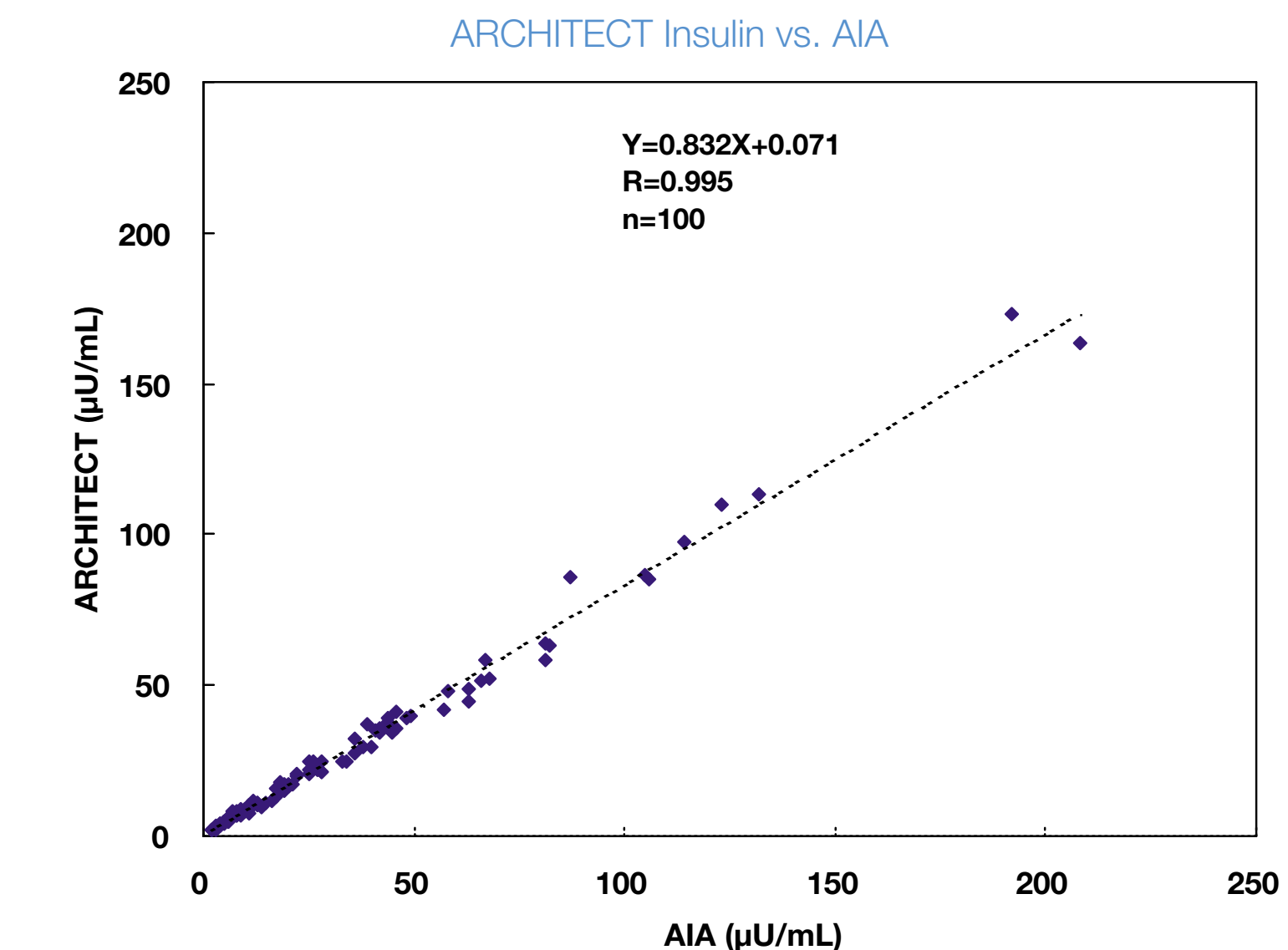
Cross-reactivity

The cross-reactivity with Proinsulin (NIBSC, Code 84/611), with C-peptide (Sigma, Code C-5051), and with Glucagon (Peptide Institute) was determined as below in the ARCHITECT Insulin assay.

Substance	Concentration	Cross-reactivity
Proinsulin	10 ⁶ pg/mL	≤ 0.1%
C-peptide	10 ⁷ pg/mL	≤ 0.001%
Glucagon	10 ⁷ pg/mL	≤ 0.001%

Correlation

100 serum samples were used in a correlation study to the Tosoh AIA-PACK IRI assay. Correlation was assessed by Least-squares regression analysis.



Conclusion

The Insulin assay developed for the ARCHITECT instrument system is:

- Sensitive: analytical sensitivity less than 0.3 µU/mL
- Precise: Within-laboratory CVs less than 6%
- Accurate: good dilution linearity and spike recovery, low interference and very low cross-reactivity, good correlation with AIA-PACK IRI (r > 0.99)
- Convenient: up to 200 tests/hour on ARCHITECT

References

Masako MORIYAMA, et al. Evaluation of insulin measurement by fully automated chemiluminescent immunoassay analyzer "ARCHITECT® I2000". *Jpn J Med Pharm Sci* 2005; 53(4): 477-482.