Insulin assays have been widely used to provide diagnostic information for diabetes mellitus and rare hypoglycemic syndromes (1–3) and to quantify insulin for pharmacokinetic evaluations. We assessed the analytical and clinical performance of the ARCHITECT® insulin assay, a chemiluminescent immunoassay recently introduced for the ARCHITECT i2000 fully automated immunoassay analyzer (Abbott Laboratories). We also tested whether major insulin analogs cross-react with the immunoassay reagents.

**Methods:** We used Clinical and Laboratory Standards Institute protocols to assess the analytical performance of the ARCHITECT insulin assay and compared its accuracy with that of the E-test TOSOH II (IRI) from TOSOH Corporation. We used 3 recombinant insulin analogs (lispro, aspart, and glargine) to evaluate the cross-reactivity of insulin analogs with the ARCHITECT immunoassay reagent.

**Results:** The total CV for the ARCHITECT assay was <5%. Correlation between the ARCHITECT insulin assay and the E-test TOSOH II (IRI) was satisfactory in the measured range, but we detected a slope deviation between the assays. The ARCHITECT insulin assay showed low cross-reactivity to the insulin analog aspart, whereas it detected the other insulin analogs, lispro and glargine, in concentrations as high as the theoretical dilution linearity, detection limit, and effects of interfering substances. When interpreting results, clinicians and laboratory pathologists should be aware of the cross-reactivity of the ARCHITECT and other immunoassays to specific insulin analogs prescribed to diabetes patients.

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**References**


15. Davignon J, Gregg RE, Sing CF. Apolipoprotein E polymorphism and athero-


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**Performance Evaluation and Cross-Reactivity from Insulin Analogs with the ARCHITECT Insulin Assay, Masako Moriyama,1 Nobuhide Hayashi,1 Chinami Ohyabu,1 Masahiko Mukai,1 Seiji Kawano,1 and Shunichi Kumagai1,2**

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**Background:** Insulin measurement is used for the diagnosis of hypoglycemia and for insulin pharmacokinetic evaluations. We assessed the analytical and clinical performance of the ARCHITECT® insulin assay, a chemiluminescent immunoassay recently introduced for the ARCHITECT i2000 fully automated immunoassay analyzer (Abbott Laboratories). We also tested whether major insulin analogs cross-react with the immunoassay reagents.

**Methods:** We used Clinical and Laboratory Standards Institute protocols to assess the analytical performance of the ARCHITECT insulin assay and compared its accuracy with that of the E-test TOSOH II (IRI) from TOSOH Corporation. We used 3 recombinant insulin analogs (lispro, aspart, and glargine) to evaluate the cross-reactivity of insulin analogs with the ARCHITECT immunoassay reagent.

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**Insulin assays have been widely used to provide diagnostic information for diabetes mellitus and rare hypoglycemic syndromes (1–3) and to quantify insulin for pharmacokinetic evaluations. The ARCHITECT® insulin assay (Abbott Laboratories) is a 1-step chemiluminescent immunoassay that uses paramagnetic microparticles coated with anti-insulin monoclonal antibody and acridinium-labeled anti-insulin monoclonal antibody conjugate. Samples, microparticles, and conjugate are added to a reaction vessel to form a particle–insulin–conjugate sandwich. After incubation, washing removes materials not bound in the 1st step. Addition of the pretrigger reagent, which includes hydrogen peroxide, and the trigger reagent, which includes sodium hydroxide, leads to acridinium-produced chemiluminescence, measured as relative light units (RLUs). The RLUs are proportional to the insulin concentration in the sample. The assay is traceable to the WHO Insulin 1st International Reference Preparation (WHO IRP 66/304; National Institute for Biological Standards and Control). The calibrators includes 6 concentrations (0, 3, 10, 30, 100, and 300 mIU/L). These are measured in duplicate to
establish the calibration curve calculated by a 4-parameter logistic curve method.

We assessed the analytical and clinical performance of the ARCHITECT insulin assay. In addition, because a recent study revealed that the insulin analogs used for patient treatment cross-reacted with reagents used for insulin measurement (4), we tested for cross-reactions between major insulin analogs and the ARCHITECT immunoassay reagents. Following the protocols of the Clinical and Laboratory Standards Institute for total imprecision, we used the ARCHITECT insulin assay to measure Bio-Rad Immunoassay Plus Controls (Bio-Rad), which include control sera containing low, moderate, and high concentrations of insulin, in duplicate in 2 runs each day for 20 days. The within-run CVs were 1.1% to ~1.8%, and the total CV did not exceed 5% in the measurement range.

We performed a dilution linearity study on 2 serum samples with increased insulin values and on the WHO standard. The samples were diluted with calibrator A (0 mIU/L). Each sample was measured twice in a 10-fold serial dilution. The ARCHITECT insulin assay was confirmed to be linear up to 273.5 mIU/L on the basis of a recovery acceptance range of 10% from the expected concentration.

Using serum pools containing low, middle, and high insulin concentrations to measure interference, we detected no interference from free and complex bilirubin (up to 194 mg/L), hemoglobin (up to 7650 mg/L), or chyle (up to 2800 formazine turbity units). In addition, a 1/10th volume of 300 mIU/L insulin solution was added to the sera with high concentrations of rheumatoid factor (223–2350 kIU/L), hepatitis B surface antigen (3798–4337 kIU/L), and hepatitis C virus RNA (2.0 × 10^6 to 3.5 × 10^6 kIU/L), respectively. These samples were measured in duplicate to obtain analytical recoveries. These potential interferents did not affect the measured insulin values.

According to the manufacturer, human anti-mouse antibody (HAMA) blockers were included in the ARCHITECT insulin. Results from the analytical recovery test showed no significant interference of HAMAs (data not shown). In addition, no high-dose hook effect was detected in 1 very highly concentrated WHO reference preparation containing 30,000 mIU/L insulin.

We assayed for insulin concentrations 5 times in solutions of proinsulin [relative molecular mass (M_r) 9390; adjusted to 1 × 10^6 ng/L (3.31 × 10^{-6} mol/L)] and C-peptide [M_r 3020; adjusted to 1 × 10^6 ng/L (0.106 × 10^{-6} mol/L)]. The cross-reactivities, defined as a percentage of the amount of insulin (M_r 5807) corresponding to the analysis results in each solution for the amount of the proinsulin or C-peptide in the sample (insulin: 1 mIU/L = 41.67 ng/L = 7.18 × 10^{-12} mol/L), were <0.005% for proinsulin and <0.00001% for C-peptide.

To determine the minimum detectable insulin concentration, we prepared several samples by diluting calibrator B (3 mIU/L) serially with calibrator A (0 mIU/L) and calculated the mean (SD) RLU values from 10 measurements of each of those samples. The detection limit was defined as the lowest measurable concentration of insulin for which the mean − 3 SD was higher than the mean + 3 SD of calibrator A, measured 20 times. In this study, the detection limit was determined to be 0.5 mIU/L.

The E-test TOSOH II (IRI) from TOSOH Corporation is an enzyme immunoassay designed for use on the AIA-21. We used this assay and the ARCHITECT assay to measure insulin concentrations in 100 hospital serum samples (Fig. 1). The regression equation between the ARCHITECT insulin (y) and the E-test TOSOH II (IRI) (x) results was: y = 0.832x + 0.068 mIU/L [95% confidence interval of the slope, 0.815–0.849; for the y-intercept; 0.775–0.912 mIU/L; correlation coefficient (r) = 0.990; and S_{yx} = 3.17 mIU/L]. Although the correlation of the ARCHITECT insulin assay with the E-test TOSOH II (IRI) was good across the clinical measurement range, we detected slope deviation. To investigate the cause of the slope deviation, we confirmed that both assays were exactly calibrated against the WHO reference preparation. We prepared 3 concentrations of insulin (50, 100, and 200 mIU/L) by diluting the WHO reference preparation with the specialized diluents provided for each assay and measured each diluted sample 5 times. The mean percentage of measured value to theoretical value was 104.5% (range, 101.5%–106.8%) for the ARCHITECT insulin and 105.4% (range, 104.9%–105.9%) for the E-test TOSOH II (IRI). These results indicated that the 2 methods were exactly calibrated against the WHO reference preparation. To examine the influence of serum effects, we added 2 different concentrations of WHO standard solution (100 and 200 mIU/L) to 8 different sera at a ratio of 1 to 9. These samples were measured 3 times to calculate recovery ratios. The mean recovery ratio was 98.7% (range, 95.5%–103.8%) for the ARCHITECT insulin assay and 111.9% (range, 105.6%–119.6%) for the E-test TOSOH II (IRI). The mean recovery ratio of the E-test TOSOH II (IRI) was thus higher than that of ARCHITECT insulin. A Bland–Altman

![Fig. 1. Comparison test.](Image)

x axis, E-test TOSOH II (IRI); y axis, ARCHITECT insulin assay. y = 0.832x + 0.068 mIU/L (r = 0.990; n = 100).
analysis indicated that proportional error accounted for the differences between these 2 assays (data not shown). Although we could not identify the causative components in sera that contributed to the slope deviation, the difference in recovery ratios suggests that some kind of matrix component in the sera affected the correlation slope.

We obtained a reference interval for fasting insulin concentrations from results of the analysis of 52 serum samples from healthy volunteers with fasting glucose concentrations within the reference interval (glucose <1 g/L after fasting for at least 8 h) defined in the American Diabetes Association revised standard criteria (5). The reference interval (mean ± 2 SD) for fasting insulin obtained with a Box–Cox power transformation and a parametric method was 2.7–10.4 mIU/L.

We obtained 3 kinds of recombinant insulin analogs, lispro (Humalog®, Eli Lilly and Company), aspart (NovoRapid® 300 FlexPen®; Novo Nordisk Pharmaceuticals), and glargine (Lantus®; Aventis Pharmaceuticals) and investigated whether they were detectable by these assays. Each of these recombinant insulin analogs has a nominal concentration of 100 kIU/L and is suitable for injection. Each was diluted volumetrically with 10 g/L bovine serum albumin to final insulin concentrations of 10 and 100 mIU/L. We measured all dilutions of the insulin preparations in triplicate and calculated the percentage cross-reactivity from the ratio of the measured and nominal concentrations. All dilutions were assayed on the ARCHITECT insulin and the E-test TOSOH II (IRI). A summary of the cross-reactivity percentages is shown in Table 1.

The ARCHITECT insulin and E-test TOSOH II (IRI) assays demonstrated similar recoveries, close to the theoretical concentration, for insulin lispro (insulin B28 Pro→Lys and B29 Lys→Pro). The ARCHITECT insulin assay had a low percentage of recovery (~76%) to insulin aspart (insulin B28 Pro→Asp), whereas the E-test TOSOH II (IRI) measured an almost nominal concentration. Insulin glargine (insulin A21 Gly→Asn, and addition of 2 arginine residues to the COOH terminus of the B chain) had ~22% cross-reactivity with E-test TOSOH II (IRI), whereas it showed concentration-dependent cross-reactivity with the ARCHITECT insulin assay (105% at 10 mIU/L and 83% at 100 mIU/L). Human insulin has 2 antigenic determinants. One is the terminus of the B chain (6–9), and the other is the immunodominant A-chain loop, which comprises residues A8–A10 (10–13)). Insulin analogs have different antigenic sites than does human insulin. These results indicated that the insulin analogs could show various degrees of cross-reactivity depending on the selection of the monoclonal antibody used in the commercial reagents.

The ARCHITECT insulin assay showed favorable results in the basic performance evaluation, including reproducibility, dilution linearity, detection limit, and effects of interfering substances. In the comparison test, however, we observed slope deviation. Standardization of immunoassays for insulin remains a problem despite the availability of large quantities of human insulin through recombinant DNA technology (2). A task force on standardization of insulin assays noted significant variability in insulin results and suggested a process for the assessment and certification of insulin assays (14). Although we could not completely explain the cause of the slope deviation, it does suggest the influence of a matrix component and the need for further investigation.

Several immunoassays are available for detecting human insulin and insulin analogs. Bowsher et al. (15) reported on a sensitive RIA specific for insulin lispro. For all such assays, users should be aware of the various cross-reactivities of insulin analogs with the immunoassays and choose the most suitable reagent.

In summary, the ARCHITECT insulin assay demonstrated good performance and is a suitable assay for the quantitative determination of insulin in human serum.

Table 1. Percentage cross-reactivity from the ratio of measured and nominal concentrations of insulin analogs.

<table>
<thead>
<tr>
<th>Insulin product</th>
<th>ARCHITECT Insulin</th>
<th>E-test TOSOH (IRI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspart (NovoRapid)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 mIU/L</td>
<td>76</td>
<td>115</td>
</tr>
<tr>
<td>100 mIU/L</td>
<td>75</td>
<td>116</td>
</tr>
<tr>
<td>Glargin (Lantus)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 mIU/L</td>
<td>105</td>
<td>16</td>
</tr>
<tr>
<td>100 mIU/L</td>
<td>83</td>
<td>22</td>
</tr>
<tr>
<td>Lispro (Humalog)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 mIU/L</td>
<td>110</td>
<td>116</td>
</tr>
<tr>
<td>100 mIU/L</td>
<td>100</td>
<td>106</td>
</tr>
</tbody>
</table>

References

Genotyping of Ovine Prion Protein Gene (PRNP) Variants by PCR with Melting Curve Analysis, Ekkehard Schütz, Melanie Scharfenstein, and Bertram Brenig (Institute of Veterinary Medicine, Georg-August-University, Burenhardtweg 2, 37077 Göttingen, Germany; * author for correspondence: fax 49-551-39-3392, e-mail eschuetz@mac.com)

Background: Scrapie is the transmissible spongiform encephalopathy in sheep. Because genetic variants of the ovine PrP gene (PRNP) can be associated with disease risk, the European Union initiated programs to eradicate high-risk PRNP genotypes from sheep livestock. For this purpose, reliable and cost-effective genotyping is needed.

Methods: We amplified DNA to cover the 3 risk codons in exon 3 encoding amino acids 136, 154, and 171. Amplicons were mixed with dye-labeled probe sets, and melting curves were recorded in a LightCycler by use of color and temperature multiplexing. Probe design was based on thermodynamic calculations to ensure unequivocal results for the 3 codons of interest, taking the additional F141 and T137 sequence variants into account.

Results: The fluorescence resonance energy transfer (FRET) method, when compared with sequencing, gave exactly the predicted melting temperatures for all possible genotypes. When we validated the method with samples from official certification programs, it showed completely matching results. Turnaround time was about 5 h after receipt of a whole-blood sample. The method detected the rare sequence variants T137 and F141, which were clearly distinguishable from the other known genotypes by melting curve analysis. One scrapie sheep was ARR/ARR, which is considered the haplotype with the lowest risk.

Conclusions: The FRET-based PRNP genotyping method for sheep is rapid and can differentiate all genotypes at each locus in 1 capillary. The assay is fast and has lower costs than restriction fragment length polymorphism analysis or sequencing.

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Scrapie was the first transmissible spongiform encephalopathy described, with reports going back to the 18th century. Although direct transmission to humans is considered impossible, scrapie has been hypothesized as the natural source of the transmissible infectious agent, the prion protein (PrP), in the epidemic of bovine spongiform encephalopathy in the United Kingdom. This raised concern for human health and led to a European Union–wide program for eradication of scrapie from sheep flocks (1, 2).

In contrast to bovine spongiform encephalopathy (3), but like human transmissible spongiform encephalopathy (4), scrapie displays a strong risk dependency on PRNP genotype (4). Three variants in the gene have been described, encoding amino acid changes at positions 136, 154, and 171, that are used for risk prediction (5). Risk groups (see Table 1 in the Data Supplement that accompanies the online version of this Technical Brief at http://www.clinchem.org/content/vol52/issue7) are used in the decision-making process for culling schemes in scrapie herds and for breeding programs (1).

Because of the small profit margins on sheep, the price for genotyping of the 3 loci must be no more than 10 € per animal. Methods available for ovPRNP genotyping are based on sequencing (6), restriction fragment analysis (5), or primer extension (7), which require several technical steps. In contrast, the technology of fluorescence resonance energy transfer (FRET) used in the LightCycler avoids additional steps, and the test can be done directly on extracted DNA (8). Our goal was to develop an inexpensive assay to identify the 15 known genotypes at the 3 positions.

We extracted genomic DNA from sheep, using the QIAamp DNA Blood Mini Kit (cat. no. 51106; Qiagen). Approximately 20 ng of DNA was used for PCR (total reaction volume, 25 µL) with 0.8 µM forward primer (5'-GGGAAGGGTGGCTACATGCT-GGGAAG-3', where * is fluorescein), 0.4 µM reverse primer (5'-TGTTGACACGT-CATGCACAAA-3'), and the PuReTaq PCR reagent set. PCR was performed in a Personal cycler (Biometra) thermocycler and included 5 min of initial denaturation at 95 °C, followed by 38 cycles of 95 °C for 1 min, 55 °C for 30 s, and 72 °C for 2 min (Amersham Biosciences). The product (19 µL) was transferred to the sample receptacle of a LightCycler capillary containing a premade probe mixture consisting of 0.25 µM of each sensor oligonucleotide (as given in Table 1) and 0.3 µM of anchor oligonucleotide. We used nearest-neighbor calculations in assay design, using MeltCalc 2.3 (9, 10) to develop an ovPRNP genotyping assay to identify all 15 known haplotypes at the 3 positions. This was accomplished by use of a combination of color and temperature multiplexing (11, 12) using in silico–designed oligonucleotide probes and anchors (13).

The placements of probes, anchor, and primers are given in Fig. 1 of the online Data Supplement. For codon 136 and 171 alleles, we used 6-carboxy-X-rhodamine (ROX)-labeled probes with one channel (channel F2: 640 nm) with temperature multiplexing, whereas the 154 allele was detected in the second channel (channel F3: 705 nm) with a Cy5.5-labeled probe (see Table 1).

The melting curve was recorded after 1 min of denaturation (95 °C) followed by reannealing at 35 °C for 1 min and detection during heating to 75 °C at 0.2 °C/s. For comparison and validation purposes, we also analyzed