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Challenges in harmonizing integrated healthcare network laboratories: multi-center evaluation of the hCG5 assay

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ABSTRACT

Background: Beckman Coulter recently introduced a new hCG assay manufactured for the Access 2 and DxI platforms. This assay is the first to use the 5th International Standard (5th IS) as its primary calibration material. Clinical laboratories are required to validate the method performance before testing and reporting patient results.

Methods: Beckman Coulter Access 2 instruments (n = 41) across Kaiser Permanente Northern California were evaluated for their performance characteristics using the hCG5 reagent. Precision, linearity, dilution verification, and patient sample comparisons were performed on each instrument.

Results: The assay was linear up to 1350 IU/L. Intra-day and inter-day precision ranged from 1.0%–3.3% and 1.8–7.3%, respectively, for the low QC material (mean concentration 4.6 IU/L). Percent bias between the previous assay (hCG2) and the hCG5 assay was 3.2 to 22.7% for hCG concentrations <1000 IU/L and −2.9 to 30% for concentrations >1000 IU/L. On board and manual dilutions agreed within 15% following proper adjustment of the instrument dilution factor.

Conclusions: Achieving Access 2 inter-instrument agreement on specimens needing dilutions (hCG > 1350 IU/L) requires validation of the on board dilution factor. Laboratories should use QC material above the linear range to monitor instrument dilution accuracy and precision.

1. Introduction

Beckman Coulter recently modified the hCG assay for the Access 2 and DxI platforms, releasing it under the name Total Beta-hCG 5th IS (hCG5). The new assay was developed to enhance precision at low concentrations of hCG. This reformulation employs the latest international standard (IS), the 5th IS, as the primary calibrant [1]. This material is more pure than the previous IS generations and was prepared to facilitate both intermethod harmonization and the reporting of hCG in molar units [1].

Accrediting agencies require laboratories to complete an internal assessment before moving new assays into production. Kaiser Permanente Northern California (KPNC) is an integrated healthcare network that provides healthcare services to 3.4 million members. hCG measurements are performed on 41 Access instruments across 21 hospital laboratories throughout Northern California. In accordance with regulatory guidelines we have validated the hCG5 assay on each of these instruments individually. The objective of this study was to evaluate the performance characteristics between instruments and hospitals by simultaneous inter-instrument comparison of precision, linearity, patient samples, dilution accuracy, and dilution precision.

2. Materials and methods

2.1. Study design and approval

Beckman Coulter Access 2 instruments (n = 41) across KPNC (21 sites) were evaluated for their performance characteristics using the Beckman hCG5 reagent. All of these instruments were in a hospital production setting. The hCG5 assay is a paramagnetic two-site immunoenzymatic assay that uses two anti-hCG antibodies for solid-phase binding (monoclonal mouse) and detection (polyclonal rabbit). While the exact epitopes on these antibodies have not been published, the assay has specificity for the free beta subunit, but not the terminal degradation product: hCG beta core fragment [2,3]. The detection antibody is conjugated to alkaline phosphatase, which catalyzes a chemiluminescent reaction upon the addition of the Lumi-Phos*530 substrate. When the chemilumiscent substrate is enzymatically dephosphorylated, it reacts to form 2-adamantanone and 3-hydroxy-methylbenzoate in an excited electronic state, which emits light as it relaxes to ground state. Since this is a two-site immunoassay format, emitted light is "proportional" to the concentration of the analyte. This study was considered a quality assessment project and was therefore

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deemed exempt from human subjects review by the KPNC Institutional Review Board.

2.2. Patient samples

Residual deidentified patient samples (serum and plasma) collected between July 15, 2014 and October 15, 2014 were used for all studies. Unless otherwise noted: specimens were stored at −20 °C for up to 10 weeks, serum specimens were collected in serum separator tubes (SST) or rapid serum tubes (RST), and plasma specimens in plasma separator tubes (PST) before being poured off into aliquot containers for storage. Serum and plasma were used interchangeably because these are the acceptable specimen types for the laboratories (as FDA approved for the hCG2 and hCG5 assays). Previous work has shown that the results between these two specimen types are comparable using the hCG2 assay when samples are free of pre-analytical errors [4]. Our analysis of paired serum and plasma samples showed similar consistency using the hCG5 assay (n = 11; concentration range = 1.2–91,000 IU/L; slope = 0.974 (95% CI 0.973–1.013); y-intercept = 0.043 IU/L (95% CI 0–0.271); average % difference = 0.03% (SD = 3.0%); R^2 = 0.9997).

All KPNC hospital laboratories contributed to the patient specimens.

2.3. Precision

Precision experiments were completed using two or three concentrations of commercial quality control (QC) material. Twenty hospital laboratories used BioRad ImmunoAssay Plus, one laboratory used Thermo Scientific MAS, and six laboratories used BioRad Fertility in addition to the ImmunoAssay Plus. Intra-day precision was completed by performing consecutive measurements (n = 20) on each level of QC material in a single day. Inter-day precision was completed by analyzing each concentration of QC material in duplicate two times per day (morning and evening) for five days (n = 20 per hCG concentration).

2.4. Linearity

The analytical measuring range was challenged with a mixing study using high and low patient samples. Each of the laboratories located a sample with a concentration of >1500 IU/L using the production hCG assay and diluted this specimen to ~1000 IU/L (high specimen); male serum with verified undetectable hCG was used as the low specimen. A series of five samples with concentrations ranging from 100% to 0% of the high sample were prepared. Each sample was analyzed in triplicate on both of the individual laboratory's instruments.

2.5. Dilution verification

The Beckman Access II instruments use a step-wise precision pump to aspirate the appropriate amount of sample to render a 1:200 dilution. However, while the pipetting mechanism is precise, it is frequently inaccurate. To address this problem, the instrument parameters have an option to change the dilution factor to a value between 160 and 230, which empirically accounts for the inaccuracy of the pipetter and maintains the claimed 1:200 dilution. The clinical reportable range was therefore evaluated on the instruments (n = 41) by comparing the results of manual dilutions to that of on board dilutions, and the dilution factor was adjusted if needed.

Samples with hCG concentration of >4000 IU/L (n = 5 per laboratory) were manually diluted 1:200. More specifically, the manual dilutions were completed using a combination of volumetric and positive displacement pipettes. The primary manufacturers of the positive displacement pipettes were MLA and Eppendorf with annual calibration verification performed in house by a licensed technologist or by a third party professional. The dilution procedure was as follows: 5 mL of wash buffer was measured using a volumetric pipette; 25 μL of wash buffer was removed using a positive displacement pipette; 25 μL of sample was added to the wash buffer; the sample was mixed well by inversion. hCG was quantified in the neat and diluted specimens on both of the individual laboratory's instruments (3 times/sample/instrument), with the instrument performing an on-board dilution to all specimens with an hCG concentration greater than the highest calibrator (~1300 IU/L). If the manual and on board dilutions disagreed by >15% the dilution factor of the instrument was evaluated and adjusted, and the verification was repeated.

2.6. Inter-instrument and inter-assay comparison

Inter-assay variability was evaluated on the 41 production instruments across 21 hospital laboratories. Of these laboratories, 20 have two instruments and therefore variability between instrument pairs (inter-instrument variability; n = 20 pairs) was also assessed. Each hospital laboratory sequestered residual specimens spanning the concentration range commonly seen in production, evaluating an average of 30 specimens per laboratory. On average, half of these specimens had detectable concentrations of hCG within the linear range (<1300 IU/L) and half had concentrations of >1300 IU/L. One site (#10) only evaluated specimens of <1300 IU/L and is therefore not represented in the figures with "high" patient sample comparisons. At the individual laboratories, specimens were analyzed on both instruments using the production hCG assay (the previous generation assay calibrated against the 3rd IS; henceforth called hCG2) and the hCG5 assays within 2 h of each other.

2.7. Statistical analysis

Statistical analyses and figures were completed using Excel, EP-Evaluator and the R statistical programming language as appropriate. Using EP-Evaluator, percent bias was calculated as the average of each individual point on the hCG2 versus hCG5 assay. Specifically, the hCG2 result was subtracted from the hCG5 result and divided by the hCG5 result. The average of all points was calculated and multiplied by 100 to generate the overall average percent bias. When calculating percent bias within the same assay between two instruments the same approach was taken, but one instrument was consistently used for the x-axis, while the other instrument was used as the y-axis.

3. Results

3.1. Precision

Intra-day precision fell within a tight range for all levels of QC material (Fig. 1A). For the low (≤10 IU/L) QC (n = 41; mean concentration 4.6 IU/L; range 3.4–9.5 IU/L) CVs ranged from 1.0–3.3% (mean 2.0%). For the mid (11–100 IU/L) control (n = 4; mean concentration 23.7 IU/L; range 22.8–25.0) CVs ranged from 1.7–2.8% (mean 2.0%). For the high (101–1000 IU/L) control (n = 37; mean concentration 348 IU/L; range 321–471 IU/L) CVs ranged from 1.0–4.0% (mean 2.2%). For the dilution control (≥1001 IU/L) (n = 6; mean concentration 16,916 IU/L; range 14,282–18,885 IU/L) CVs ranged from 2.0–3.6% (mean 2.6%).

Larger variability was observed between instruments for the inter-day precision relative to the intra-day precision (Fig. 1B), but similar to the intra-day precision, the grand mean of all sites CVs remained consistent across all levels of control material. For the low QC (n = 41; mean concentration 4.6 IU/L; range 3.3–9.6 IU/L) CVs ranged from 1.8–7.3% (mean 3.4%). For the mid control (n = 4; mean concentration 23.1 IU/L; range 22.1–24.5) CVs ranged from 1.6–4.2% (mean 3.0%). For the high control (n = 37; mean concentration 343 IU/L; range 320–465 IU/L) CVs ranged from 1.2–4.6% (mean 2.9%). For the dilution control (n = 4; mean concentration 15,306 IU/L; range 14,293–16,352 IU/L) CVs ranged from 2.9–4.9% (mean 4.1%).