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References


To the Editor:

Previously, we reported false-negative results generated by the OSOM human chorionic gonadotropin (hCG)1 Combo test (Genzyme Diagnostics)1 and certain lots of the hCG Cardinal Health Combo SP rapid test device (SP hCG rapid test; Cardinal Health)2 owing to increased concentrations of hCG β core fragment (hCGβcf). We refer to this phenomenon as the variant hook effect. In a hospital setting, these false negatives pose a substantial risk of adverse outcomes if pregnant patients are given treatments

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Point-of-Care and Over-the-counter Qualitative Human Chorionic Gonadotropin (hCG) Devices Remain Susceptible to False-Negative Results Caused by Excess hCG β Core Fragment

1 Nonstandard abbreviations: hCG, human chorionic gonadotropin; hCGβcf, hCG β core fragment; POC, point-of-care; OTC, over-the-counter.
that can affect the fetus. This observation was reported to the US Food and Drug Administration, but to our knowledge no action has been taken to change these devices. These false negatives also pose considerable risk to women at home, because a negative pregnancy test result may cause delays in prenatal care and continued engagement in behavior that can endanger the fetus. In view of the variable sensitivity of point-of-care (POC) and over-the-counter (OTC) devices to intact and variant forms of hCG as documented in the previously mentioned studies and others (3, 4), we assessed the susceptibility of OTC devices to hCG/βcf interference and compared the performance of the most susceptible OTC devices to the 2 POC devices listed above. This study had institutional review board approval.

We obtained a urine sample that reproducibly displayed a variant hook effect (i.e., weakly positive results when tested neat and strongly positive results when diluted 10-fold) from a patient without pathologically increased serum hCG concentrations. All 6 OTC devices tested (EPT; Insight Pharmaceuticals), Clearblue (Procter and Gamble), UpUp (Target), Accuclear (Procter and Gamble), First Response (Church and Dwight), and Walgreens (Walgreen) were susceptible to some degree of variant hook effect. All results were interpreted as positive, but of the 6 devices, the EPT and Clearblue devices displayed the greatest differences in test band intensity between neat and diluted hCG-positive samples and were selected for use in subsequent experiments.

Having identified the most susceptible OTC devices, we used a randomly chosen hCG-positive urine sample to dilute purified hCG/βcf purchased from the National Institute for Biological Standards and Controls (first WHO reference reagent, 2001 hCG/βcf, 99/708) to final concentrations of $5 \times 10^5$ and $1 \times 10^6$ pmol/L. Similar to our previous studies with POC devices (1, 2), a dose-dependent inhibition of positive signal was observed for both OTC devices (Fig. 1, patient A). Surprisingly, despite observable decreases in the positive signal when samples containing high concentrations of hCGβcf were tested, both OTC devices were less susceptible to hCGβcf interference than the POC devices. Indeed, both OTC devices generated clearly positive results even in the presence of $1 \times 10^6$ pmol/L hCGβcf, whereas the Cardinal Health Combo device generated a barely detectable positive signal and the OSOM device was completely inhibited with no detectable positive signal.

The susceptibility of the 2 POC and 2 OTC devices to the variant hook effect was confirmed by using all 4 devices to test a neat and 10-fold diluted second urine sample that clearly demonstrated the variant hook effect (Fig. 1, patient B). The patient was 12 weeks pregnant at the time of urine col-

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<td>Clearblue</td>
<td>EPT</td>
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<tr>
<td>0 pmol/L βcf</td>
<td>T C</td>
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<tr>
<td>$5 \times 10^5$ pmol/L βcf</td>
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<tr>
<td>$1 \times 10^6$ pmol/L βcf</td>
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<td>Variant hook neat</td>
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<td>Variant hook diluted 1:10</td>
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**Fig. 1. Demonstration of variant hook effect in OTC and POC devices.**

Clearblue (Procter and Gamble), EPT (Insight Pharmaceuticals), Cardinal Health Combo SP brand rapid test device (SP hCG rapid test; Cardinal Health) and OSOM hCG Combo Test (Genzyme Diagnostics) tested with: hCG-positive urine with various concentrations of purified hCGβcf (rows 1, 2, and 3) and patient urine (gestational age 12 weeks; serum hCG 166 659 IU/L) known to exhibit a variant hook effect neat and diluted 1:10 (rows 4 and 5). T, test line; C, control line.
Letters to the Editor

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The savings for sporadic RF quantification are most useful for low-throughput laboratories that run different low-volume tests on the same instrument. This situation is often encountered, especially in Europe, where centralized high-throughput mass spectrometry centers are the exception rather than the rule. As Olson et al. stated, their approach permitted the use of sporadic RF calibration in only 8 of 16 assay run days over a 2-month period, thus limiting the benefit.

We presently have >2 years of experience in clinical laboratory practice with LC-MS/MS quantification (Alliance HPLC 2795 separations module coupled to a Quattro Micro mass spectrometer, both from Waters Corporation) based on sporadic RF. Instead of the criteria used

1 Nonstandard abbreviations: RF, response factor; CR, calibration in each run; LS-MS/MS, liquid chromatography–tandem mass spectrometry; hRF, historical RF.

References


Historical Response Factor–Based Quantification for LC-MS/MS

To the Editor:

With great interest we read the article by Olson et al. (1), who thoroughly compared sporadic response factor (RF) calibration to calibration in each run (CR) for the therapeutic drug monitoring of nortriptyline with liquid chromatography–tandem mass spectrometry (LC-MS/MS). They found the results obtained for the 2 quantification methods to be analytically and clinically commensurate. These findings could lead to substantial reductions in costs, workload, and turnaround time.

Lection and her serum hCG concentration was 166 659 IU/L. When the neat urine sample was tested, a clear but weakly positive signal was generated by the Clearblue device, whereas the EPT and Cardinal Health Combo devices generated barely detectable positive signals. These weakly positive signals could easily have been interpreted as negative test results by personnel unfamiliar with the test devices. The OSOM device generated no positive signal and could have been interpreted only as a negative result. Importantly, when the urine sample was diluted 10-fold, strong positive signals were generated by all 4 devices, confirming their susceptibility to the variant hook effect when testing actual patient samples.

These studies demonstrate an inherent limitation of currently available qualitative POC and OTC hCG test devices. At our institution, documented susceptibility to the variant hook effect prompted a change in the manufacturer of urine qualitative hCG test devices. Our results indicate that no improvement has been made to the previously used POC device (OSOM) during the intervening 4 years and support the change in manufacturer. However, the currently used POC device (Cardinal Health Combo) also demonstrated susceptibility to the variant hook effect, highlighting the industry-wide need to address this issue. This need for improvement also applies to popular OTC devices, although surprisingly, the OTC devices we tested displayed equivalent or better performance than POC devices routinely used in a hospital setting.

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References


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