is needed to derive more robust estimates for both the 99th and 97.5th percentiles. We hope that the IFCC task force will revisit the requirements for both the number of healthy individuals needed to determine the 99th percentile and which statistical tests may be used to assess potential outliers. In the meantime, the present data represent the first attempt to characterize hs-cTnI in healthy children from ages 1 through 18 years and should be of importance to the pediatric community at large.

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Improved Performance of Point-of-Care and Over-the-Counter Qualitative Human Chorionic Gonadotropin Measurement Devices

To the Editor:

Several studies have demonstrated the susceptibility of qualitative human chorionic gonadotropin (hCG)[1] measurement devices to false-negative results caused by high concentrations of hCG β core fragment (hCGβcf) *(1–3)*. Although the prevalence of these false negative results is unknown, it is clear that increased urine hCGβcf concentrations can be observed during normal pregnancy. The use of such devices poses a risk to patients if treatment is inappropriately administered to pregnant women *(4)*.

In a recent study *(3)*, we evaluated the susceptibility of 11 point-of-care (POC) hCG devices to false-negative results due to hCGβcf. We reported that 9 of the devices were affected by hCGβcf. One of the 2 most affected devices was the Elite Plus by Cen-Med.

As a follow-up to that study, we tested 5 over-the-counter (OTC) devices (Accuclear 2 min Pregnancy Test, Lot# 3295937233, exp. 03/2016; Clearblue Plus Pregnancy Test, Lot# 3196937231, exp. 09/2015; EPT, Lot# 57477, exp. 10/2015; Equate Early Result Pregnancy Test, Lot# 65841, exp. 03/2016; First Response Early Result Lot# BU3183PA, exp. 06/2015). Intact hCG was obtained from Scripps Laboratories (C0714, lot 2436602, 11584 IU/vial). hCGβcf was purified as described previously *(3)*. hCG-negative urine was obtained from the BJH Chemistry Laboratory. Institutional review

[1] Nonstandard abbreviations: hCG, human chorionic gonadotropin; hCGβcf, hCG β core fragment; POC, point-of-care; OTC, over-the-counter.

board approval was obtained for this study. We observed a clearly detectable positive signal with a solution containing 500 pmol/L intact hCG and 5×10^5 pmol/L hCGβcf in all devices except the First Response Early Result (Fig. 1, A–E).

Recently, we were made aware of modifications to the Cen-Med Elite Plus One-Step Pregnancy Test POC device and the First Response Early Result OTC device, to minimize their susceptibility to false-negative results caused by hCGβcf. To assess the impact of these modifications, we screened both the original and modified Cen-Med devices (Original Cen-Med Lot# hCG2100115, exp. 09/2014; New Cen-Med Lot# F401004, exp. 12/2015) as well as the original and modified First Response Early Result devices (Original First Response Lot# BU3183PA, exp. 06/2015; New First Response Lot# DC40711, exp. 02/2016).

As illustrated in Fig. 1, E–H, both the original Cen-Med device and the original First Response device generated a strong positive signal with 500 pmol/L intact hCG but were completely inhibited with 500 pmol/L intact hCG and 5×10^5 pmol/L hCGβcf. With 5×10^4 pmol/L hCGβcf, the original devices generated barely detectable positive results (Cen-Med) or negative results (First Response). In contrast, the modified devices demonstrated clearly improved performance, as positive signal was observed with 500 pmol/L intact hCG and 5×10^5 pmol/L hCGβcf. In addition, both modified devices generated clearly positive signal with 5×10^4 pmol/L hCGβcf. Compared with the POC devices evaluated in our previous study, the modified Cen-Med device performs similarly to the other devices in the moderately susceptible group *(3)* and the modified First Response device performs similarly to the other OTC devices evaluated in the current study (Fig. 1).

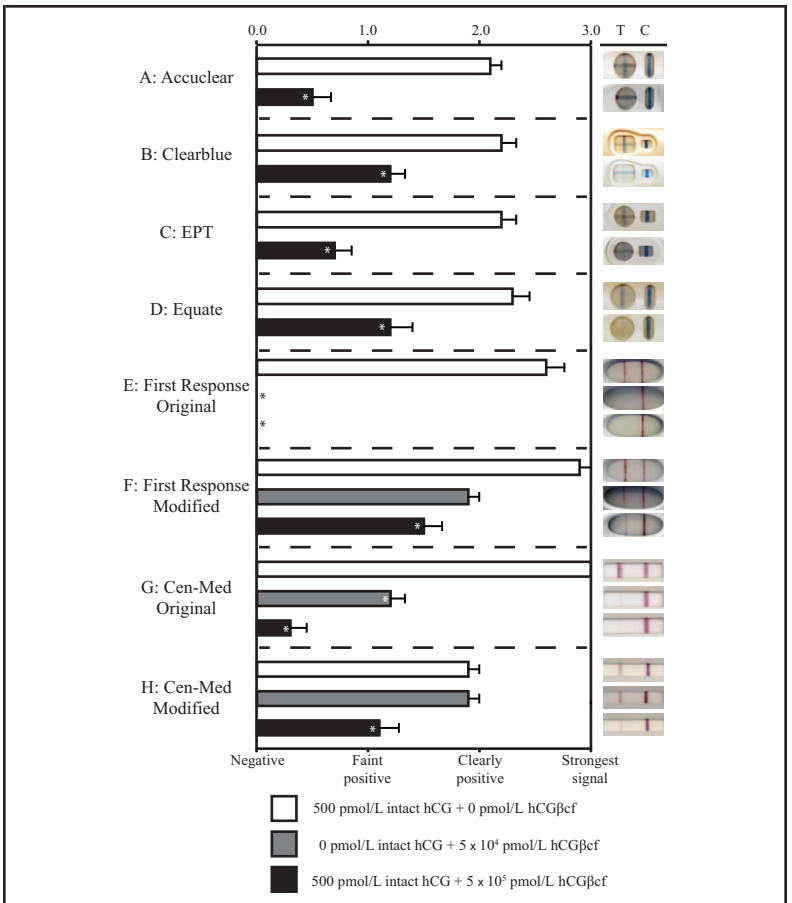

Fig. 1. Effect of hCGβcf on OTC and POC devices.

(A), Accuclear (Swiss Precision); (B), Clearblue (Swiss Precision); (C), EPT (Insight Pharmaceuticals); (D), Equate (Wal-Mart); and (E), First Response (Church & Dwight). Decreased susceptibility to hCGβcf interference in modified OTC and POC devices: original (E) and modified (F) First Response Early Result (Church & Dwight); original (G) and modified (H) Cen-Med Elite Plus (Cen-Med). Solutions contained 500 pmol/L (171 IU/L) intact hCG with 0 pmol/L hCGβcf (white bars) or 0 pmol/L intact hCG with 50 000 pmol/L hCGβcf (gray bars) or 500 pmol/L intact hCG with 500 000 pmol/L hCGβcf (black bars). Devices were tested in duplicate. Representative device images are included. Bars represent the mean result from 10 untrained readers ± SE. Statistically significant differences ($P < 0.05$) in device interpretation [(intact hCG + hCGβcf) vs intact hCG only; or hCGβcf vs intact hCG only] were calculated using Student paired *t*-test with a 2-tailed distribution and are indicated with an asterisk. T, test line; C, control line.

This study highlights the successful efforts of 2 manufacturers to improve the performance of their devices. We hypothesize that 3 approaches could be used to achieve improved device performance. In the first approach, different antibodies with greater recognition of hCGβcf could be used. In the second approach, a higher concentration of the original antibodies, if they recognize hCGβcf to some extent, could be added to the device. In the third approach, use of antibodies that recognize the intact hCG β subunit, but do not bind

hCGβcf, would eliminate negative interference due to hCGβcf and facilitate the recognition of intact hCG despite comparatively higher concentrations of hCGβcf.

Based on the 510(k) for the modified First Response device, it is clear that the antibodies were changed to recognize hCGβcf. This is supported by the fact that the original First Response device was unable to detect hCGβcf but the modified device generated positive signal when used to test both solutions containing hCGβcf. Based on the 510(k) and package insert for the Cen-Med device, the changes are unclear. The package insert for the modified Cen-Med device indicates that an anti-hCGα capture antibody is used with a gold particle–conjugated anti-hCGβ antibody. However, this antibody combination would allow for detection of intact hCG only and does not explain the ability of this device to recognize hCGβcf. The package insert also indicates that 8.53 pmol/L hCGβcf does not interfere with the performance of the modified Cen-Med device, but we demonstrate that the device gives a positive signal in the presence of 5×10^5 pmol/L hCGβcf. It is unclear why this statement is included in the package insert.

Clearly, improvement of qualitative hCG devices is possible, and we encourage other manufacturers with susceptible devices to modify their products.

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Evaluation of the CLINITEST® Human Chorionic Gonadotropin (hCG) Pregnancy Test for Susceptibility to the Hook Effect by the hCG β Core Fragment

To the Editor:

In a recent report in *Clinical Chemistry*, Nerenz et al. described a screening method to evaluate point-of-care human chorionic gonadotropin (hCG)[1] devices for susceptibility to the hook effect by the hCG β core fragment (hCGβcf) *(1)*. Among the 11 devices they evaluated was the CLINITEST® hCG pregnancy test. Nerenz et al. did not perform the testing for the CLINITEST hCG product themselves but opted to send screening samples to a colleague to perform the test on the CLINITEK® Status+ analyzer according to the manufacturer's instructions. The protocol required that the 3 screening samples be run in duplicate on a single instrument with 1 reagent lot. The results of this testing are shown in Table 1.

The interpretation of these results by Nerenz et al. was that the test detected intact hCG at 5×10^2 pmol/L, detected hCGβcf at 5×10^5 pmol/L, and gave a false-negative and a borderline result for the sample containing 5×10^2 pmol/L intact hCG + 5×10^5 pmol/L hCGβcf, indicating that this sample is at or near the threshold of a combined hCG and hCGβcf high-dose hook effect for this assay *(1)*.

We were very concerned with the indication that the CLINITEST hCG product may be moderately affected by hCGβcf or potentially provide false-negative results. Af-

[1] Nonstandard abbreviations: hCG, human chorionic gonadotropin; hCGβcf, hCG β core fragment.