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 x 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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References

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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 CLINITEST® 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 x 10^2 pmol/L, detected hCGβcf at 5 x 10^3 pmol/L, and gave a false-negative and a borderline result for the sample containing 5 x 10^2 pmol/L intact hCG + 5 x 10^3 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-
After we inspected the Materials and Methods section of the report by Nerenz et al. (1), it became clear that the same cartridges read instrumentally were shipped back to the authors and were read visually, as the authors show in Fig. 2, entitled “Hook effect due to hCGβcf in 11 POC devices.” The 2 issues of concern with this study as it relates to the CLINITEST hCG product are (a) the product does not have a visual claim and (b) the cartridges were visually read several hours after they were instrumentally read. On the basis of this information, we believe the data shown in Figs. 2 and 3 of the report, which indicate the extent to which our test detects intact hCG, hCGβcf, and the hCG/ hCGβcf hook effect, are invalid.

A cartridge read visually several hours after being tested on an instrument would compromise signal integrity/stability. The advantages of the instrument-read CLINITEST hCG assay are that it eliminates errors due to color blindness, visual acuity, and assay read time (i.e., strong positive ≥2 min and true negative = 5 min). It is well known that visual signal integrity/stability of lateral flow devices is compromised by read time (2) because strips can backflow, giving the appearance of positive results, or can dry out, changing the visual signal intensity.

Additionally, the hCGβcf concentration used in the high-dose hook challenge sample (5 × 10^5 pmol/L intact hCG + 5 × 10^5 pmol/L hCGβcf) is not supported by the literature cited by the authors (2–5). According to the data presented by McChesney et al. (3), hCGβcf represents a small portion of urine immunoreactive hCG in early pregnancy and does not become a major portion of immunoreactive hCG until week 5 (i.e., approximately 80% or 4 times the concentration of intact hCG). Stenman et al. (4) reported that the hCGβcf concentration has been shown to be similar to that of intact hCG in early pregnancy and is 8–10 times higher than the concentration of intact hCG during the remainder of a normal pregnancy. Kato and Braunstein (5) stated “The β-core fragment was a major form of immunoreactive hCG in urine throughout pregnancy and accounted for over 90% of immunoreactive hCG in urine from midpregnancy.” Again, it would appear that the concentration of intact hCG is about 8–10 times the concentration of intact hCG. Finally, one of the authors of the report that we address here published another report (2) that discussed 3 patients’ false-negative results; although the intact hCG and hCGβcf concentrations in the patients were high, the ratio of hCGβcf to intact hCG was between 2 and 4. This report goes on to show that once the ratio of hCGβcf to intact hCG exceeded 80, 2 of the tests evaluated gave false-negative results. On the basis of the existing data that the ratio of hCGβcf to intact hCG is between 4 and 10, it is difficult to understand why Nerenz et al. chose a ratio of 1000 for the high-dose hook challenge sample (1), because this value is not physiologically relevant.

As in vitro diagnostic medical device manufacturers, we take quality very seriously. In this particular case, the assertion of 4 false-negative results with the CLINITEST hCG assay was very concerning. We encourage our clinical colleagues to challenge the performance of our products. However, in this case we believe that the authors inappropriately evaluated the Clinitest hCG test (visual readings) and used a high-dose hook challenge sample (5 × 10^5 pmol/L intact hCG + 5 × 10^5 pmol/L hCGβcf), which is not physiologically relevant. The overall outcome of this flawed study protocol has cast doubt on the performance of several hCG tests exhibiting acceptable performance.

### Table 1. CLINITEST hCG performance screening method samples.

<table>
<thead>
<tr>
<th>Screening sample</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 × 10^2 pmol/L intact hCG + 0 pmol/L hCGβcf</td>
<td>Positive</td>
</tr>
<tr>
<td>5 × 10^2 pmol/L intact hCG + 0 pmol/L hCGβcf</td>
<td>Positive</td>
</tr>
<tr>
<td>5 × 10^2 pmol/L intact hCG + 5 × 10^2 pmol/L hCGβcf</td>
<td>Negative</td>
</tr>
<tr>
<td>5 × 10^2 pmol/L intact hCG + 5 × 10^2 pmol/L hCGβcf</td>
<td>Borderline</td>
</tr>
<tr>
<td>0 pmol/L intact hCG + 5 × 10^5 pmol/L hCGβcf</td>
<td>Positive</td>
</tr>
<tr>
<td>0 pmol/L intact hCG + 5 × 10^5 pmol/L hCGβcf</td>
<td>Positive</td>
</tr>
</tbody>
</table>

### References
1. Nerenz RD, Song H, Gronowski AM. Screening method to evaluate point-of-care human chorionic gonadotropin (hCG) devices for susceptibil-

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In Reply

We appreciate the opportunity to respond to the comments on our recent publication on a screening method to evaluate human chorionic gonadotropin (hCG)1 devices for susceptibility to the hook effect by hCG β core fragment (hCGβcf) (1).

The authors point out that the quantitative data shown in Figs. 2 and 3 of our paper, regarding the Clinitest hCG device, were obtained by visual reading and that (a) the Clinitest hCG product does not have a visual claim and (b) the cartridges were read 24 h after performing the test.

First, we are certainly not suggesting that clinical users read the Clinitest hCG test visually; clearly this is not advisable.

Second, when used according to the manufacturer’s instructions, the Clinitek status analyzer interpreted the devices that contained 500 pmol/L intact hCG and 50 000 pmol/L hCGβcf as negative and borderline (Fig. 2 legend). Therefore, our interpretation that the Clinitest hCG device demonstrates a hook effect due to hCGβcf is not due to misinterpretation caused by visual reading.

Third, we agree with the authors that the quantitative nature of Figs. 2 and 3 regarding the Clinitest hCG device should be interpreted with caution. However, we feel strongly that it is important for researchers to visually inspect qualitative devices that are interpreted digitally. Important trends in qualitative devices can be observed visually without the digital reader. Data by Milhorn and Korpi-Steiner also illustrate this nicely (2). In their study, they prepared samples that mimic hCG and hCGβcf concentrations found in a “representative normal patient” based on published means from a population of 37 pregnant patients. The Clinitest hCG device gave positive results for all samples corresponding to pregnancy days 16–49. Taken at face value, these results might be interpreted as showing that the Clinitest hCG device is not affected by hCGβcf. However, visual inspection of the devices showed that indeed the devices are inhibited by increasing concentrations of hCGβcf. It is clear that in patients who have different amounts of hCG and hCGβcf, the device could potentially give a negative result. In fact, the device demonstrates a hook effect due to hCGβcf alone (negative results at concentrations of 50 000 pmol/L hCGβcf). It should also be noted that the photo shown in Fig. 2 of our publication was taken immediately after the Clinitek status analyzer read the devices.

The authors also question why the concentrations of hCG and hCGβcf were chosen, as they may not represent a ratio normally seen in pregnancy. As we discuss in our article, the concentrations of hCG and hCGβcf chosen for our screening test were selected to minimize the amount of hCG and hCGβcf required for a screening test and were in no way meant to mimic hCG concentrations at any single point in pregnancy. Rather, it was meant to illustrate whether a device was in fact susceptible to the hook effect due to hCGβcf. However, Fig. 1, A and B, of our paper illustrates that concentrations of hCG and hCGβcf found at approximately 7 weeks of pregnancy (50 000 pmol/L intact hCG and 50 000 pmol/L hCGβcf) were tested on 2 devices, and even more dramatic hook effects were observed at these concentrations. Hence the concentrations chosen for our screening method are conservative and should actually underestimate, not overestimate, the hook effect due to hCGβcf. Finally, our work suggests that it is not the ratio of hCG to hCGβcf that is important for the hook effect, but rather the total amount of hCGβcf that has the most impact.

In summary, we agree that the quantitative values shown in