Analytical Performance of Home Pregnancy test that estimates time since ovulation based on hCG threshold concentration at week boundaries

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Abstract

Objective: A new urine pregnancy test is available in the USA, which consists of two immunoassay strips (one low and one high sensitivity), optical detection system and microprocessor which enables determination of pregnancy status and also estimates the number of weeks since ovulation based on hCG threshold levels. Results are displayed on an LCD as 1-2, 2-3 and 3+ weeks if a “Pregnant” result is returned. Studies have been conducted with the objective of investigating the analytical performance of this device.

Relevance: This is the first device available that estimates urinary hCG levels to time since ovulation. Therefore it is of clinical relevance to understand performance of the device with regard to accuracy, specificity, precision, batch variation and comparison to time since ovulation by a reference method.

Methodology: Quantitative measurement of hCG was conducted on all 300 urine samples by AutoDELFIA (Perkin Elmer) for comparative purposes. Laboratory testing of urine samples from pregnant (n=107) and non-pregnant volunteers (n=187) was conducted to determine accuracy of the pregnancy test (Clearblue™ Advanced Pregnancy Test with Weeks Estimator (CAPT)). Test specificity was investigated using samples from pre-, peri- and post-menopausal non-pregnant women (n=301). Precipitation was examined by testing 3 batches, across days and operators on 38 standards (0-10,807 mIU/ml) (n=50 per standard). Comparison to time since ovulation was accomplished by recruitment of women pre-conception and collection of urine samples to detect the luteinizing hormone (AutoDELFIA, with optical detection system and microprocessor which enables determination of pregnancy status and also estimates the number of weeks since ovulation based on hCG threshold levels). A minimum of 100 samples/day relative to expected period were required. A total of 2,703 devices from 9 batches were tested. Testing was randomised and blinded with a 0mIU/ml negative control included within the testing panel. The device was >99% accurate in detecting pregnancy and no test results were seen following testing of urine samples from non-pregnant and Post-menopausal pregnant women. Test specificity was 99% for day of expected period, 96% for day -1, 97% for day -2, 90% for day -3 and 65% for day -4. The precision study showed that the threshold for determining pregnancy was 15.0 mIU/ml, the 1-2-3 threshold was 15.0/15.0/mL, and the 2-3-3+ boundary was 275 mIU/mL. An ANOVA test indicated that there were no significant differences in precision and operator and day were very minor sources. In this study, agreement between weeks was determined and time since ovulation was 98%.

Conclusions: The analytical performance of this device demonstrates its ability to provide accurate pregnancy results (>99% accuracy) and provide a reasonable estimate of time since ovulation (90% agreement with LH surge reference). Due to the exponential increase in hCG during early pregnancy, the test must be capable of measuring the low and high boundary levels of hCG with precision. A single lateral flow immunoassay strip is not normally able to measure across a wide concentration range with precision, therefore, this test consists of two immunoassay strips (one low and one high sensitivity) to accommodate the required range. An optical detection system and microprocessor convert the assay signal into easy to understand results on an LCD screen. The internal architecture of this test is shown in figure 2.

Introduction

- Women who get a positive pregnancy test result wish to know quickly how far along they are.
- This knowledge allows a woman to plan and understand her pregnancy. Clinically important to access appropriate care.
- An improvement on LMP is long overdue.

- Often no better than a guess, up to 70% of women don’t know their LMP.
- Even if it’s known, assumes ovulation on day 14 – a recent study found that for half of all women, LMP prediction was 5 or more days incorrect (up to 57 days out in one case).

A new urine pregnancy test, Clearblue™ Advanced Pregnancy Test with Weeks Estimator (CAPT), is available in the USA, which as well as determining pregnancy status also estimates the number of weeks since ovulation. This analytical performance test was accomplished by recruitment of women pre-conception and collection of urine samples to detect the luteinizing hormone (AutoDELFIA, with optical detection system and microprocessor which enables determination of pregnancy status and also estimates the number of weeks since ovulation based on hCG threshold levels).}

Threshold definitions:

<table>
<thead>
<tr>
<th>Negative/Pregnant 1-2 threshold</th>
<th>Concentration of hCG at which 50% results are classified by the device as &quot;Not Pregnant&quot; and 50% of results are classified as &quot;Pregnant 1-2&quot;. This is equivalent to the device cut-off.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnant 1-2/Pregnant 2-3 threshold</td>
<td>Concentration of hCG at which 50% results are classified by the device as &quot;Pregnant 1-2&quot; and 50% of results are classified as &quot;Pregnant 2-3&quot;.</td>
</tr>
<tr>
<td>Pregnant 2-3/Pregnant 3+ threshold</td>
<td>Concentration of hCG at which 50% results are classified by the device as &quot;Pregnant 2-3&quot; and 50% of results are classified as &quot;Pregnant 3+&quot;.</td>
</tr>
</tbody>
</table>

Pregnancy Detection rate for early testing: The Pregnancy Detection rate relative to day of the expected period is shown in Table 2.

Pregnancy Detection rate for early testing: The results show agreement between Weeks Estimator results and time since ovulation 93% across all device categories, 95% CI; 91.5-94.4%. The agreement with individual device categories is shown in Table 3.

Table 1: Variance estimates

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<th>Source</th>
<th>Variability</th>
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<th>Variability</th>
<th>Variability</th>
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<tr>
<td>Day</td>
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<td>0.00000</td>
</tr>
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<td>Operator</td>
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<tr>
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</tr>
<tr>
<td>Total</td>
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<td>0.00044</td>
<td>0.00044</td>
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Conclusion:
The analytical characteristics of Clearblue™ Advanced Pregnancy Test with Weeks Estimator demonstrates that it has the necessary performance to provide accurate pregnancy results (>99% accurate) and provide a robust estimate of time since ovulation (93% agreement with LH surge reference).

Declarations of Interest:

For Men: There were no positive test results in any of the categories of weeks. All 25mIU/ml hCG standards tested (90 per batch) gave a “Pregnant” result and “Weeks Estimator” result of 1-2 weeks.

Conclusion:

Conclusions:
The mean concentration of hCG found were 0.033mIU/ml for pre-, 0.40mIU/ml for peri- and 0.50mIU/ml for the post-menopausal cohorts.

Precision Study: The precision study showed that the threshold for determining pregnancy was 10.2mIU/ml, the 1-2-3 threshold was 15.0mIU/ml, and the 2-3-3+ boundary was 275mIU/ml. Analysis of device results by hCG concentration for each batch, operator and day found very good precision around the device thresholds.

A mixed effects ANOVA model was used with standard as a fixed effect and batch, operator and day as random effects, with all two factor interactions also included as random effects. The transformation log(1+X) has been used. A log transformation is used on the ¾ values to ensure homogeneity of variance across the range of standards.

Using this mixed model approach, a components of variance analysis was performed to determine the percentage of the total sample variance attributable to each factor. The percentage of the total variation attributable to each factor is presented for the control, low and high assay sensitivity separately in table 1.

When all the variability is assigned to the different factors tested in this study, it can be seen that the majority (>94%) of the variability is device to device, such that batch <1% for batch alone and its interactions), day and operator have minimal contribution to variability. The log-difference of area samples to detect the luteinizing hormone (AutoDELFIA, with optical detection system and microprocessor which enables determination of pregnancy status and also estimates the number of weeks since ovulation based on hCG threshold levels).

References: