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 equates 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 clinical 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). Precision was examined by testing 3 batches, across days and operators on 38 standards (0-10807mIU/ml) (n=90 per standard). Comparison to time since ovulation was accomplished by recruitment of women pre-conception and collection of daily urine samples to detect the lutenizing hormone (AutoDELFIA, with ovulation defined as surge+1day). Urine sample collection continued through early pregnancy to enable laboratory comparison of device results to time since ovulation (n= 153 women). A similar sample collection protocol also enabled pregnancy detection rate to be calculated with respect to day of the expected period (n=135 pregnancy cycles).

Validation: The device was >99% accurate in detecting pregnancy and no "Pregnant" results were seen following testing of urine samples from non-pregnant Pre-, Peri- and Post-menopausal women. Pregnancy detection rate was 99% for day of expected period, 98% 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 10.2mIU/ml, the 1-2/2-3 boundary was 153mIU/ml, and the 2-3/3+ boundary was 2750mIU/ml. An ANOVA mixed effects model found batch was a minor source of variance and operator and day were very minor sources. In this study, agreement between Weeks Estimator results and time since ovulation was 93%.

Conclusions: The analytical performance of this device demonstrates 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).

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 ensure 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 known, assumes ovulation on day 14 – a recent study found that **for half of women**, LMP provided a Gestational Age (GA) that was **5 or more days incorrect** (up to 57 days out in one case)[1]
- 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 enables a woman to receive an estimation of time since ovulation at the moment of receiving her positive pregnancy result.

Test Background

The new test is a conventional digital pregnancy test that also estimates the number of weeks since ovulation. Results are displayed on an LCD as 1-2, 2-3 and 3+ weeks categories if a "Pregnant" result is returned. The basis of the weeks categorisation are the threshold level of urinary hCG that relates to the boundaries between weeks, as shown in figure 1 below.

Figure 1: Plot of Daily Rise in Urinary hCG in Early Pregnancy

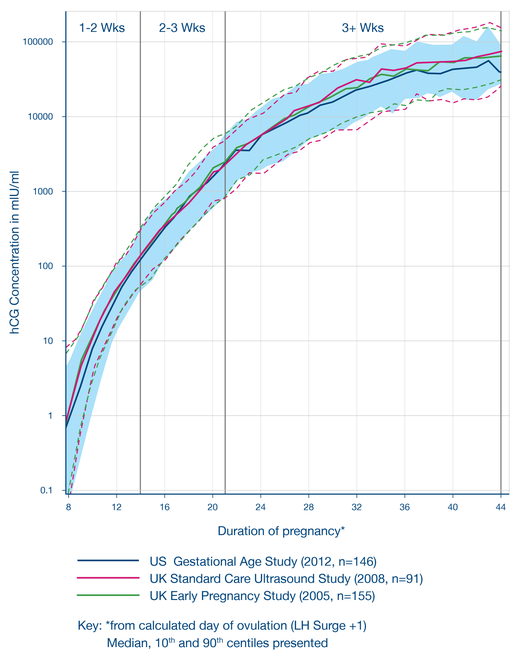

Figure 1: Median and 10th-90th centile levels of urinary hCG from 3 independent studies, with weeks categories overlain to show the derivation of thresholds relating to weeks boundaries.

Due to the exponential increase in hCG in 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.

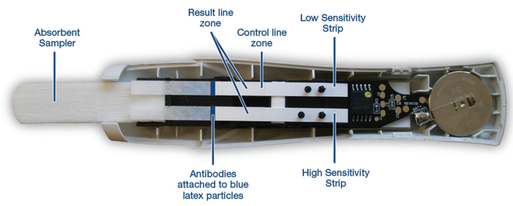

Figure 2: Internal architecture of the Clearblue Advanced Pregnancy Test with Weeks Estimator

The objective of these studies was to evaluate the analytical performance of this device.

Methods:

Quantitative measurement of hCG was conducted on all clinical samples by AutoDELFIA (Perkin Elmer) for comparative purposes. This reference assay has previously been shown to be suitable for measurement of urinary hCG[2]. All clinical samples used had been stored at -80°C prior to use (sample stability on storage and freeze/thaw had been validated under these conditions across the entire assay range). The following studies were conducted.

Accuracy Study: A total of 300 urine samples were sourced from external clinics and from internal clinical studies involving women who were attempting to conceive. Samples were collected at any time of day from women aged 18 to 45 who were seeking a pregnancy test, of which 107 were found to be from pregnant volunteers. Laboratory testing was conducted using 3 batches of Clearblue™ Advanced Pregnancy Test with Weeks Estimator (CAPT) and 1 batch of Clearblue™ Digital Pregnancy Test as comparator. Results were read and reported by 2 technicians. Comparison was also made to hCG concentration.

Specificity Study: The specificity of CAPT with urine samples from non-pregnant peri- and post-menopausal women, compared to a control group of non-pregnant women of reproductive age was examined, as elevated hCG in peri- and post- menopausal women has been reported to result in false positive results with some pregnancy tests[3]. Urine samples were sourced from the following groups:

Non-pregnant women aged 18-40 years (n=100 subjects, pre-menopausal cohort)

Non-pregnant women aged 41-55 years (n=101 subjects, peri-menopausal cohort)

Non-pregnant women aged >55 years (n=100 subjects, post-menopausal cohort)

Nine batches of CAPT were used in this study and each sample was tested on 3 of the batches. A 25mIU/ml hCG standard was randomised in the testing.

Precision Study: Precision was examined by testing 3 batches, across days and operators on 38 standards (0-10807mIU/ml) (n=90 per standard). Precision was examined both according to device result, and device Percent Attenuation (%A) i.e. the amount of light absorbed on passage through a lateral flow device.

This study also aimed to demonstrate the threshold concentration of hCG between each category.

Threshold definitions:

Negative/Pregnant 1-2 Threshold: Concentration of hCG at which 50% results are classified by the device as "Not Pregnant" and 50% of results are classified as Pregnant 1-2. This is equivalent to the device cut-off.

Pregnant 1-2/Pregnant 2-3 Threshold: Concentration of hCG at which 50% results are classified by the device as "Pregnant 1-2" and 50% of results are classified as "Pregnant 2-3"

Pregnant 2-3/Pregnant 3+ Threshold: Concentration of hCG at which 50% results are classified by the device as "Pregnant 2-3" and 50% of results are classified as "Pregnant 3+"

Pregnancy Detection rate for early testing: Pregnancy detection rate with respect to day of the expected period (LH surge + 15 days) was determined by collection of daily urine samples from women preconception in order to have accurately characterised early pregnancy samples. A minimum of 100 samples/day relative to expected period were required. A total of 2703 devices from 9 batches were tested. Testing was randomised and blinded with a 0mIU/ml negative control included within the randomisation schedule.

Agreement of "Weeks" results to time since ovulation: Comparison to time since ovulation was accomplished by recruitment of women pre-conception, with collection of daily urine samples to detect the lutenizing hormone (AutoDELFIA, with ovulation defined as surge+1day). Urine sample collection continued through early pregnancy to enable laboratory comparison of device results to time since ovulation (n=153 women). Testing was conducted using 3 batches (3654 CAPT devices).

Results

Accuracy Study: The overall agreement of CAPT compared to the comparator device results was calculated to be 99.5% (95% CI: 98.9 – 99.9%). The overall agreement of CAPT when compared to quantitative detection of hCG concentration was 99.4% (95% CI: 98.7 – 99.8%).

Specificity Study: There were no positive test results in any of the cohorts tested within any of the batches. All 25mIU/ml hCG standards tested (90 per batch) gave a 'Pregnant' result and "Weeks Estimator" result of 1-2 weeks.

The mean concentration of hCG found were 0.03mIU/ml for pre-, 0.40mIU/ml for peri-, and 0.91mIU/ml for the post-menopausal cohorts.

Precision Study: The precision study showed that the threshold for determining pregnancy was 10.2mIU/ml, the 1-2/2-3 boundary was 153mIU/ml, and the 2-3/3+ boundary was 2750mIU/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(%A+10) has been used. A log transformation is used on the %A 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 variance attributable to each factor. The percentage of the total variation attributable to each factor is presented for the control, low assay and high assay seperately in table 1.

When all the variability is assigned to the different factors tested in this study, it can be seen that the majority (>84%) of the variability is device to device, such that batch (<11% for batch alone and its interactions), day and operator have minimal contribution to variability. This result would be expected of a device that can provide consistent results between users, occasions and batches.

Table 1: Variance estimates

Source	Control		High		Low	
	Variability	% of Total	Variability	% of Total	Variability	% of Total
Batch	0.00023	10.9	0.00020	8.3	0.00032	7.7
Operator	0.00001	0.4	0.00000	0.1	0.00015	3.6
Day	0.00001	0.2	0.00000	0.0	0.00000	0.1
Operator/Batch	0.00001	0.2	0.00000	0.1	0.00003	0.8
Day*Batch	0.00001	0.3	0.00001	0.3	0.00008	1.9
Day*Operator	0.00000	0.1	0.00001	0.5	0.00008	1.9
Standard*Batch	0.00001	0.2	0.00004	1.6	0.00000	0.0
Standard*Operator	0.00000	0.0	0.00002	1.0	0.00000	0.0
Day*Standard	0.00000	0.0	0.00001	0.6	0.00000	0.0
Device	0.00186	87.7	0.00211	87.6	0.00347	84.0
TOTAL	*0.00212*	*100.0*	*0.00241*	*100.0*	*0.00414*	*100.0*
CV(%)	4.7	-	5.0	-	6.6	-

CV (%) from back transform: (exp(sqrt(TOTAL)-1))*100

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

Table 2: Pregnancy detection rate of CAPT on days before expected period

Day in Cycle Relative to Expected Period	Number of CAPT Positive Results	Number of CAPT Negative Results	Total number of Pregnant samples tested	Estimated pregnancy detection rate (%)	95% confidence interval	
					Lower	Upper
-4 days	228	120	348	65.52	60.26	70.50
-3 days	318	33	351	90.60	87.05	93.44
-2 days	323	7	330	97.88	95.68	99.14
-1 day	323	4	327	98.78	96.90	99.67
0 (day of expected period)	297	3	300	99.00	97.11	99.79

All negative controls gave a "Not Pregnant" result.

Agreement of "Weeks" results to time since ovulation: Agreement between Weeks Estimator results and time since ovulation was 93% across all device categories, 95% CI; 91.5-94.4%. The agreement with individual device categories is shown in table 3.

Device result	Agreement (%)	95% CI for level of agreement
1-2 Weeks	96.9	(93.8, 99.6)
2-3 Weeks	84.6	(80.6, 88.3)
3+ Weeks	97.4	(96.4, 98.3)

Agreement with ultrasound derived GA has previously been reported as 97%[4].

Conclusions

The analytical characteristics of Clearblue™ Advanced Pregnancy Test with Weeks Estimator demonstrates 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).

References:

1: Johnson *et al* (2011) Curr Med Res Opin 27:393-401
2: Johnson *et al* (2011) Clin Chem 57:A188;E45
3: Snyder *et al*. (2005) Clin. Chem. 51, 1830-1835
4: Johnson *et al* (2013) Human Reproduction 28:i5;O-012. Agreement for all device results with Crown Rump Length 10-13+6 weeks GA was 97%, agreement by weeks category varied from 45-99% according to whether ultrasound bias and variability was accommodated.

Declaration of Interest:

These studies were funded by SPD Development Company Ltd., a fully owned subsidiary of SPD Swiss Precision Diagnostics GmbH, the manufacturers of Clearblue™ products. S Johnson and P Perry are all employees of SPD Development Company Ltd. T Alonzo and M Zinaman received consultancy from SPD Development Company for their involvement with this research.