Clin Chem Lab Med 2011;49(8):xxx-xxx © 2011 by Walter de Gruyter • Berlin • New York. DOI 10.1515/CCLM.2011.211

The untility of six over-the-counter (home) pregnancy tests

Laurence A. Cole*

Department of Obstetrics and Gynecology, USA hCG Reference Service, University of New Mexico, Albuquerque, NM, USA

Abstract

Background: The home pregnancy market is rapidly evolving. It has moved from detection of pregnancy on the day of missed menstrual bleeding, to detection claims 4 days prior. It is moving from all manual tests to digital tests, with a monitor reading the bands and informing women they are pregnant. A thorough study is needed to investigate the validity of claims and evolving usefulness of devices.

Methods: Studies were proposed to examine the sensitivity and specificity of home tests and their abilities to detect pregnancy. Methods examined the abilities of tests to detect human chorionic gonadotropin (hCG), hyperglycosylated hCG, free β-subunit, a mixture of these antigens in 40 individual early pregnancy urines and 80 individual early pregnancy urines.

Results: Using a mixture of hCG, hyperglycosylated hCG and free β-subunit typical for early pregnancy, the sensitivity of the First Response manual and digital tests was 5.5 mIU/mL, while the sensitivities of the EPT and ClearBlue brand manual and digital tests was 22 mIU/mL. On further evaluation, the First Response manual and digital tests both detected 97% of 120 pregnancies on the day of missed menstrual bleeding. The EPT manual and digital devices detected 54% and 67% of pregnancies, respectively, and the ClearBlue manual and digital devices detected 64% and 54% of pregnancies, respectively.

Conclusions: First Response manual and digital claim >99% detection on the day of missed menses. The results here suggest similar sensitivity for these two tests. The EPT and ClearBlue manual and digital test make similar >99% claims, the data presented here disputes their elevated claim.

Q1: Author: The sentence '40 individual early pregnancy urines .... urines' makes no sense. Please clarify

Introduction

The first home pregnancy test was EPT (1). Since then, devices have undergone many changes, including the adaption of an immunometric assay format (2). Currently, manual tests are interpreted as a faint test line present on a device showing pregnancy. Digital devices, in contrast, display ''yes'' or ''no'' or ''pregnant'' or ''not pregnant'' on an LCD screen.

This article examines the advantages and disadvantage of different home pregnancy tests available in the USA and Western World today, and their utility in detecting pregnancy. This article compares digital and manually read home pregnancy tests. It also examines the claim that devices can detect pregnancies 4 days prior to missed menstrual bleeding. This article focuses on the manual and digital devices First Response, EPT and ClearBlue Easy, the principal devices used today (3). An extensive study is described describing the sensitivity of devices for detection of hCG and specificity for detecting human chorionic gonadotropin (hCG), hyperglycosylated hCG and the free β-subunit. The abilities of over-the-counter devices to detect pregnancy in 120 urines in the days leading up to and following the time of missed menstrual bleeding are carefully assessed.

Q2: Reference 3 and 4 reordered in text and references. Please confirm

Materials and methods

Collection of urine by the USA hCG Reference Service was conducted according to a protocol approved by the Human Research Review Committee at the University of New Mexico (Protocol 04-132). All results from this study were accumulated and analyzed in an Microsoft Excel 2007 spreadsheet (Microsoft, Richmond, WA, USA).

Calibration units

The Siemens Immulite assay has been shown to equally detect hCG, hyperglycosylated hCG, hCG free β-subunit, and nicked hCG (3, 4), and to function equally well in analyzing serum and urine samples (5, 6). From multiple studies performed using this test calibrated against WHO 4th IS, 1 ng/mL of this standard has always read as 11 mIU/mL, 2 ng/mL as 22 mIU/mL, 5 ng/mL as 55 mIU/mL, and 10 ng/mL as 110 mIU/mL. Similarly, using this automated assay, 1 ng/mL of our hyperglycosylated hCG standard C5 has always read as 11 mIU/mL, 2 ng/mL as 22 mIU/mL, 5 ng/mL as 55 mIU/mL, and 10 ng/mL as 110 mIU/mL. As such, we consider 1 ng/mL of hCG or hyperglycosylated hCG to be the equivalent of 11 mIU/mL of hCG (3–6). Similarly, hCG β-subunit standard (WHO 1st RR), 1 ng/mL has always yielded 18 mIU/mL in the Siemens Immulite test, and 2 ng/mL as 36 mIU/mL, 5 ng/mL as 90 mIU/mL, and 10 ng/mL as 180 mIU/mL. Thus, we consider 1 ng/mL of hCG β-subunit to be the equivalent of 18 mIU/mL. On a molar basis, considering the molecular weights of β-subunit and hCG, the β-subunit value should be 36,700÷22,200 or 1.65× greater than the hCG value, 1.65×11=18. This shows that these conversion factors, 11× and 18× are molar equivalents (5, 6).

*Corresponding author: Laurence A. Cole, Department of Obstetrics and Gynecology, USA hCG Reference Service, MSC10-5580, University of New Mexico, Albuquerque, NM 87131, USA
Phone: +1-505-272-6137,
E-mail: lac@unm.edu, larry@hcglab.com
Received October 28, 2010; accepted March 13, 2011

Study 1, standard hCG

In Study 1, standard hCG (WHO 1st RR, WHO IRR 99/688) was reconstituted in phosphate buffered saline 0.1% bovine serum albumin at a concentration of 100 ng/mL. This was added to a pool of normal female urine (pooled from six non-pregnant females). hCG standard was added to urine at concentrations of 5.0 ng/mL (55 mIU/mL), 3.5 ng/mL (38 mIU/mL), 2 ng/mL (22 mIU/mL), 1.0 ng/mL (11 mIU/mL), 0.5 ng/mL (5.5 mIU/mL) and 0.3 ng/mL (3.3 mIU/mL). Six devices of each of six brands of test were evaluated with each concentration of urine. The lowest concentration at which all devices were positive was considered the analytical sensitivity. This study and all five other studies listed below were performed blinded in that urine samples were randomly coded. Two senior laboratory technologists and one nurse read the over-the-counter devices in this study and the five other studies listed below.

Study 2, hyperglycosylated hCG

In Study 2, standard hyperglycosylated hCG (C5 standard) (7) was reconstituted in phosphate buffered saline 0.1% bovine serum albumin at a concentration of 100 ng/mL. This was added to the same pool of normal female urine. The concentrations were 5.0 ng/mL (55 mIU/mL), 3.5 ng/mL (38 mIU/mL), 2 ng/mL (22 mIU/mL), 1.0 ng/mL (11 mIU/mL), 0.5 ng/mL (5.5 mIU/mL) and 0.3 ng/mL (3.3 mIU/mL). Six devices of each of six brands of test were evaluated with each concentration of urine. The lowest concentration at which all devices were positive was considered as the analytical sensitivity.

Study 3, hCG β-subunit

In Study 3, standard hCG free β-subunit (WHO 1st RR, WHO IRR 99/650) was reconstituted in phosphate buffered saline 0.1% bovine serum albumin at a concentration of 100 ng/mL. This was added to the same pool of normal female urine. The concentrations were 5.0 ng/mL (90 mIU/mL), 3.5 ng/mL (63 mIU/mL), 2 ng/mL (36 mIU/mL), 1.0 ng/mL (18 mIU/mL), 0.5 ng/mL (9 mIU/mL) and 0.3 ng/mL (5.4 mIU/mL). The different dilutions of urine were tested with six devices using each of six brands of test. The lowest concentration at which all devices were positive was considered as the analytical sensitivity.

Study 4, mixture of hCG forms

In early pregnancy, urine total hCG is comprised primarily of hyperglycosylated hCG (3, 5). A mixture was prepared of hCG and hyperglycosylated hCG to mimic early pregnancy hCG. In Study 4, funded by the USA hCG Reference Service, hCG, hyperglycosylated hCG and free β-subunit were mixed in a ratio of 40%:40%:20% (by weight) in urine, generating a mixture consistant with the day of the missed menstruation (3–5). Normal female urines were prepared containing an equivalent of 5.0 ng/mL (55 mIU/mL), 3.5 ng/mL (38 mIU/mL), 2 ng/mL (22 mIU/mL), 1.0 ng/mL (11 mIU/mL) and 0.5 ng/mL (5.5 mIU/mL). The different dilutions in urine were each tested with six devices of each of the six brands of test. The lowest concentration at which all devices were positive was considered the analytical sensitivity.

Study population

In a clinical trial, 215 women eager to achieve pregnancy volunteered to collect daily urine samples through the length of their menstrual cycle. All collections were collected at random times of the day. Daily urines were collected in up to five menstrual cycles until pregnancy was achieved. When pregnancy was achieved, collection was then continued until 6 weeks of pregnancy (weeks since last menstrual period). This clinical trial lasted 4 years. Each volunteer was supplied with a home ovulation kit to aid in achieving pregnancy. A total of 137 of the 215 women achieved pregnancy within five menstrual cycles.

In each menstrual cycle, the luteinizing hormone (LH) peak was detected in the urine samples (urine samples days 10–20 of each cycles tested) using the Immulite 1000 LH test. The day that menstrual bleeding started was also carefully recorded. In each case, 3–5 menstrual cycles passed before pregnancy was achieved. The timing of LH peak to start of menstrual bleeding, and menstrual bleeding to menstrual bleeding was recorded. All urines were frozen after collection, and maintained frozen until testing. Urines were brought to 37°C in a 37°C water bath prior to testing with the over-the-counter devices.

All urines were tested daily for hCG using the Siemens 1000 total hCG test, and tested for free β-subunit using our FBT11/anti-β peroxidase microtiter plate assay. Hyperglycosylated hCG was measured using our B152/anti-β peroxidase specific microtiter plate assays, as described previously (5, 8).

Study 5 and 6, evaluation of pregnant women

Forty of the 137 women (randomly selected, all had clinical pregnancies) provided urine for Study 5. In Study 5, 480 of the over-the-counter devices were purchased by the USA hCG Reference Service from local pharmacies. No external company interest or external finances or financial interests were involved. Over-the-counter devices were tested in singlicate with each individual's daily urine sample on the day of the missed menstrual period, and at 3 days after the day of the missed menstrual period. The day of the missed menstrual period was calculated from the average length of the 3–5 prior menstrual cycles before achieving pregnancy.

In a further study, Study 6, eighty separate volunteers (no overlap) from the 137 participants with clinical pregnancies in the clinical trial (randomly selected) provided daily urine during pregnancy. In this study, 80 volunteer's urines were tested daily with all six over-the-counter devices from 6 days prior to missing the menstrual period up to 4 days after missing the menstrual period, or over 11 days. The day of the missed menstrual period was calculated from the average length of the 3–5 prior menstrual cycles before achieving pregnancy. For this study, 80 of each of the six devices was tested on 11 days, a total of 5280 over-the-counter devices were purchased by Church and Dwight Inc. They were supplied with the understanding that the study would be performed blinded (all urines coded), and that they would have no say in the data, could not change the data, nor change the content of the manuscript.

Results and discussion

Two hundred and fifteen women eager to achieve pregnancy were monitored daily over six menstrual cycles. The average length of the menstrual cycle was determined. One hundred and thirty-seven of these volunteers achieved pregnancy, the remainder either dropped out of the program or never became clinically pregnant. We measured total hCG (Siemens Immulite 1000 assay) daily in urine samples until pregnancy was detected (defined as three consecutive days of rising hCG results), or detected pregnancy prospectively, then for three further weeks after detecting pregnancy (to

approximately 6 weeks of gestation as measured from the date of last menses). A total of 136 of 137 of these women achieved pregnancy (first day to detect >1 mIU/mL total hCG) prior to the expected day of missing menstrual bleeding (day determined from average menstrual cycle length) or at 28.6±3.8 (standard deviation) days. One hundred and thirty-five women had achieved pregnancy one day prior to missing their menstrual cycle, 126 two days prior, 121 three days prior, 104 four days prior, 55 five days prior, 40 six days prior, and 32 seven days prior to missed menstrual bleeding.

Median urine hCG was calculated for 3 weeks (3 weeks 0 days to 3 weeks 6 days), 4 weeks, 5 weeks and 6 weeks of pregnancy. As shown in Table 1, hCG concentrations rise weekly in an exponential manner, 2.2, 21, 175 and 2075 ng/mL, respectively, or the equivalent of 24, 231, 1925 and 22,803 mIU/mL. The proportion of hyperglycosylated hCG as a percentage of total hCG declines accordingly with advancing weeks, 88%±90% (mean±standard deviation), 75%±53%, 51%±48%, and 23%±20%, respectively. The proportion of free β-subunit also declined with advancing weeks, 9.0%±12.7%, 6.1%±6.3%, 5.4%±3.0%, and 4.1%±3.4%. Clearly, hyperglycosylated hCG is the principal hCG component produced in the first 3 weeks of gestation (3 weeks–5 weeks of gestation).

Clearly, based on these results (Table 1), most over-the-counter pregnancy tests should be designed to detect hCG in the 3rd week (week since last menses) of pregnancy, and have a sensitivity of at least 2.2 ng/mL or 24 mIU/mL (median total hCG at 3 weeks pregnancy). Considering the 88% hyperglycosylated hCG, tests should equally or better detect hyperglycosylated hCG than regular hCG, and considering the 9.0% free β-subunit should ideally also detect free β-subunit.

First Response manual and Gold digital over-the-counter products are produced by Church and Dwight Inc. ClearBlue manual and digital products are made and marketed by Inverness Medical Innovation. The EPT manual and EPT Certainty digital are marketed by Pfizer Consumer Healthcare. For Studies 1–5, funded entirely by the USA hCG Reference Service, 1272 over-the-counter devices were purchased at 12 pharmacies in the Albuquerque area as detailed under Methods. For Study 6, a total of 5280 products, were purchased by Church and Dwight with an understanding that the project would be performed in blinded fashion, and that they had no say in the study, study results or the study design.

Study 1 (Table 2) was designed to determine the sensitivity of devices for detecting pure hCG. The concentration at which a device yielded six of six positive results was considered the sensitivity of the device (Table 2). We found the First Response manual to have a sensitivity of <3.3 mIU/mL (no lower concentration tested), EPT manual of 5.5–11 mIU/mL and ClearBlue Easy manual of 5.5–11 mIU/mL. For the digital devices, similar sensitivities were observed. The First Response Gold digital was 3.3–5.5 mIU/mL, the EPT Certainty digital was 5.5–11 mIU/mL and the ClearBlue Easy digital was 5.5–11 mIU/mL.

In Study 2 (Table 2), we considered how well the over-the-counter tests detected hyperglycosylated hCG, the principal form of hCG in urine in early pregnancy (Table 1). In Study 2, we repeated Study 1 using hyperglycosylated hCG standard (C5). As shown in Table 2, the First Response manual and digital were found to have similar sensitivities for hCG and hyperglycosylated hCG. As reported previously, EPT and ClearBlue tests detected hyperglycosylated hCG with low sensitivity, one quarter of that of hCG (5, 8). This problem seems to have been partly resolved by Inverness Medical Innovations and Pfizer Consumer Healthcare, UK. The EPT manual now has similar sensitivity for hCG and hyperglycosylated hCG, EPT Certainty digital has a 11–22 mIU/mL sensitivity for hyperglycosylated hCG, ClearBlue Easy manual now has similar sensitivity for hCG and hyperglycosylated hCG, and ClearBlue Easy digital has a 11–22 mIU/mL sensitivity for hyperglycosylated hCG.

In Study 3 (Table 2), we examined the sensitivity of tests for detecting free β-subunit, a third major component of urine in early pregnancy, accounting for as much as 9.0%±12.7% of total hCG (Table 1). This was again a repeat of Study 1, using free β-subunit instead of hCG standards. We found that the First Response and First Response digital detected free β-subunit, while EPT, EPT Certainty digital, ClearBlue Easy manual and ClearBlue Easy digital did not detect free β-subunit. A recently published report by Cervinski et al. (9) indicates that these tests do detect free β-subunit. Clearly, from the data in Table 2, if they do detect free β-subunit, it is detected with very low affinity.

Table 1 Detection of hCG variants in urine from pregnant females. Total hCG was measured using the Siemens Immulite hCG test (detects hCG, hyperglycosylated hCG and free β-subunit on an equimolar basis), hyperglycosylated hCG and free β-subunit were measured using antibodies B152 and FBT11 in microtiter plate specific immunometric assays. The proportion of free β-subunit and hyperglycosylated hCG were calculated as the proportion of total hCG in urine samples. SD is standard deviation. Evaluation of 137 women on 7 days means that 959 urine samples were tested.

Gestation age (weeks since last menses)	Evaluation	Median total hCG, ng/mL (mIU/mL)	Hyperglycosylated hCG (% mol/mol) mean±SD, %	Free β-subunit (% mol/mol) mean±SD, %
3 weeks 0 days–3 weeks 6 days	136 women on 7 days	2.2 (24)	88±90	9.0±12.7
4 weeks 0 days–4 weeks 6 days	137 women on 7 days	21 (231)	75±53	6.1±6.3
5 weeks 0 days–5 weeks 6 days	137 women on 7 days	175 (1925)	51±48	5.4±3.0
6 weeks 0 days–6 weeks 6 days	137 women on 7 days	2073 (22,803)	23±20	4.1±3.4

Table 2 Evaluation of over-the-counter tests with synthetic non-pregnant urine mixtures containing pure hCG, pure free β-subunit and pure hyperglycosylated hCG standards.

Concentration	First Response manual	First Response Gold digital	EPT manual	EPT Certainty digital	ClearBlue Easy manual	ClearBlue Easy digital
Study 1. Non-pregnant female urine containing hCG dimer 1st RR standard						
5.0 ng/mL or molar equivalent of 55 mIU/mL	6 of 6	6 of 6	6 of 6	6 of 6	6 of 6	6 of 6
3.5 ng/mL or molar equivalent of 38 mIU/mL	6 of 6	6 of 6	6 of 6	6 of 6	6 of 6	6 of 6
2.0 ng/mL or molar equivalent of 22 mIU/mL	6 of 6	6 of 6	6 of 6	6 of 6	6 of 6	6 of 6
1.0 ng/mL or molar equivalent of 11 mIU/mL	6 of 6	6 of 6	6 of 6	6 of 6	6 of 6	6 of 6
0.5 ng/mL or molar equivalent of 5.5 mIU/mL	6 of 6	6 of 6	2 of 6	2 of 6	3 of 6	2 of 6
0.3 ng/mL or molar equivalent of 3.3 mIU/mL	6 of 6	4 of 6	0 of 6	0 of 6	0 of 6	0 of 6
Sensitivity	3.3	5.5	11	11	11	11
Study 2. Non-pregnant female urine containing C5 hyperglycosylated hCG standard						
5.0 ng/mL or molar equivalent of 55 mIU/mL	6 of 6	6 of 6	6 of 6	6 of 6	6 of 6	6 of 6
3.5 ng/mL or molar equivalent of 38 mIU/mL	6 of 6	6 of 6	6 of 6	6 of 6	6 of 6	6 of 6
2.0 ng/mL or molar equivalent of 22 mIU/mL	6 of 6	6 of 6	6 of 6	6 of 6	6 of 6	6 of 6
1.0 ng/mL or molar equivalent of 11 mIU/mL	6 of 6	6 of 6	6 of 6	2 of 6	6 of 6	4 of 6
0.5 ng/mL or molar equivalent of 5.5 mIU/mL	6 of 6	6 of 6	0 of 6	0 of 6	0 of 6	0 of 6
0.3 ng/mL or molar equivalent of 3.3 mIU/mL	6 of 6	2 of 6	0 of 6	0 of 6	0 of 6	0 of 6
Sensitivity	3.3	5.5	11	22	11	22
Study 3. Non-pregnant female urine containing free β-subunit 1st RR standard						
5.0 ng/mL or molar equivalent of 90 mIU/mL	6 of 6	6 of 6	0 of 6	0 of 6	0 of 6	0 of 6
3.5 ng/mL or molar equivalent of 63 mIU/mL	6 of 6	6 of 6	0 of 6	0 of 6	0 of 6	0 of 6
2.0 ng/mL or molar equivalent of 36 mIU/mL	6 of 6	6 of 6	0 of 6	0 of 6	0 of 6	0 of 6
1.0 ng/mL or molar equivalent of 18 mIU/mL	6 of 6	6 of 6	0 of 6	0 of 6	0 of 6	0 of 6
0.5 ng/mL or molar equivalent of 9 mIU/mL	6 of 6	6 of 6	0 of 6	0 of 6	0 of 6	0 of 6
Sensitivity	9.0	9.0	>90	>90	>90	>90
Study 4. Non-pregnant female urine containing 40% hCG, 40% hyperglycosylated hCG, 20% free β-subunit combination						
5.0 ng/mL or molar equivalent of 55 mIU/mL	6 of 6	6 of 6	6 of 6	6 of 6	6 of 6	6 of 6
3.5 ng/mL or molar equivalent of 38 mIU/mL	6 of 6	6 of 6	6 of 6	6 of 6	6 of 6	6 of 6
2.0 ng/mL or molar equivalent of 22 mIU/mL	6 of 6	6 of 6	6 of 6	6 of 6	6 of 6	6 of 6
1.0 ng/mL or molar equivalent of 11 mIU/mL	6 of 6	6 of 6	4 of 6	1 of 6	2 of 6	3 of 6
0.5 ng/mL or molar equivalent of 5.5 mIU/mL	6 of 6	6 of 6	0 of 6	0 of 6	0 of 6	0 of 6
Sensitivity	5.5	5.5	22	22	22	22

We then examined in Study 4 (Table 2), a combination of components resembling early pregnancy urine. As found, First Response manual and First Response digital had sensitivities of <5.5 mIU/mL. However, the EPT manual and digital and ClearBlue Easy manual and digital all had sensitivities of 11–22 mIU/mL. This suggested that failure to detect free β-subunit, and the improvement in hyperglycosylated hCG sensitivity were still limited by the early pregnancy combinations yielding sensitivities of 11–22 mIU/mL rather than 5.5–11 mIU/mL. However, further improvements are needed in the detection of both hyperglycosylated hCG and free β-subunit to optimally detect early pregnancies.

It became clear that the only way to truly evaluate the utility of over-the-counter devices was to test them with real urine samples and not random mixes (like Study 4) in early pregnancy. For this reason, the USA hCG Reference Service tested daily urine from 137 women achieving pregnancy. In Study 5, 40 individuals were randomly selected from the 137 women, and their urine was tested on the day of missed menstrual bleeding. The average start of menstrual bleeding was 28.6 ± 3.8 days since the start of last menstrual bleeding, or 13.1 ± 1.7 days since the LH peak. Urine was also tested 3 days after the day of missing start of menstrual bleeding (Table 3). It was shown that all 40 women had achieved pregnancy the day of missed menses.

As found in Study 5 (Table 3), on the day of missed menses 100% of urine samples tested with First Response manual devices and 98% of urine samples tested with First Response Gold digital devices were positive. In contrast, just 55% of the 40 urines from pregnant women tested with EPT manual, 65% with EPT Certainty digital, 58% with ClearBlue Easy manual and 60% with ClearBlue Easy digital were positive. This difference in First Response, EPT and ClearBlue tests reflected the differences seen in sensitivity, ability to detect hyperglycosylated hCG and the free β-subunit observed in Study 1–4.

We examined urine 3 days after missing the start of menstrual bleeding. With First Response manual and First Response Gold digital we observed 100% and 95% detection, respectively. With EPT manual and EPT Certainty digital devices we observed 80% and 80% detection, respectively. With ClearBlue manual and digital we observed 75% and 75%. All these test manufacturers make the same claim of >99% accuracy on the day of missed menstrual bleeding. It appears that Church and Dwight Inc., manufacturers of First Response devices, can rightfully make this >99%

Table 3 Evaluation of over-the-counter tests with urine from pregnant women.

Day relative to missed period	Achieved pregnancy, %	hCG median, mIU/mL	First Response manual, %	First Response Gold digital, %	EPT manual, %	EPT Certainty digital, %	ClearBlue Easy manual, %	ClearBlue Easy digital, %
Study 5. Pregnant woman, 40 total								
0	100	41	100	98	55	65	58	60
+3	100	154	100	95	80	80	75	75
Study 6. Pregnant woman, 80 total								
−6	29	2.1	25	25	0	0	0	0
−5	40	2.9	33	25	5.0	5.0	5.0	5.0
−4	76	5.2	58	42	6.3	6.3	8.8	3.8
−3	88	12	74	68	14	18	27	12
−2	92	21	76	81	29	31	29	28
−1	99	40	93	91	42	55	57	51
0	99	70	96	96	53	68	67	51
+1	100	143	100	96	64	71	74	69
+2	100	227	100	99	77	79	81	77
+3	100	302	100	100	80	86	87	84
+4	100	534	100	100	100	100	100	100

claim, but Pfizer Consumer Healthcare manufacturers of EPT products and Inverness Medical Innovations manufacturers of First Response products cannot make this claim.

We were able to repeat Study 5 using 80 of the remaining 137 pregnant volunteer's urine samples (Study 6). Study 6 was funded by Church and Dwight Inc. It yielded virtually identical results to Study 5, showing high detection on the day of missed menstrual bleeding for First Response manual and digital products (96% detection), and not good results for EPT and ClearBlue manual and digital products (53%, 68%, 67% and 51% detection). Combining the two studies together (Study 5 and Study 6, examining results from 120 individuals, the First Response manual and digital products both detected 97% of pregnancies on the day of missed menstrual bleeding. The EPT manual and digital devices detected 54% and 67% of pregnancies and the ClearBlue Easy manual and digital products detected 64% and 54% of pregnancies on the day of missed menstrual bleeding.

Study 6 investigated the utility of over-the-counter tests from 6 days prior to day of missing the menstrual period (determined from an average length of menstrual cycle, over 3–5 cycles) until 4 days after missing the menstrual cycle. This study allowed us evaluate the comment made by First Response and ClearBlue Easy devices, ''use up to 4 days prior to missing menstrual period''. The EPT tests boast on its packaging ''no other brand is more accurate.''

As shown in Table 3, at 4 days prior to the missed menstrual period, only 76% of women achieving pregnancy have detectable pregnancy by this time. As shown in Study 6 (Table 3), at this time, 4 days prior to missed menstrual bleeding, the First Response manual and digital tests detected 58% and 42% of pregnancies. The test manufacturer's claim slightly better detection at this time, 69% and 58% detection. However, the EPT manual and digital tests detected just 6.3% of pregnancies, and the ClearBlue manual and digital tests detected just 8.8% and 3.8% of pregnancies, respectively. The manufacturer claims a detection rate of 53% and 51%, or 6.0-fold and 13-fold better results than what we found. Unfortunately, the consumer has to deal with this. It is concluded that the First Response manual and digital tests seemingly have limited value prior to the date of missed menstrual bleeding. These tests should make no claim about early detection as they are insensitive.

As shown, new digital devices are virtually as sensitive as manual devices, detecting 97%, 67% and 54% (mean 73%) of the 120 pregnancies in Study 5 and Study 6 on the day of missed menstrual bleeding, compared with 97%, 54% and 64% (mean 72%) of pregnancies. At times, the manual devices evaluated in this study were difficult to read with very faint lines. When this happened, all three readers had to agree on a result and to whether the test was positive or negative. If this happens with member of the public it may lead to confusion. The digital tests, in contrast gave clear and indisputable result, ''yes'' or ''no'' or ''pregnant'' or ''not pregnant''.

We asked ourselves whether there is any possibility that our calculation of the individual day of missed menstrual bleeding could be wrong. This was an important consideration when considering our claim that EPT and ClearBlue East products are misleading to the public. We understand from talking with on line technical support at Inverness Medical Innovation Ltd. (the manufacturer of ClearBlue Easy), that they calculate the day of a missed menstrual period as LH peak plus 17 days. They calculate the sensitivity of their test accordingly. Among the 137 pregnant individuals examined, the average time to missed menstrual bleeding was 13.1 ± 1.7 days following the LH peak. In a large study by Wilcox et al. (10), it was 13 ± 1.9 days following the LH peak. In our own publication investigating the timing of missed menses, we investigated 408 menstrual cycles and observed an average of 13.2 ± 2.0 days after the LH peak (6). In a recent large study of 895 menstrual cycles organized by Inverness Medical Innovation Ltd., it was found to be 13.2 ± 1.9 days post LH peak to the start of menstrual bleeding (11). It clearly appears that our calculation and our timing is correct and confirmed by Inverness Medical

Innovation Ltd. However, Inverness Medical Innovations Ltd. calibrates their home pregnancy test based on the LH peak plus 17 days, or 4 days longer than that shown by scientific investigation. This may explain the lack of sensitivity, shown here, of their tests to detect pregnancy on the actual day of a missed menstrual period, LH peak plus 13 days.

Overall our conclusion is that early pregnancy over-the-counter pregnancy test methods need to detect the three important early pregnancy ingredients, hCG, hyperglycosylated hCG and free β-subunit equally. A test specifically designed for detecting early pregnancy, making claims of detection 4 days prior to the day of a missed menstrual period, must also be highly sensitive for detecting hCG-related molecules. While these criteria are seemingly met by First Response manual and digital, they are clearly not met by EPT and ClearBlue devices based on the 120 pregnancies examined here. EPT and ClearBlue make the claims, such as ''more than 99% accurate,'' ''no other brand is more accurate'' and ''used 4 days before the expected period'' seemingly with FDA approval. However, these claims are not supported by our study of 120 pregnant women.

Care is needed in purchasing over-the-counter pregnancy tests. Claims made by ClearBlue and EPT easy, such as $>99\%$ accuracy on the day of a missed menstrual period, and by ClearBlue for use 4 days prior to missing menses are, based on Study 5 and 6, without foundation. The claims made by First Response devices for detection of all pregnancies on the day of missing menstrual bleeding, while slightly off, may however, be considered valid. This study is the largest and most comprehensive study of over-the-counter tests ever published, examining urine spiked with hCG, hyperglycosylated hCG, free β-subunit, and a mixture of all three, and 120 individual urines from pregnant females.

Conflict of interest statement

Authors' conflict of interest disclosure: The authors stated that there are no conflicts of interest regarding the publication of this article.

Research funding: None declared.
Employment or leadership: None declared.
Honorarium: None declared.

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