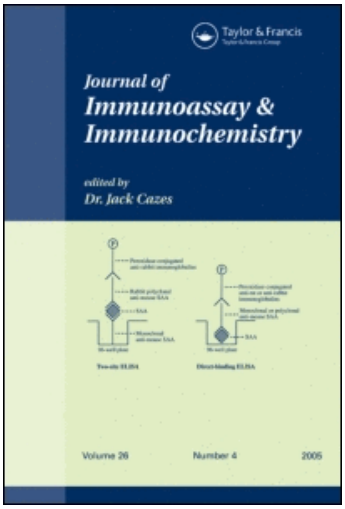

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ASSESSMENT OF FOUR ENZYME IMMUNOASSAY KITS FOR SERUM β-hCG SCREENING

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ABSTRACT

This pilot study evaluates four qualitative serum β-human chorionic gonadotropin (β-hCG) enzyme immunoassay kits for both routine pregnancy testing and screening for ectopic pregnancy. We found that the Tandem-E HCG assay had the lowest limit of detection, and longest turnaround time. Tandem ICON HCG assay had the shortest turnaround time. The Vis-Con II was the most economical batch test. The Quest assay kit was both fast and simple and had the lowest cost for stat testing.

KEY WORDS: β-hCG, pregnancy testing, ectopic pregnancy screening, enzyme immunoassay.

INTRODUCTION

Human chorionic gonadotropin (hCG), a glycoprotein hormone comprised of alpha and beta subunits, is produced in normal and ectopic pregnancies, and by a variety of tumours including seminomas, trophoblastic tumours, teratomas and embryonal carcinomas. It is also present at low levels in 3-14% of healthy males and non-pregnant females (1,2).

Qualitative hCG is clinically useful in the distinction of ectopic pregnancy from other abdominal disorders (3,4).

Latex or hemagglutination inhibition assays for hCG in urine have a detection limit of 750 IU/L due to cross reactivity with luteinizing hormone (LH). Direct agglutination assays using antibodies against the beta sub-unit of hCG are more specific and have detection limits of 200-400 IU/L of β-hCG, but they provide a reliable detection of pregnancy only 4-5 weeks after fertilization. These tests are prone to erroneous results due to high protein concentration, drug interferences (phenothiazines), low ionic strength and semen contamination (5,6).

Monoclonal antibodies to β-hCG have no cross reactivity with LH and two site enzyme immunoassays using these antibodies have reduced the detection limit for β-hCG to 50 IU/L in urine and 25 IU/L in serum, levels that occur 1-2 weeks after conception.

For the present study, serum was used because β-hCG levels rise in serum before urine, serum assays are more sensitive than urine assays, matrix problems may occur with urine specimens (7), and a blood sample is easily obtained from a patient presenting with an acute abdomen whereas a urine sample may require catheterization.

MATERIALS AND METHODS

Four kits for β-hCG were evaluated: Tandem-E HCG (Hybritech Inc., San Diego, CA), Tandem ICON HCG (Hybritech Inc., San Diego, CA), Vis-Con II Concep-7-BHCG (ICN Canada Ltd., Montreal, Canada). and Quest (Quidel, La Jolla, CA). All four kits are solid phase, two site enzyme immunoassays employing monoclonal antibodies to β-hCG, (Table 1). All assays were performed according to the manufacturers' instructions, using the quality control materials

TABLE 1

KIT DESCRIPTIONS*

	Tandem-E HCG	Tandem ICON HCG	Vis-Con II	Quest
Solid Phase	Bead	Membrane	Tube	Dipstick pad
Detection Limit (IU/L)	25	25	25	50
Serum volume (uL)	100	500	100	500
Assays per kit	100	48	50	50
Warm up time (min)	30	20	20	0
Storage (^{o}C)	2-8	2-8	2-8	<30
Quality Control/ Calibrators	Zero Calibrator Positive ref. control 300 IU/L 25 IU/L	Negative Control Positive Control 25 IU/L	Positive Control 25 IU/L Positive reference 50 IU/L	Negative Control on dipstick
Interpretation	A405 nm	Blue dot	Blue solution	Blue pad

* from the Kit Instructions

provided. For the Tandem-E HCG and Vis-Con II kits, the qualitative rather than quantitative procedures were performed.

The 4 kits were evaluated with 30 serum samples with β-hCG concentrations of 0-94 IU/L, as determined by the quantitative immunoradiometric Tandem-R-HCG kit assay (Hybritech Inc., San Diego, CA). The standards in this method are calibrated against the WHO First International Reference Preparation IRP #75/53. Between-day standard deviations were 1.2 and 2.9 IU/L at levels of 24.0 and 79.7 IU/L respectively. Samples were stored frozen at -20°, thawed once and stored at 4° for 48 h during analysis by the four procedures. Samples with concentrations below 65 IU/L were assayed in duplicate by each kit procedure.

Results were interpreted as follows. For the Tandem-E HCG procedure, a result equal to or greater than the 25 IU/L positive reference was considered positive. In the Tandem ICON HCG method, a result was considered positive if the blue dot was of intensity equal to or greater than the 25 IU/L control cylinder. With the Vis-Con II method, a colour intensity of a specimen greater than the 25 IU/L control indicated a positive result. For the Quest method, a result was considered positive if the indicator pad was darker blue than the negative reference pad on the same dipstick.

RESULTS

Tandem-E HCG: There was a reagent warm up time of 30 min. The maximum number of samples in a run was limited to 24 plus 6 calibrants and controls which was the number that could be pipetted in 10 min. Positive results were seen down to 21 IU/L The colour intensity was stable for at least 1 h.

Tandem ICON HCG: Although the stated detection limit was 25 IU/L, a faint blue dot was seen at concentrations as low as 10 IU/L. No dot was seen with the negative control provided with the kit or with patient sera less than 10 IU/L β-hCG. The sensitivity of this method was 82% with six false negatives on samples with concentrations between 27 and 32 IU/L. The test has to be read at 3 min and it was noted that colour intensity continues to increase slowly over one hour. The maximum permitted time between steps allowed a maximum batch size of 5 tests plus 2 controls. Turnaround time for a batch of 5 samples was 20 min for reagent warm up and 11 min for assay. One cylinder could be saved by running first the negative control and then the positive control on the same cylinder, but with added delay of 11 min.

VIS-Con II: Negative controls and all patient samples of 25 IU/L or less gave negative results. Two false negatives occurred with patient samples of 27 IU/L. The inclusion of a 25 IU/L β-hCG control is essential to interpret results at the stated lower limit of detection. The tubes must be read against the reference tube during the 5-15 min incubation time as the colour slowly increases with time.

Quest: All patient samples of 32 IU/L or greater gave a positive result. With the stated detection limit of 50 IU/L, the sensitivity was 100%. Positive results were also seen in 5 out of 9 samples with concentrations between 25 and 30 IU/L. There were no positive results with samples having less than 23 IU/L. With this procedure it is essential that the indicator and reference pads be blotted completely to prevent false negative results.

DISCUSSION

None of the assays produced a positive result with a negative control or a patient sample with less than 21 IU/L. At their stated detection limits, all four kits are suitable for routine screening for pregnancy, the Quest kit at 10-16 days and the rest at 6-9 days post-fertilization. At the stated detection limits, the sensitivities of the Tandem-E HCG and Quest assays were 100%, while those of the Vis-Con II and the Tandem ICON HCG were 94 and 82% respectively. The false negative results using the Vis-Con II and Tandem ICON HCG suggest their consistent detection limit is closer to 30 than 25 IU/L while the Quest assay with a 'detection limit' of 50 IU/L had false positive results which suggested its detection limit is about 35 IU/L.

The Tandem ICON HCG assay produced a faint blue dot at β-hCG concentrations of 10-24 IU/L which must be distinguished from a positive result, since up to 14% of healthy nonpregnant subjects have β-hCG concentrations in the range of 5-25 IU/L (2).

Our institution carries out approximately 1400 tests for pregnancy and 600 stat tests for ectopic pregnancy yearly. The four kits were evaluated with respect to their suitability for this workload. Table 2 summarizes the total turnaround time and the estimated costs of batch and stat testing.

Batch Testing: The batch size chosen for comparison was the maximum possible for the Tandem ICON HCG assay (five patient samples and two controls). Rapid turnaround time is not critical for routine pregnancy testing, but it does influence the cost of labour even though other tasks may be undertaken during incubation periods. The costs used here have to be interpreted in the context of competitive

TABLE 2

COMPARATIVE COSTS[a] AND TURNAROUND TIMES

	TANDEM-E HCG	TANDEM ICON HCG	VIS-CON II	QUEST
Cost per kit ($)	271.23[b]	220.00	80.00	195.00
Cost per assay	2.71	4.58	1.60	3.90
No. of standards/ controls	6	2	1	0
Reagent Cost				
Batch of 5 patient samples	29.80	32.05	10.10	19.50
Single patient sample	18.97	13.74	3.70[b]	3.90
Assay Time (min)				
Batch/Stat	120	11/10	20	18/16
Including warm-up	150	31	40	
Total Cost per Patient ($)[c]				
Batch	11.96	6.96	3.02	4.80
Stat	48.97	16.24	8.70	7.90

[a] Canadian dollars in June 1985

[b] Includes cost of substrate kit ($73.97)

[c] Salary rate $15.00/h; calculations exclude warm-up time

pricing policies for pregnancy test kits with frequently available discounts.

The Quest assay was considered by routine technologists to be the most robust and simplest to perform, while increasingly greater attention to time and technique was required with the Vis-Con II, Tandem ICON HCG and Tandem-E HCG assays.

Stat Testing: Rapid turnaround time and a robust assay are important for stat β-hCG testing in suspected ectopic pregnancy. All kits, with the exception of the Tandem-E HCG, which had a turnaround time in excess of 2 h, appeared to perform well when judged against these criteria. The absence of a warm-up period was an advantage of the Quest assay, but it should be noted that the latest version of the Tandem ICON HCG can also be stored at ambient temperature.

Although distinction of ectopic pregnancy from other intra-abdominal disorders is greatly assisted by qualitative measures of β-hCG, distinction from the threatened abortion of an intrauterine pregnancy often requires ultrasound or the quantitative estimation of β-hCG on several occasions to estimate the time for doubling of the β-hCG concentration. The qualitative test has its main role in excluding pregnancy in both routine and critical care situations, and the kits reviewed here are all capable of performing reliably in this context.

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