K961351

Attachment 3

JUN - 3 1996

Summary of Safety and Effectiveness

hCG Extended Range (hCG ER) Method for the Technicon Immuno 1° system

Listed below is a comparison of the performance of the Technicon Immuno 1° hCG ER method and a similar method granted clearance of substantial equivalence (standard Technicon Immuno 1° hCG method). The information below was extracted from the Technicon Immuno 1 hCG ER method sheet, the Technicon Immuno 1 hCG method sheet, and data on file at Bayer Corporation.

Intended Use

This *in vitro* diagnostic procedure is intended to quantitatively measure human chorionic gonadotropin (hCG) in human serum on the Technicon Immuno 1° system. Measurements of human chorionic gonadotropin are used for the detection of pregnancy. This method is an adaptation of an existing Technicon Immuno 1 hCG method and has an extended range from 800 mIU/mL to 100,000 mIU/mL. This diagnostic method is not intended for use on any other system.

Part Number	Technicon Immuno 1° hCG ER T01-2966-51		Technicon Immuno 1° hCG T01-2966-51	
Reference Range	800 - 100,000 mIU/mL		0.5 - 1000 mIU/mL	
Precision, within-run (n = 130 over 20 days)	mean (mIU/mL)	%CV	mean (mIU/mL)	%CV
	978	3.8%	18.3	2.4%
	9,707	3.5%	55.7	2.2%
	26,746	3.6%	195.8	2.1%
	28,658	3.8%		
	83,756	3.2%		
Precision, total (n = 130 over 20 days)	mean (mIU/mL)	%CV	mean (mIU/mL)	%CV
	978	5.5%	18.3	4.0%
	9,707	6.2%	55.7	3.7 %
	26,746	4.8%	195.8	3.7%
	28,658	6.8%		
	83,756	6.2%		

Regression Equation	**Y = 1.11 X + 323** **where Y = Immuno 1 hCG ER** **X = Immuno 1 hCG** **n = 104 sera** **r = 0.998**

Assay Description

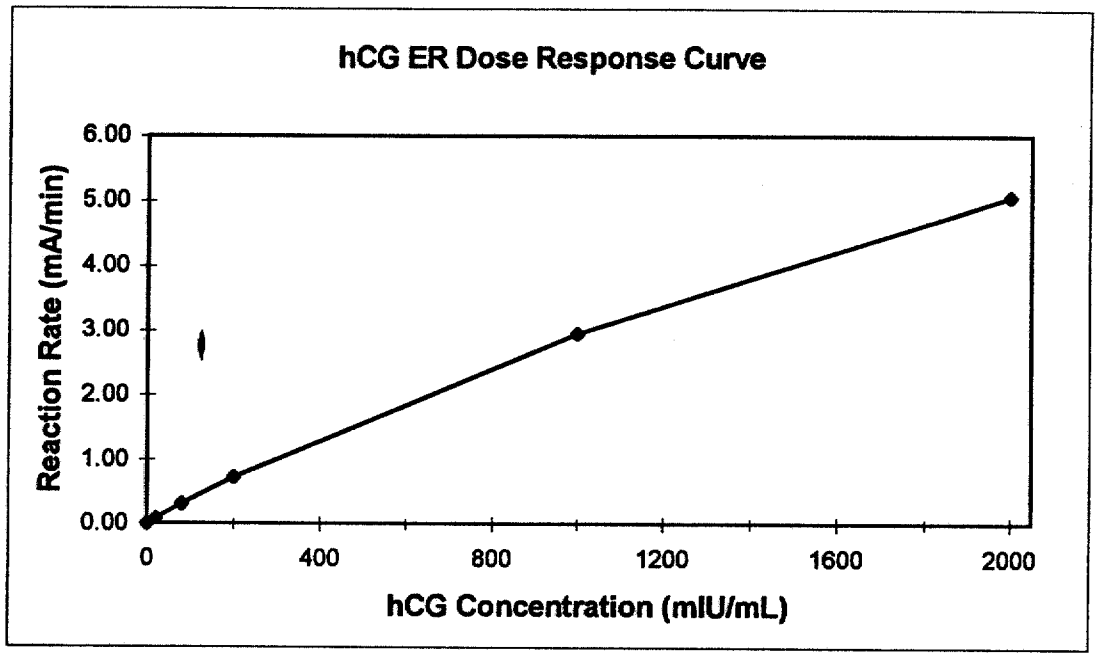
This method is a double antibody immunoassay. hCG Antibody Conjugate 1 (R1) and hCG Antibody Conjugate 2 (R2) are reacted with patient sample (or calibrator containing hCG) and incubated on the Technicon Immuno 1 system at 37°C. The mIMP Reagent (monoclonal ImmunoMagnetic Particle) is added and a second incubation occurs during which the antibody complex is bound. The mIMP/antibody complex is then washed and the pNPP (para nitrophenylphosphate) substrate is added. The alkaline phosphatase (ALP) in the antibody conjugate reacts with the pNPP to form para-nitrophenoxide and phosphate. Increasing absorbance, due to the formation of para-nitrophenoxide, is monitored at 405 nm and 450 nm. A sample having no hCG will have the minimum label bound, while samples containing high hCG concentrations will have maximum label bound. Thus the dose/response curve is directly proportional to the hCG in the sample.

The hCG ER method is an adaptation of the existing Technicon Immuno 1 hCG method. They both use the identical hCG Reagent, Part No. T01-2966-51. The hCG ER Calibrators, Part No. T03-3668-01, are identical in formulation to the Technicon SETpoint Reproductive Calibrators, Part No. T03-3148-01, which are used for the standard hCG method. There are no changes in either reagent or calibrator formulation. The hCG ER method differs from the standard hCG method in three ways:

1. The hCG ER calibrators, are reconstituted in 1.0 mL of Reproductive Calibrator Diluent while the Reproductive Calibrators are reconstituted in 2.0mL of Reproductive Calibrator Diluent. This doubles the concentrations of hCG in the calibrator from 0 - 1000 mIU/mL to 0 - 2000 mIU/mL.

2. 65µL of calibrator are reacted with reagent, while 1.3µL of any other sample, serum or control, are reacted. 65µL of the highest calibrator, 2000 mIU/mL, corresponds to 130 total mIU of hCG. A corresponding serum sample with 130 total mIU of hCG, at 1.3µL volume, would have 100,000 mIU/mL concentration. Thus a serum sample at 100,000 mIU/mL reacts the same total quantity of hCG, and produces the same amount of p-nitrophenoxide, as does calibrator at 2,000 mIU/mL.

3. The concentrations of all samples other than calibrator are automatically multiplied by a factor of 50, which is the difference in volume between 65µL and 1.3µL.



Conc. mIU/mL	Rate mA/min
0	0.0042
20	0.0812
80	0.3074
200	0.7286
1000	2.9533
2000	5.0696

Imprecision

Within run and total imprecision were evaluated by testing two commercial controls and three serum pools for twenty days on two systems using two lots of hCG reagent. For both systems, twenty days of data, one run a day, were collected with one reagent lot and 17 days of data were collected with the other reagent lot . The mean rate for each reagent lot was used to generate calibration curves, and concentrations for each lot were calculated from their respective calibration curves. The within-run variance was calculated daily. The among day variance was equal to the variance of the daily means minus the within-run variance divided by the number of reps per day. Total variance was equal to the among day variance plus the within-run variance. Standard deviations were the square roots of the variances.

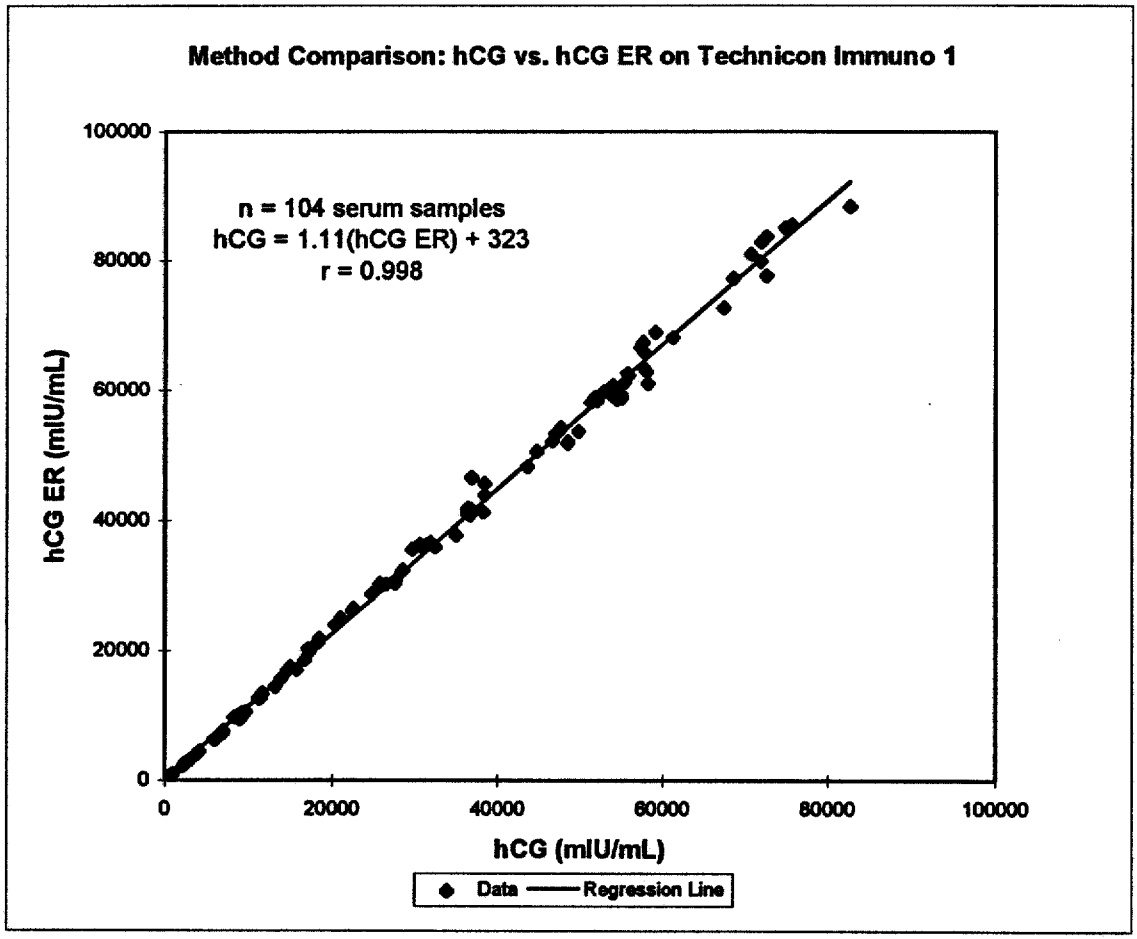
Imprecision Results

Product	Number of Replicates	Mean mIU/mL	Within Run S.D. mIU/mL	Within Run C.V.	Total S.D. mIU/mL	Total C.V.
Serum Pool 1	129	978	38	3.8%	54	5.5%
Control 1	147	9707	343	3.5%	585	6.2%
Control 2	130	28658	1083	3.8%	1926	6.8%
Serum Pool 2	132	26746	955	3.6%	1262	4.8%
Serum Pool 3	129	83756	2732	3.2%	5212	6.2%

Correlation

104 serum samples were assayed for hCG on the Technicon Immuno 1 with the hCG and hCG ER methods. All samples were assayed undiluted in the hCG ER method. Samples that read between 800 and 1000 mIU/mL were assayed undiluted in the standard hCG method. Those that read greater than 1000 mIU/mL were manually diluted with Technicon Immuno 1 Sample Diluent B (Product # T03-3574-01) before assaying. Two researchers separately diluted each sample in order to minimize individual biases in dilution.

hCG values ranged from 840 mIU/mL to 88,500 mIU/mL. Results were calculated using linear regression.



Interfering Substances/ Specificity

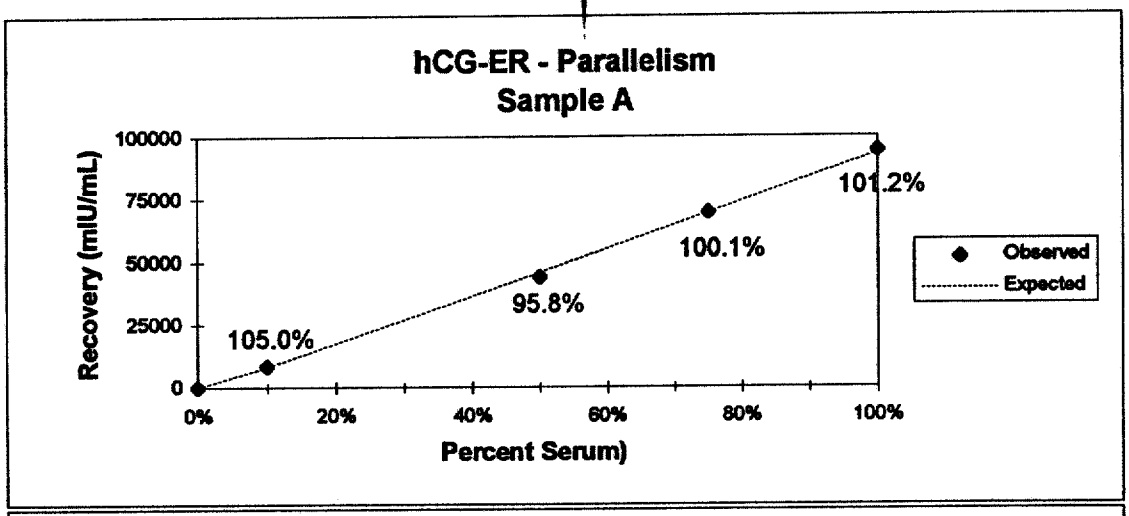
The hCG ER method has the identical reagent and calibrator formulation as the standard hCG method. However the hCG ER method uses 1.3µL of sample while the standard method uses 50µL . With 1/38th the sample volume of the standard method, the hCG ER method has 1/38th the amount of interfering or cross-reacting substances added to the reaction. Assay specificities are expected to be the same as for the standard method.

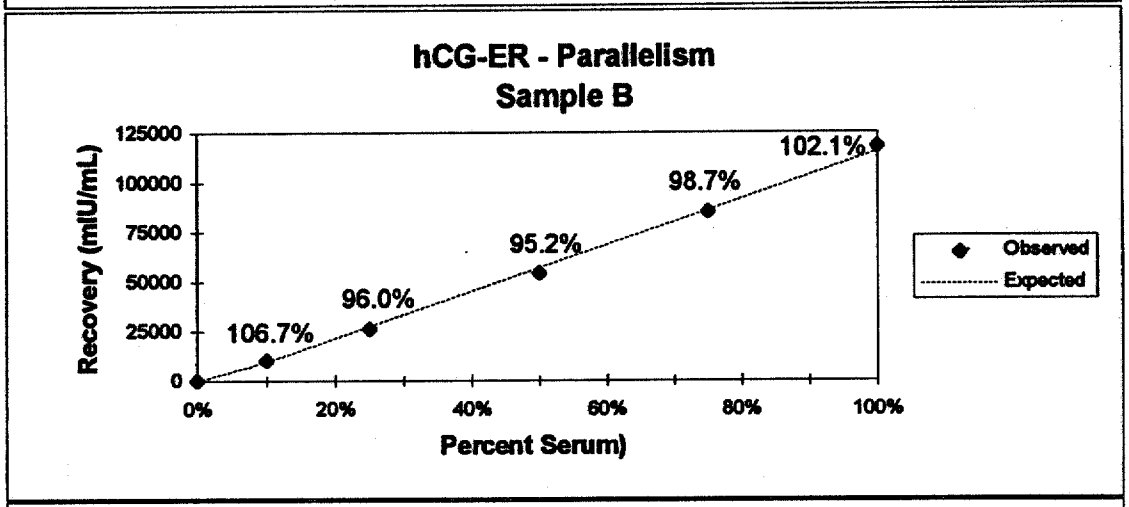
Expected Values

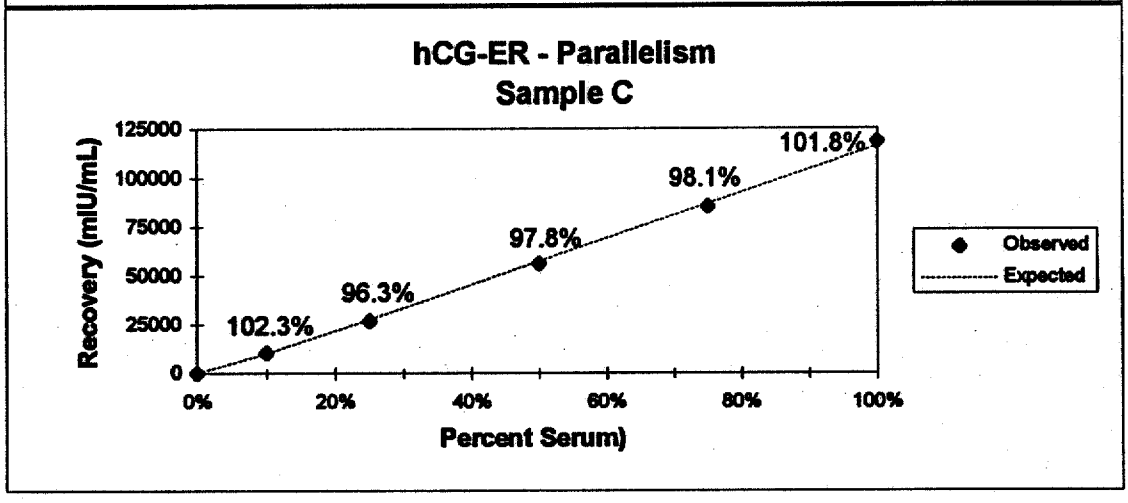
The hCG ER method has the same expected values as the standard hCG method. The only difference between the two methods is that the standard hCG method reports hCG values between 0.5 and 1000 mIU/mL and the hCG ER method reports hCG values between 800 and 100,000 mIU/mL.

Sample Dilution / Parallelism

For parallelism, three unique serum samples with hCG values close to 100,000 mIU/mL were diluted with Sample Diluent B. The mean observed values were all within 95%-107% of expected.

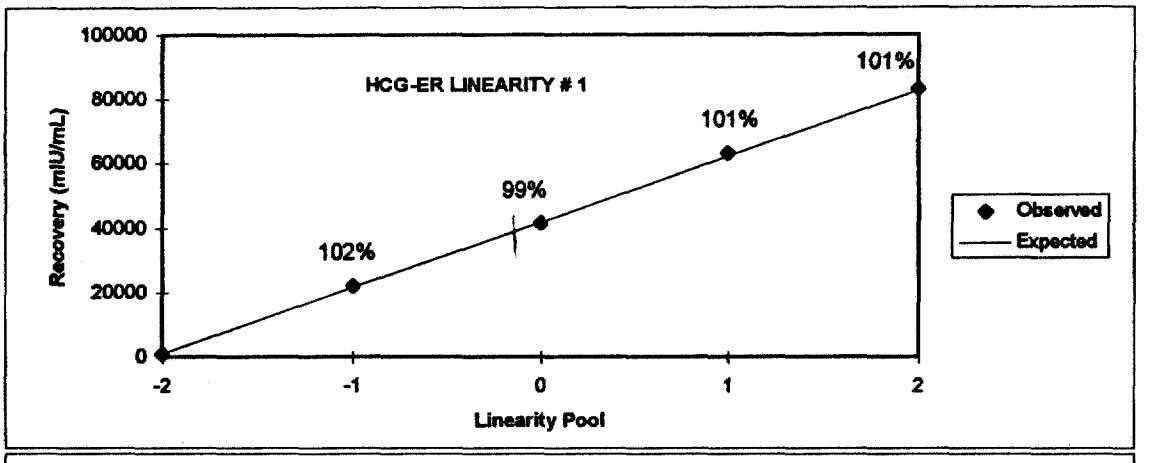


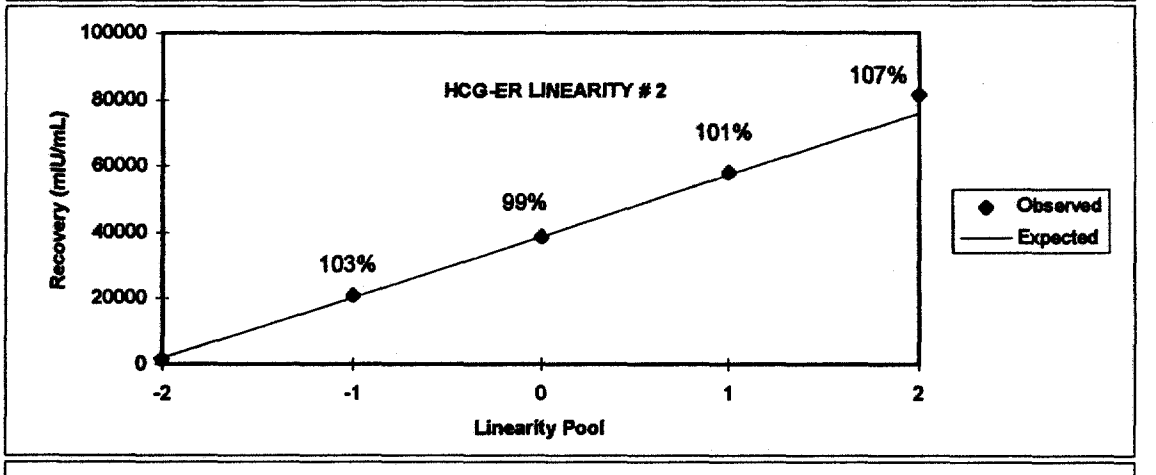


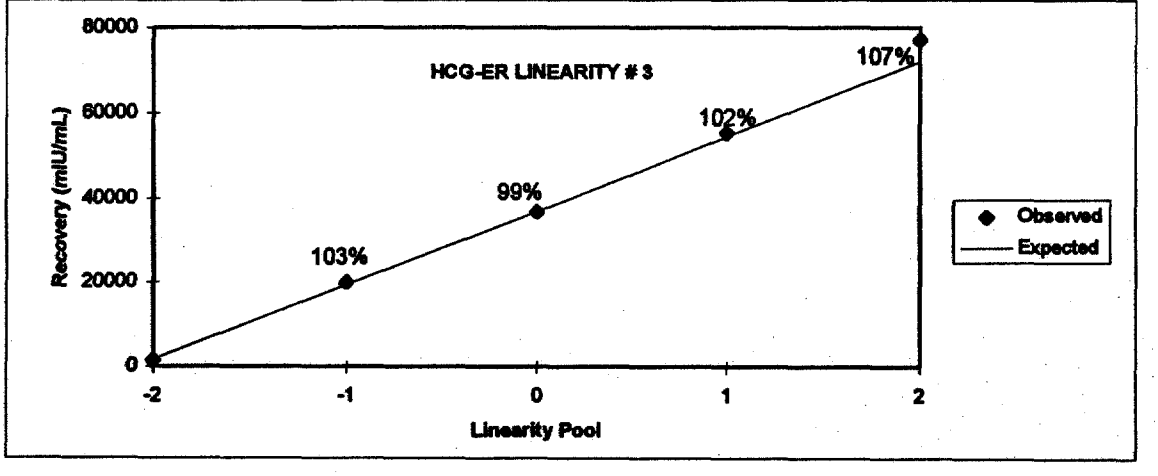


Sample Dilution / Linearity

Three unique pairs of high and low hCG serum samples were co-diluted and assayed. The observed recoveries were all within 99%-107% of expected.





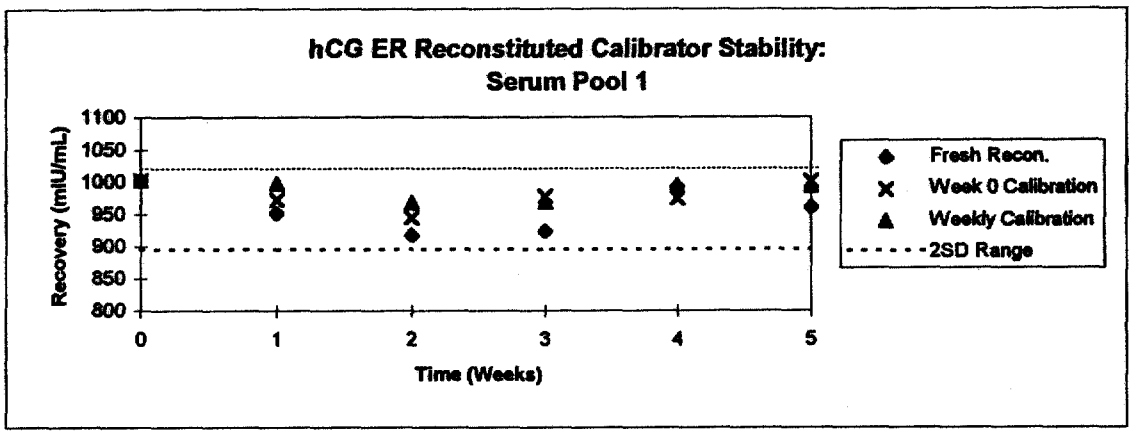


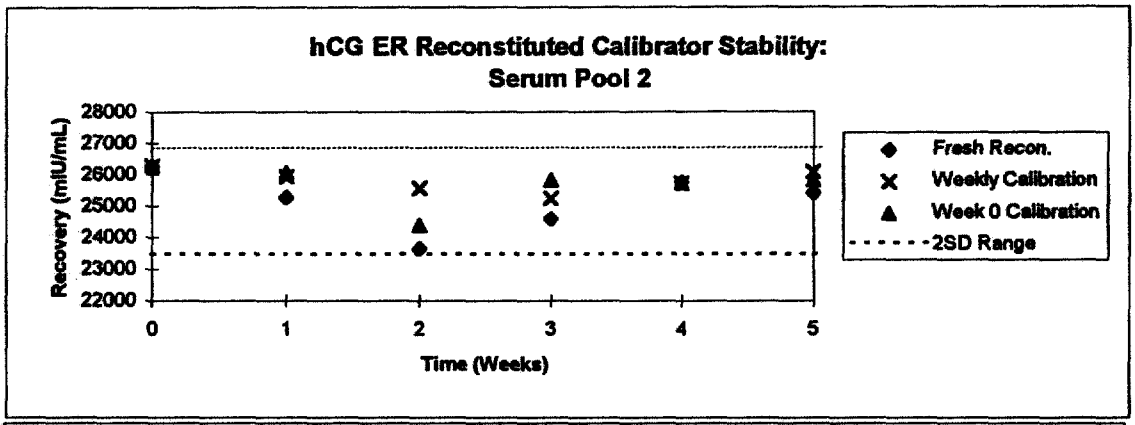
Reagent & Calibrator Stability

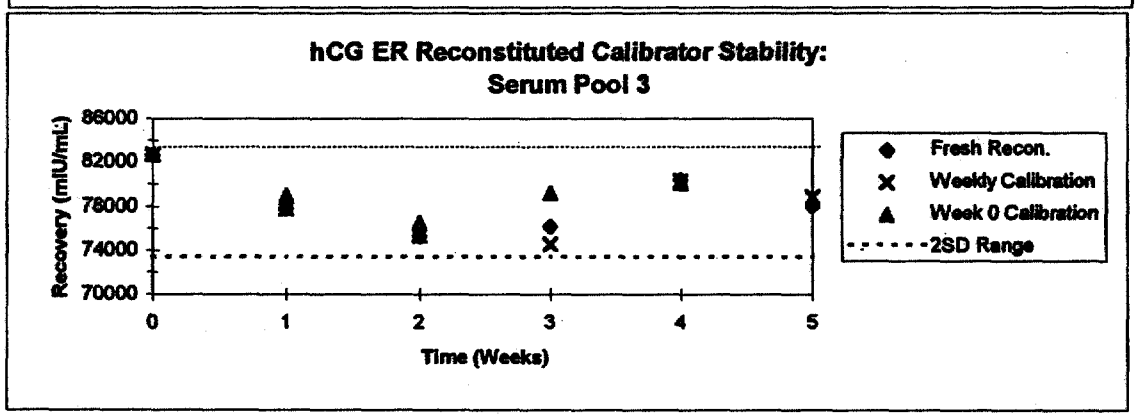
The same hCG reagent is used for the hCG ER method as for the standard method. No additional stability studies are necessary.

The hCG ER calibrators, Part No. T03-3668-01, are identical in formulation to the Technicon SETpoint Reproductive Calibrators, Part No. T03-3148-01. Shelf life and shipping stability are not necessary.

The hCG ER calibrators are stable for 30 days after reconstitution. Three sets of calibrators were reconstituted and tested individually at Time 0. The three sets were <u>not</u> pooled. Their rates were combined and used for "Week 0" calibration. At weekly intervals only one set of open vial calibrator was tested along with a freshly reconstituted set. A different set was used weekly for five weeks. Serum pool recoveries were calculated from week 0, weekly, and fresh reconstituted calibrations.


hCG ER Reconstituted Calibrator Stability:
Serum Pool 1
Recovery (mIU/mL)
1100
1050
1000
950
900
850
800
0
1
2
3
4
5
Time (Weeks)
Fresh Recon.
Week 0 Calibration
Weekly Calibration
2SD Range


hCG ER Reconstituted Calibrator Stability:
Serum Pool 2
Recovery (mIU/mL)
28000
27000
26000
25000
24000
23000
22000
0
1
2
3
4
5
Time (Weeks)
Fresh Recon.
Weekly Calibration
Week 0 Calibration
2SD Range


hCG ER Reconstituted Calibrator Stability:
Serum Pool 3
Recovery (mIU/mL)
86000
82000
78000
74000
70000
0
1
2
3
4
5
Time (Weeks)
Fresh Recon.
Weekly Calibration
Week 0 Calibration
2SD Range

Minimal Detectable Concentration

The hCG ER method has a lowest reportable result of 800 mIU/mL. Minimal detectable dose is not determined. The lowest reportable result is used to determine the lower limit of detection rather than minimal detectable dose.