Feasibility of applying manual microplate ELISAs to general purpose automated instruments

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Poster B-35, AACC Annual Meeting, July 25-29, 2004, Los Angeles, CA

INTRODUCTION:

Clinical laboratories are growing less and less dependent upon manual techniques. Today several user-programmable instrument systems (open) are commercially available for automating micro-well enzyme immuno assays (EIAs). The aim of this study was to assess the feasibility of adapting quantitative EIAs to such an instrument system, identify and reduce the major sources of variation, and determine the precision, accuracy, and correlation of EIA results obtained on an automatic open analyzer.

ABSTRACT:

Method: A variety of different analytes were measured using EIA microwell kits manufactured by BioCheck (Foster City, CA) according to the manual techniques described in the kit package inserts, and again using ChemWell®, a single-plate user-programmable automated EIA analyzer manufactured by Awareness Technology (Palm City, FL). Timing, mixing, and other user-programmable factors were modified to optimize assay precision, accuracy, standard value recovery, and correlation with results from the manual method.

Results: Once instrument programming parameters were set for each assay, ChemWell results using the BioCheck ELISA kits were found to be equivalent or better than results from the manually run assay. Precision obtained using two to four different control levels, and sample correlation data for 11 selected assays run on ChemWell are shown below:

Assay	Precision Range (%CV)		R-squared Correlation to Manually Run Assay Results (n=30)
	Intra-Assay (n=10)	Inter-Assay (n=20)	
Estradiol (E2)	4.9-7.1	5.6-6.2	0.965
Ferritin	1.7-3.3	2.0-3.1	0.995
FSH	1.2-5.1	1.6-4.5	0.998
hCG	1.9-5.2	3.0-4.2	0.991
hGH	1.7-4.8	3.9-5.3	0.990
IgE	3.1-5.2	6.6-9.0	0.994
LH	1.1-11.2	2.0-10.4	0.996
Progesterone	2.6-4.9	4.6-6.4	0.996
Prolactin	1.4-3.2	2.7-3.0	0.997
Testosterone	2.8-6.7	3.0-6.6	0.995
Troponin I	1.3-4.6	2.8-5.2	0.990

Conclusion: Processing BioCheck ELISAs on ChemWell yielded accurate and precise results with improved throughput and equivalence with manually obtained results. The programmable parameters with the greatest effect on assay results were incubation timing and degree of mixing. For example, results obtained running an entire 96-well plate with one timer, were generally superior to results obtained when running a separate timer for each strip. This was attributed to eliminating mixing duration differences between strips, and to reducing strip-to-strip timing conflicts. Microwells processed automatically generally move about and mix more than wells processed manually. Several of the manual assay incubation times could be significantly shortened without compromise to performance, by using the automatic instrument's mixer. Each reagent and instrument system requires similar study to determine optimal programming parameters for reliability in clinical diagnostics

METHOD:

1. Optimization of Programming Parameters: ChemWell ® (Awareness Technology, Palm City, FL) is a single-plate analyzer that can be used at ambient temperature or 37C. It uses a single dispense probe, 8-channel washing system, and a filter-photometer for absorbance reading. Flexible user software requests various programming parameters. A variety of parameter settings were evaluated in order to obtain optimal assay results from ChemWell. Such parameters include: reagent dispense accuracy, assay timing, plate mixing and sample predilution method. Assays with incubation times of 1 hour or greater were evaluated for equivalent performance using continuous plate mixing and shorter incubation times.

2. Evaluation of ChemWell Assay Performance:

A. Precision & Accuracy: Intra-assay and inter-assay precision was determined using the optimized ChemWell assay protocols. Two to four levels of control material were assayed in two separate runs of 10 replicates each. Assay precision and accuracy were determined from these runs. The ChemWell precision was compared to the published manual precision to assess equivalence.

B. Manual to ChemWell Result Correlation: Thirty serum samples covering each assay range were assayed manually and using ChemWell. Each sample was assayed singularly using a standard curve run in duplicate. Sample correlation was calculated and plotted.

C. Standard Recovery: Standard recovery was evaluated by running a complete 96 well plate with standards in strips 1, 6 and 12. Standards run in the center of the plate (strip 6) and at the end of the plate (strip 12) were compared to those run in strip 1 to calculate concentration recovery.

RESULTS:

1. Optimization of Programming Parameters:

A. Reagent Dispense Accuracy: A solution of p-nitrophenol (PNP) in 0.5M NaOH with 0.01% Triton X-100 was used to optimize the pipetting speed and airgap values in order to determine the best settings to use for the volume sensitive enzyme conjugate solution addition. It was determined for the ChemWell instrument, that the slowest aspiration speed (speed 0), medium dispense speed (speed 2) and an airgap setting of 25µl yielded the best 8-well reagent dispense precision. Using these settings the conjugate could be dispensed with a precision of 1-2 %CV based on 405/630 nm absorbance readings of 100µl PNP solution.

B. Assay Timing: Timing assay steps with a separate timer for each 8-well strip versus timing assay steps with a single timer for the whole 96-well plate was evaluated. It was determined that using a single assay timer which results in some timing differences between different strips, yielded better automated processing than using individual timers for each strip. This was attributed to a reduction of timer conflicts and resulting delays in assay steps occurring between individual assay strips during the shorter assay steps such as the substrate incubation.

C. Plate Mixing: Plate mixing was found to have significant effects on assay results. Microwells processed automatically generally move about and mix more than wells processed manually. Processing assays in a stripwise manner in the ChemWell instrument could cause cumulative mix effects to occur between assay strips. When this happened, early assay strips experienced mixing for a longer time period than latter strips. Since the assay calibrators are typically run first, this could affect the assay accuracy and precision.

Assays with manual method incubation times greater than 1 hour were evaluated for incubation time shortening by using continuous plate mixing during all assay steps. The following assays were found to run successfully with continuous plate mixing: beta-2 microglobulin, C-reactive protein, myoglobin, and troponin. Running with continuous mixing reduced the average assay completion time by approximately one half. The following assays were found to not be suitable for running with continuous mixing: estradiol, T3, T4, and TSH. These assays showed concentration differences in some serum samples when run with and without continuous plate mixing.

D. Sample Predilution: The following assays require a sample predilution prior to loading onto the assay plate: beta-2 microglobulin (1:101), c-reactive protein (1:100) and myoglobin (1:10). It was found that aspiration of the sample diluent, followed by aspiration of 5ul of air, then aspiration of the serum sample and dispensing the sample and diluent together into a sample cup resulted in the best initial sample mixing. In order to achieve the best predilution mixing and thus the best assay precision, it was found that the predilution had to be aspirated and dispensed 3 times to facilitate mixing before the sample is loaded on the assay plate.

2. Evaluation of ChemWell Assay Performance:

A. Precision & Accuracy:

Control Values and Range	ChemWell Results					
	Intra-Assay (n=10)			Inter-Assay (n=20)		
	Mean	S.D.	%CV	Mean	S.D.	%CV
Beta-2 Microglobulin (µg/mL)						
0.7 (0.5-0.8)	0.70	0.00	0.0	0.70	0.00	0.0
1.0 (0.8-1.3)	1.14	0.05	4.5	1.14	0.05	4.3
2.5 (1.5-3.4)	2.76	0.10	3.5	2.85	0.14	4.8
4.9 (3.0-6.8)	5.31	0.31	5.9	5.45	0.37	6.9
C-Reactive Protein (CRP) (mg/L)						
1.6 (1.1-2.1)	1.82	0.06	3.5	1.82	0.06	3.2
2.8 (2.3-3.3)	3.31	0.07	2.2	3.26	0.09	2.9
5.9 (3.5-8.3)	6.26	0.22	3.5	6.19	0.32	5.3
Estradiol (E2) (pg/mL)						
27 (13-57)	25.8	1.4	5.4	25.8	1.6	6.2
286 (220-482)	265.9	13.1	4.9	265.3	15.0	5.6
Ferritin (ng/L)						
60 (39-90)	79.5	1.4	1.7	78.9	1.6	2.0
125 (97-180)	143.5	3.7	2.6	144.1	4.2	2.9
301 (231-426)	372.5	11.8	3.2	375.1	11.8	3.1
Follicle-Stimulation Hormone (FSH) (mIU/mL)						
7.0 (5.2-10.4)	5.31	0.21	4.0	5.29	0.24	4.5
14.6 (12.7-22.2)	14.19	0.26	1.8	14.00	0.41	2.9
53 (41-80)	48.05	0.57	1.2	48.35	0.76	1.6
Human Chorionic Gonadotropin (hCG) (mIU/mL)						
5.5 (2.8-6.4)	5.33	0.28	5.2	5.40	0.23	4.2
16 (12-18)	14.89	0.29	1.9	14.66	0.44	3.0
220 (168-237)	201.00	7.05	3.5	198.08	8.02	4.1
Human Growth Hormone (hGH) (ng/mL)						
4.4 (3.1-5.2)	3.93	0.07	1.7	4.11	0.19	4.7
10.2 (7.5-11.4)	10.33	0.31	3.0	10.59	0.41	3.9
18.5 (13.1-20.8)	20.80	0.49	2.4	20.05	1.06	5.3
Immunoglobulin E (IgE) (IU/mL)						
233 (175-320)	211.7	10.3	4.9	222.2	14.6	6.6
39 (39-62)	41.9	1.3	3.1	44.8	3.3	7.3
44 (41-62)	47.2	1.3	2.8	51.2	4.6	9.0
Luteinizing Hormone (LH) (mIU/mL)						
1.9 (1.0-2.5)	2.18	0.20	9.4	2.24	0.23	10.4
19 (17-22)	20.24	0.41	2.0	20.27	0.10	2.0
48 (43-52)	43.99	0.47	1.1	44.42	0.90	2.0
Myoglobin (ng/mL)						
38 (31-45)	41.1	1.3	3.1	40.1	1.5	3.8
90 (77-103)	102.6	1.5	1.5	98.9	4.3	4.4
298 (258-338)	335.9	5.8	1.7	335.6	9.9	2.9
496 (386-607)	568.0	22.8	4.0	562.3	21.6	3.8
Progesterone (ng/mL)						
5.2 (3.5-7.5)	4.52	0.22	4.9	4.73	0.30	6.4
31.7 (20.0-39.5)	28.79	0.75	2.6	27.85	1.29	4.6
Prolactin (PRL) (ng/mL)						
10.0 (7.4-12.8)	9.37	0.30	3.2	9.37	0.27	2.9
24 (23-27)	22.84	0.33	1.4	23.00	0.62	2.7
53 (47-54)	45.63	1.28	2.8	46.53	1.38	3.0
Testosterone (ng/mL)						
1.2 (0.0 - 2.0)	1.03	0.07	6.6	1.02	0.07	6.6
13.1 (7.3-17.0)	12.76	0.35	2.8	12.73	0.38	3.0
Thyroid Stimulation Hormone (TSH) (µIU/mL)						
0.53 (0.34-0.61)	0.41	0.03	7.7	0.42	0.04	8.8
5.9 (4.7-7.3)	4.72	0.13	2.8	4.79	0.14	3.0
34 (21-36)	22.82	0.38	1.7	22.57	0.46	2.0
Ultrasensitive Thyroid Stimulation Hormone (U-TSH) (µIU/mL)						
0.42 (0.34-0.61)	0.40	0.00	0.0	0.40	0.04	9.8
5.9 (4.7-7.3)	5.83	0.11	1.8	5.93	0.15	2.5
Thyroxine (T4) (µg/dL)						
3.8 (2.4-4.8)	3.66	0.14	3.9	3.69	0.16	4.3
8.3 (5.1-11.2)	8.15	0.33	4.1	8.17	0.36	4.4
13.6 (9.6-17.80	12.92	0.48	3.7	12.83	0.41	3.2
Triiodothyronine (T3) (ng/mL)						
1.1 (0.25-1.63)	0.75	0.05	6.5	0.81	0.08	9.8
2.5 (1.1-3.7)	2.24	0.05	2.3	2.25	0.07	2.9
4.2 (2.1-6.1)	3.27	0.06	1.7	3.28	0.09	2.7
Troponin-I (ng/mL)						
1.7 (1.3-2.1)	1.83	0.07	3.7	1.87	0.09	4.6
7.4 (6.2-8.7)	7.80	0.11	1.4	8.18	0.41	5.0
27.3 (22.7-32.0)	29.02	0.39	1.3	30.36	1.59	5.2
54.8 (42.5-67.2)	63.60	1.01	1.6	64.80	1.79	2.8

B. Manual to ChemWell Result Correlation:

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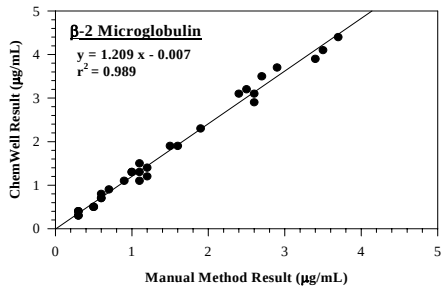

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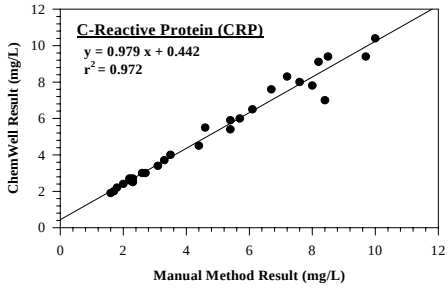

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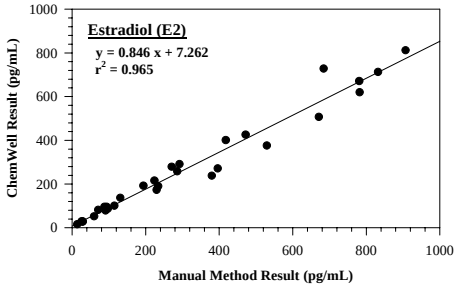

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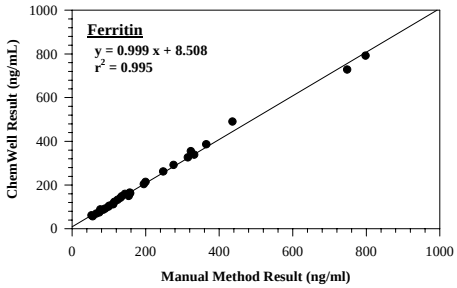

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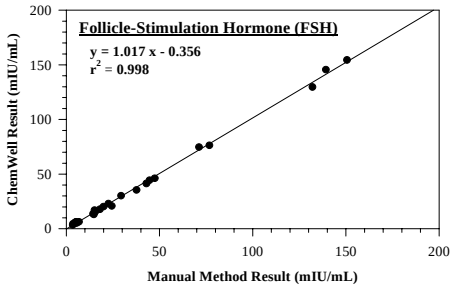

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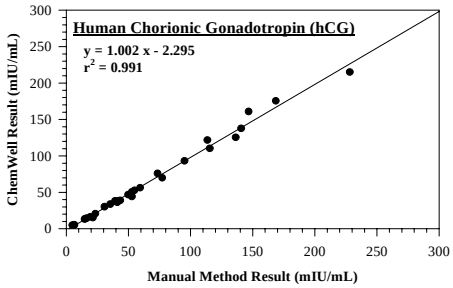

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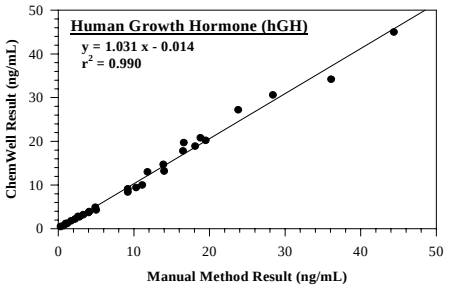

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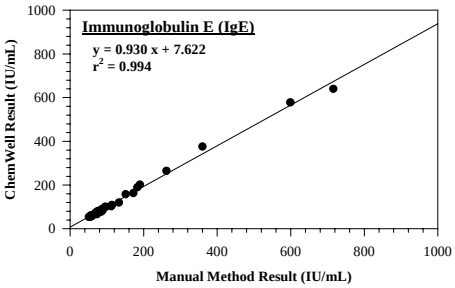

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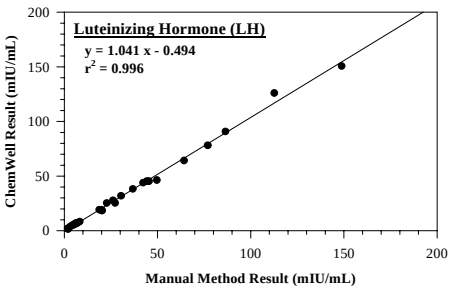

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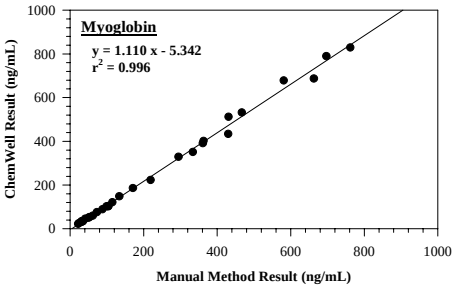

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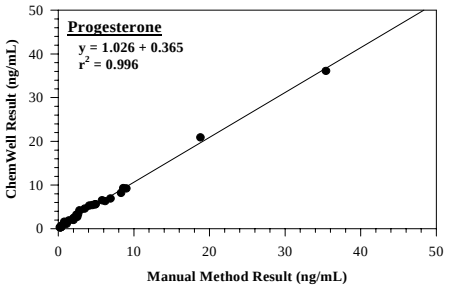

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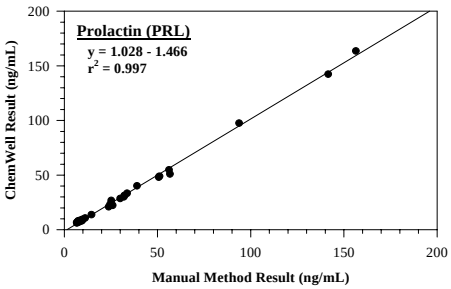

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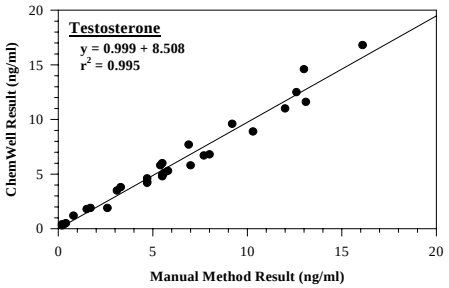

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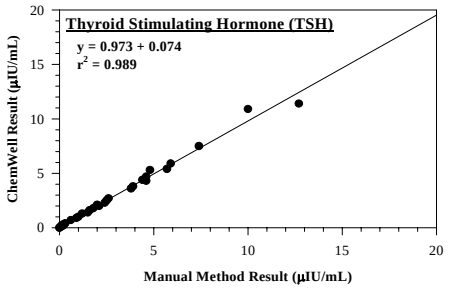

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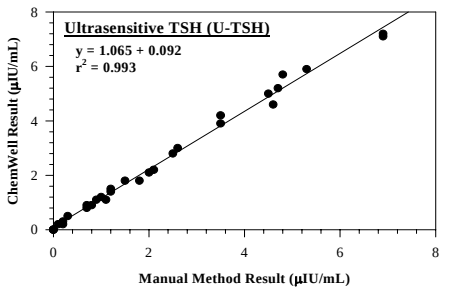

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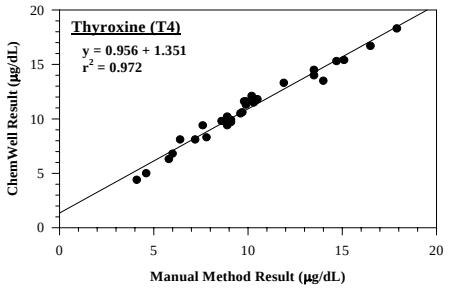

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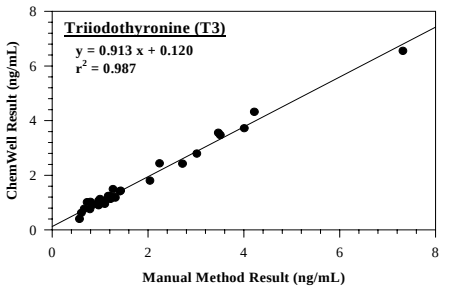

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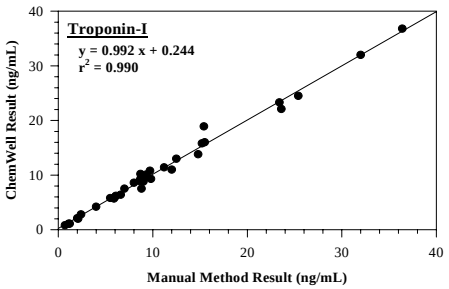

C. Standard Recovery:

Assay	Standard Recovery	
	Range (%)	Average (%)
Beta-2 Microglobulin	100.0 – 111.0	103.8
C-Reactive Protein (CRP)	100.0 – 110.0	105.3
Estradiol (E2)	70.0 – 98.5	91.6
Ferritin	94.3 – 103.3	97.8
Follicle-Stimulation Hormone (FSH)	100.2 – 104.0	102.2
Human Chorionic Gonadotropin (hCG)	92.6 – 102.0	97.7
Human Growth Hormone (hGH)	90.0 – 100.0	95.9
Immunoglobulin E (IgE)	95.0 – 102.5	98.6
Luteinizing Hormone (LH)	96.7 – 101.3	98.3
Myoglobin	96.0 – 103.0	99.5
Progesterone	96.7 – 101.0	99.1
Prolactin (PRL)	97.4 – 100.0	98.6
Testosterone	97.5 – 100.0	99.4
Thyroid Stimulating Hormone (TSH)	91.2 – 100.0	96.6
Ultrasensitive Thyroid Stimulating Hormone (U-TSH)	100.0 – 105.0	102.0
Thyroxin (T4)	99.7 – 110.0	103.2
Triiodothyronine (T3)	90.7 – 100.8	96.7
Troponin-I	95.1 – 114.3	107.7

CONCLUSION:

This study shows that manual ELISA kits can be adapted to run on open programmable automated instruments with equivalent or better performance. With proper instrument programming, excellent assay precision can be achieved as well as good correlation to the manual processing methods. Enzyme conjugate dispense accuracy, plate mixing and incubation timing were the significant factors in achieving good automated processing performance. By processing assays using continuous plate mixing, incubation times for some assays could be shortened without compromising performance. Using the ChemWell instrument's sample dilution feature, assays requiring sample dilutions were also shown to yield good automated performance provided that the dilutions were aspirated and dispensed 3 times to facilitate mixing before loading the diluted sample to the assay plate.

Processing BioCheck ELISAs using the ChemWell instrument yielded an average intra-assay precision of 3.2 %CV and an average inter-assay precision of 4.4 %CV. The manual to ChemWell concentration correlation was also very good, yielding an average r-squared correlation coefficient of 0.989. No significant standard recovery bias was seen when processing these assays using ChemWell, the average standard recovery was 99.7 %.

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