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Laboratory evaluation of the UniCel DxI 800 analyser (Beckman Coulter) for detecting HBV and HCV serological markers

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ABSTRACT

Background: Due to their high prevalence, hepatitis B virus (HBV) and hepatitis C virus (HCV) infections need accurate and rapid diagnosis tools.
Objectives: Technical performances of the UniCel DxI 800 analyser (Beckman Coulter) and a comparison with the Vitros ECi (Ortho Clinical Diagnostics) were performed for five serological markers: HBsAg, total anti-HBc, anti-HBc IgM, anti-HBs and anti-HCV.
Study design: Reproducibility was determined by repeated tests on the manufacturers' controls. The performance of the UniCel DxI 800 was assessed by testing negative and positive samples previously analysed with the Vitros ECi. The accuracy and linearity of anti-HBs assay were evaluated using the WHO international standard (W1042).
Results: The intra-assay and inter-assay coefficients of variation were: 0.8% and 4.4% for HBsAg, 2.4% and 6.2% for anti-HBc, 5% and 8.7% for anti-HBc IgM, 2.1% and 5.1% for anti-HBs and 3.7% and 7.4% for anti-HCV. The two methods were concordant: 100% agreement for the five markers except for the negative HBsAg sera (99%). The anti-HBs results correlated well with the Vitros ECi ($r = 0.925$ with $p < 0.0001$) and the WHO standard ($r^2 = 0.9996$). Throughput was 216 tests/h.
Conclusion: The high throughput, specificity and sensitivity make UniCel DxI 800 assays useful for routine diagnoses of HCV and HBV infections.

1. Introduction

Hepatitis B virus (HBV) and hepatitis C virus (HCV) infections are a great public health problem because of their prevalence worldwide. Thus, screening for these infections is an important part of routine laboratory activity. Quantitative testing for anti-HBs antibodies is crucial for follow-up of HBV vaccination. A threshold of 10 mIU/mL is widely accepted as giving protection against HBV infection.[1] Monitoring anti-HBs is also useful for managing HBV immune globulin prophylaxis in, for example, liver transplant recipients.

Beckman Coulter introduced the UniCel DxI 800 analyser in 2004. This study evaluates its clinical performance using five assays for detecting HBV surface antigen (HBsAg), anti-HBs antibodies, and antibodies against HBV core antigen (total anti-HBc and IgM anti-HBc) and HCV (anti-HCV) in the serum. We compared the accuracy between the UniCel DxI 800 and the Vitros ECi (Ortho Clinical Diagnostics) previously evaluated for HBV and HCV markers.[2,3]

2. Methods

2.1. Immunoassays

UniCel DxI 800 is a multiparametric immunoassay system with random access that uses magnetic particle separation and indirect chemiluminescent technology. Four assays were qualitative: HBsAg, Total anti-HBc, IgM anti-HBc and anti-HCV. Results were calculated as normalized S/CO value, which is the ratio of the sample rate to the cut-off rate. A ratio over 1 is considered to be positive, and a ratio of 0.9–1 doubtful. The measuring range of the anti-HBs quantitative assay was 2–800 mIU/mL.

The Vitros ECi has been used in our laboratory since 2001 and previous studies have validated its performance for HBV and HCV serological testing.[2,3] We therefore used the Vitros ECi as reference in this evaluation of the HBV and HCV assays recently developed for the Beckman Coulter UniCel DxI 800. The VITROS ECI analyser also uses chemiluminescent technology. The range of the anti-HBs assay was 8–1000 mIU/mL. Both anti-HBs assays used inactivated HBsAg of human origin (subtypes ay and ad).

Vitros ECi measurements were performed in our routine practice by experimented technicians. UniCel DxI 800 assays were performed in March 2007 by two technicians. Their training was

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Table 1
Intra-assay precision of UniCel DxI 800 assays.

	HBsAg		Anti-HBs		Anti-HBc		Anti-HBc IgM		Anti-HCV	
	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos
n	20	20	20	20	20	20	20	20	20	20
Mean	0.28	884.13	0.64	58.16	0.16	3.18	0.01	1.64	0.10	3.23
S.D.	0.01	7.36	0.07	1.19	0.01	0.08	0.00	0.08	0.00	0.12
%CV	4.46	0.83	10.56	2.05	4.97	2.38	0.00	5.00	4.56	3.67

Table 2
Inter-assay precision of UniCel DxI 800 assays.

	HBsAg		Anti-HBs		Anti-HBc		Anti-HBc IgM		Anti-HCV	
	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos
n	26	27	33	33	36	36	36	36	31	31
Mean	0.25	7.5	0.55	57.08	0.18	3.02	0.01	1.85	0.08	2.80
S.D.	0.07	0.33	0.15	2.93	0.03	0.19	0.00	0.16	0.01	0.20
%CV	29.77	4.35	26.86	5.13	18.61	6.22	34.99	8.73	7.54	7.40

performed and validated by Beckman Coulter technical assistance on site during 3 days. Both analysers were used following manufacturer's recommendations. The results of the Vitros ECi tests were not available to the personnel reading the UniCel DxI 800 results.

2.2. Study design

Intra-assay variations were determined by measuring 20 replicates of the manufacturer's positive and negative controls in a single run. The inter-assay precision was determined using the same controls; they were tested each day throughout the evaluation period. Means, standard deviations (S.D.) and coefficients of variation (CV) were then calculated.

The two analysers were compared using a retrospective data collection of patients' sera that had been previously assayed in our laboratory with the Vitros ECi and stored at $-23 \pm 5\,^{\circ}C$. Storage did not exceed 2 years which warranted the stability of antibody concentrations. Demographic or clinical information were not available. The numbers of negative and positive samples compared were: 104 and 108 for HBsAg, 225 and 36 for total anti-HBc, 103 and 21 for anti-HBc IgM, 120 and 124 for anti-HBs and 109 and 26 for anti-HCV.

The accuracy and linearity of the anti-HBs assay were evaluated using the WHO international reference anti-HBs standard (code W1042, provided by NISBC, Hertfordshire, UK, enquiries@nibsc.ac.uk) by testing five replicates of serial dilutions (500, 250, 100, 50, 25 and 10 mIU/mL).

The throughput was determined using samples tested for any of the five analytes. Thus, two series of 116 samples were loaded onto the UniCel DxI 800, 5 min apart. These samples underwent 407 tests, each of which included tests for at least HBs Ag, anti-HBc and anti-HCV.

2.3. Statistical analysis

The quantitative anti-HBs assay was analysed by the Spearman regression test and differences by Student's *t*-test or the non-parametric Mann–Whitney *U*-test. Statistical significance was set at $p < 0.05$. Anti-HBs concentrations were also compared by Bland–Altman analysis.[4]

3. Results

3.1. Intra-assay and inter-assay precision

The intra-assay and inter-assay precision data are summarized in Tables 1 and 2. They showed excellent reproducibility for positive samples, with intra-assay coefficients of variation less than 5% and inter-assay coefficients of less than 8.73%.

3.2. Laboratory evaluation on routine samples

Table 3 summarizes the qualitative results obtained with the two analysers. Concordance was nearly perfect. There was only one discordant result for HBsAg. A negative serum was assayed positive with the UniCel DxI 800, but the antigen response was very low, close to the threshold. Additional serum was not available to check this result. Thus, the relative sensitivities and specificities (including 95% confidence interval) were 100% (96.6–100.0) and 99% (94.8–99.8) for HBsAg, 100% (97.0–100.0) and 100% (97.0–100.0) for anti-HBs, 100% (90.0–100.0) and 100% (98.3–100.0) for total anti-HBc, 100% (84.5–100.0) and 100% (96.4–100.0) for anti-HBc IgM, 100% (87.1–100.0) and 100% (96.6–100) for anti-HCV.

3.3. Evaluation of anti-HBs quantitative results

The serial dilutions of the WHO anti-HBs standard were measured by the UniCel DxI 800 (Table 4). The percentage of recovery was good ranging from 83.0% to 93.2%. The five replicates of the 10 mIU/mL standard were all detected with excellent reproducibility. The regression curve obtained by testing serial dilutions of the standard was linear (Fig. 1).

Table 3
Comparison of UniCel DxI 800 and Vitros ECi results.

		UniCel DxI									
		HBsAg		Anti-HBs		Anti-HBc		Anti-HBc IgM		Anti-HCV	
		Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg	Pos
Vitros	Neg	103	1	120	0	225	0	103	0	109	0
	Pos	0	108	0	124	0	36	0	21	0	26

Table 4
Results for dilutions of WHO anti-HBs standard tested with the UniCel DxI 800 anti-HBs assay.

Theoretical concentration	DxI results				
	Minimum	Maximum	Mean	%CV	Difference
500	441.0	449.0	444.8	0.7	55.2
250	224.0	239.7	230.6	2.5	19.4
100	89.9	96.5	93.2	2.8	6.8
50	45.4	47.7	46.3	2.0	3.7
25	21.0	23.6	21.8	4.9	3.2
10	8.0	9.0	8.3	4.8	1.7

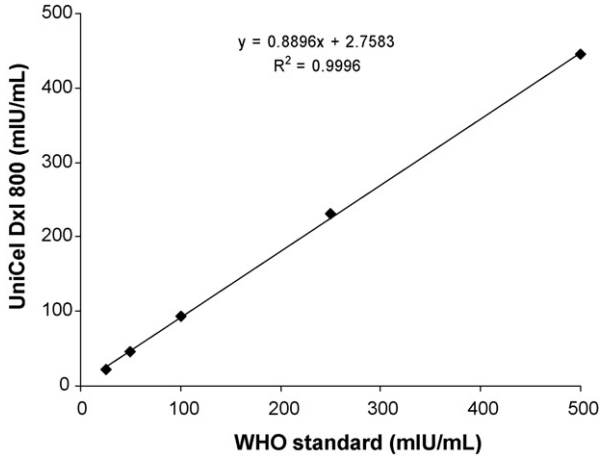

Fig. 1. Evaluation of linearity by testing serials dilutions of the WHO standard.

The regression curve obtained with samples within the measuring range of both analysers (110/124 sera) is shown in Fig. 2. The Spearman test coefficient was 0.925 ($p < 0.0001$). The correlation was kept for results <100 mIU/mL. The anti-HBs concentrations were also compared by Bland–Altman analysis (Fig. 3). The mean bias, expressed as a percentage, was very low (1.5%), although some samples differed markedly.

3.4. Throughput

The performance of the UniCel DxI 800 was evaluated with reference to two different criteria. The first criteria were pipetting

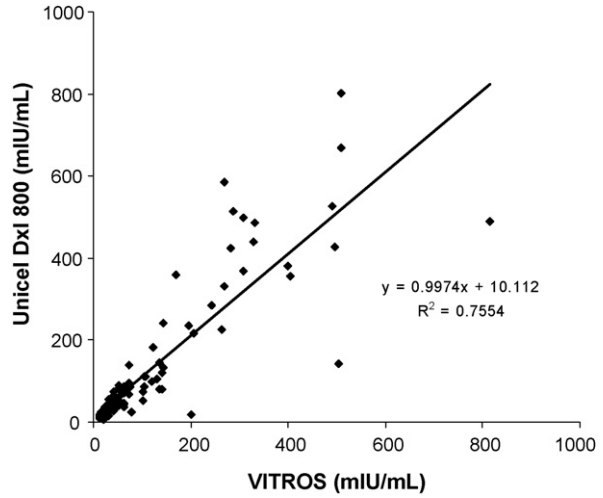

Fig. 2. Correlation between UniCel DxI and VITROS ECi for anti-HBs data.

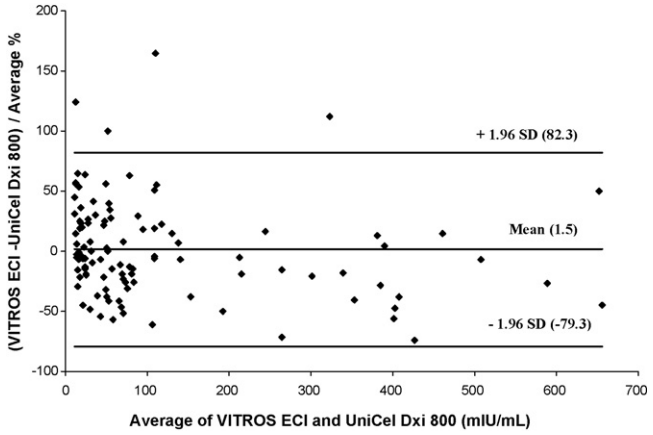

Fig. 3. Bland–Altman distributions of VITROS ECi and UniCel DxI 800 anti-HBs results. Difference is expressed as a percentage versus average expressed in mIU/mL.

throughput. The 116 samples were processed in 32 min, giving an average pipetting throughput of 217 samples/h. Concerning the second criteria, all the 407 results were produced in 113 min, giving an average throughput of 216 tests/h.

4. Discussion

The majority of recently developed automated immunoassays uses chemiluminescent technology and provides high throughput, sensitivity and specificity. Several such systems are now available for virological diagnosis. Five of the systems tested in the very recent comparison of nine anti-HBs assays[5] used chemiluminescence (Vitros ECi from Ortho Clinical Diagnosis, Architect from Abbott Diagnostics, Advia Centaur from Siemens Diagnostics, Liaison from Diasorin, and Elecsys from Roche Diagnostics).

The technical performances of all the UniCel DxI 800 assays tested were excellent, giving intra-assay coefficients of variation of less than 5%, and inter-assay coefficients of 8.7%. These results were obtained for both positive controls and the dilutions of the WHO anti-HBs standard. They are consistent with the published Beckman specifications and with the anti-HBs assay developed for the Beckman Coulter Access analyser.[6]

Concerning the clinical performance, agreement rates between Vitros ECi and UniCel DxI 800 assays were very high. With reference to Vitros ECi, relative sensitivity and specificity were 100% except for HBsAg where one discrepant result was obtained. Hence, we confirmed that in a routine laboratory testing, all the assays meet the criteria of the common technical specifications of the European Union's Directive on In Vitro Diagnostic Medical Devices[7] in terms of specificity and sensitivity. Previous studies have investigated the sensitivity and specificity for HBV and HCV testing on various analysers: Access,[6] Vitros,[2,3] Advia Centaur,[8,9] Architect.[10] Overall results ranged from 97.4% to 100% for sensitivity and from 92.8% to 100% for specificity. Huzly et al. compared nine available assays for quantitative anti-HBs and found sensitivities of 93.5–100% and specificities of 96.8–100%.[5] These data are not in agreement with the common technical specifications of the European Union's Directive. They questioned the problem of the "reference value" for comparing all these assays and the need for multicentre studies.

The problem of quantitative assays like anti-HBs measurements is more complex. We find that the UniCel DxI 800 assay was closely calibrated upon WHO standard. We confirmed that UniCel DxI 800 detects samples of 10 mIU/mL, in agreement with the specifications of the assay. This point is crucial, since this concentration is assumed to protect against HBV infection.[1] The widely accepted

International and European standard for quality in medical laboratories, ISO 15 189,[11] recommends that laboratories determine the uncertainty associated with their quantitative results. This uncertainty should be useful for determining the grey zone around this critical value of 10 mIU/mL. Our results, good reproducibility and little variation from the WHO standard, indicate that this grey zone will be very narrow for the UniCel DxI 800 assay. This approach is also critical in some clinical situations, where medical decisions are based on anti-HBs thresholds. For example, some authors have proposed giving anti-HBs immune globulin when anti-HBs titers drop below 70 mIU/mL in the follow-up of HBV-infected liver transplant recipients.[12] Some countries have also introduced a 100 mIU/mL concentration in their recommendations for HBV vaccination.[5]

The UniCel DxI 800 and Vitros ECi anti-HBs assays appeared to be significantly correlated, with a very little difference in Bland–Altman analysis. The mean difference of 1.5% we obtained is similar to the mean difference of 2.9% recently found between the Vitros ECi and Architect.[5] However, this study found mean differences of over 50% for some pairs of analysers, and the mean coefficient of variation was 47.1% (15.0–201.0). The authors concluded that the results were not reliable, even though an international standard was used for calibrations. There may also be large discrepancies between data for individual samples, even when the assays are well correlated, and this must be taken into account if long-term monitoring of anti-HBs is needed. The situation is similar for anti-rubella IgG assays, where improved standardization is also required.[13] The origin of these discrepancies is unclear, although several hypotheses have been advanced. The WHO standard, which is an immunoglobulin preparation rather than a serum, could generate a matrix effect. Heterogeneity could be due to the origin of the HBsAg used in the assays (human or recombinant). The quality of the immune response may also vary depending on the clinical situation or on the vaccine antigen, so making assays unreliable.[14] Thus, when a long-term and accurate follow-up of anti-HBs levels is required, the use of the same assay should be recommended.

The UniCel DxI 800 appears to be easy to use in routine practice, even for technicians with little experience. It is provided with cutting-edge equipment: clot detection, liquid level sensing, reflex testing and reduced daily (10 min) and weekly (30 min) maintenance. It can store up to 50 assays at 2–8 °C and requires sample volumes of 5–110 μL. Its maximum throughput is 400 tests/h. We routinely obtain a throughput of 217 samples/h and 216 tests/h. This is higher than previously published data; the throughputs of the Architect, Elecsys and Vitros ECi under routine conditions were 123, 97 and 46 tests/h, respectively,[15] and 102 for the Advia Centaur.[16]

Finally, the UniCel DxI 800 fulfills the requirements of the Common Technical Specifications of the European Union for the HCV and HBV serological markers tested in our laboratory evaluation. It also combines practical convenience and probably the best throughput for this type of analyser.

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