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Evaluation of a kinetic rate assay method for serum alpha-L-fucosidase activity in patients with hepatocellular carcinoma using a novel substrate. J. Wang[1], E. Cao[2]. [1]Beijing Bei Hua Fine Chemicals Co. LTD., Beijing 100022 China, Beijing, China, [2]Institute of Biophysics, Chinese Academy of Sciences, Beijing 100101 China, Beijing, China.

Objective: Developing kinetic rate assay kit for alpha-L-fucosidase (AFU) using a novel substrate CNPF(2-chloro-4-nitrophenyl-alpha-L-fucopyranoside) and clinical implication in the diagnosis of hepatocellular carcinoma (HCC).
Methods: Evaluating the developing kinetic rate assay kit by determining the enzyme activity levels in patients with HCC, other malignant neoplasm and healthy subjects.
Results: We have evaluated the kinetic rate assay kit for serum AFU using a novel substrate without the need of any sample blank determination employing Hitachi 7170 automated analyzer. The reaction was carry out at 37°C, monitoring the wavelength at 405 nm. The linearity was observed up to 300 U/L. The intra-assay precision (n=10), SD 0.47; CV 2.6 (control, 17.8 U/L) SD 0.31; CV 0.79% (control, 39.71 U/L) and SD 0.79; CV 0.77% (control, 102 U/L). Interference of various substances such as ascorbic acid (6 g/L), hemoglobin (220 mg/L), bilirubin (200 mg/L) showed no significance effects on the assay.
Serum samples from 884 Chinese people including 518 healthy adults and 366 patients were determined using the developing rate assay kit. The normal values in healthy adults was estimated at 22.8 + 7.1 U/L. No significant difference was found between male and female groups ($p > 0.05$). The mean value of serum AFU activity in patients with HCC was significantly higher than that found in patients with chronic hepatitis, other malignant neoplasm, cirrhosis, other sickness and control subjects. No significant differences were found between controls and patients with cirrhosis or patients with other malignant neoplasm or chronic hepatitis or other sickness. Using this kit, AFU sensitivity and specificity is found to be 82.5 % and 88.2 %.
Conclusions: The new kinetic rate kit surmount the defects of previous methods for determining AFU activity and has higher sensitivity and specificity and This kit can be used in large-scale screening for AFU activity without the need of any sample blank determination and adapted to variety of automated clinical analyzers.

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ARCHITECT ® CA 15-3, a chemiluminescent assay. J. G. Hofler[1], T. R. Kettlety[1], D. L. Wolaniuk[1], S. B. Smith[1], E. M. Schmidt[1], G. A. Smith[1], W. E. Covert[1], J. A. Friese[2]. [1]Fujirebio Diagnostics, Inc, Malvern, PA, [2]Abbott Laboratories, Abbott Park, IL.

Previously treated breast cancer is managed with the aid of the measurement of CA 15-3 assay values. The ARCHITECT CA 15-3 Assay is a chemiluminescent automated assay (CMIA) using the CHEMIFLEX® technology which allows for excellent sensitivity, precision and accuracy. The assay described in this abstract is a two step assay utilizing paramagnetic microparticles coated with monoclonal antibody 115D8 and acridinium labeled monoclonal antibody DF3 conjugate. Sample, microparticles and diluent are combined in the first step. The reaction mixture is incubated. Following a wash, the DF3 conjugate is added to the mixture in the second step. Pre-trigger and Trigger solutions are added to the reaction mixture. The chemiluminescence produced is measured as relative light units (RLUs). The RLUs generated are directly proportional to the amount of CA 15-3 antigen in the sample. Preliminary precision studies were completed over 20 days with 2 runs per day and 2 replicates per run (80 total replicates) on two separate instruments using two separate lots of reagents. For buffer-based panels with concentrations of 40 to 250 U/mL, total CV's ranged from 3.2 to 4.9%. For serum-based panels with concentrations of 27.0 to 683.2 U/mL, total CV's ranged from 2.2 to 5.1%. The analytical sensitivity was <0.1 U/mL. Patient samples containing high levels of CA 15-3 were diluted with Multi-Assay Manual Diluent. The mean dilution recovery for 10 samples was 92% with a range of 85%-98%. Interference for protein, lipid, bilirubin and hemoglobin was less than 12%. One hundred fifty-three samples, within the dynamic range of the assay, were analyzed on the ARCHITECT and AxSYM for the presence of CA 15-3. The calculated linear regression produced a slope of 0.94, an intercept of +1.37 and an r-value of 0.973. The addition of the CA 15-3 assay will expand the cancer menu on the ARCHITECT System. In conclusion, an accurate, sensitive and precise CA 15-3 assay has been developed for the ARCHITECT instrument system.

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Evaluation of latex-enhanced turbidimetric reagents for measuring free immunoglobulin light-chains on the Bayer Advia 1650. H. D. Carr-Smith[1], B. Harland[2], J. Anderson[2], J. Overton[1], G. Wieringa[2], A. R. Bradwell[3]. [1]The Binding Site Ltd., Birmingham, United Kingdom, [2]Christie Hospital NHS Trust, Manchester, United Kingdom, [3]University of Birmingham, Birmingham, United Kingdom.

Assays specific for serum immunoglobulin free light chains (FLC) that are compatible with commonly used laboratory nephelometric and turbidimetric analyzers have recently become available. Studies with these assays have shown that serum FLC measurement is useful for the diagnosis and monitoring of patients with primary amyloidosis, non-secretory myeloma and light chain myeloma. Here we describe development of serum FLC assays for use on the Bayer Advia 1650 and evaluate their performance. The main assay characteristics are summarised in the table below.
Interference was within ±3.5% when bilirubin (200mg/L), hemoglobin (5g/L) or chyle (1,930 formazine turbidity units) were added to serum samples with known FLC concentrations. Linearity was assessed by measurement of serially diluted serum samples and comparison of expected with actual results:- kappa free: y=0.98x-3.38mg/L, R^2=0.995; lambda free: y=1.03x+1.44, R^2=0.98. Both assays were linear over the range tested. These assays were compared with the FLC assays for the Hitachi 911. Serum samples from normal subjects, patients with systemic lupus erythematosus and multiple myeloma were assayed for FLC on both systems and the results were compared by regression analysis:- kappa free y=1.13x-0.87mg/L (R=0.94, n=46); lambda free y=1.08x-39.50mg/L (R=0.96, n=50). The assays for the Bayer Advia 1650 provide a rapid, precise method of measuring FLC in serum and show good agreement with existing assays.

Main assay characteristics

Assay	Free Kappa	Free Lambda
Range (mg/L)	3.75-619	7.0-933
Sample dilution	1/5	1/5
Sensitivity (mg/L)	1.2	1.4
Assay time (minutes)	13.5	13.5
Intra-assay precision (n=15)	5.1 (5.82)	1.7 (4.66)
%CV (Mean mg/L)	2.8 (18.0)	4.3 (12.3)
	3.9 (22.5)	2.6 (34.4)
	3.2 (34.9)	1.6 (65.7)
Inter-assay precision (n=15)	5.0 (13.9)	3.8 (26.3)
%CV (Mean mg/L)	4.4 (30.5)	2.7 (50.4)

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Analytical and clinical performance of Beckman Coulter's Access® BR Monitor (CA 15-3* Antigen) immunoassay. K. G. Loveland[1], C. Carollo-Neumann[2], R. E. Parson[1], T. Smith[2], J. Todtleben[2], I. A. Mizrahi[1]. [1]Beckman Coulter, Inc, San Diego, CA, [2]Beckman Coulter, Inc, Chaska, MN.

The Access BR Monitor assay is a fully automated, random access chemiluminescent assay developed for the quantitative measurement of CA15-3 antigen levels in serum and plasma (heparin) using the Access® Immunoassay Systems. This assay is intended for use as an aid in the management of patients diagnosed with breast cancer.
Methods: The assay is a one-step immunoenzymatic sandwich assay utilizing monoclonal anti-CA15-3 coated paramagnetic particles, alkaline phosphatase conjugated anti-CA15-3 and chemiluminescent detection. The chemiluminescence signal of analyte in the sample is read from a stored, 6-point calibration curve spanning 0-1000 U/mL.
Studies: Studies included: 1) Analytical performance (analytical sensitivity, precision, linearity upon dilution (LUD), and interfering substances); 2) Beckman Coulter's Access vs. Abbott AxSYM CA15-3 methods comparison (n=435); 3) Expected values for apparently healthy females (n=304); 4) Distribution of CA15-3 concentrations from patients with various non-malignant and malignant conditions (n=1295); 5) Serial monitoring samples from patients (n=36) diagnosed with breast cancer (n=140 samples); 6) Relative (n=140) and clinical (n=64) sensitivity and specificity for Access and AxSYM assays.
Results: Analytical sensitivity, the lowest detectable level of CA15-3 antigen distinguishable from zero with 95% confidence, is 0.5 U/mL. The assay exhibits within-run, between-run, and total imprecision ranging from 1.4 to 4.6%CVs for Access assay concentrations ranging from 14.7 to 661.8 U/mL. LUD recoveries for six elevated samples ranged from 88.9% to 112.5%. No significant interference was observed with common chemotherapeutic agents and other potential interferents with Access assay recoveries ranging from 96% to 107%. Methods comparison using Deming regression analysis gave the following results (0-250 U/mL range, n=435): slope=0.82, Y-intercept=1.92, r=0.91. Comparable CA15-3 results were obtained with