Clinical Chemistry and Immunoassay Testing Supporting the Individual Healthy Life

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OVERVIEW: Clinical laboratory testing provides the information needed for the diagnosis and treatment of disease. Hitachi's automated analyzers for clinical testing have undergone rapid advancement since the shipping of the first system manufactured (Japan) in 1971. Efforts to increase the reliability of clinical data and measurement sensitivity have rapidly increased the test menu that can be measured in clinical chemistry testing and immunoassay. Furthermore, the integration of automated chemistry analyzers and immunoassay systems has made it possible to do both chemistry testing and immunoassay with a single system, thus reducing the workload in clinical laboratories. Now, as policies for control of medical expenses are becoming increasingly strict, there is a demand for even more efficiency in the automated analyzer and for higher quality in the test data. To meet that demand, Hitachi continues to improve the functionality of the automated analyzer.

INTRODUCTION

TESTING for the constituents of blood and urine provides evidence for diagnosis, and has been effective in the early detection and prediction of illness. Hitachi extended spectrometer technology to clinical testing, and in 1971 it shipped the 400 model, Japan's first domestically produced automated chemistry analyzer. The automated chemistry analyzer rapidly measures enzymes, lipids, electrolytes, proteins and other such components in blood and urine, a process known as biochemical testing. The system is widely used by many hospitals and clinics for diagnosis and comprehensive health examinations.

In the 1980s, there was a high demand for measurement of hormones and cancer markers and other such disease-specific labels. Many of such components exist in the blood serum only in very small amounts. For that reason, immunoassay techniques were developed. Immunoassay uses the highly specific antigen-antibody reaction, and thus features highly sensitive measurement. Immunoassay was initially positioned as special testing. A special automated analyzer was developed for immunology and its use rapidly spread. With the increasing use of immunoassay, a high demand developed for a system that integrates biochemical testing and immunoassay to reduce the clinical testing workload. In response to that demand, Hitachi led the world in developing an integrated automated analyzer product.

Fig. 1—Next-generation cobas 6000 Analyzer Series Automated Analyzer.
The cobas c 501 analyzer for clinical chemistry testing (left) and the cobas e 601 analyzer for immunoassay (right) are combined. The system is marketed worldwide by Roche Diagnostics.

The current environment of strict policies concerning the cost of medical care demands improvements in the automated analyzer for even higher efficiency and test data quality. Hitachi continues to improve automated analyzer functionality to meet that demand.

NEXT-GENERATION AUTOMATED ANALYZER WITH EXPANDED TEST MENU

Hitachi, in collaboration with Roche Diagnostics, has developed the biochemical and immunoassay automated analyzer for clinical testing, and that system holds the top share of the world market. The most recent next-generation automated analyzer, the cobas* 6000 analyzer series[1], is shown in Fig. 1.

The cobas 6000 analyzer series combined the cobas c 501 analyzer for biochemical testing and the cobas e 601 analyzer for immunoassay into a single integrated system. It was equipped with new technology and new functions, and the number of test menus that it could test rapidly increased. Now, in October 2007, the system can measure over 150 test menus in biochemical and immunoassay testing.[2] This single system covers about 95% of the workload for biochemistry and immunoassay testing.

The progression in the number of test menus that can be analyzed for the Hitachi model 400 automated analyzer and the Hitachi model 705 automated analyzer[3], a world-wide best seller that was first shipped in 1980, is shown in Table 1. With the most recent generation cobas 6000 analyzer series, there was a very large increase in analysis test menus.

*cobas is a registered trademark of Roche Diagnostics.

INCREASING CLINICAL DATA RELIABILITY

We take the cobas 6000 analyzer series as an example of our efforts to increase the reliability of clinical data.

Reduction of Sample Carry-over

The concentration range of blood constituents may extend to the 6th decimal place, so measures to reduce

TABLE 1. Changes in Test Menus

Each generation of automated analyzer adds new test menus.
The latest cobas 6000 analyzer series can measure over 150 biochemical and immunoassay test menus with a single system.

Substrates	Model 400	Model 705	cobas 6000
Albumin	○	○	○
Ammonia			○
Bicarbonate			○
Bilirubin-direct		○	○
Bilirubin-total	○	○	○
Calcium		○	○
Cholesterol		○	○
HDL-Cholesterol			○
LDL-Cholesterol			○
Creatinine enz.			○
Creatinine Jaffe		○	○
Fructosamine			○
Glucose		○	○
Iron		○	○
Lactate		○	○
Magnesium		○	○
Phosphorus	○	○	○
Total Protein	○	○	○
Total Protein U/CSF			○
Triglycerides		○	○
Triglycerides GB			✱
UIBC		○	○
Urea/Bun BUN	○	○	○
Uric Acid	○	○	○

Drugs of Abuse	Model 400	Model 705	cobas 6000
Amphetamines			○
Barbiturates			○
Benzodiazepines			○
Cannabinoids			○
Cocain Metabolite			○
Ethanol		○	○
Methadone			○
Methaqualone			○
Opiates			○
Phencyclidine			○
Propoxyphene			○
LSD			○

Enzymes	Model 400	Model 705	cobas 6000
ACP			○
ALP	○	○	○
ALT/GPT	○	○	○
Amylase-tot.		○	○
Amylase-pancr.		○	○
AST/GOT	○	○	○
Cholinesterase		○	○
CK		○	○
CK-MB		○	○●
GGT		○	○
GLDH		○	○
HBDH		○	○
LDH		○	○
Lipase			○

Proteins	Model 400	Model 705	cobas 6000
α1-Acid Glycoprotein			○
α1-Antitrypsin		○	○
α1-Microglobuline			○
β2-Microglobuline			○
Albumin (immuno)			○
APO A1			○
APO B			○
ASLO		○	○
ATⅢ		○	○
C3c		○	○
C4		○	○
Ceruloplasmin		○	○
CRP		○	○
CRP High Sensitivity		○	○
D-Dimer			○
Ferritin			○●
Haptoglobin		○	○
HbA1c (whole blood)			○
IgA		○	○
IgG		○	○
IgM		○	○
Kappa Light Chains			○
Lambda Light Chains			○
Lipoprotein (a)			○
Myoglobin			○●
Prealbumin			○
RF		○	○
Soluble Transferrin Receptor			○
Transferrin		○	○

TDM, medication monitoring	Model 400	Model 705	cobas 6000
Acetaminophen			○
Amikacin		○	✱
Carbamazepine		○	○
Cyclosporine			✱
Digitoxin			○
Digoxin		○	○●
Gentamicin		○	○
Lidocaine		○	○
Lithium			○
MPA			○
NAPA			○
Phenobarbital		○	○
Phenytoin		○	○
Procainamide			○
Quinidine			✱
Salicylate			○
Theophylline		○	○
Tobramycin		○	✱
Valproic Acid		○	○
Vancomycin			○

Electrolytes	Model 400	Model 705	cobas 6000
Chloride		○ISE	○ISE
Potassium		○ISE	○ISE
Sodium		○ISE	○ISE

Animia	Model 400	Model 705	cobas 6000
Ferritin			●
Folate			●
Iron		○	○
Transferrin		○	○
Vitamin B12			●

Bone Metabolism	Model 400	Model 705	cobas 6000
β-CrossLaps			●
Calcium		○	○
Osteocalcin			●
25- (OH) 2 Vitamin D3			✱
P1NP			●
PTH			●

Thyroid Function	Model 400	Model 705	cobas 6000
Anti-Tg			●
Anti-TPO			●
FT3			●
FT4			●
T3			●
T4		○	●
T-uptake			●
TG		○	●
TSH			●
TSHr Ab			●

Diabetes	Model 400	Model 705	cobas 6000
C-Peptide			●
Glucose		○	○
HbA1c (whole blood)			○
Insulin			●

Tumor Markers	Model 400	Model 705	cobas 6000
AFP			●
CA 125			●
CA 15-3			●
CA 19-9			●
CA 72-4			●
CEA			●
CYFRA 21-1			●
Free PSA			●
NSE			●
S-100			●
Total PSA			●

Rheumatoid Arthritis	Model 400	Model 705	cobas 6000
Anti-CCP			✱

Cardiac	Model 400	Model 705	cobas 6000
CK-MB (mass)			●
CK-MB (mass) STAT			●
CRP High Sensitivity			○
Myoglobin			●
Myoglobin STAT			●
NT proBNP			●
Troponin T			●
Troponin T STAT			●

Featility/Hormones	Model 400	Model 705	cobas 6000
ACTH			●
Cortisol			●
DHEA-S			●
Estradiol			●
FSH			●
HCG+β			●
HCG STAT			●
LH			●
PAPP-A			✱
Progesterone			●
Prolactin			●
SHBG			●
Testosterone			●

Infectious Disease	Model 400	Model 705	cobas 6000
Anti-HAV			●
Anti-HAV IgM			●
Anti-HBc			●
Anti-HBc IgM			●
Anti-HBe			●
Anti-HBs			●
CMV IgG			✱
CMV IgM			✱
HBeAg			●
HBsAg			●
HIV Ag			●
HIV combi			●
Rubella IgG			●
Rubella IgM			●
Toxo IgG			●
Toxo IgM			●

Others	Model 400	Model 705	cobas 6000
IgE			●
Serum Index		○	○
RBC Folate Hemolyzing Reagent			●
Total Mycophenolic Acid			○

○ : clinical chemistry　○ISE: biochemical ion-selective electrode　● : immunoassay testing, cobas 6000 e 601 analyzer　✱ : currently under development

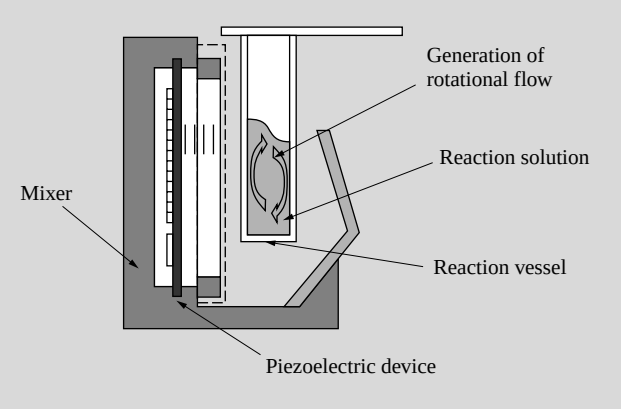


Fig. 2—Ultrasonic Mixer.[4]
The reaction solution is mixed by a vertical rotational flow induced within the reaction vessel.

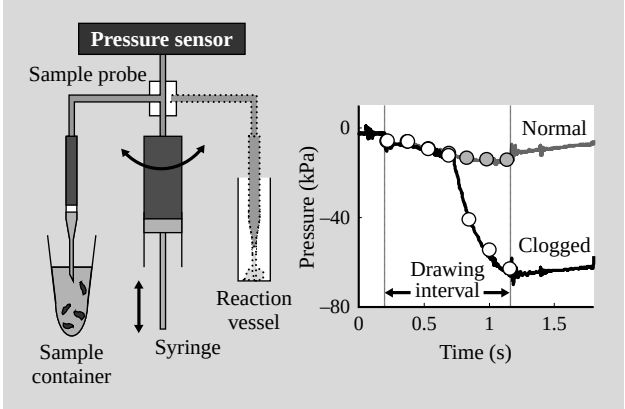


Fig. 4—Pressure Monitoring as Sample is Drawn.[9]
The pressure waveform within the sample probe is compared to a normal waveform to detect clogging in the sample probe.

Fig. 3—Non-contact Mixing of the Reaction Solution.
High-speed photographs of a pigmented aqueous solution being mixed in special reaction incubator and reaction vessel.

carry-over between samples are required when samples from many patients are processed consecutively through the same sample pipette mechanism or the same reagent mixing mechanism in an automated analyzer.

To reduce sample carry-over, the cobas c 501 analyzer implements a non-contact type of reagent mixing[4]. A piezoelectric device is used to subject the side of the reaction vessel to high-frequency ultrasonic sound waves, which create a rotating flow within the reaction vessel to mix the reagents (see Fig. 2).

In earlier systems, the mixing of sample and reagent was done by inserting a stirring rod into the liquid. The non-contact method eliminates sample carry-over by liquid adhering to the stirring rod[5], [6], [7]. Stirring by ultrasonic waves[8] is illustrated in Fig. 3.

Immunoassay requires even more rigorous reduction of sample carry-over than does biochemical testing. For example, in the case of test items that have a wide range of concentrations in blood, such as the HCG (human chorionic gonadotropin stimulating hormone) used in pregnancy testing, a large carry-over from a highly concentrated sample to the next patient sample could lead to false results. The same is true for items that are evaluated as positive or negative, such as infectious items.

The parts of the cobas e 601 analyzer that come in contact with the sample are disposable. Disposable sample pipette tips and reaction vessel are used and the mixing method involves no contact, so sample carry-over is eliminated.

Reduction of Problems Caused by Sample Intake

Patient samples may contain fibrin or other solid materials that can clog the sample probe and prevent the drawing in of the prescribed amount of sample. To detect the drawing in of foreign material or other such abnormalities, a pressure sensor is placed in the flow path between the sample probe and the syringe to monitor the flow pressure (see Fig. 4). The change in pressure within the sample probe is analyzed to automatically detect abnormalities such as clogging of the sample probe[9]. Confirming that the specified amount of sample is drawn ensures the reliability of test results.

Reduction of Problems Caused by Reagents

To reduce the problems caused by the handling of reagents, and thus ensure the reliability of analysis data, we use premeasured reagent packs[1] (see Fig. 5). The prepared reagent packs eliminate human error in the preparation of reagents, and so reduce problems. Also, because the packs can be simply set into the system, the work of preparing the reagents and inserting them into the system can be eliminated. The reagent pack is fitted with a cover called the lid so that it is always sealed. Optimization of the reagent pipette probe structure allows direct drawing of the reagent

Fig. 5—Cobas c Packs.
Cobas c 501 analyzer reagent packs are shown. Reagents are drawn directly from the sealed reagent pack.

TABLE 2. Improved Test Sensitivity of Immunoserological TSH
The development of an analytical method increases the sensitivity of immunoserological TSH (thyroid stimulating hormone).

Generation	Sensitivity uIU/mL	Measurement method
First generation	1.0	Radioisotope-immunoassay (RIA)
Second generation	0.1	Enzyme immunoassay (EIA)
Third generation	0.01	Chemical luminescence immunoassay (CLIA)
Fourth generation	0.005	Electrogenerated chemiluminescence immunoassay (ECL)

from the sealed reagent pack. That improves the stability of the reagent after the seal of the pack is broken, thus ensuring the reliability of the test data.

RAISING THE SENSITIVITY OF CLINICAL DATA MEASUREMENT

Immunoassay Techniques

The concentration of blood constituents varies greatly with the type of constituent. Examples for items measured in biochemical testing and immunoassay are presented in Fig. 6. Most components are measured by biochemical analysis. On the other hand, hormones, tumor labels and other such disease-specific components are present in the blood only in minute quantities. For that reason, analytical techniques that are more highly sensitive and highly specific were required. Immunoassay methods were developed to meet that need.

Various immunoassay methods have been developed. Divided into four generations, there are RIA (radioisotope-immunoassay), EIA (enzyme immunoassay), CLIA (chemical luminescence immunoassay) and ECL (electrogenerated chemiluminescence immunoassay). In ECL, electrical stimulation causes a bound label reagent to emit light. Hitachi and Roche Diagnostics collaborated in the development of the cobas e 601 analyzer for immunoassay based on electrochemical luminescence.

Taking the TSH (thyroid stimulating hormone) as an example, the evolution of serological testing methods are presented in Table 2[10]. After the first generation, the measurement sensitivity increased with each new analytical method.

ECL Technology

The cobas e 601 analyzer combines ECL and magnetic particles to achieve highly sensitive analysis. Magnetic particles are reacted with the sample or an

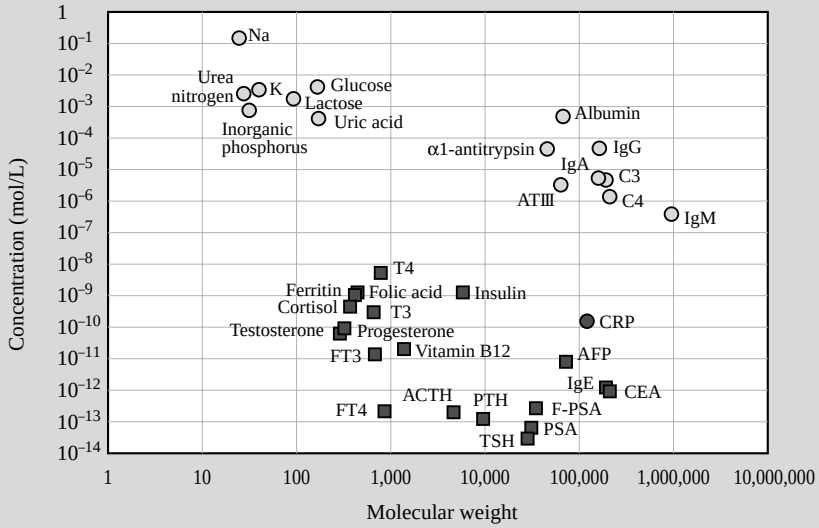
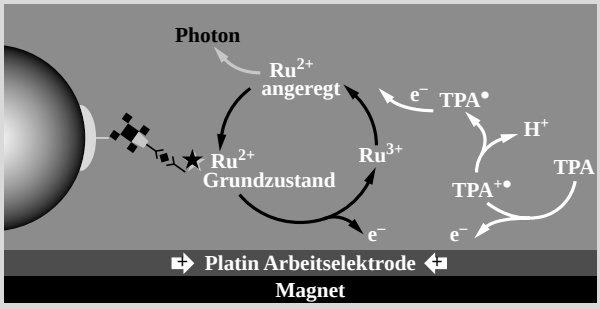


Fig. 6—Examples of Target Constituents for Biochemical and Immunoassay Testing.
The concentration of blood constituents varies with the type of constituent. Most constituents are measured by biochemical analysis (○). Measurement of constituents that have low concentrations requires highly-sensitive analysis. For measurement of concentrations below 10^{-6} mol/L, immunoassay (■) is most often used.



Ru2: tris-bipyridyl ruthenium metal cation TPA: tripropylamine
Grundzustand: ground state angeregt: excited state
Platin Arbeitselektrode: Pt working state

Fig. 7—Principle of the Electrogenerated Chemiluminescence Reaction.
The immunocomplex that is captured on the electrode emits light when a certain voltage is applied.

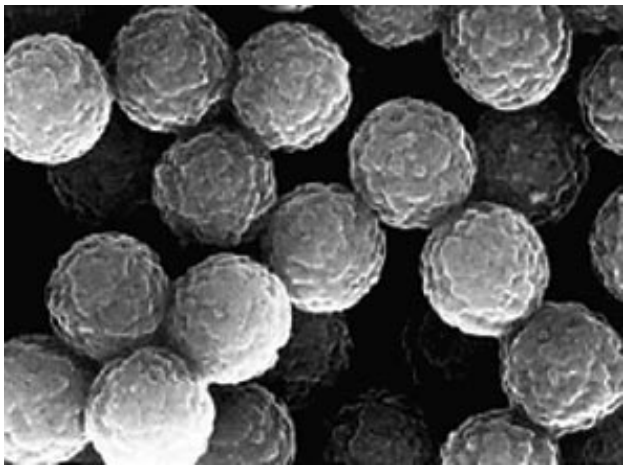


Fig. 8—Magnetic Particles Captured on the Electrode.
The electrogenerated chemical label reagent bound to the magnetic particles emits light by an electrochemical reaction.

ECL label reagent to form an immunocomplex. A reaction solution that contains the immunocomplex is drawn to an electrode by the action of magnetism.

The principle of the ECL reaction is illustrated in Fig. 7[11]. To increase luminescence efficiency, the Ru^{2+} (tris-bipyridyl ruthenium metal cation) complex is used as the chemical luminescent label[12],[13],[14] and TPA (tripropylamine) is used as the emitter[15]. Ru^{2+} undergoes an electrochemical oxidation reaction on the electrode surface and transitions to an excited state via Ru^{3+}. When the excited state returns to the ground state, light is emitted. The magnetic particles that are captured on the electrode are immunocomplexes that consist of sample and Ru metal complex (Ru^{2+}) and emit light at a specified voltage. The amount of light emitted is proportional to the weight of the immunocomplex and thus the weight of the sample. It can therefore be used for quantitative measurement.

The collection of magnetic particles on the electrode[15] is illustrated in Fig. 8.

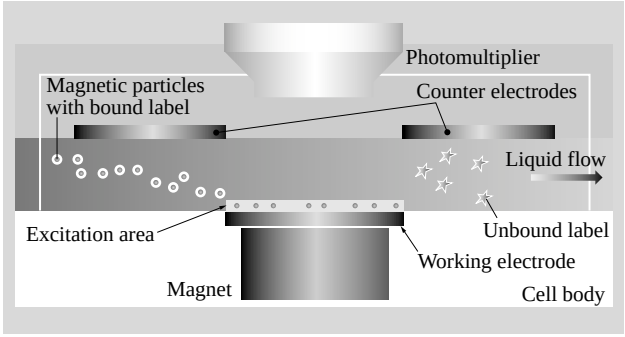


Fig. 9—ECL Measuring Cell.
The ECL (electrogenerated chemiluminescence) measuring cell has a simple flow-through structure.

ECL Measuring Cell

The patient sample includes high concentrations of a large variety of foreign substances other than the target of the analysis. To achieve highly sensitive analysis, it is necessary to separate the target substances from the other constituents and also exclude the non-bound labels from the measurement system. To implement this process, which is called B/F (bound/free) separation, with a simple system that does not require a special mechanism, we adopted a detector that uses the flow-through method. The detector, which is called the ECL measuring cell[15], is shown in Fig. 9.

The magnetic particles that are bound to the immunocomplex are captured uniformly on the surface of the electrode with a magnet. A buffer solution that contains TPA is introduced into the flow path with strict control of the flow rate. In that way, the extraneous materials and separation labels in the sample are removed from the detection and measurement system. When a certain voltage is applied to the magnetic particles that are bound to the immunocomplex and collected on the electrode, the light emitted by immunocomplex due to the electrochemical luminescence reaction can be measured.

INTEGRATED AUTOMATED ANALYZER

What has impelled progress in the automation of clinical testing was the demand for systems that can reduce laboratory workload. Previously, laboratory testing involved the separate operation of an automated

biochemical analyzer and an automated analyzer for immunoassay. When multiple systems were used, test reliability required that patient samples be divided into a portion for use in biochemical testing and a portion for use in immunoassay. That complicated the flow of patient samples. The separate systems required respective specialist for operation and maintenance. The cobas 6000 analyzer series addressed the problem of laboratories that operated separate automated analyzer systems for biochemical testing and immunoassay by providing an integrated system.

Integrated Workflow

Biochemical testing accounts for about 90% of the total for both biochemical and immunoassay testing. For that reason, we constructed a workflow in which biochemical testing occupies the central position in an overall laboratory workflow that integrates biochemical testing and immunoassay.

Integrating the testing workflow eliminated the need to divide samples for biochemical testing and immunoassay and reduced the number of times the samples had to be subdivided by pipetting by about 80%. It also reduced by about 30% the use of consumables such as the test tubes needed for dividing samples. The integrated system allowed unified management of the testing workflow, including sample placement, test specification, test data management, and creation of test reports, and thus greatly reduced the laboratory workload.

Combining Modules

The cobas 6000 analyzer series adopts a module combination approach to system configuration. The cobas c 501 analyzer for biochemical testing and the cobas e 601 analyzer for immunoassay were flexibly combined into a single integrated system. The optimum module combination can be selected to match the laboratory scale, and modules can easily be expanded to meet future increases in number of tests conducted. Laboratory administration using a single integrated platform contributes to increased efficiency in testing work, thus improving laboratory services.

CONCLUSIONS

Clinical testing has the important role of providing the information needed for the diagnosis and treatment of illness. Since the shipment of the first automated analyzer manufactured (Japan) in 1971, Hitachi has made rapid progress in this field. Our efforts toward increased reliability of clinical data and measurement sensitivity have resulted in a rapid increase in the number of test items for clinical biochemistry and immunoassay. At the same time, construction of an integrated system made it possible to handle both biochemical testing and immunoassay with a single automated analyzer system and greatly advanced the efficiency of testing tasks.

Clinical testing is of great importance, and the development of technology for increased sensitivity, automation, and higher reliability of analysis and detection and its application in products are also increasing in importance. In the future, we plan to steadily broaden the scope of possible testing to include new analytical methods such as genetic testing and methods for simultaneous analysis of multiple test items aiming at tailor-made medical care. We will also continue with technical innovation for faster and more efficient testing.

ACKNOWLEDGMENTS

We wish to express our deep gratitude to Mr. T. Hartke, Ms. S. Rosenblatt and other members of Roche Diagnostics for their cooperation in the writing of this paper.

For information on the cobas 6000 analyzer series, please contact Roche Diagnostics.

REFERENCES

(1) cobas 6000 analyzer series, http://www.mylabonline.com/products/cobas6000/c6000.php
(2) cobas 6000 analyzer series Test Menu, http://www.labsystems.roche.com/content/products/cobas6000/test_menu.html
(3) Application Cases (II) Model 705 Hitachi Automated Analyzer, *Technical Data ACA* **No. 16** (1983) in Japanese.
(4) T. Mimura et al., "Clinical Chemistry Analyzer for Supporting the Clinical Laboratory," *Hitachi Hyoron* **85**, pp. 623–626 (Sep. 2003) in Japanese.
(5) M. Hanawa et al., "Introducing the Hitachi LABOSPECT Series Automated Biochemical Analyzer," *Journal of the Japan Society for Clinical Laboratory Automation* **30**, p. 351 (2005) in Japanese.
(6) T. Ida et al., "Setting of Ultrasonic Mixing Conditions of the Hitachi LABOSPECT Automated Analyzer with Respect to Reagent pH," *Journal of the Japan Society for Clinical Laboratory Automation* **31,** p. 761 (2006) in Japanese.
(7) M. Yamaguchi et al., "Performance of the 9000 Series Hitachi Automated Analyzer in Application to Regular Testing," *Journal of the Japan Society for Clinical Laboratory Automation* **31**, p. 742 (2006) in Japanese.
(8) 9000 Series Hitachi Automated Analyzer Product Catalog, HTM-048P (2004) in Japanese.

(9) M. Iijima et al., "The LABOSPECT Series: Hitachi's Automatic Clinical Chemistry Analyzer to Realize the High Quality Laboratory Testing," *Hitachi Hyoron* **88**, pp. 702–707 (Sep. 2006) in Japanese.
(10) Y. Niiyama et al., "Multi-function Immunoassay System Suitable for Various Analysis," *Hitachi Hyoron* **79**, pp. 767–770 (Oct. 1997) in Japanese.
(11) Elecsys Prinzip, http://www.roche.de/diagnostics/lerncenter/index.htm
(12) W. L. Wallace et al., "Electrogenerated Chemiluminescence. 35. Temperature Dependence of the ECL Efficiency of Tris (2,2'-bipyridine) Rubidium$^{(2+)}$ in Acetonitrile and Evidence for Very High Excited State Yields from Electron Transfer Reactions," *The Journal of Physical Chemistry* **83**, pp. 1350-1357 (1979).
(13) J. K. Leland et al., "Electrogenerated Chemiluminescence: An Oxidative-reduction Type ECL Reaction Sequence Using Tripropyl Amine," *Journal of the Electrochemical Society* **137**, pp. 3127-3131 (1990).
(14) J. N. Demas et al., "Quantum Efficiencies on Transition Metal Complexes. II. Charge-transfer Luminescence," *Journal of the American Chemical Society* **93**, pp. 2841-2847 (1971).
(15) K. Erler et al., "Elecsys Immunoassay System Using Electro-Chemiluminescence Detection," *HITACHI REVIEW* **47**, pp. 21-26 (1998).

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