Special Topic for Vol. 36, No.1: Approaches to Highly Sensitive Rapid Examination
: For quick and accurate clinical assay

QuickGene Series: Rapid and simple system for DNA/RNA extraction which uses polymer porous membrane

Toshihiro Mori*, Yoshihide Iwaki*, Rie Hando*, Hiroyuki Komazawa*, Hiroko Otomo*, Tasuku Sasaki*, Tomoko Mori*, Hideyuki Kanehara*, Katsuya Inana*, Yumiko Takeshita**, Yasuhito Momoki**, Yoshihiko Makino***

1.Background

Recent progress in molecular biology has rapidly advanced the analysis of various genes, bringing the advent of tailor-made medical treatments which are adapted to patients' individual genetic properties into the near future. With the current trend of conducting genetic examinations on site, demand will increase for a quick, simple, automated means of conducting highly accurate examinations. To meet those needs, it is vital that we develop technologies to speed up, simplify, automate, and improve the accuracy of the preparation process in which nucleic acid (DNA or RNA) samples are extracted for examination.

In this article, we introduce a nucleic acid extraction technique developed by FUJUFILM Corporation. Using a newly developed porous polymer membrane (hereinafter, simply 'membrane' which selectively traps nucleic acids, this technique enables nucleic acid extraction under low pressure. Because this techniques does not require centrifugation, it results in a speedy, simple, and highly accurate way to automatically extract nucleic acids from a various of biological samples.1-3) We will now describe the characteristics of the QuickGene series, the nucleic acid extraction system which uses this novel extraction technique and present some results from nucleic acid extraction experiments using the series.

2. Novel Nucleic Acid Extraction Technique

2.1 Physical Properties of the Porous Membrane

The membrane is mounted inside a cartridge in the nucleic acid extraction kit. As shown in Figure 1, the membrane is extremely thin (80 μm) compared to a conventional glass fiber filter (1000 μm), and has a homogeneous inner structure. These properties allow filtration under low pressure, and thus miniaturization and automation of the device. They also do not retain left-over fluid in the membrane, allowing nucleic acids to be obtained with fewer contaminating impurities.

2.2 Surface and Nucleic Acid-Immobilizing Properties of the Membrane

Fig. 2 shows the relationship between the hydrophilicity of the membrane surface and yield when extracting genome DNA from a whole blood sample (200 μl). As shown in the results, as we increased the surface hydrophilicity of the membrane, the yields of genome DNA increased. It is difficult to control the surface properties of a glass fiber membrane, but an organic polymer membrane's surface can be modified with various functional groups. This allows control of adsorption or desorption of nucleic acids to and from the membrane.

*Life Science Research Laboratories, FUJIFILM Corporation
**Life Science Products Division, FUJIFILM Corporation
***Business Development Division, FUJIFILM CMIC HEALTHCARE Co, LTD

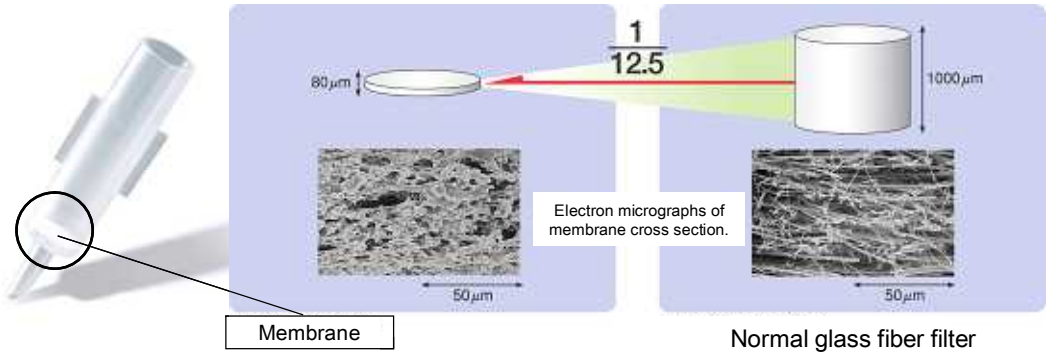

Fig.1 Electron micrographs (SEM) of the membrane and glass fiber filter

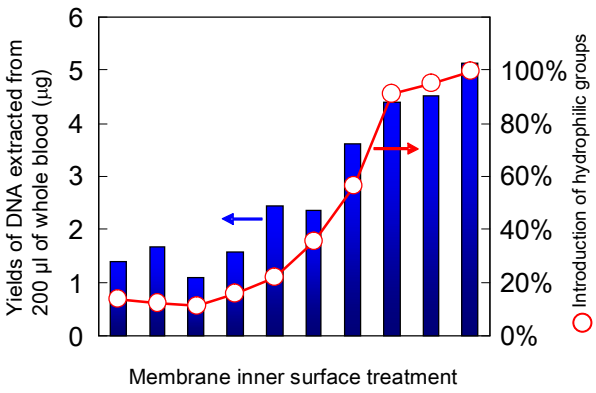

Fig.2 Effects of hydrophilization of membrane inner surface on yields of DNA

2.3 Nucleic Acids Extraction Process

The nucleic acid extraction technique consists of adsorption or desorption of nucleic acids or from the membrane surface. This is achieved by appropriately controlling the surface properties of the membrane and the polarities of lysates containing nucleic acids, wash solution, and elute solution (collection solution) (Fig. 3).

1) When an organic solvent such as ethanol is added to a lysate to reduce its polarity, nucleic acids adsorb to the membrane (Fig. 3a).

2) Prior to elution, the membrane is washed with a low polarity solution to remove components other than the nucleic acids contained in the membrane, without desorbing nucleic acids of interest (Fig. 3b). Fig. 4 shows the electron micrographs (SEM) of the lyophilized membrane surface, taken immediately after the washing process.

3) Finally, nucleic acids are eluted from the membrane with a high polarity solution. (Fig. 3c)

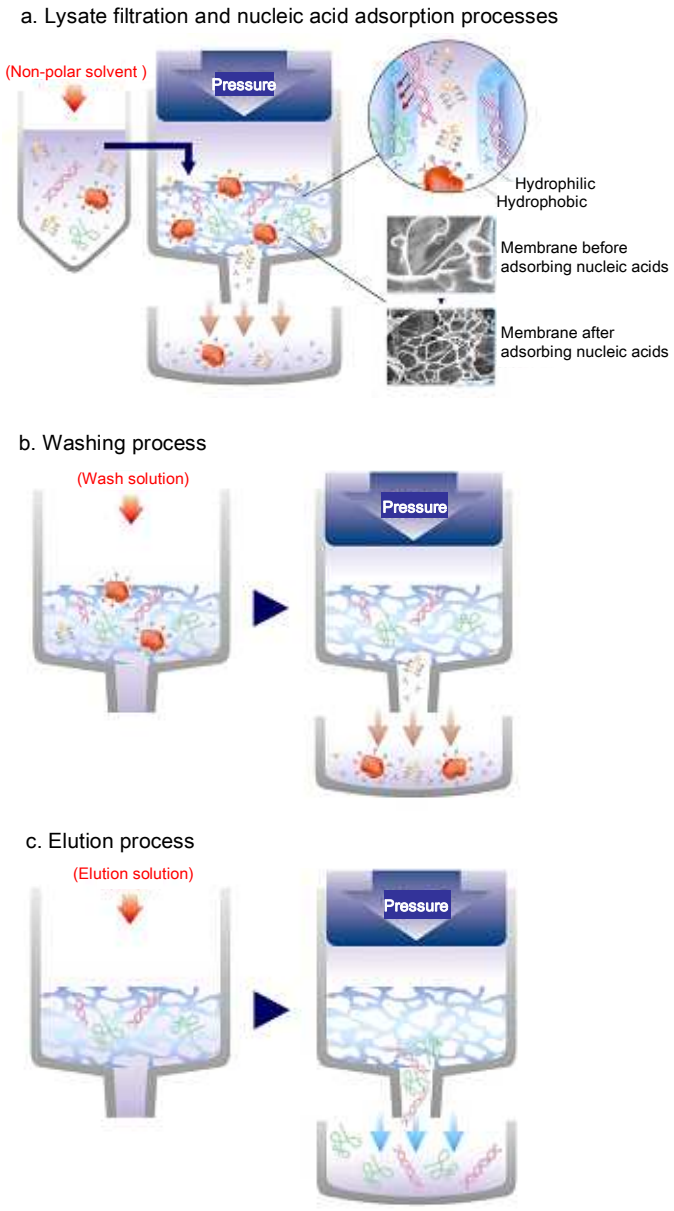

Fig. 3 Nucleic acid extraction process using the membrane

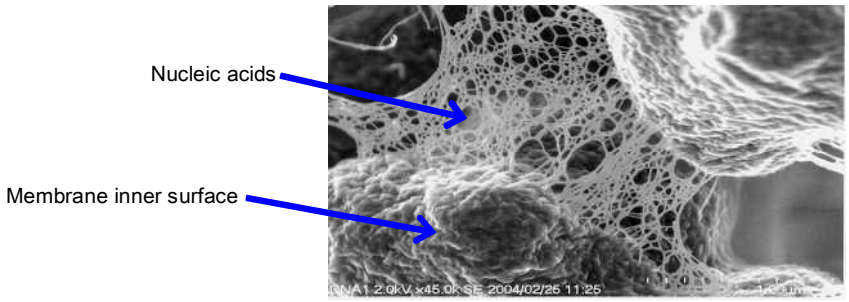

Fig. 4 Nucleic acids adsorbed to the membrane (SEM photograph of lyophilized membrane, after washing)

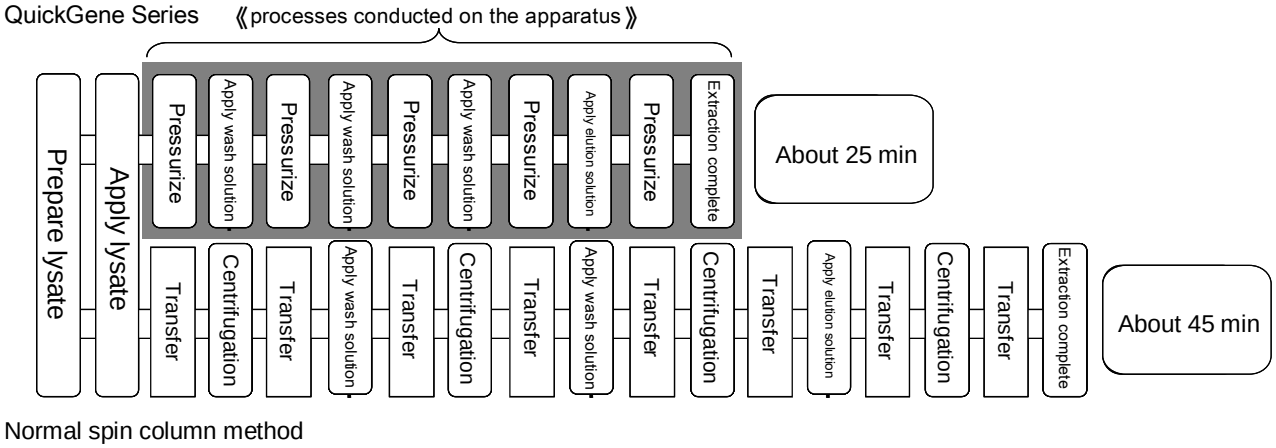

Fig. 5 Comparison of nucleic acid extraction processes (extracting genome DNA from whole blood)
Upper row: QuickGene Series using nucleic acid extraction method with the membrane
Lower row: Conventional nucleic acids extraction methods using glass fiber filter (spin column method)

Fig. 5 shows a comparison of processes in which genome DNA was extracted from whole blood. In one process, we used the QuickGene Series with the membrane, and in the other, we used a conventional spin column method with a glass fiber filter. Time from lysate preparation to the end of extraction is 25 minutes with QuickGene Series. This is about 20 minutes faster than the commonly used spin column method which takes 45 minutes and requires centrifugation. Furthermore, because the QuickGene Series uses a dedicated apparatus, processes following lysate application can be performed sequentially through to the end of extraction without removing the cartridge from the apparatus. Time required from lysate application to the end of extraction is about six minutes for eight samples.

3. An Example of Nucleic Acids Extraction

(Genome DNA Extraction from Whole Blood)

3.1 Sample Preparation

Whole blood, protease (EDB) and lysis buffer (LDB) are mixed to degrade proteins, disperse lipids and disrupt nuclear membrane in whole blood and thereby release genome DNA. Ethanol is added to the mixture to complete lysis. (Fig. 6).

3.2 Extraction

After passing lysate through the membrane, a wash solution containing ethanol is used to flush away unwanted substances, leaving the genome DNA adsorbed to the membrane. Genome DNA is then eluted from the membrane. The operations are performed on a dedicated QuickGene Series apparatus. Using the QuickGene-810, the extraction process takes six minutes for eight samples, the fastest time ever achieved.

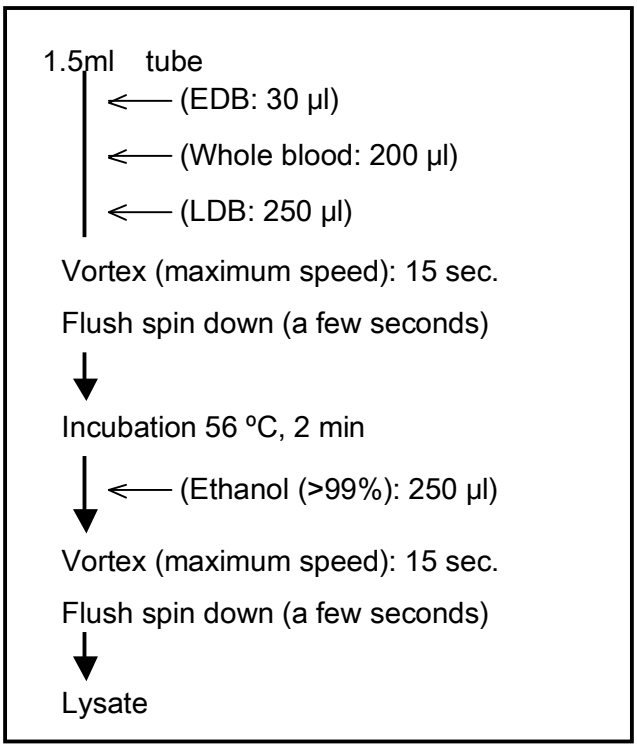

Fig. 6 Sample preparation of genome DNA extraction from human whole blood

3.3 Evaluation Results of Extracted DNA

Table 1 shows extraction results comparing the dedicated QuickGene kit (whole blood DNA kit) and apparatus (QuickGene-810), a spin column kit (using a glass fiber filter), and an automated nucleic acid extraction system (magnetic beads method), both from Company A.
The QuickGene-810's recovery of genome DNA was equivalent to that of the spin column kit from Company A. A ratio of 260/280 indicates no difference in protein contamination between those kits. However at a ratio of 260/230, which indicates chaotropic salts, the QuickGene-810 yielded a better result than both the spin column kit and the automated extractor from Company A, extracting extremely pure genome DNA. Although the automated extractor from Company A has a good recovery, the product is contaminated by a noticeably large quantity of chaotropic salts. For this reason, it seems the yield does not reflect the actual recovery of genome DNA. Furthermore, the QuickGene-810 product had little absorption at 400 nm, which shows contamination of hemoglobin, indicating high purity as well.

Table 1 Yields and purities of genome DNA extracted from whole blood (µg)

	DNA yields (µg/1 x 10^5 WBC)	260nm/280nm OD ratio	260nm/230nm OD ratio	400nm OD ratio
QuickGene-810	**0.48**	**1.97**	**1.77**	**0.01**
Company A's spin column	**0.49**	**1.92**	**1.61**	**0.03**
Company A's automated extractor	**0.56**	**1.93**	**1.93**	**0.92**	**0.03**

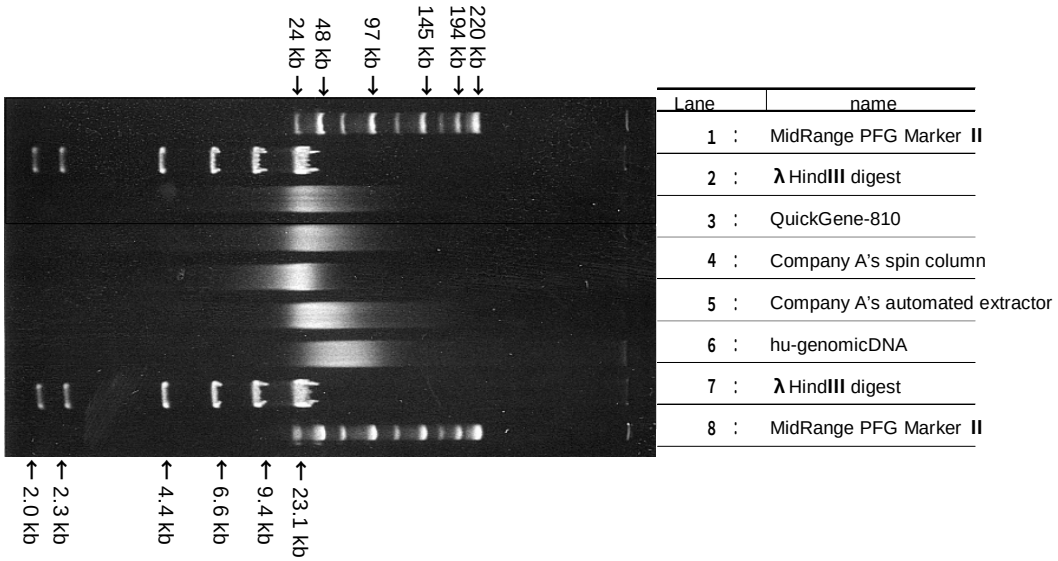

Fig. 7 Pulsed-field electrophoretogram of genome DNA extracted from whole blood

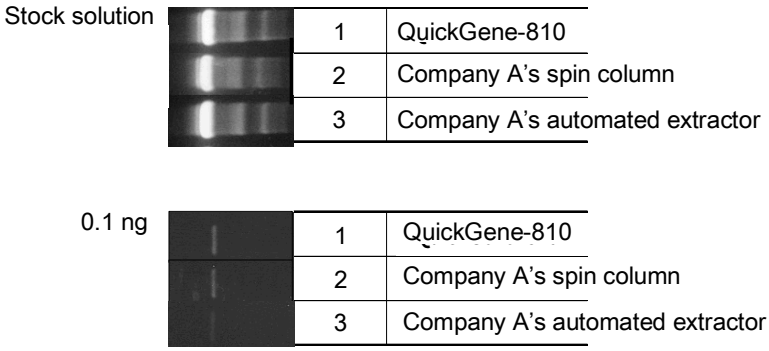

Fig. 8 Electrophoretogram of PCR products obtained from genome DNA extracted from whole blood

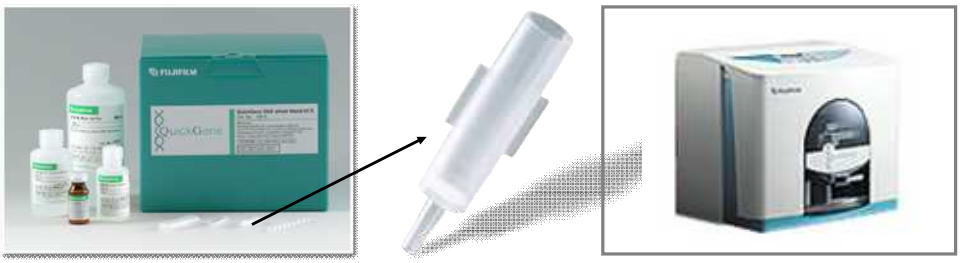

Fig. 9 Dedicated kit, enlarged image of cartridge, dedicated automated extractor (right, the QuickGene-810)

Fig. 7 shows the results of applying pulsed-field electrophoresis to extracted genome DNA. With Company A's spin column kit, DNA from about 7 to about 60 kb was recovered. On the other hand, the QuickGene-810 could recover DNA ranging from about 10 to about 140 kb. In other words, the QuickGene-810 could yield longer DNA samples. Using both the QuickGene-810 system and Company A's spin column kit, we performed PCR on genome DNA extracted from whole blood. For the PCR, we used genome DNA extracts, both undiluted and diluted to 0.1 ng, and G3PDH primer. Fig. 8 shows the results of agarose electrophoresis of the PCR products. Electrophoresis bands detected were similar for PCR products from both undiluted extract and diluted (0.1 ng) genome DNA, using either kit.

4. QuickGene Series

The QuickGene Series is comprised of a dedicated kit, consisting of a cartridge equipped with a membrane and process solution, and a dedicated extractor (Fig. 9).

4.1 Dedicated kits

The QuickGene Series provides various dedicated kits to extract DNA or RNA from a variety of samples depending on their intended use. Table 2 shows examples of yields and purities when extracting DNA or RNA from various samples using dedicated QuickGene Series kits.

4.2 Dedicated apparatuses

We provide the following user-friendly QuickGene Series apparatuses:
1) QuickGene-800 and 810 are standard automated apparatuses which automatically inject wash solution and eluent and exert pressure. They treat up to eight samples at a time (Dimensions: Width 450 mm; Depth 330 mm; Height 400 mm. Weight: about 21 kg).

Table 2. Dedicated kits for QuickGene Series and examples of yields and purities of DNA or RNA extracted from various samples

Dedicated kits	Sample	Type	Maximum tissue volume to be treated	Yield examples (μg)	A260/ A280
DNA tissue kit	Liver (RNase treated)	DNA	5 mg	4.5	1.88
	Tail (RNase treated)		5 mg	4.0	1.92
DNA whole blood kit	Whole blood		200 μl	6.5	1.98
Plasmid kit	*E. coli* pBS II/GAPDH/DH5 α		1×10^9	19.3	1.98
RNA tissue kit II	Liver (DNase treated)	RNA	30 mg	122	2.21
	Spleen (DNase treated)		30 mg	48	2.05
	Heart (DNase treated)		30 mg	21	2.37
RNA cultured cell kit	HL60 (DNase treated)		1×10^6	9.7	1.94
RNA cultured cell HC kit	HEK293 (DNase treated)		6 cm dish	79.1	1.86
	HL60 (DNase treated)		15×10^6	167.3	1.92
	HeLa (DNase treated)		10 cm dish	129	2.20
	HEK293 (DNase treated)		10 cm dish	**175.3**	**2.29**
RNA blood cell kit	Leukocyte (DNase treated)		1×10^7	4.6	2.20

Fig. 10 QuickGene-Mini80 and its dedicated kit

2) The QuickGene-610L is an automated apparatus which is capable of extracting nucleic acid from 2 ml of whole blood, realizing large volume nucleic acid recovery (Dimensions: Width 450 mm; Depth 330 mm; Height 400 mm. Weight: about 24 kg).
3) The QuickGene-HT/Biomek NX is a simple, convenient and fully automated high-throughput apparatus which treats 96 whole blood samples concurrently in a short period (Dimensions: Width 1,070 mm; Depth 820 mm; Height 1,120 mm. Weight: about 100 kg).
4) The QuickGene-Mini80 is a simplified apparatus which makes it possible to install one per bench and is easily operated even on a clean bench (Dimensions: Width 280 mm; Depth 220 mm; Height 180 mm. Weight: about 3 kg).(Fig. 10).

5. Summary

Here we have introduced a membrane, which is a key material for nucleic acid extraction technology, and which provides the following advantages:
1) The membrane can add chemical reactivity to nucleic acids and can be modified with optional functional groups.
2) It can be manufactured in various sizes, for processes ranging from large scale to micro-sized.
We hope to exploit these advantages to speed up, simplify, automate, and improve the accuracy of gene examinations, and develop products which meet a range of needs.

■References

1) Proceedings I of the 85th Spring Annual Meeting of The Chemical Society of Japan, Presentation 473 3H6-31, 2005
2) European Patent 1382677
3) Makino, Mori, Takeshita, Hando, Komazawa, Otomo, Sasaki, Watanabe and Momoki: QuickGene-800: Development of rapid and simple system for DNA/RNA extraction using polymer porous membrane. Membrane, 31(3), 174-177, 2006

QuickGene Series: Rapid and simple system for DNA/RNA extraction which uses polymer porous membrane

Toshihiro Mori*, Yoshihide Iwaki*, Rie Hando*, Hiroyuki Komazawa*, Hiroko Otomo*, Tasuku Sasaki*, Tomoko Mori*, Hideyuki Kanehara*, Katsuya Inana*, Yumiko Takeshita**, Yasuhito Momoki**, Yoshihiko Makino***

*Life Science Research Laboratories, FUJIFILM Corporation
**Life Science Products Division, FUJIFILM Corporation
***Business Development Division, FUJIFILM CMIC HEALTHCARE Co., Ltd

Footnote: This review is the English translation from the original Japanese version appeared in a Japanese journal “Rinsho Kagaku” (Clinical Chemistry), 33-39, No. 1, Vol. 36, 2007, published by Japan Society of Clinical Chemistry, Tokyo, Japan.