2-アミノエタノールのマウスを用いた
経口投与によるがん原性試験（混水試験）報告書

試験番号：0642

APPENDICES

APPENDICES

APPENDIX 1-1

IDENTITY OF 2-AMINOETHANOL

IN THE 2-YEAR DRINKING WATER STUDY

IDENTITY OF 2-AMINOETHANOL IN THE 2-YEAR DRINKING WATER STUDY

Test Substance : 2-Aminoethanol (Wako Pure Chemical Industries, Ltd.)

A. Lot No. : LTQ4405

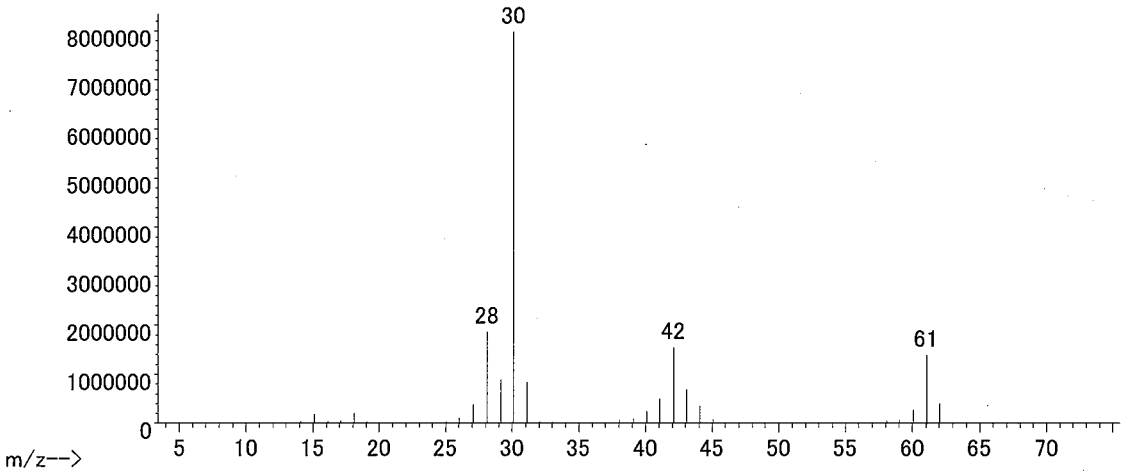
1. Spectral Data

Mass Spectrometry

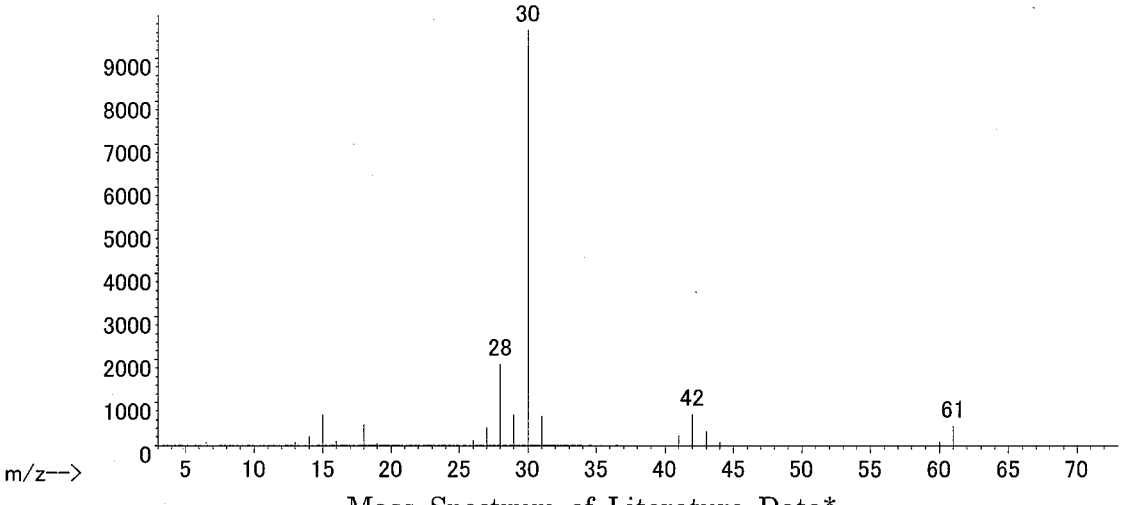
Instrument : Agilent Technologies 5973N Mass Spectrometer

Ionization : EI (Electron Ionization)

Ionization Voltage : 70eV



Mass Spectrum of Test Substance



Mass Spectrum of Literature Data*

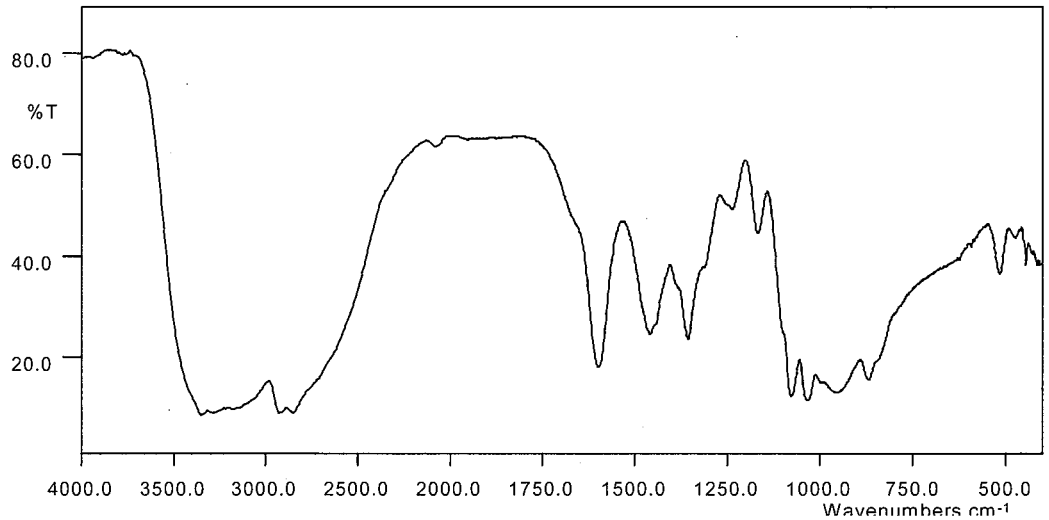
Result: The mass spectrum was consistent with literature spectrum.
(*McLafferty FW, ed. 1994. Wiley Registry of Mass Spectral Data. 6th ed. New York, NY : John Wiley and Sons.)

Infrared Spectrometry

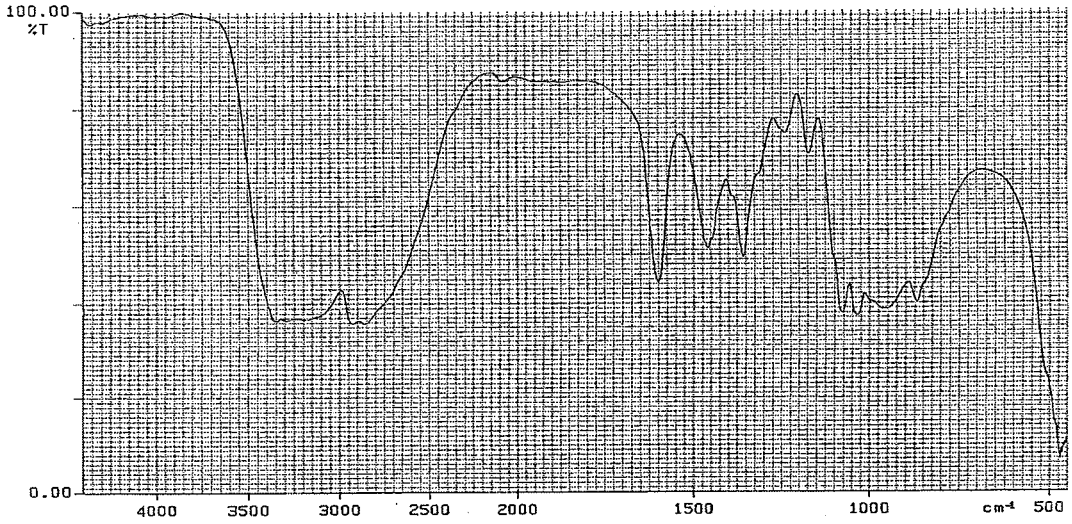
Instrument : Shimadzu FTIR-8200PC Infrared Spectrometer

Cell : KBr Liquid Cell

Resolution : 2 cm^{-1}



Infrared Spectrum of Test Substance



Infrared Spectrum of Literature Data*

Result: The infrared spectrum was consistent with literature spectrum.
(*Performed by Wako Pure Chemical Industries, Ltd.)

2. Conclusion: The test substance was identified as 2-aminoethanol by mass spectrum and infrared spectrum.

B. Lot No. : TSK2767

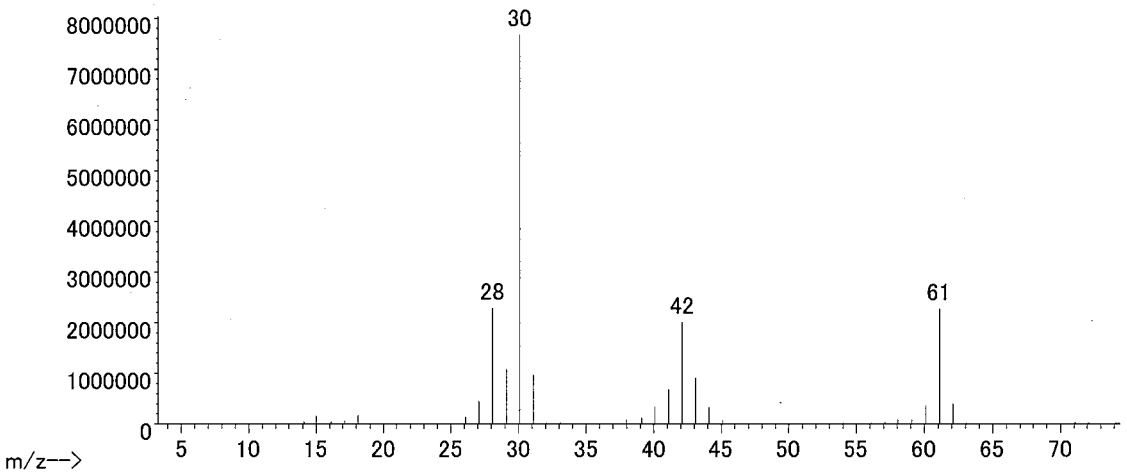
1. Spectral Data

Mass Spectrometry

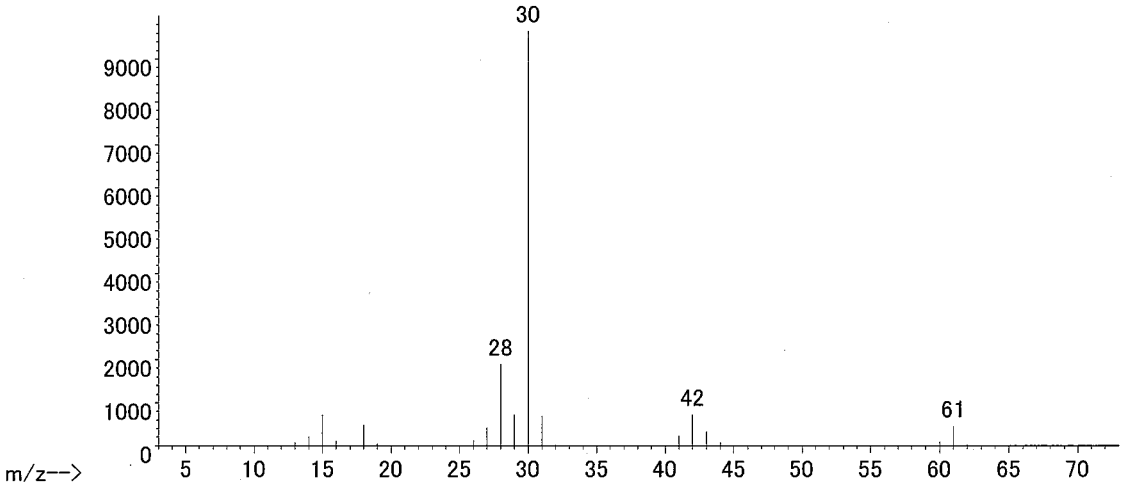
Instrument : Agilent Technologies 5973N Mass Spectrometer

Ionization : EI (Electron Ionization)

Ionization Voltage : 70eV



Mass Spectrum of Test Substance



Mass Spectrum of Literature Data*

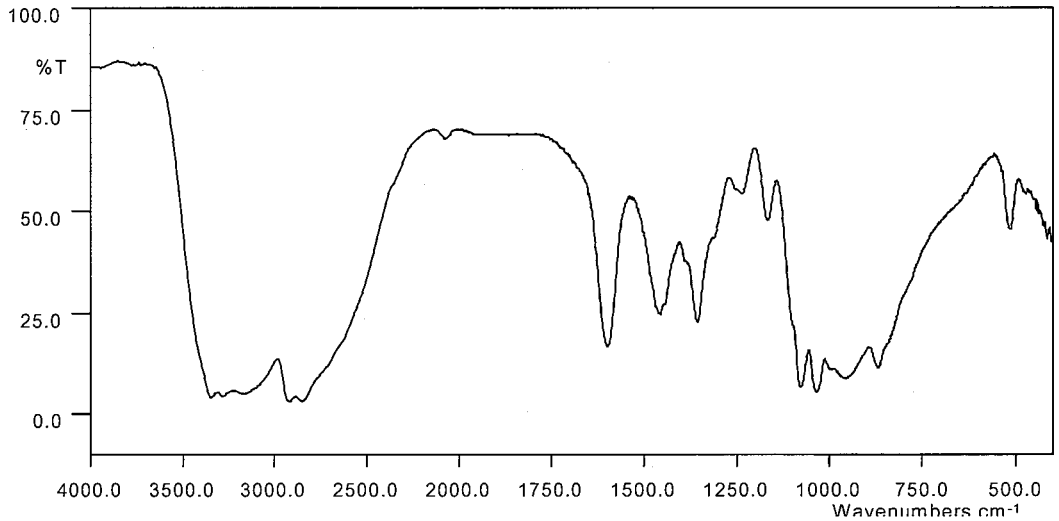
Result: The mass spectrum was consistent with literature spectrum.
(*McLafferty FW, ed. 1994. Wiley Registry of Mass Spectral Data. 6th ed. New York, NY : John Wiley and Sons.)

Infrared Spectrometry

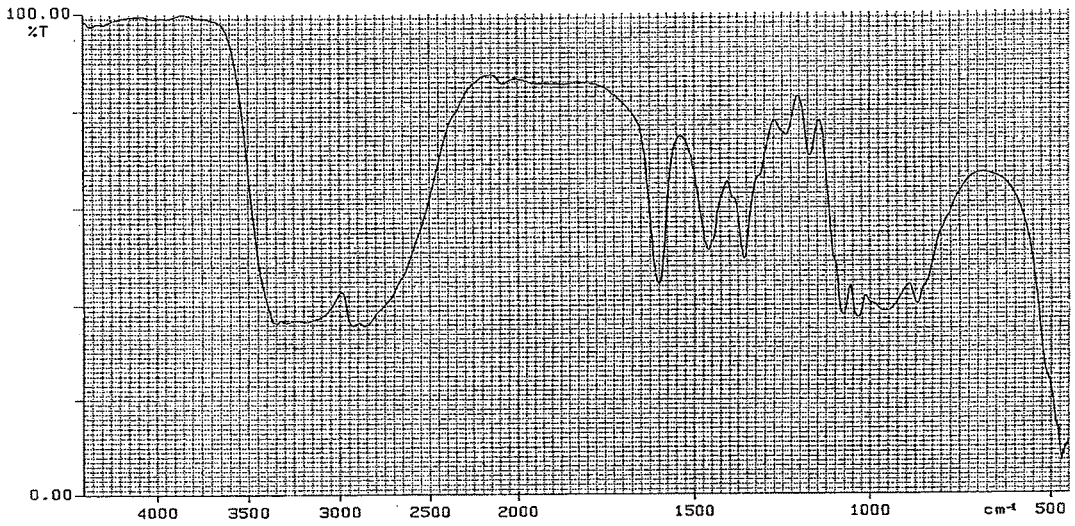
Instrument : Shimadzu FTIR-8200PC Infrared Spectrometer

Cell : KBr Liquid Cell

Resolution : 2 cm^{-1}



Infrared Spectrum of Test Substance



Infrared Spectrum of Literature Data*

Result: The infrared spectrum was consistent with literature spectrum.
(*Performed by Wako Pure Chemical Industries, Ltd.)

2. Conclusion: The test substance was identified as 2-aminoethanol by mass spectrum and infrared spectrum.

APPENDIX 1-2

STABILITY OF 2-AMINOETHANOL

IN THE 2-YEAR DRINKING WATER STUDY

STABILITY OF 2-AMINOETHANOL IN THE 2-YEAR DRINKING WATER STUDY

Test Substance : 2-Aminoethanol (Wako Pure Chemical Industries, Ltd.)

A. Lot No. : LTQ4405

1. High Performance Liquid Chromatography

Instrument : Agilent Technologies 1090 High Performance Liquid Chromatograph

Column : TSK-GEL ODS-80TM (4.6 mmϕ × 15 cm)

Column Temperature: 40 °C

Flow Rate : 1 mL/min

Mobile Phase : Acetonitrile : Distilled water = 7 : 3

Detector : UV (470 nm)

Injection Volume : 10 μL

Pre-treatment : 2-Aminoethanol was allowed to react with derivative of 2-fluoro-7-nitrobenzofurazan, and analyzed. The derivative of 2-fluoro-7-nitrobenzofurazan was reacted according to the method of Dojindo Molecular Technologies, Inc.* based on the reaction of the appropriate 2-aminoethanol, 2-fluoro-7-nitrobenzofurazan, boric acid and hydrochloric acid with borate buffer solution.

(*Dojindo Molecular Technologies, Inc. http://dominoweb.dojindo.co.jp/goodsr5.nsf/View_Display/N020?OpenDocument [accessed 10 April 2006])

Date Analyzed	Peak No.	Retention Time (min)	Area (%)
2006.06.05	1	1.775	100
2008.01.25	1	1.781	100

Result: High performance liquid chromatography indicated one major peak (peak No.1) analyzed on 2006.6.5 and one major peak (peak No.1) analyzed on 2008.1.25. No new trace impurity peak in the test substance analyzed on 2008.1.25 was detected.

2. Conclusion: The test substance was stable for the period that the test substance had been used for the study.

B. Lot No. : TSK2767

1. High Performance Liquid Chromatography

Instrument : Agilent Technologies 1090 High Performance Liquid Chromatograph

Column : TSK-GEL ODS-80TM (4.6 mmϕ × 15 cm)

Column Temperature: 40 °C

Flow Rate : 1 mL/min

Mobile Phase : Acetonitrile : Distilled water = 7 : 3

Detector : UV (470 nm)

Injection Volume : 10 μL

Pre-treatment : 2-Aminoethanol was allowed to react with derivative of 2-fluoro-7-nitrobenzofurazan, and analyzed. The derivative of 2-fluoro-7-nitrobenzofurazan was reacted according to the method of Dojindo Molecular Technologies, Inc.* based on the reaction of the appropriate 2-aminoethanol, 2-fluoro-7-nitrobenzofurazan, boric acid and hydrochloric acid with borate buffer solution.

(*Dojindo Molecular Technologies, Inc. http://dominoweb.dojindo.co.jp/goodsr5.nsf/View_Display/N020?OpenDocument [accessed 10 April 2006])

Date Analyzed	Peak No.	Retention Time (min)	Area (%)
2008.01.11	1	1.775	100
2008.07.09	1	1.781	100

Result: High performance liquid chromatography indicated one major peak (peak No.1) analyzed on 2008.1.11 and one major peak (peak No.1) analyzed on 2008.7.9. No new trace impurity peak in the test substance analyzed on 2008.7.9 was detected.

2. Conclusion: The test substance was stable for the period that the test substance had been used for the study.

APPENDIX 1-3

CONCENTRATION OF 2-AMINOETHANOL IN FORMULATED WATER IN THE 2-YEAR DRINKING WATER STUDY

CONCENTRATION OF 2-AMINOETHANOL IN FORMULATED WATER IN THE 2-YEAR DRINKING WATER STUDY

Analytical Method : The samples were analyzed by high performance liquid chromatography.

Instrument : Agilent Technologies 1090 High Performance Liquid Chromatograph

Column : TSK-GEL ODS-80TM (4.6 mmϕ × 15 cm)

Column Temperature: 40 °C

Flow Rate : 1 mL/min

Mobile Phase : Acetonitrile : Distilled water = 7 : 3

Detector : UV (470 nm)

Injection Volume : 10 μL

Pre-treatment : 2-Aminoethanol was allowed to react with derivative of 2-fluoro-7-nitrobenzofurazan, and analyzed. The derivative of 2-fluoro-7-nitrobenzofurazan was reacted according to the method of Dojindo Molecular Technologies, Inc.* based on the reaction of the appropriate 2-aminoethanol, 2-fluoro-7-nitrobenzofurazan, boric acid and hydrochloric acid with borate buffer solution.

(*Dojindo Molecular Technologies, Inc. http://dominoweb.dojindo.co.jp/goodsr5.nsf/View_Display/N020?OpenDocument [accessed 10 April 2006])

	Target Concentration		
Date Analyzed	800[a]	2000	5000
2006.06.30	763[b] (95.4)[c]	1910 (95.5)	4880 (97.6)
2006.09.01	760 (95.0)	2020 (101)	4870 (97.4)
2006.11.24	822 (103)	2090 (105)	5300 (106)
2007.02.16	802 (100)	1970 (98.5)	4920 (98.4)
2007.05.11	857 (107)	2010 (101)	4970 (99.4)
2007.08.03	798 (99.8)	1980 (99.0)	5140 (103)
2007.10.26	789 (98.6)	2020 (101)	5020 (100)
2008.01.18	801 (100)	2020 (101)	5070 (101)
2008.04.11	798 (99.8)	2150 (108)	5300 (106)

[a] ppm
[b] ppm (Mean measured concentration.)
[c] % (Mean measured concentration/target concentration × 100.)

APPENDIX 1-4

STABILITY OF 2-AMINOETHANOL

IN FORMULATED WATER

STABILITY OF 2-AMINOETHANOL IN FORMULATED WATER

Analytical Method : The samples were analyzed by high performance liquid chromatography.

Instrument : Agilent Technologies 1090 High Performance Liquid Chromatograph

Column : TSK-GEL ODS-80TM (4.6 mmϕ × 15 cm)

Column Temperature: 40 °C

Flow Rate : 1 mL/min

Mobile Phase : Acetonitrile : Distilled water = 7 : 3

Detector : UV (470 nm)

Injection Volume : 10 µL

Pre-treatment : 2-Aminoethanol was allowed to react with derivative of 2-fluoro-7-nitrobenzofurazan, and analyzed. The derivative of 2-fluoro-7-nitrobenzofurazan was reacted according to the method of Dojindo Molecular Technologies, Inc.* based on the reaction of the appropriate 2-aminoethanol, 2-fluoro-7-nitrobenzofurazan, boric acid and hydrochloric acid with borate buffer solution.

(*Dojindo Molecular Technologies, Inc. http://dominoweb.dojindo.co.jp/goodsr5.nsf/View_Display/N020?OpenDocument [accessed 10 April 2006])

	Target Concentration	
Date Analyzed	800[a]	5000
2006.05.08	833 (100)[b]	5000 (100)
2006.05.12[c]	792 (95.1)	4970 (99.4)

[a] ppm
[b] % (Percentage was based on the concentration at the date of preparation.)
[c] Animal room samples

APPENDIX 2

METHODS, UNITS AND DECIMAL PLACE FOR HEMATOLOGY AND BIOCHEMISTRY IN THE 2-YEAR DRINKING WATER STUDY OF 2-AMINOETHANOL

METHODS, UNITS AND DECIMAL PLACE FOR HEMATOLOGY AND BIOCHEMISTRY IN THE 2-YEAR DRINKING WATER STUDY OF 2-AMINOETHANOL

Item	Method	Unit	Decimal place
Hematology			
Red blood cell (RBC)	Light scattering method [1]	$\times 10^6/\mu L$	2
Hemoglobin(Hgb)	Cyanmethemoglobin method [1]	g/dL	1
Hematocrit(Hct)	Calculated as RBC×MCV/10 [1]	%	1
Mean corpuscular volume(MCV)	Light scattering method [1]	fL	1
Mean corpuscular hemoglobin(MCH)	Calculated as Hgb/RBC×10 [1]	pg	1
Mean corpuscular hemoglobin concentration (MCHC)	Calculated as Hgb/Hct×100 [1]	g/dL	1
Platelet	Light scattering method [1]	$\times 10^3/\mu L$	0
Reticulocyte	Light scattering method [1]	%	1
White blood cell(WBC)	Light scattering method [1]	$\times 10^3/\mu L$	2
Differential WBC	Light scattering method [1]	%	0
Biochemistry			
Total protein(TP)	Biuret method [2]	g/dL	1
Albumin (Alb)	BCG method [2]	g/dL	1
A/G ratio	Calculated as Alb/(TP−Alb) [2]	−	1
T-bilirubin	Azobilirubin method [2]	mg/dL	2
Glucose	GlcK·G-6-PDH method [2]	mg/dL	0
T-cholesterol	CE·COD·POD method [2]	mg/dL	0
Triglyceride	MGLP·GK·GPO·POD method [2]	mg/dL	0
Phospholipid	PLD·ChOD·POD method [2]	mg/dL	0
Aspartate aminotransferase (AST)	JSCC method [2]	IU/L	0
Alanine aminotransferase (ALT)	JSCC method [2]	IU/L	0
Lactate dehydrogenase (LDH)	SFBC method [2]	IU/L	0
Alkaline phosphatase (ALP)	GSCC method [2]	IU/L	0
γ-Glutamyl transpeptidase (γ-GTP)	JSCC method [2]	IU/L	0
Creatine kinase (CK)	JSCC method [2]	IU/L	0
Urea nitrogen	Urease·GLDH method [2]	mg/dL	1
Sodium	Ion selective electrode method [2]	mEq/L	0
Potassium	Ion selective electrode method [2]	mEq/L	1
Chloride	Ion selective electrode method [2]	mEq/L	0
Calcium	OCPC method [2]	mg/dL	1
Inorganic phosphorus	PNP·XOD·POD method [2]	mg/dL	1

1) Automatic blood cell analyzer (ADVIA120 : Siemens Healthcare Diagnostics Inc.)
2) Automatic analyzer (Hitachi 7080 : Hitachi,Ltd.)