4-*tert*-ブチルカテコールのラットを用いた
経口投与によるがん原性試験（混餌試験）報告書

試験番号：0739

APPENDICES

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APPENDIX 1-1

IDENTITY OF 4-*tert*-BUTYLCATECHOL IN THE 2-YEAR FEED STUDY

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Test Substance : 4-*tert* -Butylcatechol (Wako Pure Chemical Industries, Ltd.)

A.

Lot No. : PEK3567

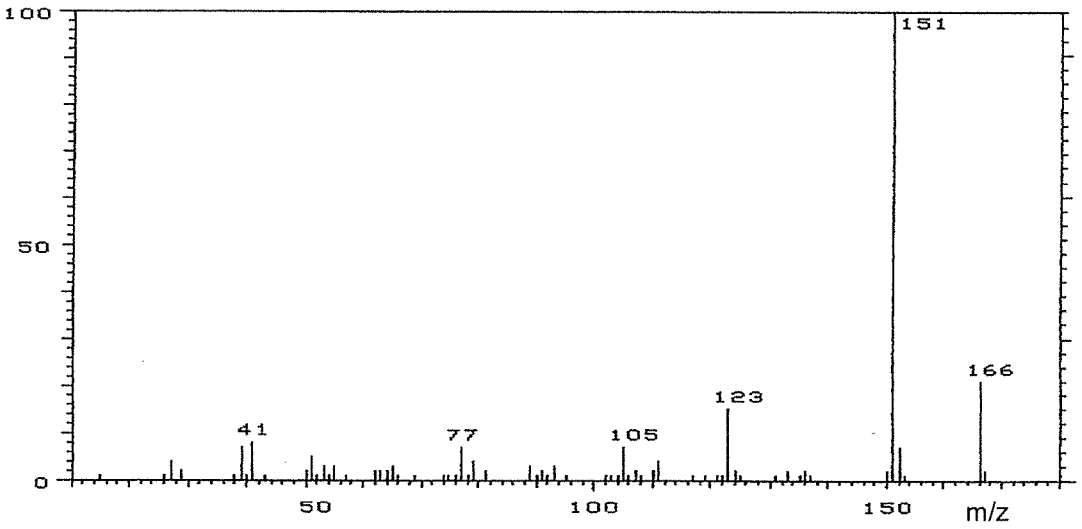
1. Spectral Data

Mass Spectrometry

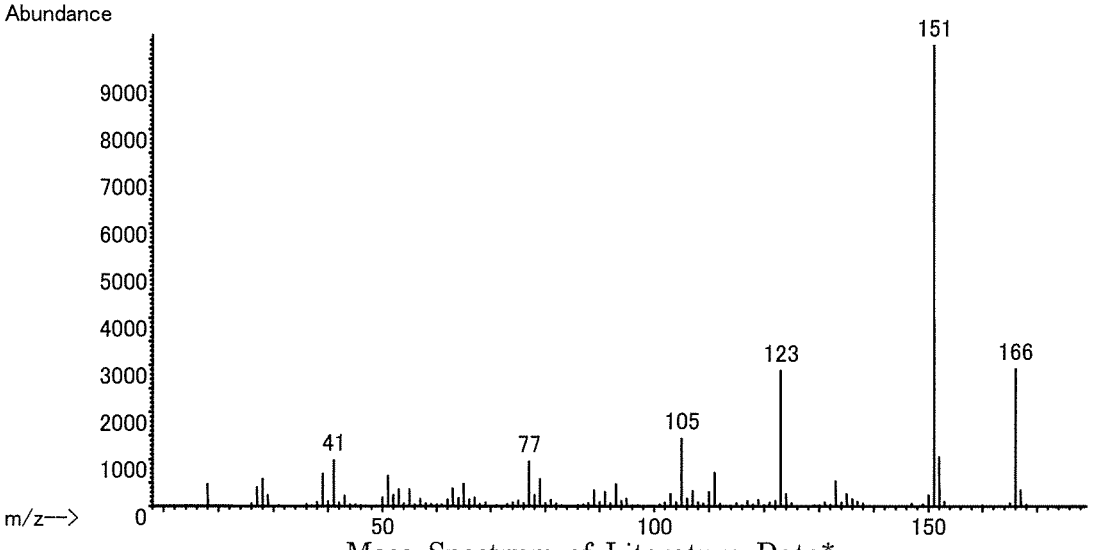
Instrument : Hitachi M-80B Mass Spectrometer

Ionization : EI (Electron Ionization)

Ionization Voltage : 70eV



Mass Spectrum of Test Substance



Mass Spectrum of Literature Data*

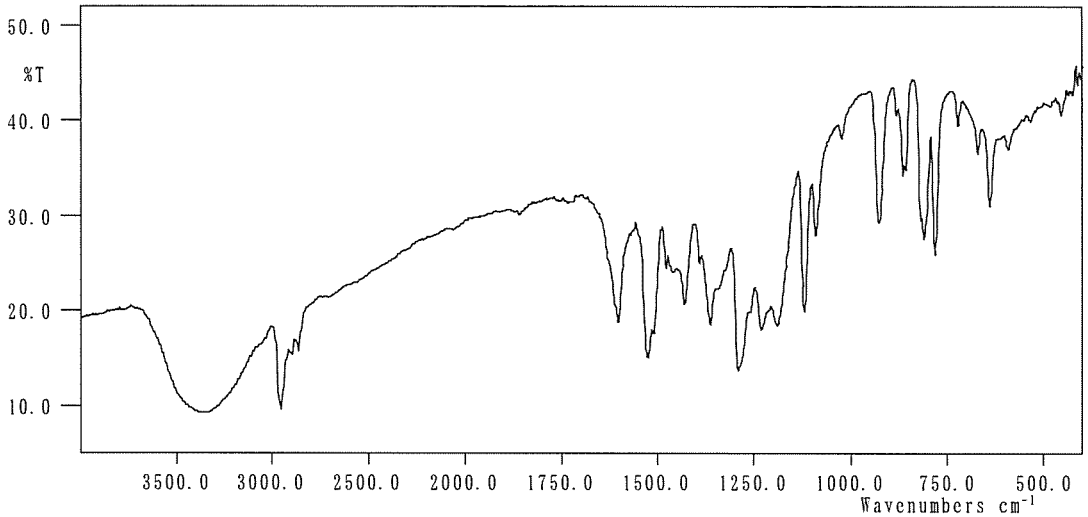
Result: The mass spectrum was consistent with literature spectrum.
(*McLafferty FW, ed. 1994. Wiley Registry of Mass Spectral Data. 6th ed. New York, NY:John Wiley and Sons.)

Infrared Spectrometry

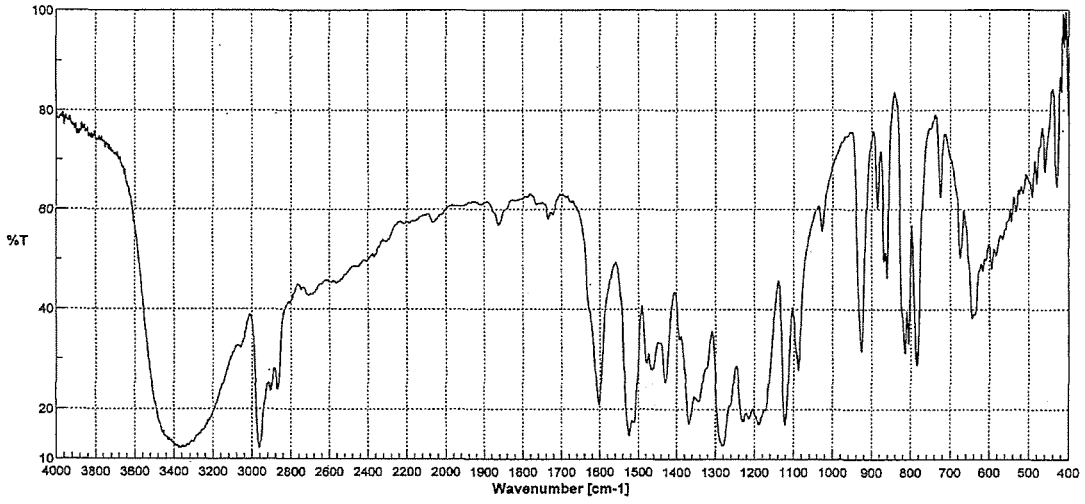
Instrument : Shimadzu FTIR-8200PC Infrared Spectrometer

Cell : KBr

Resolution : 4 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 4-*tert*-butylcatechol by mass spectrum and infrared spectrum.

B.

Lot No. : STL0824

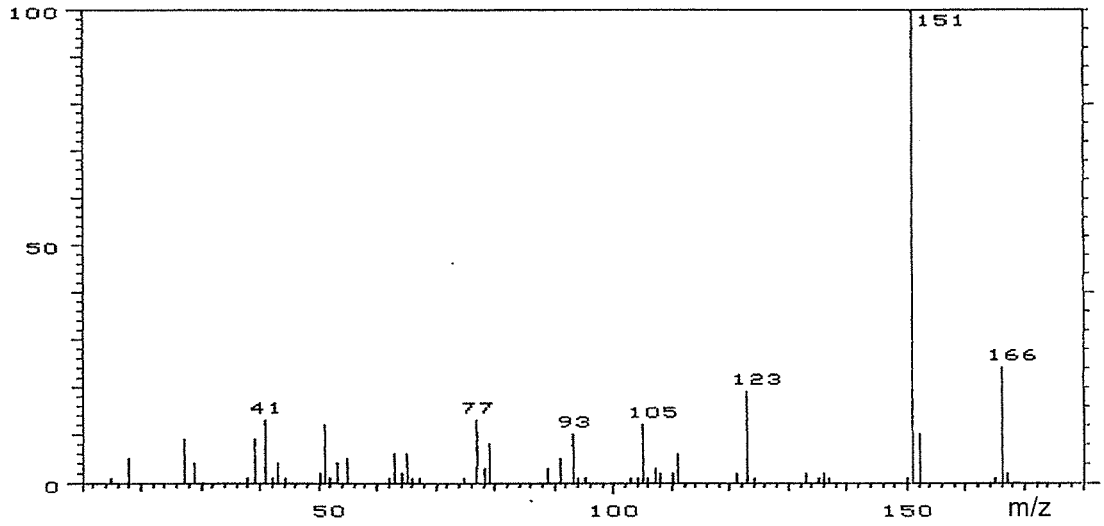
1. Spectral Data

Mass Spectrometry

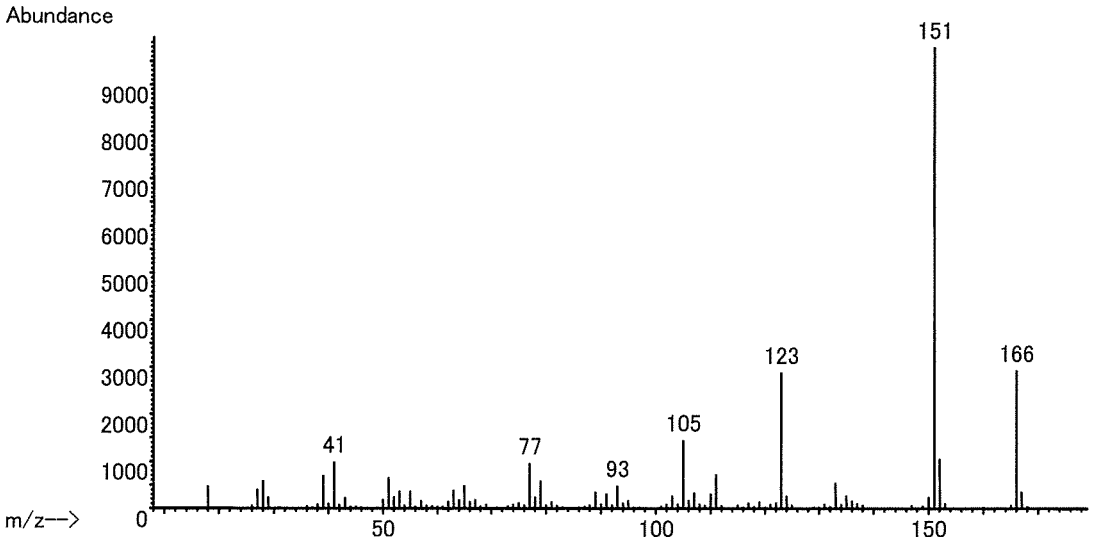
Instrument : Hitachi M-80B Mass Spectrometer

Ionization : EI (Electron Ionization)

Ionization Voltage : 70eV



Mass Spectrum of Test Substance



Mass Spectrum of Literature Data*

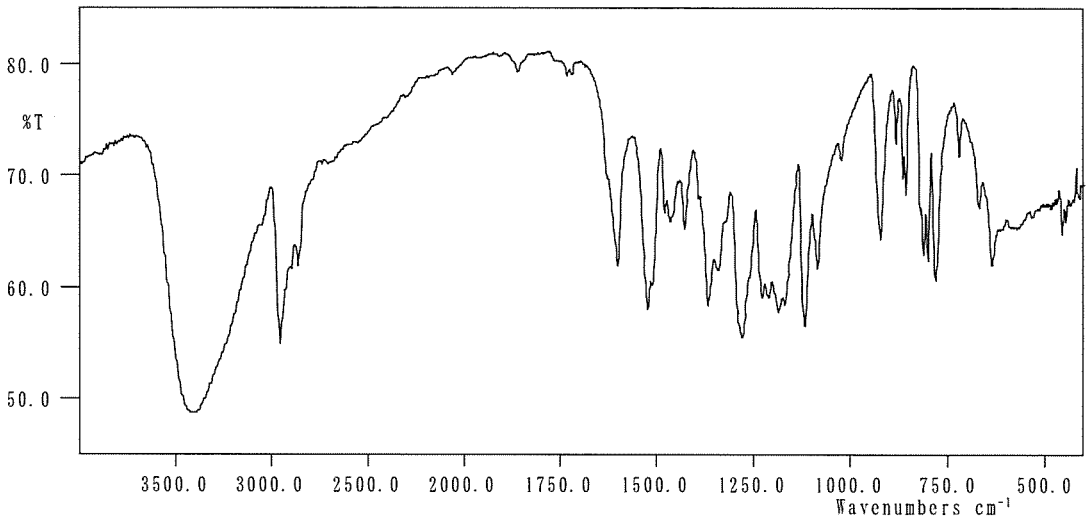
Result: The mass spectrum was consistent with literature spectrum.
(*McLafferty FW, ed. 1994. Wiley Registry of Mass Spectral Data. 6th ed. New York, NY:John Wiley and Sons.)

Infrared Spectrometry

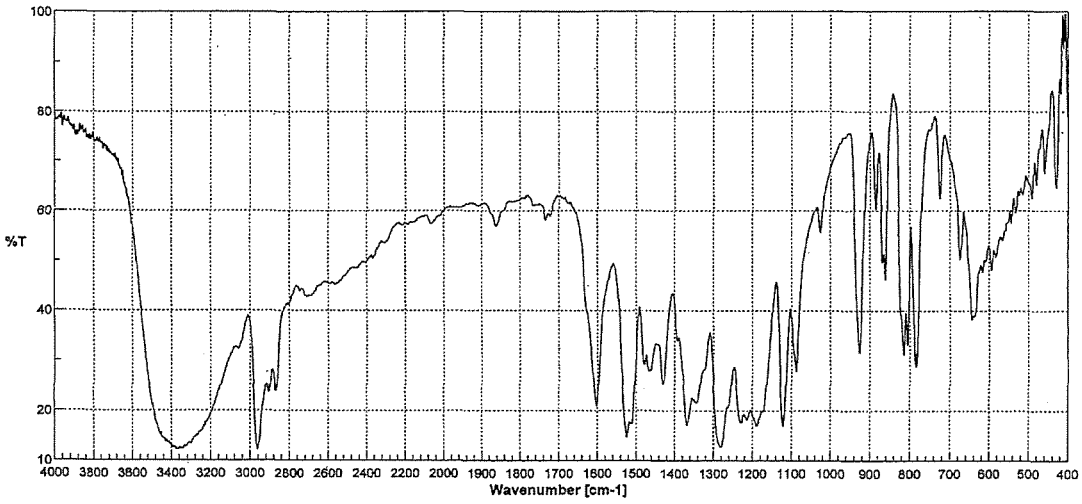
Instrument : Shimadzu FTIR-8200PC Infrared Spectrometer

Cell : KBr

Resolution : 4 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 4-*tert*-butylcatechol by mass spectrum and infrared spectrum.

APPENDIX 1-2

STABILITY OF 4-*tert*-BUTYLCATECHOL IN THE 2-YEAR FEED STUDY

STABILITY OF 4-*tert*-BUTYLCATECHOL IN THE 2-YEAR FEED STUDY

Test Substance : 4-*tert* -Butylcatechol (Wako Pure Chemical Industries, Ltd.)

A.

Lot No. : PEK3567

1. High Performance Liquid Chromatography

Instrument : Shimadzu LC-10 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 : 5mmol SDS solution (Phospholic acid pH2.2) = 50 : 50

Detector : UV (285 nm)

Injection Volume : 10 μL

Date analyzed	Peak No.	Retention Time (min)	Area (%)
2009.07.31	1	4.378	100
2010.10.29	1	4.317	100

Result: High performance liquid chromatography indicated one major peak (peak No.1) analyzed on 2009.7.31 and one major peak (peak No.1) analyzed on 2010.10.29. No new trace impurity peak in the test substance analyzed on 2010.10.29 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. : STL0824

1. High Performance Liquid Chromatography

Instrument : Shimadzu LC-10 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 : 5mmol SDS solution (Phospholic acid pH2.2) = 50 : 50

Detector : UV (285 nm)

Injection Volume : 10 μL

Date analyzed	Peak No.	Retention Time (min)	Area (%)
2010.10.22	1	4.322	100
2011.09.01	1	4.373	100

Result: High performance liquid chromatography indicated one major peak (peak No.1) analyzed on 2010.10.22 and one major peak (peak No.1) analyzed on 2011.9.1. No new trace impurity peak in the test substance analyzed on 2011.9.1 was detected.

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

APPENDIX 2-1

CONCENTRATION OF 4-*tert*-BUTYLCATECHOL IN FORMULATED DIETS IN THE 2-YEAR FEED STUDY

CONCENTRATION OF 4-*tert*-BUTYLCATECHOL IN FORMULATED DIETS IN THE 2-YEAR FEED STUDY

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

Instrument : Shimadzu LC-10 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 : 5mmol SDS solution (Phospholic acid pH2.2) = 50 : 50

Detector : UV (285 nm)

Injection Volume : 10 μL

	Target Concentration		
Date Analyzed	444[a]	1333	4000
2009.8.4	452[b] (102)[c]	1370 (103)	3920 (98.0)
2009.10.27	476 (107)	1400 (105)	4200 (105)
2010.1.19	479 (108)	1380 (104)	4110 (103)
2010.4.13	483 (109)	1400 (105)	4190 (105)
2010.7.6	462 (104)	1440 (108)	3670 (91.8)
2010.9.28	470 (106)	1350 (101)	4140 (104)
2010.12.21	475 (107)	1370 (103)	3970 (99.3)
2011.3.15	460 (104)	1400 (105)	4240 (106)
2011.6.7	434 (97.7)	1360 (102)	3800 (95.0)

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

APPENDIX 2-2

HOMOGENEITY OF 4-*tert*-BUTYLCATECHOL IN FORMULATED DIETS IN THE 2-YEAR FEED STUDY

HOMOGENEITY OF 4-*tert*-BUTYLCATECHOL IN FORMULATED DIETS IN THE 2-YEAR FEED STUDY

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

Instrument : Shimadzu LC-10 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 : 5mmol SDS solution (Phospholic acid pH2.2) = 50 : 50

Detector : UV (285 nm)

Injection Volume : 10 μL

	Target Concentration		
	444[a]	1333	4000
Coefficient Variation	8.02[b]	4.13	2.47

[a] ppm
[b] % (n=7)

APPENDIX 2-3

STABILITY OF 4-*tert*-BUTYLCATECHOL IN FORMULATED DIETS

STABILITY OF 4-*tert*-BUTYLCATECHOL IN FORMULATED DIETS

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

Instrument : Shimadzu LC-10 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 : 5mmol SDS solution (Phospholic acid pH2.2) = 50 : 50

Detector : UV (285 nm)

Injection Volume : 10 μL

	Target Concentration	
Date Analyzed	150[a]	15000
2008.10.28	144 (100)[b]	14200 (100)
2008.11.05[c]	139 (96.5)	14000 (98.6)
2008.11.05[d]	139 (96.5)	13800 (97.2)

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

APPENDIX 3

METHODS, UNITS AND DECIMAL PLACE FOR HEMATOLOGY AND BIOCHEMISTRY IN THE 2-YEAR FEED STUDY OF 4-*tert*-BUTYLCATECHOL

METHODS, UNITS AND DECIMAL PLACE FOR HEMATOLOGY AND BIOCHEMISTRY IN THE 104- WEEK FEED STUDY STUDY OF 4-*tert*-BUTYLCATECHOL

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]	U/L	0
Alanine aminotransferase (ALT)	JSCC method [2]	U/L	0
Lactate dehydrogenase (LDH)	JSCC method [2]	U/L	0
Alkaline phosphatase (ALP)	JSCC method [2]	U/L	0
γ-Glutamyl transpeptidase (γ-GTP)	JSCC method [2]	U/L	0
Creatine kinase (CK)	JSCC method [2]	U/L	0
Urea nitrogen	Urease·GLDH method [2]	mg/dL	1
Creatinine	Jaffé 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.)