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[redacted]のウサギを用いた急性眼刺激性／腐食性試験（試験番号：[redacted]】
の英文翻訳報告書

（試験番号：[redacted]）

株式会社ＬＳＩメディエンス

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Statement of Accurate Translation

Title: Acute Eye Irritation/Corrosion Study of [redacted] in Rabbits

Study number: [redacted]

I, the undersigned, hereby certify that the attached English report is a complete and accurate translation of the final report.

Signature: [redacted] August 23, 2017

Hitoshi Katou
Kumamoto Safety Assessment Department,
Nonclinical Research Center,
Drug Development Service Segment,
LSI Medience Corporation

Date:

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[redacted]

Final Report (Translated version)

Acute Eye Irritation/Corrosion Study of [redacted] in Rabbits

(Study number: [redacted])

LSI Medience Corporation

[redacted]

1. Statement

Title: Acute Eye Irritation/Corrosion Study of [redacted] in Rabbits

Study number: [redacted]

This study was conducted in compliance with the following Good Laboratory Practice Standard.

OECD Principles of Good Laboratory Practice (as revised in 1997)

Study director: *Signed in the original* *August 23, 2017*

Hitoshi Katou
Kumamoto Safety Assessment Department,
Nonclinical Research Center,
Drug Development Service Segment,
LSI Medience Corporation

Date

[redacted]

2. Contents

[redacted]

[redacted]

[redacted]

3. Study Outline

3.1 Title

Acute Eye Irritation/Corrosion Study of [redacted] in Rabbits

3.2 Study No.

[redacted]

3.3 Purpose

The purpose of this study is to assess the eye irritation or corrosion potential of [redacted] in rabbits.

3.4 Applied GLP

OECD Principles of Good Laboratory Practice (as revised in 1997)

3.5 Applied guideline

OECD Guideline for the Testing of Chemicals (No. 405, 2012)

3.6 Sponsor

[redacted]

3.7 Organization under contract

LSI Medience Corporation
1-13-4, Uchikanda, Chiyoda-ku, Tokyo 101-8517, Japan

3.8 Testing facility

Kumamoto Laboratory, LSI Medience Corporation
1285, Kurisaki-machi, Uto-shi, Kumamoto 869-0425, Japan

3.9 Study director

Hitoshi Katou
Kumamoto Safety Assessment Department,
Nonclinical Research Center,
Drug Development Service Segment,
LSI Medience Corporation
1285, Kurisaki-machi, Uto-shi, Kumamoto 869-0425, Japan

3.10 Main study contributors

Preparation of dosing formulations: Maki Ishimoto

[redacted]

Animal receipt:	Ryuji Yoshida
Animal care:	Ryuji Yoshida
Grouping:	Ryuji Yoshida
Application:	Ryuji Yoshida
Clinical observation:	Ryuji Yoshida
Body weight measurement:	Ryuji Yoshida
Observation of eye:	Ryuji Yoshida

3.11 Study schedule

Study initiation:	May 12, 2017
Animal receipt:	May 16, 2017
Grouping:	May 22, 2017
Initiation of the experiment:	May 23, 2017
Initiation test:	
Application	May 23, 2017
Observation of eye	May 23, 2017 to May 26, 2017
Confirmatory test:	
Application	May 24, 2017
Observation of eye	May 24, 2017 to May 27, 2017
Completion of the study:	Date signed on the final report by the study director

3.12 Retention

The materials listed in the following section will be retained in the archives of the testing facility. These materials will be retained for 5 years after submission of the final report. Further retention will be determined in consultation with the sponsor.

3.13 Retention materials

(1) Study protocol
(2) Documents on test substance
(3) Documents on used animals
(4) Documents on test results
(5) Correspondence documents
(6) Final report

3.14 Others

Conduct of this study was reviewed by Animal Experimentation Committee and approved by the director of Nonclinical Research Center (Approval number: 2017-0290), according to "Guidelines for Animal Studies (Guidelines of Nonclinical Research Center)."

[redacted]

4. Study Director Signature

Title: Acute Eye Irritation/Corrosion Study of [redacted] in Rabbits

Study number: [redacted]

Study director: *Signed in the original* *August 23, 2017*

Hitoshi Katou
Kumamoto Safety Assessment Department,
Nonclinical Research Center,
Drug Development Service Segment,
LSI Medience Corporation

Date

[redacted]

5. Summary

The acute eye irritation or corrosion potential of [redacted] was assessed in male New Zealand White rabbits. The eye reaction of the cornea, iris and conjunctivae of the administrated eye was observed in detail 1, 24, 48 and 72 hours after administration, and eye irritation was graded in accordance with Association Française de Normalisation. In addition, the observation of the cornea using fluorescein sodium was conducted in detail 24, 48 and 72 hours after administration.

No ocular reaction was observed on the cornea, iris, or conjunctivae in any animal during the observation period. The I.A.O.I. was 0, and evaluated as "Non-irritant". In the observation of the cornea using fluorescein sodium, no stain spot was observed in any animal during the observation period.

In conclusion, [redacted] had no irritation or corrosion potential on the rabbit eye under the conditions of this study.

[redacted]

6. Materials and Methods

6.1 Test substance

6.1.1 Name

[redacted]

6.1.2 Chemical name

2,3,3,3-tetrafluoro-2-(trifluoromethyl) propanenitrile

6.1.3 CAS No.

42532-60-5

6.1.4 Appearance

Colorless gas

6.1.5 Lot No.

[redacted]

6.1.6 Amount received

2 bottles

6.1.7 Supplier

[redacted]

6.1.8 Storage conditions and area

Room temperature (actual temperature: 19.3°C to 22.8°C; acceptable range: 1°C to 30°C), in Test Substance Storage Room A046

6.1.9 Stability confirmation

The sponsor indicates that the expiry date of the test substance in this study is 5 years from the shipping date (April 5, 2017).

6.1.10 Handling precautions

The following items were worn: safety glasses, a mask, and rubber gloves.

6.1.11 Handling of remaining test substance

All remaining test substance was discarded at the testing facility.

[redacted]

6.2 Preparation of dosing formulations

6.2.1 Preparation method

The test substance supplied from the supplier was used as is.

6.2.2 Frequency of preparation

At the time of use

6.2.3 Identification of dosing formulations

A label indicating the study number, substance name, preparation date and name of preparer was attached to a bottle.

6.3 Test system

6.3.1 Species

Rabbit

6.3.2 Strain

Kbl: NZW

6.3.3 Rationale for selection

This is widely used in acute eye irritation/corrosion studies, and there is abundant historical data.

6.3.4 Microbial level

SPF

6.3.5 Number and sex of animals purchased

4 males

6.3.6 Supplier (Breeding facility)

Kitayama Labes Co., Ltd.

6.3.7 Age

At receipt:	10 weeks old
At application:	11 weeks old

6.3.8 Body weight range at receipt

1924 to 2085 g (males, acceptable range: 1100 to 2700 g)

[redacted]

6.3.9 Quarantine and acclimation

At animal receipt, species, strain, age in weeks, number and sex were confirmed, and observation for clinical signs and body weight measurement were performed. A quarantine and acclimation period was set for 6 days from the day of receipt. During this period, all animals were examined for clinical signs once daily, and body weights were measured twice (receipt day and 6 days after receipt). Animals without any abnormalities were used for grouping.

6.3.10 Grouping

On the day before the application for initiation test, the cornea, iris and conjunctivae of both eyes were observed macroscopically and then with a slit lamp (SL-5, Kowa). Approximately 0.1 mL of Japanese pharmacopoeia physiological saline (Otsuka Pharmaceutical Factory, Inc., lot No. 4A85N) was dropped on the inclusion site of fluorescein sodium on FLUORES Ocular Examination Test Paper 0.7 mg (Showa Yakuhin Kako Co., LTD, lot No. 5134R), and dabbed on the cornea. The cornea was washed with about 20 mL of distilled water (Otsuka Pharmaceutical Factory, Inc., lot No. 6F78) 2 to 3 seconds later, and both eyes were examined with a slit lamp. Three animals without any abnormality in the cornea, iris or conjunctivae were selected, and assigned to the group by the total randomization method using a computer system (Provantis®, Instem LSS Limited).

6.3.11 Identification of animals

Before grouping:

At the animal receipt, numbers marked on the auricle by the breeder were used as the quarantine animal number (No. 1001 - 1004), and a label indicating the study number for computer system, quarantine animal number and sex was attached to the front of each cage.

After grouping:

The animal number (No. 1 - 3) was inscribed inside the left auricular by a black oil felt pen, and a label indicating the study number, animal number and sex was attached to the front of each cage.

6.3.12 Handling of remaining animals

The remaining animal was excluded from this study and euthanized by exsanguination under thiopental sodium overdose to the earlobe vein on May 30, 2017.

[redacted]

6.4 Animal management

6.4.1 Animal room

B303

6.4.2 Environmental conditions

6.4.2.1 Room temperature

Actual temperature: 22.9°C to 23.8°C (acceptable range: 20.0°C to 26.0°C)

6.4.2.2 Relative humidity

Actual humidity: 45.5% to 75.1%* (acceptable range: 35.0% to 75.0%)

*: transient humidity rise [75.1%, 10:42 to 10:43] due to cleaning and sanitation occurred on May 22, 2017)

6.4.2.3 Ventilation

10 to 20 times per hour

6.4.2.4 Lighting period

7:00 to 19:00 light and 19:00 to 7:00 dark cycles

6.4.3 Animal accommodation

6.4.3.1 Rack

Stainless cascade rack

6.4.3.2 Cage

Aluminum cage (W650 × D570 × H413 mm, with a resting board on the floor)

6.4.3.3 Feeder

Stainless feeder

6.4.3.4 Environmental enrichment

Toys (plastic water bottle and dumbbell) were used for improvement of animal welfare.

6.4.3.5 Number of animals per cage

1 animal/ cage

6.4.4 Diet

6.4.4.1 Description

Pellet diet (RC4, Oriental Yeast Co., Ltd.)

[redacted]

6.4.4.2 Lot number
170125, 170215

6.4.4.3 Feeding method
Ad libitum

6.4.4.4 Analysis
Analytical data for each lot were provided by Oriental Yeast Co., Ltd., and the contaminants in the diet were confirmed to be within the acceptable limits established by the testing facility.

6.4.5 Water
6.4.5.1 Description
Well water

6.4.5.2 Method of sanitization
Admixed with sodium hypochlorite (free residual chlorine concentration: about 0.2 ppm)

6.4.5.3 Water supply method
Ad libitum (water was supplied with an automatic dispenser)

6.4.5.4 Analysis
The water was analyzed twice a year at Nichigo Kyushu Co., Ltd. From the analytical data, it was confirmed that the quality of the water met the specifications of the testing facility.

6.5 Group composition

Dosing foumulation	Dosage	Sex	Animal Nos.
[redacted]	Single burst* / eye	Male	1 - 3

*: 0.7 to 0.8 g

6.6 Application
6.6.1 Application route
Administration into the eye

6.6.2 Application method
The administration was conducted in the portable draft. The pursuer of administration worn with a gas mask.

[redacted]

The initiation test was performed using one animal (animal No. 1).

6.6.2.1 Initiation test

(1) Sixty minutes prior to test substance administration (acceptable range: ±6 minutes), 0.01 mg/kg of buprenorphine (Lepetan injection 0.2 mg, lot No. 4G99L2, Otsuka Pharmaceutical Co., Ltd.) was administrated by subcutaneous injection.
(2) Five minutes prior to test substance administration (acceptable range: ±1 minute), one drop of the 0.4% oxybuprocaine hydrochloride (Oxybuprocaine hydrochloride minims® ophthalmic solution 0.4% SENJU®, lot No. H011, Senju Pharmaceutical Co., Ltd.) was administrated to both eyes.
(3) The animal was fixed in a retainer.
(4) The test substance was administered in a single burst about 1 second from a distance of about 10 cm directly in front of the eye by gently drawing the lower eyelid of the right eye. The left eye served as the control.
(5) About eight hours after test substance application (acceptable range: 8 to 9 hours), 0.01 mg/kg of buprenorphine and 0.5 mg/kg of meloxicam (Metacam® 0.5% injection, lot No. J20807C-24, Boehringer Ingelheim Vetmedica Japan Co., Ltd.) were administrated by subcutaneous injection.
(6) Since no eye irritation reactions were observed at 24 hours after test substance administration, administration of these analgesics was terminated.

Since no severe impairment was observed at 1 hour after application, a confirmatory test (section 6.6.2.2) was conducted for 2 animals on the following day.

6.6.2.2 Confirmatory test

(1) Sixty minutes prior to test substance administration (acceptable range: ±6 minutes), 0.01 mg/kg of buprenorphine (Lepetan injection 0.2 mg, lot No. 4G99L2, Otsuka Pharmaceutical Co., Ltd.) was administrated by subcutaneous injection.
(2) Five minutes prior to test substance administration (acceptable range: ±1 minute), one drop of the 0.4% oxybuprocaine hydrochloride was administrated to both eyes.
(3) The animal was fixed in a retainer.
(4) The test substance was administered in a single burst about 1 second from a distance of about 10 cm directly in front of the eye by gently drawing the lower eyelid of the right eye. The left eye served as the control.
(5) About 8 hours after test substance administration (acceptable range: 8 to 9 hours), 0.01 mg/kg of buprenorphine and 0.5 mg/kg of meloxicam (Metacam® 0.5% injection, lot No. J20807C-24, Boehringer Ingelheim Vetmedica Japan Co., Ltd.) were administrated by subcutaneous injection.
(6) Since no eye irritation reactions were observed at 24 hours after test substance

[redacted]

administration, administration of these analgesics was terminated.

6.6.3 Rationale for selection of application route, method, dosage, frequency and period

In accordance with the applied guideline

6.7 Frequency and method of observation and measurement

6.7.1 Observation of eye

6.7.1.1 Observation

The eye conditions of the cornea, iris and conjunctivae of the administrated eye were observed macroscopically and then with a slit lamp in detail 1, 24, 48 and 72 hours after administration and the reactions of the eye were scored based on the OECD guideline (Text-table 1) and Draize's evaluation criteria (Text-table 2, area of cornea involved and discharge only excerpt for only relevant section) [1]. In the observations at 24 hours after administration and onward, the administered eye was observed first by a slit lamp, and one drop of about 0.1 mL of Japanese pharmacopoeia physiological saline (Otsuka Pharmaceutical Factory, Inc.) was dropped on the inclusion site of fluorescein sodium on FLUORES Ocular Examination Test Paper 0.7 mg, and dabbed on the cornea. The cornea was washed with distilled water. Subsequently, the presence or absence of any injury in the corneal epithelium was examined again by a slit lamp.

Text-table 1 Grading of ocular lesions

A: Cornea	
Opacity: degree of density (readings should be taken from most dense area)	
No ulceration or opacity	0
Scattered or diffuse areas of opacity (other than slight dulling of normal lustre); details of iris clearly visible	1
Easily discernible translucent area; details of iris slightly obscured	2
Nacrous area; no details of iris visible; size of pupil barely discernible	3
Opaque cornea; iris not discernible through the opacity	4
Maximum possible:	4
C: Iris	
Normal	0
Markedly deepened rugae, congestion, swelling, moderate circumcorneal hyperaemia; or injection; iris reactive to light (a sluggish reaction is considered to be an effect)	1
Hemorrhage, gross destruction, or no reaction to light	2
Maximum possible:	2
Conjunctivae	
D: Redness (refers to palpebral and bulbar conjunctivae; excluding cornea and iris)	
Normal	0
Some blood vessels hyperaemic (injected)	1

[redacted]

Diffuse, crimson colour; individual vessels not easily discernible	2
Diffuse beefy red	3
Maximum possible:	3

E: Chemosis, swelling (refers to lids and/or nictitating membranes)

Normal	0
Some swelling above normal	1
Obvious swelling, with partial eversion of lids	2
Swelling, with lids about half closed	3
Swelling, with lids more than half closed	4
Maximum possible:	4

Text-table 2 Draize's evaluation criteria (opacity area and discharge)

Cornea

B: Area of cornea involved

No opacity area	0
One quarter (or less) but not zero	1
Greater than one quarter, but less than half	2
Greater than half, but less than three quarters	3
Greater than three quarters, up to whole area	4
Maximum possible:	4

Conjunctivae

F: Discharge

No discharge	0
Any amount different from normal (does not include small amounts observed in inner canthus of normal animals)	1
Discharge with moistening of the lids and hairs just adjacent to lids	2
Discharge with moistening of the lids and hairs, and considerable area around the eye	3
Maximum possible:	3

6.7.1.2 Evaluation

Eye irritation was graded in accordance with Association Française de Normalisation (Text-table 3) [2]. The total score of individual animal (Individual Index of Ocular Irritation, I.I.O.I.) was calculated by the following formula from the Grading of ocular lesions by OECD guideline (Text-table 1) and Draize's evaluation criteria (Text-table 2) [1].

$$\text{Total score (I.I.O.I)} = (A \times B \times 5) + (C \times 5) + [(D + E + F) \times 2]$$

Next, the total score of all animals was calculated, and the obtained value was divided by the number of animals to obtain the mean total score (Mean Index of Ocular Irritation,

M.I.O.I.). The eye irritation was evaluated from the maximum mean total score (Index of Acute Ocular Irritation, I.A.O.I.).

Text-table 3 Evaluation criteria (AFNOR, partly modified)

Values of indices			Conclusion
I.A.O.I.	M.I.O.I.	I.I.O.I.	The test substance is:
0 to 5	= 0 after 48 hours		non irritant
5 to 15	< 5 after 48 hours		slightly irritant
15 to 30	< 5 after 4 days		irritant
30 to 60	≤ 20 after 7 days	after 7 days: ≤ 30 in 3 rabbits out of 3 and ≤ 15 in at least 2 rabbits out of 3	very irritant
60 to 80	≤ 40 after 7 days	after 7 days: ≤ 60 in 3 rabbits out of 3 and ≤ 30 in at least 2 rabbits out of 3	severely irritant
80 to 110			extremely irritant

6.7.1.3 Photography

A photograph of a representative animal was taken at each observation day.

6.7.2 Clinical observation

An observation was performed twice before and after application on the administration day, in the morning and afternoon on the following day of application and two days later, and once daily on the other days. The administration day was defined as day 0 (Day 0).

6.7.3 Body weight measurement

Body weight was measured before application on application day and 72 hours observation day after administration.

6.8 Handling of dead and moribund animals during study period

Not applicable

6.9 Final disposal of animals

The animals were euthanized by exsanguination under thiopental sodium overdose to the earlobe vein on May 30, 2017.

[redacted]

6.10 Statistical analysis

No statistical analysis was performed.

6.11 Computer system

6.11.1 Computer system for used

Safety study support system (Provantis®, Instem LSS Limited)

6.11.2 Computer protocol numbers

Before grouping:
Grouping and thereafter: [redacted]
The inspection items and a schedule of the data collection were registered with the present computer system protocol.

6.11.3 Registry of the study into a computer system

(1) Grouping and calculation of dose volume of analgesic
(2) Data collection and tabulation (On-line data collection)
Body weight

7. Results

7.1 Eye reaction

The results of eye reactions are shown in Table 1, Table 2 and Appendix 1.
In [redacted] no ocular reaction was observed on the cornea, iris, or conjunctivae in any animal at 1, 24, 48 or 72 hours after administration. The M.I.O.I. were 0, and evaluated as "Non irritant". In the observation of the cornea using fluorescein sodium, no stain spot was observed in any animal at 24, 48 or 72 hours after administration.

7.2 Clinical signs

The data are shown in Table 3.
No abnormal clinical sign was observed in any animal.

7.3 Body weight

The data are shown Table 4.
No abnormal body weight gain was observed in any animal.

8. Discussion

No ocular reaction was observed on cornea, iris, or conjunctivae in any animal during the observation period. The I.A.O.I. was 0, and evaluated as "Non-irritant". In the observation of the cornea using fluorescein sodium, no stain spot was observed during the observation period.

[redacted]

In conclusion, [redacted] had no irritation or corrosion potential on the rabbit eye under the conditions of this study.

9. References

[1] Draize, J.H. (1959): "Dermal toxicity", Appraisal of the safety of chemicals in foods, drugs and cosmetics, pp46-59, Association of Food & Drug Officials of the United States, Austin, Texas.

[2] J.P. Guillot, L. Caillard, J.F. Gonnet et C. Clement (1980): IFREB-RP, Produits Chimiques, Tolérance locale oculaire et cutanée, Protocoles "Cosmétique" AFNOR et OCDE. (IFREB-RP, Chemicals, Ocular and Cutaneous Local Tolerance, "Cosmetic", A.F.N.O.R. and O.E.C.D. Protocols.)

10. Study Note

10.1 Deviations from the protocol

None

[redacted]

Table 1 Mean total scores

Test substance	Number of animals	Mean Index of Ocular Irritation (M.I.O.I.) 1hr [a)]	24hr	48hr	72hr	Classification
[redacted]	3	0*	0*	0*	0*	Non irritant

* Index of Acute Ocular Irritation (I.A.O.I.)
a) Time after application

Table 2 Fluorescein staining area of the cornea

Test substance	Animal No.	Staining area 24hr [a)]	48hr	72hr
[redacted]	1	- [b)]	-	-
	2	-	-	-
	3	-	-	-

a) Time after application
b) -: No change

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Table 3 Clinical signs

Animal No.	Days of application						
	0		1		2		3
	BFR	AFT	AM	PM	AM	PM	DAY
1	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-
3	-	-	-	-	-	-	-

Abbreviation: BFR, before application; AFT, after application
Clinical sign: -, No abnormality

Table 4 Body weights

Animal No.	Body weight (g) on day	
	Days of application	
	0	3
1	2201	2247
2	2318	2340
3	2355	2359

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[redacted]

Appendix 1 Individual eye irritation scores

Test substance	Animal No.	Tissue		Eye irritation score			
				1 hr [a]	24 hr	48 hr	72 hr
[redacted]	1	Cornea	(A)	0	0	0	0
			(B)	0	0	0	0
		Iris	(C)	0	0	0	0
		Conjunctivae	(D)	0	0	0	0
			(E)	0	0	0	0
			(F)	0	0	0	0
		I.I.O.I. [b]		0	0	0	0
	2	Cornea	(A)	0	0	0	0
			(B)	0	0	0	0
		Iris	(C)	0	0	0	0
		Conjunctivae	(D)	0	0	0	0
			(E)	0	0	0	0
			(F)	0	0	0	0
		I.I.O.I.		0	0	0	0
	3	Cornea	(A)	0	0	0	0
			(B)	0	0	0	0
		Iris	(C)	0	0	0	0
		Conjunctivae	(D)	0	0	0	0
			(E)	0	0	0	0
			(F)	0	0	0	0
		I.I.O.I.		0	0	0	0
	M.I.O.I. [c]			0	0	0	0

a) Time after application
b) I.I.O.I.: Individual Index of Ocular Irritation = (A×B×5)+(C×5)+[(D+E+F)×2]
(A) Opacity - degree of density, (B) Area of cornea involved, (C) Values, (D) Redness, (E) Chemosis, (F) Discharge
c) M.I.O.I.: Mean Index of Ocular Irritation

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[redacted]

Quality Assurance Statement

Title: Acute Eye Irritation/Corrosion Study of [redacted] in Rabbits
Study No.: [redacted]

This study was carried out in accordance with the following standard. I hereby certify that this final report faithfully describes the methods and results in this study. The inspection and reporting are as follows.

OECD Principles of Good Laboratory Practice (as revised in 1997)

	Inspection Date	Reporting Date to the Study Director	Reporting Date to the Management
Study Protocol			
Study Protocol	May 15, 2017	May 15, 2017	May 15, 2017
Computer Protocol	May 19, 2017	May 19, 2017	May 19, 2017
Study Procedure			
Receipt of animals, Body weight measurement	May 16, 2017	May 16, 2017	May 16, 2017
Application, Observation of eye	May 23, 2017	May 23, 2017	May 23, 2017
Raw Data, Final Report			
Raw Data, Draft Report	Jul. 06, 2017	Jul. 06, 2017	Jul. 06, 2017
Raw Data, Final Report	Aug. 22, 2017 to Aug. 23, 2017	Aug. 23, 2017	Aug. 23, 2017

Signed on behalf of Quality Assurance Manager

Quality Assurance Manager: *Signed by Kimitoshi Hirose* in the original* August 23, 2017

*: QAM Provisional Representative
Fumi Cho
Kumamoto Laboratory,
LSI Medience Corporation

Date