For blinded substances

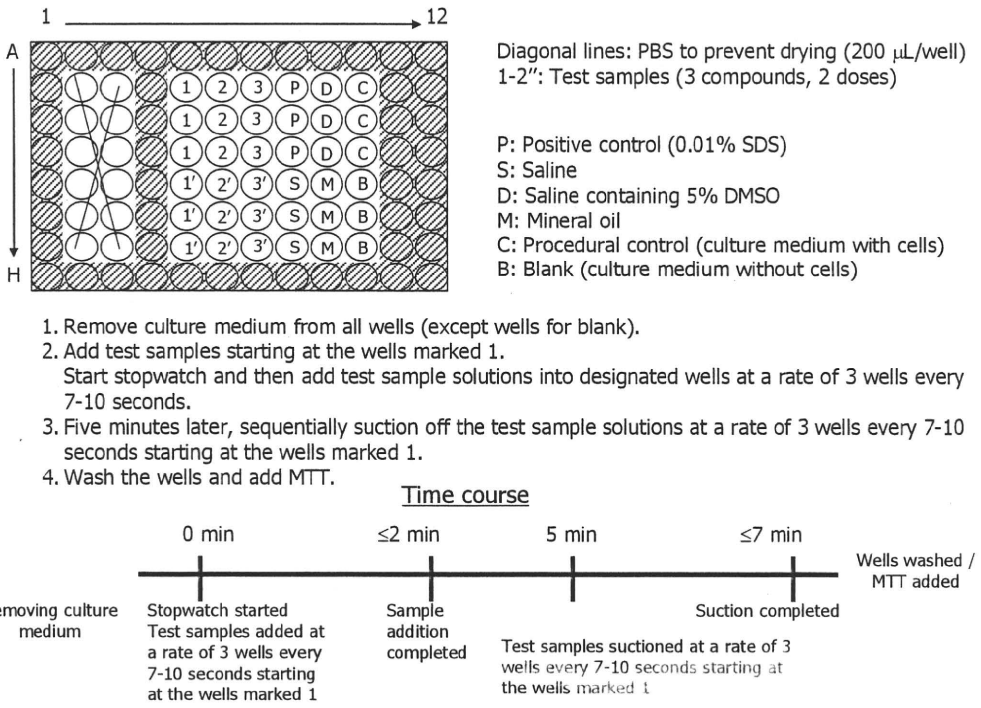

Figure 3.4. Method of test substance exposure

* Two hundred (200) μL of 0.5 mg/mL[Note 3)] of MTT (CAS No. 298-93-1) was added per well and incubated for 2 h in an incubator (37°C, 5% CO_2).

Note 3): To prepare MTT solution, 21 mg of MTT was combined with 42 mL of the MEM medium, shaken to mix, and let stand for 20 min. MTT solution was well shaken again, placed in a 20 mL syringe, and filtered through a 0.45 μm microfilter. Steps were taken to protect the MTT solution from light between the time of weighing and the time of use.

* Following the 2 hr incubation period, plates were removed from the incubator and medium was discarded. Plates were tapped lightly on a paper towel to sufficiently remove liquid.

* Two hundred (200) μL of 0.04N HCl-isopropanol (isopropanol 96 mL + 1N HCl 4 mL) were added to each well and were let stand for 60 min.

* Plate was placed in reader following the homogenous mixing of cell suspension in the plate, and the absorbance was measured at a wavelength of 570 nm.

6) Calculation of Cell viability

* A cell viability was calculated by taking the ratio of the absorbance of each substance to the absorbance of the solvent control group (%):

$$\text{Cell Viability} = \frac{\text{Absorbance of Test Sample (Absorbance of Test Sample - Blank)}}{\text{Absorbance of Solvent Control (Absorbance of Solvent Control - Blank)}} \times 100$$

* For each substance concentration, three wells per experiment were used and the mean value was calculated to obtain the cell viability.

* If the mean value of the test substance absorbance of three wells is lower than the mean value of the solvent control absorbance and the cell viability value is negative, the cell viability is 0.

* The mean value of three independent[Note 4)] experiments was used as the final cell viability for each substance concentration.

Note 4): For each substance concentration, three wells per plate were used for the evaluation. When criterion 1, 2, and 3 given below—which are the conditions for completing the experiment—are met, the mean value is becomes the result for one independent test.

3.5. Acceptance Criteria

* Criterion 1: The absorbance after subtracting the blank of the medium operation control is 0.3 or higher.

* Criterion 2a: The cell viability in physiological saline is 80% or higher when the cell viability in the medium control is 100%.

* Criterion 2b: The cell viability in 5% (w/w) DMSO is 80% or higher when the cell viability in the medium control is 100%.

* Criterion 2c: The cell viability in mineral oil is 80% or higher when the cell viability in the medium control is 100%.

* Criterion 3: The cell viability of positive control (SLS 0.01%) is in the range of 21.1–62.3 (i.e., within the range of the mean cell viability ± 2 standard deviation, $41.7 \pm 10.3 \times 2$).

* Criterion 4: The standard deviation of the cell viability obtained with three experiments performed independently for each test substance concentration is less than 15[Note 5)].

Note 5): If the standard deviation of the cell viability obtained with three experiments performed independently is greater than 15 (SD>15), a new experiment is preformed and a new determination is made as to whether criterion 4 for the experiment has been met, on the basis of the results of three other independent experiments.

3.6. Prediction model

1) Criteria for irritants and non-irritants

* The mean cell viability after adding the test substance at 5% solution and exposing for 5 min is the endpoint of the STE test. Substances with the mean cell viability values greater than 70% were considered non-irritants, while those with the cell viability values of 70% or less were considered irritants. We defined this rule with the STE irritation classifications (STE classification)

2) Rank classification of eye irritation in STE tests

* The eye irritation property rank classifications for STE tests are shown in Figure 3.5. We defined this rule with the STE rank.

* Under the 5% exposure condition of STE testing, a score of 0 is given when the cell viability is greater than 70%, and a score of 1 is given when the cell viability is 70% or less. Under the 0.05% exposure condition of

STE testing, when the cell viability is greater than 70%, the score is 1; when the cell viability is 70% or less, the score is 2. The 5% and 0.05% scores are added together, and the strength of the substance's eye irritation is classified based on the value obtained. A score of 1 was classified as weak irritation and given a rank of 1; a score of 2 was rank 2 or classified as medium irritation, and a score of 3 resulted in a classification of strong irritation, a rank of 3.

STE irritation score

Test sample solution 5%	Score	Test sample solution 0.05%	Score
If CV > 70%,	0	If CV > 70%,	1
If CV ≤ 70%,	1	If CV ≤ 70%,	2

CV: Cell viability

5% score + 0.05% score = STE rank

STE rank	Eye irritation potential
1	Minimally irritant
2	Moderately irritant
3	Severely irritant

Fig. 3.5 The STE rank

3.7 Schedule of experiments

The technical workshop held at Kanebo Cosmetics Inc.(Odawara) on Thursday, May 29, 2008 in the 1st phase validation study. A workshop focusing was on the technical transfer of the principles and experimental procedures of the STE test. The three laboratories participating in the previous validation study participated in the 2nd phase validation study. Therefore, we deleted the training course and preliminary testing of this test for technical transfer.

Duration of this validation study was six-month from June 2010 to December 2010 on schedule. The meetings of VMT were held at Kyoto University on Friday, June 11 and Wednesday, 24 November, 2010.

3.8 Test substances (blinded test substances)

In this phase validation study, the 40 test substances finally selected by the Chemical Management group including two substances (C07 and C37) used in the 1st phase validation study (Table 3.1). The selected test substances had been previously evaluated using the Draize test and had been classified using GHS categories. The other information is described at the report on selection of test substances for the 2nd phase validation study. Ten test substances were sent to three laboratories and twenty test substances from the others forwarded to two laboratories. Therefore, each laboratory received thirty of forty substances.

All test substances are blinded, coded, rotated and distributed by JaCVAM by the middle of August according to coding list by data analyzing group. The test substances were encoded and blinded according to data analysis

group and the blinded substances were distributed a chemical master of each participating laboratory.

In consideration of the safety of the individuals in charge of the experiment, JaCVAM forward the information of appropriate environmental conditions and the Material Safety Data Sheet (MSDS) of blinded test substances only for the chemical master. We gave instructions to handle the all test substances as Toxic and Deleterious Substances, to store the test substance under appropriate environmental conditions and the enclosed MSDS can be referred for the study manager in case of an accident only.

Table 3.1 shows the code numbers, names of the substances, GHS categories and distributed laboratories. The other information is described at the report on selection of test substances for the validation study such as CAS numbers, supplier, molecular weight, description for each of the 40 blinded test substance substances.

In an additional study, JaCVAM distributed the three non-coded test substances (No. C01, C02 and C25) at each laboratory after the 2nd phase validation study.

Table 3.1 Code numbers, substance names, CAS numbers, and GHS categories of the 40 test substances.

code	Chemical Name	GHS	Lab.		
			1	2	3
c06	m-phenylenediamine	1	○	○	○
c27	2-methylbutanoic acid	1	○	○	○
c37	1-Octanol	2A	○	○	○
c17	isopropyl myristate	NI	○	○	○
c32	imidazole	1	○	○	○
c40	isobutanal	2B	○	○	○
c07	2-Ethylhexyl p-dimethyl-amino benzoate	NI	○	○	○
c15	butanol	2A	○	○	○
c14	n-lauroylsarcosine sodium salt	2B	○	○	○
c28	ethyl-2-methylacetoacetate	2B	○	○	○
c22	isopropyl bromaide	NI	○	○	-
c30	cyclohexanone	NI	○	-	○
c08	ethyl acetate	2B	○	○	-
c33	ammonium nitrate	2B	○	○	-
c12	camphen	2B	○	○	-
c04	n-butanal	2B	-	○	○
c38	Triton X-100 (5%)	2B	-	○	○
c21	propasol solvent P	2B	-	○	○
c23	di(propylene glycol) propyl ether	2B	○	-	○
c20	hexyl cinnamic aldehyde	2B	○	-	○
c13	sodium monochloroacetate	2B	○	-	○
c25	methyl cyanoacetate	2A	○	○	-
c26	calcium thioglycollate	2A	○	○	-
c05	cyclopentanol	2A	○	○	-
c18	citric acid	2A	-	○	○
c11	butyrolactone	2A	-	○	○
c29	potassium sorbate	2A	-	○	○
c24	isopropyl alcohol	2A	-	○	○
c34	methyl acetate	2A	○	-	○
c31	2-benzyloxyethanol	2A	○	-	○
c19	isobutyl alcohol	2A	○	-	○
c35	myristyl alcohol	2A	○	-	○
c01	distearyldimethylammonium chloride	1	○	○	-
c39	pyridine	1	○	○	-
c03	sodium lauryl sulfate	1	○	○	-
c10	methoxyethyl acrylate	1	-	○	○
c36	sodium salicylate	1	-	○	○
c16	2,5-dimethyl-2,5-hexanediol	1	-	○	○
c09	monoethanolamine	1	○	-	○
c02	promethazine hydrochloride	1	○	-	○

3.9 Data management

1) Data set and management

To analyze the data for the 40 test substances, the data analyzing group prepared a program wherein data were entered into a database from individual data sheets upon completion of data review. Summary statistics were provided on the basis of data sheet (STE Datasheet(20100625).xls) obtained from each participating laboratory as well as the 1st phase validation study.

After experiments, the recorded experimental results (on recording sheets) were forwarded to the record management group by the study director at each laboratory. The copies of the raw data and data sheets (electronic files) were also sent to the manager of data analysis group. Here, "raw data" were considered to be

data printouts of the absorbance measurements and the date on which each measurement was taken; each piece of data was signed by the study director. The data from the participated laboratories must satisfy with the criteria 1-3 for experimental validation prior to submission. If they are not satisfied with the criteria, the record management group or data analysis group can request an additional data before finishing the validation study.

The data summary and analysis from the 40 test substances were performed on the basis of all dataset at each laboratory.

2) Handling of data in the dataset of the investigation for the 40 test substances

* Absorbance

Although not reflected in the data sheet, absorbance values less than 0 had been set to 0.

* The cell viability

Although not reflected in the data sheet, mean viability values less than 0 of three experiments had been set to 0, which was based on a decision made at the1st phase validation study.

3) Analyze

The intra-laboratory reproducibility at each laboratory were analyzed with all data which or not depended the acceptance criterion 4. The accepted data with accordance to criteria were used to evaluate inter-laboratory reproducibility. After decoding, the differences in the STE rank between laboratories were confirmed.

For the predicitivity, the 40 blinded substances plus 25 substances were used at the 1st phase validation study (total 63: two substances (C07: 2-ethylhexyl p-dimethyl-amino benzoate and C37: 1-octanol) were used in both validation studies) are as shown in Table 3.1. For each laboratory, the primary analysis provided the correspondence between the STE classification, as determined by the STE test at the 5% concentration and the GHS classification (UN GHS Categories 1 and No). The sensitivity, specificity, and accuracy (Accuracy A) values were outlined through the 2 × 2 contingency table at total laboratories.

For the secondary analysis, the correspondence between the STE rank and the GHS categories (i.e., UN GHS Category 1, Category 2, and No) was provided. The accuracy (Accuracy B) value was outlined through the 3 × 3 contingency table at total laboratories.

3.10 Additional study

After the validation study, three test substances (C01, C02 and C25) were re-evaluated by three laboratories to be clear the reason of difference results between laboratories. Using non-coded test substance, these laboratories re-tested with accordance to the same protocol. However, preparation methods of test substances were proposed with the protocol by lead laboratories. Using saline and not used 5% DMSO solution as the solvent, especially, test substance C01 (distearyldimethylammonium chloride) should be prepared in condition with heat up at 50 ℃ hot water.

Table 3.2 Test substance lists used in the 1st Phase and 2nd phase validation studies.

Code	Substance	GHS
Alcohols (13 substance)		
Q/c37	1-octanol	2A
c31	2-benzyloxyethanol	2A
R	2-ethyl-1-hexanol	2A
K	2-methyl-1-pentanol	2B
c15	butanol	2A
T	cyclohexanol	1
c05	cyclopentanol	2A
E	ethanol	2A
c19	isobutyl alcohol	2A
c24	isopropyl alcohol	2A
c35	myristyl alcohol	2A
L	n-hexanol	2A
c21	propasol solvent P	2B
Aldehydes (3 substance)		
c20	hexyl cinnamic aldehyde	2A
c40	isobutanal	2B
c04	n-butanal	2B
Amines (2 substances)		
c09	monoethanolamine	1
c06	m-phenylenediamine	1
Alcane and Cycloalcane (2 substances)		
M	3,3-dimethylpentane	NI
N	methyl cyclopentane	NI
Esters (6 substances)		
c08	ethyl acetate	NI
c28	ethyl-2-methylacetoacetate	2B
c17	isopropyl myristate	NI
c10	methoxyethyl acrylate	1
c34	methyl acetate	2A
c25	methyl cyanoacetate	2A

Code	Substance	GHS
Heterocyclics (2 substances)		
c32	imidazole	1
c39	pyridine	1
Hydrocarbons (3 substances)		
c12	camphen	2B
c22	isopropyl bromaide	NI
P	toluene	NI
Ketones and Lactones (7 substances)		
S	acetone	2A
c11	butyrolactone	2A
c30	cyclohexanone	NI
W	gluconolactone	NI
J	methyl amyl ketone	NI
X	methyl ethyl ketone	2A
O	methyl isobutyl ketone	NI
Organic acids (2 substances)		
c27	2-methylbutanoic acid	1
c18	citric acid	2A
Organic salts (5 substances)		
c26	calcium thioglycollate	2A
U	n,n-dimethylguanidine sulfate	NI
c29	potassium sorbate	2A
c13	sodium monochloroacetate	2B
c36	sodium salicylate	1
Polyols (5 substance)		
16	2,5-dimethyl-2,5-hexanediol	1
A	3-methoxy-1.2-propanediol	NI
C	glycerol	NI
B	polyethylene glycol 400	NI
Y	propylene glycol	NI

Table 3.2 Test substance lists used in the 1st Phase and 2nd phase validation studies (continued)

Code	Substance	GHS
Surfactants (7 substances, 8 tests)		
I	benzalkonium chloride	1
H	cetylpyridinium bromide	1
c01	distearyldimethylammonium chloride	1
c14	n-lauroylsarcosine sodium salt	2A
c03	sodium lauryl sulfate	1
G	Triton X-100	1
c38	Triton X-100 (5%)	2A
D	Tween20	NI
Other substances (5 substances)		
V/c07	2-ethylhexyl p-dimethyl-amino benzoate	NI
c33	ammonium nitrate	2A
c23	di(propylene glycol) propyl ether	2B
c02	promethazine hydrochloride	1
F	sodium hydroxide	1

Substances selected for 1st validation study (25 substances)

4. Results

4.1 Quality of the study

To ensure the quality of the study, the following steps were performed:

*VMT recorded the process by which the test protocol would be revised

* The record management group verified record sheets

* The data analyzing group checked between raw data and data sheets

4.2. Data Handling

1) Data review

The Data analyzing group confirmed the consistency of the data printed out with the data inputted into the electronic file. All data and the value including invalid data, which were not satisfied with the criteria, were necessary no change to reflect the correct value and request no additional experiments.

2) Defective records

The chair of VMT confirmed the all records record sheets at each laboratory. The record from Lab 2 showed that test substance C01 detected incrustation at well surface in 96 well plate which was difficult to eliminate with washing a test substance and they appeared yellow color in isopropanol. Furthermore, the test substance C26 caused attachment at the well surface in plastic tube to prepare the test solution. The technician of lab 2 changed it from plastic tube to glass tube.

*3) Number of experiments performed

For any experiment performed in any laboratory, that did not satisfy the all criteria for validity as described in 3.6 was determined not to be valid or whenever an obvious error in an experimental procedure occurred, the experiment was repeated.

In the mean absorbance after subtracting the blank of the medium control (Table 4.1), the cell viability with saline (Table 4.2), 5% (w/w) DMSO in saline (DMSO: Table 4.3), or mineral oil (Table 4.4), there were no errant data from criteria. However, deviant six data (two plates) of the cell viability with saline were detected at Lab 3 as shown in Table4.2. Furthermore, deviant 19 data (seven plates) and 21 data (seven plates) of the cell viability with positive control (SLS) were confirmed with Lab 2 and 3, respectively. From the overlap of errant six data at lab3, total 40 data (14 plates), each 19 data (seven plates) and 21 data (seven plates) at Lab 2 and 3 by the criterion 3. These invalid data which is not satisfied with criteria 1-3 were re-tested by all laboratories.

After the validation study, the total in valid data were 15 data (5 test substances) in all data, each 12 data (4 test substances; C01, C25 twice and C40) and 3 data (C07) at Lab 2 and 3 by the criterion 4. That is, the invalid data of criterion 4 were SD> 15 of the cell viability obtained with three experiments performed independently for each test substance. The total number of experiments for the 40 substances in three laboratories, including the numbers of experiments that were not valid, is shown in Table 4.5. There were 0, 31 and 24 data at lab 1, lab2 and lab3, respectively.

4.3 Intra-laboratory reproducibility

The total number of experiments for the 95 substances (30 results for 40 substances) in three laboratories, including the numbers of experiments that were not five valid, is shown in Table 4.6 and 4.7. Each 12 invalid data (4 test substances; C01, C25 twice and C40) and 3 data (C07) at Lab 2 and 3 are shown due to SD>15 in this table.

The ratio of valid results are 100% (0/30) at lab 1 , 88.2% (30/34) at lab 2 and 96.8% (30/31) at lab 3. We consider Intra-laboratory reproducibility of the STE test is high. Absolutely, the data of three independent tests were accepted in order to satisfy the conditions for test validity, and valid data were used with all the substances for predicitivity.

Table 4.1 Medium OD and passing status for Criteria 1

		Lab.					
		1		2		3	
File No.	Round	Medium	Criteria 1	Medium	Criteria 1	Medium	Criteria 1
1	0					0.51	OK
	1	0.54	OK	0.48	OK	0.61	OK
	2	0.38	OK	0.52	OK	0.51	OK
	3	0.5	OK	0.46	OK	0.5	OK
2	0					0.47	OK
	0			0.47	OK	0.59	OK
	1	0.84	OK	0.45	OK	0.64	OK
	2	0.46	OK	0.5	OK	0.6	OK
	3	0.41	OK	0.44	OK	0.55	OK
3	0					0.55	OK
	0					0.64	OK
	1	0.54	OK	0.46	OK	0.63	OK
	2	0.45	OK	0.4	OK	0.48	OK
	3	0.48	OK	0.42	OK	0.56	OK
4	0			0.44	OK		
	0			0.53	OK		
	1	0.85	OK	0.47	OK	0.52	OK
	2	0.43	OK	0.43	OK	0.54	OK
	3	0.45	OK	0.44	OK	0.7	OK
5	0					0.57	OK
	1	0.43	OK	0.42	OK	0.45	OK
	2	0.32	OK	0.45	OK	0.53	OK
	3	0.38	OK	0.53	OK	0.65	OK
6	0			0.48	OK		
	1	0.42	OK	0.46	OK	0.58	OK
	2	0.43	OK	0.39	OK	0.51	OK
	3	0.47	OK	0.44	OK	0.65	OK
7	0			0.58	OK		
	1	0.42	OK	0.49	OK	0.65	OK
	2	0.47	OK	0.52	OK	0.49	OK
	3	0.41	OK	0.5	OK	0.65	OK
8	1	0.42	OK	0.52	OK	0.65	OK
	2	0.43	OK	0.59	OK	0.61	OK
	3	0.41	OK	0.57	OK	0.66	OK
9	0			0.48	OK		
	1	0.44	OK	0.56	OK	0.65	OK
	2	0.45	OK	0.57	OK	0.6	OK
	3	0.42	OK	0.41	OK	0.66	OK
10	0					0.55	OK
	1	0.4	OK	0.61	OK	0.61	OK
	2	0.82	OK	0.52	OK	0.6	OK
	3	0.64	OK	0.4	OK	0.61	OK
11	1			0.42	OK	0.66	OK
	2			0.42	OK	0.64	OK
	3			0.54	OK	0.74	OK
12	0			0.51	OK		
	1			0.46	OK		
	2			0.41	OK		
	3			0.56	OK		
13	1			0.56	OK		
	2			0.48	OK		
	3			0.37	OK		

Round 0: Invalid data at any criteria in a laboratory

OK: valid

X: Invalid

Table 4.2 The cell viability by saline and passing status for Criteria 2a

		Lab.					
		1		2		3	
File No.	Round	Cell Viability	Criteria 2a	Cell Viability	Criteria 2a	Cell Viability	Criteria 2a
1	0					89.8	OK
	1	103.5	OK	93.6	OK	95.0	OK
	2	91.3	OK	92.3	OK	97.3	OK
	3	89.6	OK	87.6	OK	96.8	OK
2	0					91.8	OK
	0			104.9	OK	94.4	OK
	1	88.4	OK	104.8	OK	93.3	OK
	2	87.1	OK	91.4	OK	105.8	OK
	3	90.1	OK	94.3	OK	86.4	OK
3	0					90.3	OK
	0					71.4	×
	1	92.1	OK	95.8	OK	95.8	OK
	2	85.0	OK	104.3	OK	98.1	OK
	3	87.6	OK	93.5	OK	81.5	OK
4	0			97.1	OK		
	0			85.9	OK		
	1	89.9	OK	101.6	OK	98.4	OK
	2	89.5	OK	103.0	OK	103.3	OK
	3	87.9	OK	116.2	OK	82.9	OK
5	0					72.4	×
	1	93.0	OK	99.9	OK	105.8	OK
	2	90.1	OK	100.4	OK	85.4	OK
	3	94.4	OK	102.3	OK	91.7	OK
6	0			93.8	OK		
	1	95.1	OK	104.0	OK	96.2	OK
	2	91.2	OK	118.6	OK	93.2	OK
	3	86.1	OK	94.0	OK	91.6	OK
7	0			82.7	OK		
	1	97.6	OK	93.4	OK	106.6	OK
	2	86.0	OK	93.2	OK	99.1	OK
	3	91.5	OK	84.0	OK	94.3	OK
8	1	87.1	OK	94.8	OK	92.7	OK
	2	91.3	OK	84.0	OK	87.1	OK
	3	94.1	OK	85.8	OK	94.5	OK
9	0			95.4	OK		
	1	86.0	OK	88.5	OK	100.0	OK
	2	85.6	OK	90.0	OK	88.0	OK
	3	101.5	OK	89.6	OK	96.1	OK
10	0					93.4	OK
	1	94.7	OK	83.1	OK	89.4	OK
	2	91.5	OK	86.3	OK	92.7	OK
	3	111.0	OK	105.4	OK	98.3	OK
11	1	.	.	99.5	OK	93.8	OK
	2	.	.	98.8	OK	93.8	OK
	3	.	.	87.4	OK	94.2	OK
12	0			83.8	OK		
	1	.	.	101.4	OK	.	.
	2	.	.	82.5	OK	.	.
	3	.	.	86.6	OK	.	.
13	1	.	.	86.6	OK	.	.
	2	.	.	100.9	OK	.	.
	3	.	.	94.0	OK	.	.

Round 0: Invalid data at any criteria in a laboratory

OK: valid

X: Invalid

Table 4.3 The cell viability by 5% DMSO and passing status for Criteria 2b

		Lab.					
		1		2		3	
File No.	Round	Cell Viability	Criteria 2b	Cell Viability	Criteria 2b	Cell Viability	Criteria 2b
1	0					89.5	OK
	1	94.1	OK	93.1	OK	93.9	OK
	2	86.3	OK	86.7	OK	96.5	OK
	3	83.1	OK	92.8	OK	95.0	OK
2	0					95.0	OK
	0			89.0	OK	94.9	OK
	1	91.6	OK	95.3	OK	87.8	OK
	2	83.2	OK	81.6	OK	99.2	OK
	3	91.5	OK	80.2	OK	90.1	OK
3	0					90.3	OK
	0					95.8	OK
	1	85.0	OK	95.2	OK	95.2	OK
	2	82.0	OK	99.8	OK	102.1	OK
	3	83.1	OK	87.7	OK	84.8	OK
4	0			105.8	OK		
	0			91.1	OK		
	1	84.4	OK	90.0	OK	95.3	OK
	2	95.5	OK	99.8	OK	102.8	OK
	3	92.2	OK	99.6	OK	87.5	OK
5	0					95.6	OK
	1	86.4	OK	103.1	OK	98.4	OK
	2	92.0	OK	96.9	OK	90.9	OK
	3	88.9	OK	96.7	OK	89.1	OK
6	0			98.2	OK		
	1	90.5	OK	91.9	OK	92.0	OK
	2	84.2	OK	110.2	OK	92.9	OK
	3	82.4	OK	95.3	OK	91.3	OK
7	0			86.9	OK		
	1	92.7	OK	90.0	OK	104.0	OK
	2	89.4	OK	88.2	OK	94.4	OK
	3	86.6	OK	85.9	OK	98.9	OK
8	1	88.9	OK	86.5	OK	88.7	OK
	2	92.2	OK	84.6	OK	92.7	OK
	3	99.3	OK	81.7	OK	97.6	OK
9	0			85.6	OK		
	1	84.0	OK	83.5	OK	100.2	OK
	2	86.9	OK	82.2	OK	85.0	OK
	3	95.6	OK	87.1	OK	98.6	OK
10	0					97.5	OK
	1	86.6	OK	91.6	OK	89.8	OK
	2	91.2	OK	80.1	OK	93.4	OK
	3	99.8	OK	94.5	OK	95.6	OK
11	1	.	.	96.4	OK	95.8	OK
	2	.	.	110.6	OK	94.3	OK
	3	.	.	80.1	OK	96.5	OK
12	0			90.5	OK		
	1	.	.	98.6	OK	.	.
	2	.	.	93.6	OK	.	.
	3	.	.	90.7	OK	.	.
13	1	.	.	90.7	OK	.	.
	2	.	.	97.6	OK	.	.
	3	.	.	85.0	OK	.	.

Round 0: Invalid data at any criteria in a laboratory

OK: valid

X: Invalid

Table 4.4 The cell viability by mineral and passing status for Criteria 2c

		Lab.					
		1		2		3	
File No.	Round	Cell Viability	Criteria 2c	Cell Viability	Criteria 2c	Cell Viability	Criteria 2c
1	0					98.4	OK
	1	107.7	OK	84.2	OK	90.2	OK
	2	96.6	OK	80.3	OK	102.7	OK
	3	84.6	OK	86.6	OK	103.2	OK
2	0					97.2	OK
	0			101.4	OK	88.5	OK
	1	98.0	OK	107.5	OK	87.1	OK
	2	88.7	OK	86.6	OK	106.3	OK
	3	95.7	OK	96.3	OK	92.1	OK
3	0					94.7	OK
	0					98.2	OK
	1	104.6	OK	96.8	OK	92.7	OK
	2	86.9	OK	98.8	OK	112.7	OK
	3	86.9	OK	94.2	OK	104.2	OK
4	0			110.2	OK		
	0			92.3	OK		
	1	92.6	OK	86.6	OK	89.7	OK
	2	96.0	OK	108.1	OK	101.0	OK
	3	97.9	OK	112.4	OK	92.3	OK
5	0					97.6	OK
	1	98.1	OK	99.8	OK	106.6	OK
	2	96.0	OK	95.2	OK	94.8	OK
	3	107.0	OK	99.3	OK	93.7	OK
6	0			99.7	OK		
	1	106.0	OK	94.6	OK	98.6	OK
	2	103.0	OK	109.1	OK	101.7	OK
	3	94.2	OK	112.4	OK	100.2	OK
7	0			89.7	OK		
	1	101.0	OK	96.5	OK	95.7	OK
	2	102.2	OK	111.7	OK	102.4	OK
	3	99.7	OK	84.3	OK	94.3	OK
8	1	101.0	OK	90.4	OK	97.5	OK
	2	98.8	OK	92.3	OK	94.3	OK
	3	101.2	OK	82.0	OK	97.5	OK
9	0			101.6	OK		
	1	96.4	OK	84.9	OK	104.1	OK
	2	92.7	OK	90.7	OK	89.9	OK
	3	114.6	OK	103.6	OK	101.9	OK
10	0					86.9	OK
	1	97.8	OK	88.9	OK	91.1	OK
	2	101.2	OK	81.6	OK	91.9	OK
	3	103.6	OK	107.2	OK	96.8	OK
11	1	.	.	103.5	OK	94.4	OK
	2	.	.	105.2	OK	93.8	OK
	3	.	.	96.5	OK	96.0	OK
12	0			94.6	OK		
	1	.	.	109.5	OK	.	.
	2	.	.	97.9	OK	.	.
	3	.	.	97.0	OK	.	.
13	1	.	.	97.0	OK	.	.
	2	.	.	108.3	OK	.	.
	3	.	.	96.9	OK	.	.

Round 0: Invalid data at any criteria in a laboratory

OK: valid

X: Invalid

Table 4.5 The cell viability by positive control and passing status for Criteria 3

		Lab					
		1		2		3	
File No	Round	Cell Viability	Criteria 3	Cell Viability	Criteria 3	Cell Viability	Criteria 3
1	0					20.4	x
	1	21.4	OK	49.1	OK	36.6	OK
	2	30.2	OK	53.3	OK	28.3	OK
	3	40.7	OK	48.9	OK	34.7	OK
2	0					12.1	x
	0			66.1	x	17.2	x
	1	21.4	OK	41.6	OK	28.6	OK
	2	30.6	OK	38.7	OK	41.7	OK
	3	44.9	OK	54.9	OK	47.3	OK
3	0					16.0	x
	0					62.9	x
	1	30.7	OK	60.6	OK	26.4	OK
	2	37.2	OK	47.2	OK	44.6	OK
	3	43.2	OK	57.4	OK	49.7	OK
4	0			70.1	x		
	0			67.5	x		
	1	32.1	OK	53.8	OK	40.5	OK
	2	34.1	OK	39.6	OK	48.7	OK
	3	42.9	OK	47.2	OK	32.9	OK
5	0					62.8	x
	1	41.3	OK	57.6	OK	30.0	OK
	2	57.4	OK	55.5	OK	40.0	OK
	3	49.2	OK	59.6	OK	39.9	OK
6	0			71.8	x		
	1	45.9	OK	47.6	OK	28.6	OK
	2	56.4	OK	40.7	OK	37.7	OK
	3	39.0	OK	47.2	OK	36.8	OK
7	0			77.7	x		
	1	53.9	OK	33.9	OK	29.9	OK
	2	43.6	OK	27.7	OK	23.2	OK
	3	48.0	OK	54.2	OK	47.1	OK
8	1	44.0	OK	39.6	OK	40.1	OK
	2	52.9	OK	36.6	OK	53.6	OK
	3	34.6	OK	48.2	OK	55.4	OK
9	0			65.3	x		
	1	50.8	OK	60.9	OK	48.4	OK
	2	52.7	OK	50.8	OK	39.7	OK
	3	54.6	OK	61.9	OK	57.0	OK
10	0					19.4	x
	1	44.1	OK	36.9	OK	36.6	OK
	2	37.0	OK	43.5	OK	44.3	OK
	3	32.9	OK	47.3	OK	50.3	OK
11	1			54.8	OK	40.2	OK
	2			56.1	OK	37.7	OK
	3			56.3	OK	24.3	OK
12	0			62.3	x		
	1			56.8	OK		
	2			51.8	OK		
	3			43.6	OK		
13	1			43.6	OK		
	2			50.9	OK		
	3			32.0	OK		

Round 0: Invalid data at any criteria in a laboratory

OK: valid

X: Invalid

Table 4.6 Total numbers of experiments and numbers of invalid experiments at each laboratory

Code	Lab1		Lab2		Lab3	
	Complete	Incomplete	Complete	Incomplete	Complete	Incomplete
C01	3	0	7	4(3)		
C02	3	0			4	1
C03	3	0	3	0		
C04			4	1	4	1
C05	3	0	3	0		
C06	3	0	3	0	4	1
C07	3	0	4	1	8	5(3)
C08	3	0	4	1		
C09	3	0			5	2
C10			4	1	5	2
C11			4	1	5	2
C12	3	0	3	0		
C13	3	0			5	2
C14	3	0	4	1	5	2
C15	3	0	3	0	3	0
C16			3	0	3	0
C17	3	0	3	0	3	0
C18			3	0	4	1
C19	3	0			4	1
C20	3	0			4	1
C21			5	2	3	0
C22	3	0	3	0		
C23	3	0			3	0
C24			5	2	3	0
C25	3	0	11	8(6)		
C26	3	0	3	0		
C27	3	0	3	0	3	0
C28	3	0	3	0	3	0
C29			3	0	3	0
C30	3	0			3	0
C31	3	0			3	0
C32	3	0	3	0	3	0
C33	3	0	4	1		
C34	3	0			3	0
C35	3	0			3	0
C36			4	1	3	0
C37	3	0	3	0	4	1
C38			4	1	4	1
C39	3	0	4	1		
C40	3	0	8	5(3)	4	1
Total	90	0	121	31	114	24

( ): in valid data for criterion 4

Table 4.7 Invalid experiments of criterion 4 at each laboratory

Lab.	Substance	Conc.	Round	Cell viability	MEAN± SD
2	c01	High	1	33.7	**56.9±32.1**
			2	43.4	
			3	93.6	
	C25	High	1	110.5	**89.4±17.4**
			2	66.7	
			3	91.1	
	C25	High	1	110.6	**99.8±19.3**
			2	111.3	
			3	77.4	
	C40	High	1	56.9	**35.8±18.7**
			2	21.3	
			3	29.3	
3	C07	High	1	98.4	**80.1±18.7**
			2	61.0	
			3	81.0	

4.4 The cell viability of 40 blinded test substance substances

The cell viabilitys at two concentrations (high: 5%; low: 0.05%) for each test substance used are shown in Table 4.8. All data in this table are the valid data.

Only mean cell viabilitys of each test substance and the STE rank are shown in Table 4.9.

Table 4.8 The cell viabilitys of 40 blinded test substance substances and solvents used

			1			2			3		
Chemical	Conc.	Round	Cell Viability	MEAN	Solvent	Cell Viability	MEAN	Solvent	Cell Viability	MEAN	Solvent
c01	High	1	28.5	33.1	Saline	65.5	70.1	5% DMSO	.		
		2	33.4			81.7			.		
		3	37.3			62.9			.		
	Low	1	78.0	83.0		98.8	88.5		.		
		2	94.5			80.5			.		
		3	76.5			86.2			.		
c02	High	1	0.2	0.9	Saline	.		.	2.4	1.5	Saline
		2	1.2			.			0.0		
		3	1.2			.			2.2		
	Low	1	50.5	58.5		.			72.9	77.8	
		2	52.0			.			72.7		
		3	72.9			.			87.9		
c03	High	1	0.0	0.0	Saline	0.0	0.0	Saline	.		.
		2	0.0			0.0			.		
		3	0.0			0.0			.		
	Low	1	0.4	0.2		0.0	0.0		.		
		2	0.0			0.0			.		
		3	0.2			0.0			.		
c04	High	1	.		.	10	10	Mineral	1.0	1.0	Mineral
		2	.			13			0.0		
		3	.			8			1.8		
	Low	1	.			94	95		84.1	82.1	
		2	.			85			91.8		
		3	.			105			70.4		
c05	High	1	9.0	8.0	Saline	7.1	9.7	Saline	.		.
		2	8.6			10.5			.		
		3	6.5			11.4			.		
	Low	1	100.5	92.7		99.6	95.6		.		
		2	92.8			95.2			.		
		3	84.9			91.9			.		
c06	High	1	0.9	3.9	Saline	6.2	7.5	Saline	5.1	4.0	Saline
		2	4.7			8.4			2.7		
		3	6.1			8.1			4.2		
	Low	1	102.5	101.7		104.2	106.0		95.7	94.9	
		2	103.2			108.2			88.4		
		3	99.5			105.6			100.6		
c07	High	1	102.3	104.6	Mineral	100.9	95.8	Mineral	79.0	89.5	Mineral
		2	117.0			92.5			98.0		
		3	94.6			94.1			91.5		
	Low	1	100.1	102.7		108.2	100.2		94.5	93.0	
		2	107.8			88.6			97.2		
		3	100.2			103.9			87.3		
c08	High	1	4.5	16.9	Saline	20.9	17.2	Saline	.		.
		2	15.0			17.4			.		
		3	31.1			13.4			.		
	Low	1	85.5	91.2		103.2	102.8		.		
		2	90.1			104.1			.		
		3	98.2			101.3			.		

Table 4.8 The cell viabilitys of 40 blinded test substance substances and solvents used (continued)

			Lab.								
			1			2			3		
Chemical	Conc.	Round	Cell Viability	MEAN	Solvent	Cell Viability	MEAN	Solvent	Cell Viability	MEAN	Solvent
c09	High	1	1.4	1.2	Saline	.	.	.	1.1	1.7	Saline
		2	0.0			.			0.6		
		3	2.1			.			3.6		
	Low	1	93.6	91.5		.	.		85.9	89.5	
		2	90.4			.			88.7		
		3	90.4			.			94.0		
c10	High	1	.	.	.	7.2	7.7	Saline	2.7	4.8	Saline
		2	.			7.7			4.7		
		3	.			8.3			6.9		
	Low	1	.	.		83.0	91.8		98.5	98.9	
		2	.			89.2			97.7		
		3	.			103.1			100.4		
c11	High	1	.	.	.	44.5	46.0	Saline	63.4	63.2	Saline
		2	.			40.8			69.0		
		3	.			52.9			57.2		
	Low	1	.	.		87.6	92.1		91.5	100.4	
		2	.			93.7			104.4		
		3	.			94.9			105.2		
c12	High	1	96.5	102.0	Mineral	69.7	75.1	Mineral	.	.	.
		2	106.5			82.7			.		
		3	103.1			73.0			.		
	Low	1	90.1	97.9		93.8	93.1		.	.	
		2	103.1			88.1			.		
		3	100.4			97.5			.		
c13	High	1	28.1	25.8	Saline	.	.	.	6.4	6.7	Saline
		2	36.9			.			12.6		
		3	12.3			.			1.1		
	Low	1	83.2	94.3		.	.		93.1	101.6	
		2	96.7			.			103.9		
		3	103.0			.			108.0		
c14	High	1	1.5	0.5	Saline	0.8	0.3	Saline	1.9	1.0	Saline
		2	0.0			0.0			1.0		
		3	0.0			0.0			0.0		
	Low	1	11.5	3.8		7.4	7.1		2.3	1.3	
		2	0.0			5.2			0.2		
		3	0.0			8.7			1.4		
c15	High	1	4.6	6.8	Saline	8.2	7.4	Saline	3.9	3.7	Saline
		2	7.2			6.7			4.7		
		3	8.7			7.4			2.6		
	Low	1	98.5	101.4		105.8	95.6		92.3	87.7	
		2	99.5			93.3			92.3		
		3	106.3			87.9			78.6		
c16	High	1	.	.	.	88.8	85.3	Saline	80.0	73.6	Saline
		2	.			87.7			66.6		
		3	.			79.4			74.0		
	Low	1	.	.		102.0	106.3		99.6	99.3	
		2	.			111.5			99.0		
		3	.			105.5			99.3		

Table 4.8 The cell viabilitys of 40 blinded test substance substances and solvents used (continued)

			Lab.								
			1			2			3		
Chemical	Conc.	Round	Cell Viability	MEAN	Solvent	Cell Viability	MEAN	Solvent	Cell Viability	MEAN	Solvent
c17	High	1	90.6	99.1	Mineral	88.9	88.6	Mineral	113.6	100.8	Mineral
		2	109.1			85.4			101.0		
		3	97.6			91.5			87.8		
	Low	1	91.5	95.5		100.9	91.4		107.3	100.8	
		2	100.8			89.1			97.6		
		3	94.2			84.2			97.7		
c18	High	1	.	.	.	4.8	6.5	Saline	1.4	3.0	Saline
		2	.			7.0			0.8		
		3	.			7.7			6.6		
	Low	1	.	.		84.0	79.9		88.4	90.1	
		2	.			87.3			85.8		
		3	.			68.5			96.1		
c19	High	1	5.1	2.7	Saline	.	.	.	3.0	3.4	Saline
		2	0.9			.			0.0		
		3	2.0			.			7.3		
	Low	1	91.8	97.1		.	.		97.7	97.4	
		2	101.3			.			97.4		
		3	98.3			.			97.1		
c20	High	1	99.4	101.2	Mineral	.	.	.	87.7	90.0	Mineral
		2	110.8			.			84.4		
		3	93.3			.			97.9		
	Low	1	101.1	102.2		.	.		108.8	100.4	
		2	108.5			.			89.2		
		3	97.0			.			103.1		
c21	High	1	.	.	.	32.7	19.1	Saline	6.6	4.4	Saline
		2	.			11.6			3.3		
		3	.			12.9			3.2		
	Low	1	.	.		77.1	80.9		96.0	98.0	
		2	.			85.8			92.3		
		3	.			79.9			105.6		
c22	High	1	87.1	88.6	Mineral	107.1	95.94	Mineral	.	.	.
		2	85.8			89.1			.		
		3	93.0			91.7			.		
	Low	1	84.8	85.6		105.3	98.59		.	.	
		2	77.9			93.2			.		
		3	94.0			97.3			.		
c23	High	1	16.3	5.9	Saline	.	.	.	0.7	1.2	Saline
		2	1.4			.			1.4		
		3	0.0			.			1.6		
	Low	1	93.0	95.9		.	.		95.3	99.3	
		2	98.6			.			97.6		
		3	96.1			.			105.0		
c24	High	1	.	.	.	87.9	88.7	Saline	98.3	93.4	Saline
		2	.			89.8			91.2		
		3	.			88.4			90.6		
	Low	1	.	.		92.1	95.7		100.2	100.7	
		2	.			101.8			99.0		
		3	.			93.0			102.9		