7-3. Difference between LabCyte EPI-MODEL 24 protocol and EPISKIN protocol

The differences between the LabCyte EPI-MODEL 24 protocol and EPISKIN protocol are summarized in Table 3. Although the amount of medium (Table 3(A)), amount of test chemicals (Table 3(B)), and threshold of IL-1α content (Table 3(C)) for the LabCyte EPI-MODEL 24 protocol are different from the EPISKIN protocol, their conditions meet the descriptions of the Performance Standards.

Table 3. Differences between the LabCyte EPI-MODEL 24 protocol and EPISKIN protocol.

(A) Amount of medium.

	LabCyte EPI-MODEL 24 SOP	EPISKIN SOP	Reason
Pre-incubation	0.5 mL	2 mL	LabCyte EPI-MODEL 24 cultures are performed in 24-well culture plates. A medium volume of 0.5 mL to 1 mL is appropriate to add to the 24-well culture plate. A medium volume of 1 mL is necessary for a 42-hour culture.
Post-incubation	1 mL	2 mL	
MTT assay	0.5 mL	2 mL	

These conditions meet the descriptions of the Performance Standards.

(B) Amount of test chemicals.

Test chemical	LabCyte EPI-MODEL 24 SOP	EPISKIN SOP	Reason
Liquid	25 μL (75 μL/cm^2)	10 μL (25 μL/cm^2)	The lowest amount of the test chemical that spread uniformly was applied to the test model.
Solid	25 mg+25 μL DW (75 μL/cm^2)	10 mg+10 μL DW (25 μL/cm^2)	

These conditions meet the descriptions of the Performance Standards.

(C) Amount of test chemicals.

LabCyte EPI-MODEL 24 SOP	EPISKIN SOP	Performance Standards (EPISKIN)
IL-1α content ≥ 120 pg/tissue (IL-1α content ≥ 120 pg/mL)	IL-1α content ≥ 100 pg/tissue (IL-1αcontent ≥ 50 pg/mL)	IL-1α content ≥ 120 pg/tissue (IL-1α ≥ 60 pg/mL)

The threshold of IL-1α released in LabCyte EPI-MODEL was set based on the conditions for EPISKIN described in the Performance Standards.

7-4. Data collection, handling, and analysis

The independent biostatisticians for the study collected and organised the data using specific data collection software (Datasheet4.0:20080910.xls). They will work in close collaboration with the biostatisticians, (Mr. Takashi Omori, Mr.Etsuyoshi Miyaoka, and Mr. ken-

ya Ishiyama). After decoding the data, they will perform statistical analyses. The data management procedures and statistical tools applied will be approved by the VMT.

7-5. Quality assurance, GLP

Laboratories

All participating laboratories worked in the spirit of OECD GLP-like principles.

QA aspects

Mr. Kenya Ishiyama and Dr. Hajime Kojima assured the quality of all the data and records.

8. Results

8-1.Phase I

8-1-1 Negative control

In Phase I data, Table 4 shows the absorbance values for the negative control. All data for the negative control met the acceptance criteria.

Table 4. Absorbance of negative control by phase I.

	Exp.				
	1	2	3		
Lab.	Value	Value	Value	Mean	SD
a	1.073	0.928	1.007	1.003	0.073
b	0.93	1.245	1.042	1.072	0.16
c	0.96	0.869	0.761	0.863	0.1
d	0.987	0.928	0.939	0.951	0.031
e	0.84	0.884	0.973	0.899	0.068
f	1.049	0.934	0.968	0.984	0.059
g	1.147	1.159	1.074	1.127	0.046

8-1-2 Positive control and test chemicals

Table 5 shows the testing chemicals did not show any great score when the scores on tests were repeated in each laboratory. Furthermore, there was no significant inter-laboratory variation. These experiments suggested the feasibility of the LabCyte EPI-MODEL24 through the experiment. All laboratories were judged to participate at the Phase II by the validation management team.

Table 5. Viability of the positive control and three coded chemicals by phase I

		1	2	3		
Chem.	Lab.	Viability	Viability	Viability	Mean	SD
PC	a	6.35	27.55	15.67	16.52	10.63
	b	3.94	3.51	3.97	3.81	0.26
	c	5.45	4.81	3.49	4.58	1
	d	11.74	7.22	14.08	11.02	3.49
	e	31.6	9.76	38.61	26.66	15.05
	f	3.1	2.89	2.93	2.97	0.11
	g	4.46	7.17	2.62	4.75	2.29
P01	a	62.67	39.12	46.61	49.46	12.03
Ethanol	b	41.08	50.86	86.58	59.51	23.95
	c	68.13	34.13	67.31	56.53	19.4
	d	68.57	40.52	33.03	47.37	18.73
	e	54.19	72.08	60.55	62.27	9.07
	f	.	64.16	47.98	56.07	11.44
	g	4.68	5.23	6.67	5.53	1.03
P02	a	103.63	104.17	98.48	102.09	3.14
Glycerol	b	85.5	100.58	67.97	84.68	16.32
	c	101.24	99.41	104.84	101.83	2.76
	d	103.3	101.35	89.73	98.13	7.34
	e	101.75	98.06	99.04	99.62	1.91
	f	.	97.23	96	96.62	0.87
	g	94	98.16	103.6	98.59	4.82
P03	a	109.13	90.73	97.78	99.22	9.28
naphtalen acetic acid	b	93.96	103.91	103.96	100.61	5.76
	c	103.66	102.11	117.3	107.69	8.36
	d	102.28	98.15	94.56	98.33	3.86
	e	107.11	104.39	97.36	102.95	5.03
	f	.	101.34	102.07	101.7	0.52
	g	92.2	101.04	105.52	99.59	6.78

8-2. Phase II

8-2-1. Comments on the datasheets by phase II

Tables 6 and 7 show the comments from each laboratory. The 1st test from Lab. a did not meet the acceptance criteria. Therefore, an additional test was performed and then submitted to the biostatisticians.

Table 6. Comments on the datasheets (Viability) by phase II.

Lab ID	Exp.No.	Lot	Date	Comments
a	Main-2	LCE24-081013-B	2008/10/20	This test was recorded as the Main-1.
a	Main-3	LEC24-081117-B	2008/11/1	This test was recorded as the Main-2.
a	Main-4	LCE24-081117-B	2008/11/22	This test was recorded as the Main-3.
b	Main-1	LCE24-081013-B	2008/10/20	
b	Main-2	LCE24-081027-B	2008.11.04	
b	Main-3	LCE24-081117-B	2008/11/25	
c	1	LCE24-080929-B	2008.10.6	
c	2	LCE24-081020-B	2008/10/27	
c	3	LCE24-081027-B	2008.11.3	
d	81021	LCE24-081020-B	2008/10/27	
d	81028	LCE24-081027-B	2008/11/4	
d	81118	LCE24-081117-B	2008/11/25	
e	Main-1	LCE24-081006-B	2008/10/14	
e	Main-2	LCE24-081013-B	2008/10/20	
e	Main-3	LCE24-081020-B	2008/10/27	
f	LAB-08VAL	LCE24-080929-B	2008/10/6	
f	Maruishi	LCE24-081013-B	2008/10/20	
f	LAB-08VAL	LCE24-081103-B	2008/11/10	
g	Main-1	LCE24-080929-B	2008.10.06	By an application of G49,G53,G55, the model's cap was discolored.
g	Main-2	LCE24-081013-B	2008.10.20	By an application of G49,G53,G55, the model's cap was discolored.
g	Main-3	LCE24-081027-B	2008.11.03	By an application of G49,G53,G55, the model's cap was discolored.

Table 7. Comments on the datasheets (ELISA) by phase II.

Lab ID	Exp.No.	Lot	Date	Comments
a	Main-2	LCE24-081013-B	2008/10/20	This test was recorded as the Main-1.
a	Main-3	LEC24-081117-B	2008/11/1	This test was recorded as the Main-2.
a	Main-4	LCE24-081117-B	2008/11/22	This test was recorded as the Main-3.
b	Main-1	LCE24-081013-B	2008/12/12	
b	Main-2	LCE24-081027-B	2008/12/12	
b	Main-3	LCE24-081117-B	2008.12.26	
c	1	LCE24-080929-B	2008/10/7	
c	2	LCE24-081020-B	2008/10/30	
c	3	LCE24-081027-B	2008.11.3	
d	81021	LCE24-081020-B	2008/11/11	
d	81028	LCE24-081027-B	2008/11/26	
d	81118	LCE24-081117-B	2009/1/7	
e	Main-1	LCE24-081006-B	2008/12/2	
e	Main-2	LCE24-081013-B	2008/12/2	
e	Main-3	LCE24-081020-B	2008/12/19	
f	Maruishi	LCE24-081013-B	2008/11/25	
f	Maruishi	LCE24-081013-B	2008/11/27	
f	LAB-08VAL	LCE24-081103-B	2008/12/25	
g	Main-1	LCE24-080929-B	2008.10.09	
g	Main-2	LCE24-081013-B	2008.10.22	
g	Main-3	LCE24-081027-B	2008.11.05	

8-2-2. Negative control

Table 8 shows the absorbance values for the negative control. Excluding the results of Lab. a, 1st of 4th (data not shown), all data for the negative control met the acceptance criteria.

Table 8. Absorbance of negative control by phaseII.

	Lab.						
Exp.	a	b	c	d	e	f	g
1	0.75	0.93	0.91	0.82	0.80	0.84	1.13
2	0.86	0.85	1.01	0.90	0.90	0.79	1.18
3	0.82	0.84	0.93	0.96	0.91	0.83	1.05
Mean	0.81	0.88	0.95	0.89	0.87	0.82	1.12
Sd	0.06	0.05	0.05	0.07	0.06	0.02	0.06
Min	0.75	0.84	0.91	0.82	0.80	0.79	1.05
Max	0.86	0.93	1.01	0.96	0.91	0.84	1.18

8-2-3. Positive control

Table 9 and Figure 2 show three independent viabilities and summary statistics for the positive control from each laboratory. All data were sufficient and met the acceptance criteria for the positive control. In Table 10, the viabilities of No. 13, which is a positive control (5% SLS solution), are shown. These data were similar to that of positive control.

Table 9. Viability of the positive control by phase II.

	Lab.						
Exp.	a	b	c	d	e	f	g
1	5.9	5.2	4.1	5.7	4.1	3.5	3.1
2	8.8	12.3	5.4	2.6	12.6	2.9	10.8
3	2.5	7.8	3.8	3.3	5.6	3.2	4.2
Mean	5.7	8.4	4.4	3.9	7.4	3.2	6.0
Sd	3.1	3.6	0.8	1.6	4.5	0.3	4.1
Min	2.5	5.2	3.8	2.6	4.1	2.9	3.1
Max	8.8	12.3	5.4	5.7	12.6	3.5	10.7

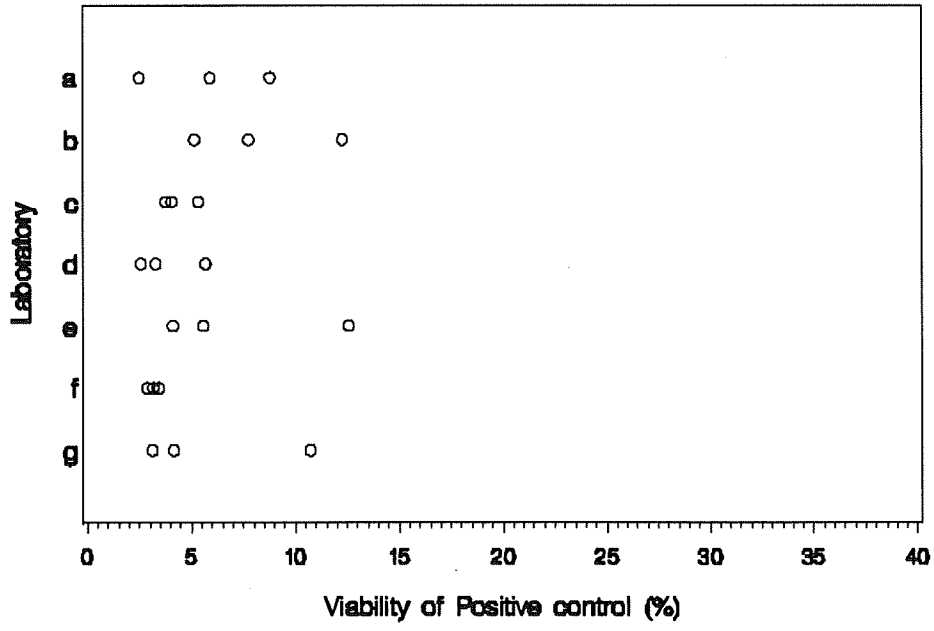


Figure 2. Viability of the positive control at each laboratory by phase II.

Table 10. Viability of chemical No. 13 (5% SLS solution).

			Lab.							
Chem.	Vivo	Score	Exp.	A	b	c	d	e	f	g
13			1	12.2	5.2	9.9	3.8	12.9	12.0	10.7
			2	3.6	3.2	5.0	3.6	6.7	3.1	8.0
			3	2.2	12.5	3.3	2.5	4.7	7.4	3.3

8-2-4. Skin irritation test by cell viability

The results of the LabCyte EPI-MODEL 24 skin irritation test when it was only evaluated based on cell viabilities as an indicator are shown in Table 11. A summary of the statistical analysis of the viability for each chemical is shown in Table 12 and Figure 3 .

Table 11. Viability of chemicals at each laboratory.

				Lab.						
Chem.	Vivo	Score	Exp.	a	b	c	d	e	f	g
01	no	0	1	31.0	47.1	10.6	14.3	38.1	14.3	10.6
			2	11.2	10.4	20.3	9.1	25.2	11.2	10.6
			3	11.6	16.1	12.4	9.6	32.3	10.4	14.0
02	no	0	1	79.8	66.9	88.1	102.3	101.8	75.3	96.0
			2	76.5	61.7	89.7	89.8	76.4	67.2	94.8
			3	65.2	88.7	85.8	67.6	85.8	75.7	103.3
03	no	0	1	109.1	93.3	94.6	105.1	129.6	94.2	100.5
			2	103.9	99.8	93.1	112.8	106.6	97.9	93.4
			3	100.9	102.3	95.7	101.4	103.9	92.5	111.1
04	no	0	1	106.3	94.4	97.1	106.1	127.1	100.1	104.8
			2	95.2	100.2	99.9	100.9	113.6	92.8	103.3
			3	96.5	98.6	97.8	98.4	105.2	92.7	109.8
05	no	0.3	1	78.5	61.7	91.4	79.4	103.0	71.9	96.8
			2	78.5	71.9	95.2	70.5	90.3	39.3	89.9
			3	74.1	84.5	89.2	66.1	89.6	55.1	88.4
06	no	0.3	1	92.5	77.9	81.0	91.3	97.0	87.8	87.2
			2	79.4	83.5	79.1	102.4	81.5	94.4	81.2
			3	82.4	80.5	83.6	82.7	90.7	81.1	54.1
07	no	1	1	24.1	10.8	20.8	21.7	17.5	15.8	31.5
			2	12.6	12.6	16.2	13.8	22.2	31.1	22.5
			3	17.8	13.2	15.2	19.8	21.3	15.6	19.9
08	no	1	1	111.9	86.7	75.3	109.4	114.9	89.7	101.1
			2	90.2	100.6	82.3	107.5	100.9	97.8	100.9
			3	95.3	104.8	77.2	103.0	100.9	96.5	109.0
09	no	1.7	1	112.8	96.7	106.6	105.0	115.8	98.8	102.3
			2	97.1	110.1	96.8	103.4	108.6	86.5	103.4
			3	101.1	109.5	93.5	98.1	103.9	97.7	112.1
10	no	1.7	1	115.9	115.4	107.5	114.3	132.0	104.0	107.9
			2	104.1	110.1	103.6	108.2	117.0	101.2	108.4
			3	86.5	111.3	103.7	105.5	107.5	101.2	113.1

Table 11. continued

				Lab.						
Chem.	Vivo	Score	Exp.	a	b	c	d	e	f	g
11	R38	2	1	113.7	105.0	101.0	102.4	123.1	103.1	102.8
			2	98.1	106.6	94.6	105.8	110.4	98.0	100.5
			3	112.6	103.7	94.1	102.7	105.5	94.6	109.0
12	R38	2	1	28.2	24.6	24.9	54.3	55.6	27.2	87.7
			2	18.4	24.6	44.8	76.2	57.8	65.2	98.0
			3	15.3	15.9	28.1	27.4	57.2	66.0	112.6
14	R38	2.3	1	11.1	12.1	14.7	10.7	14.2	13.1	13.5
			2	6.6	8.3	9.5	11.7	12.0	16.7	12.0
			3	6.8	8.8	9.1	10.2	10.4	17.0	10.6
15	R38	2.3	1	11.1	9.3	13.1	8.0	11.0	8.6	9.2
			2	7.1	10.2	19.3	8.6	11.3	5.9	24.7
			3	8.2	9.9	8.1	9.2	8.7	7.1	9.2
16	R38	2.7	1	67.9	92.0	51.5	18.1	98.2	59.6	64.9
			2	32.2	54.1	86.3	79.2	90.6	50.4	79.6
			3	59.8	98.3	81.7	37.7	78.7	67.5	86.5
17	R38	2.7	1	6.1	4.5	5.3	6.6	8.9	6.9	6.2
			2	4.8	4.7	6.0	5.3	6.3	5.5	5.3
			3	5.6	5.7	5.9	3.9	5.4	4.5	5.3
18	R38	3	1	82.1	46.5	91.2	83.7	98.9	69.2	92.4
			2	78.3	50.6	87.3	69.9	87.2	80.6	85.9
			3	25.3	100.0	87.5	59.0	69.1	71.9	94.4
19	R38	3	1	15.0	74.6	10.0	30.4	83.1	40.1	35.8
			2	19.9	10.9	22.4	28.3	26.1	87.0	44.7
			3	51.1	32.0	35.0	18.2	69.4	71.8	38.7
20	R38	4	1	31.1	24.8	10.4	9.6	10.7	8.1	8.8
			2	9.3	8.0	7.6	16.9	8.2	7.8	6.7
			3	29.5	9.3	7.6	30.9	6.2	8.2	8.6

Table 12. Summary of the statistical analysis of the viability for each chemical.

		Lab.						
Chem.	Stat.	a	b	c	d	e	f	g
01	Mean	17.9	24.5	14.4	11.0	31.9	12.0	11.7
	Sd	11.3	19.8	5.2	2.9	6.4	2.0	2.0
	Min	11.2	10.4	10.6	9.1	25.2	10.4	10.6
	Max	31.0	47.1	20.3	14.3	38.1	14.3	14.0
02	Mean	73.8	72.4	87.8	86.6	88.0	72.7	98.0
	Sd	7.7	14.3	1.9	17.6	12.8	4.8	4.6
	Min	65.2	61.7	85.8	67.6	76.4	67.2	94.8
	Max	79.8	88.7	89.7	102.3	101.8	75.7	103.3
03	Mean	104.7	98.5	94.5	106.4	113.3	94.8	101.7
	Sd	4.1	4.6	1.3	5.8	14.1	2.7	8.9
	Min	100.9	93.3	93.1	101.4	103.9	92.5	93.4
	Max	109.1	102.3	95.7	112.8	129.6	97.9	111.1
04	Mean	99.3	97.8	98.2	101.8	115.3	95.2	105.9
	Sd	6.1	3.0	1.5	3.9	11.0	4.2	3.4
	Min	95.2	94.4	97.1	98.4	105.2	92.7	103.3
	Max	106.3	100.2	99.9	106.1	127.1	100.1	109.8
05	Mean	77.0	72.7	91.9	72.0	94.3	55.4	91.7
	Sd	2.5	11.4	3.1	6.8	7.6	16.3	4.5
	Min	74.1	61.7	89.2	66.1	89.6	39.3	88.4
	Max	78.5	84.5	95.2	79.4	103.0	71.9	96.8
06	Mean	84.8	80.7	81.2	92.1	89.7	87.8	74.2
	Sd	6.9	2.8	2.3	9.9	7.8	6.7	17.7
	Min	79.4	77.9	79.1	82.7	81.5	81.1	54.1
	Max	92.5	83.5	83.6	102.4	97.0	94.4	87.2
07	Mean	18.2	12.2	17.4	18.4	20.3	20.8	24.6
	Sd	5.7	1.3	3.0	4.2	2.5	8.9	6.1
	Min	12.6	10.8	15.2	13.8	17.5	15.6	19.9
	Max	24.1	13.2	20.8	21.7	22.2	31.1	31.5
08	Mean	99.1	97.4	78.3	106.6	105.6	94.7	103.7
	Sd	11.4	9.5	3.6	3.3	8.1	4.4	4.6
	Min	90.2	86.7	75.3	103.0	100.9	89.7	100.9
	Max	111.9	104.8	82.3	109.4	114.9	97.8	109.0
09	Mean	103.7	105.4	98.9	102.2	109.4	94.3	105.9
	Sd	8.2	7.6	6.8	3.6	6.0	6.8	5.4
	Min	97.1	96.7	93.5	98.1	103.9	86.5	102.3
	Max	112.8	110.1	106.6	105.0	115.8	98.8	112.1
10	Mean	102.1	112.2	104.9	109.3	118.8	102.1	109.8
	Sd	14.8	2.8	2.2	4.5	12.3	1.6	2.9
	Min	86.5	110.1	103.6	105.5	107.5	101.2	107.9
	Max	115.9	115.4	107.5	114.3	132.0	104.0	113.1

Table 12. continued.

		Lab.						
Chem.	Stat.	a	b	c	d	e	f	g
11	Mean	108.1	105.1	96.6	103.6	113.0	98.6	104.1
	Sd	8.7	1.4	3.8	1.9	9.0	4.3	4.4
	Min	98.1	103.7	94.1	102.4	105.5	94.6	100.5
	Max	113.7	106.6	101.0	105.8	123.1	103.1	109.0
12	Mean	20.7	21.7	32.6	52.6	56.9	52.8	99.5
	Sd	6.7	5.0	10.7	24.4	1.1	22.2	12.5
	Min	15.3	15.9	24.9	27.4	55.6	27.2	87.7
	Max	28.2	24.6	44.8	76.2	57.8	66.0	112.6
14	Mean	8.2	9.7	11.1	10.9	12.2	15.6	12.0
	Sd	2.6	2.1	3.1	0.8	1.9	2.2	1.4
	Min	6.6	8.3	9.1	10.2	10.4	13.1	10.6
	Max	11.1	12.1	14.7	11.7	14.2	17.0	13.5
15	Mean	8.8	9.8	13.5	8.6	10.3	7.2	14.4
	Sd	2.1	0.4	5.6	0.6	1.4	1.4	9.0
	Min	7.1	9.3	8.1	8.0	8.7	5.9	9.2
	Max	11.1	10.2	19.3	9.2	11.3	8.6	24.7
16	Mean	53.3	81.4	73.1	45.0	89.1	59.1	77.0
	Sd	18.7	23.9	18.9	31.2	9.8	8.6	11.0
	Min	32.2	54.1	51.5	18.1	78.7	50.4	64.9
	Max	67.9	98.3	86.3	79.2	98.2	67.5	86.5
17	Mean	5.5	4.9	5.8	5.3	6.9	5.6	5.6
	Sd	0.7	0.6	0.4	1.4	1.8	1.2	0.5
	Min	4.8	4.5	5.3	3.9	5.4	4.5	5.3
	Max	6.1	5.7	6.0	6.6	8.9	6.9	6.2
18	Mean	61.9	65.7	88.7	70.9	85.1	73.9	90.9
	Sd	31.7	29.8	2.2	12.4	15.0	6.0	4.5
	Min	25.3	46.5	87.3	59.0	69.1	69.2	85.9
	Max	82.1	100.0	91.2	83.7	98.9	80.6	94.4
19	Mean	28.7	39.2	22.5	25.6	59.5	66.3	39.8
	Sd	19.6	32.4	12.5	6.5	29.7	23.9	4.5
	Min	15.0	10.9	10.0	18.2	26.1	40.1	35.8
	Max	51.1	74.6	35.0	30.4	83.1	87.0	44.7
20	Mean	23.3	14.0	8.6	19.2	8.4	8.0	8.1
	Sd	12.1	9.3	1.6	10.8	2.3	0.2	1.2
	Min	9.3	8.0	7.6	9.6	6.2	7.8	6.7
	Max	31.1	24.8	10.4	30.9	10.7	8.2	8.8

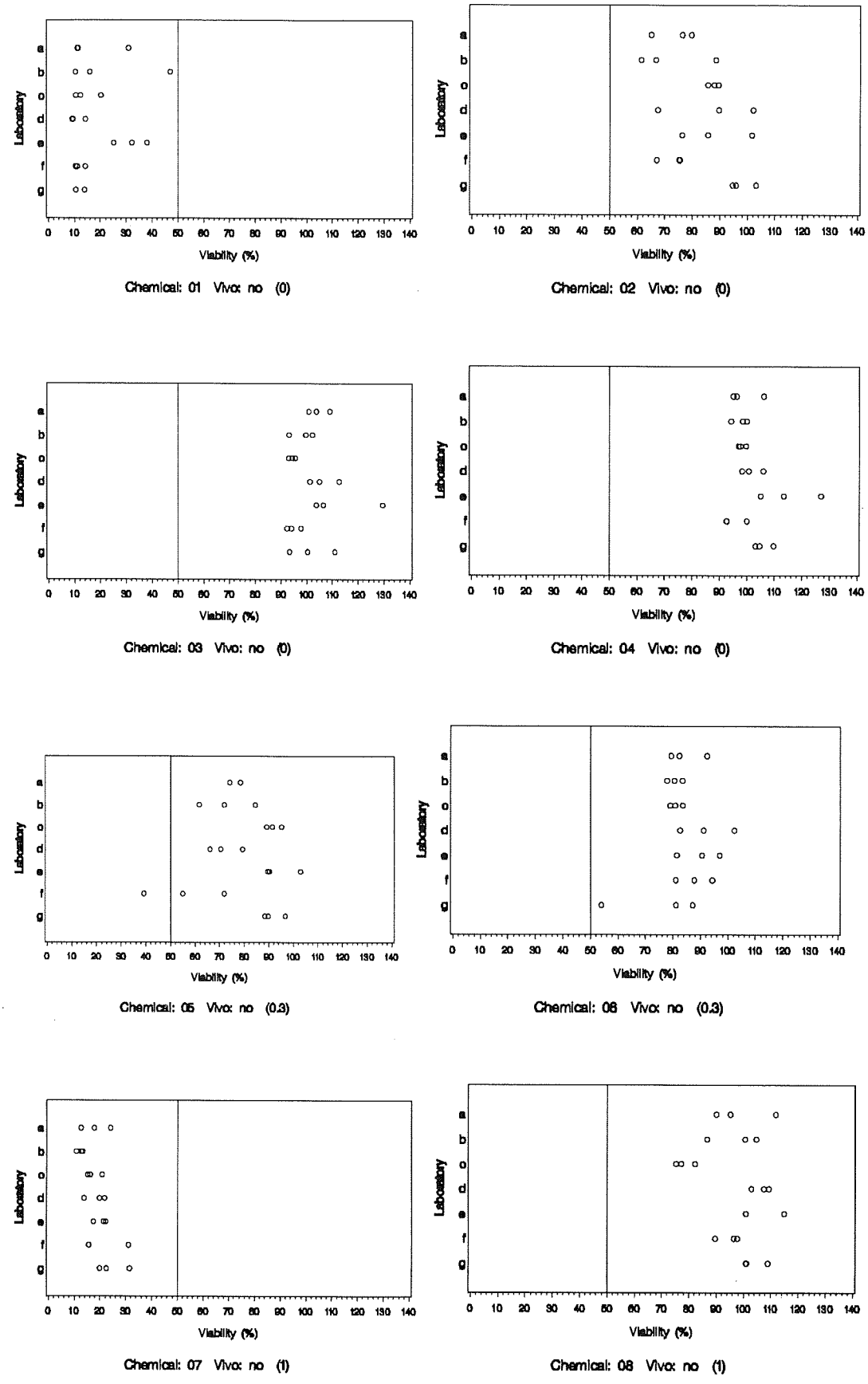
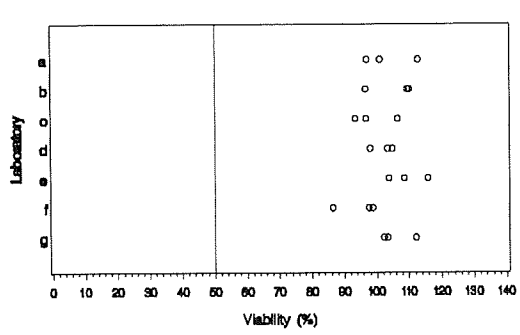
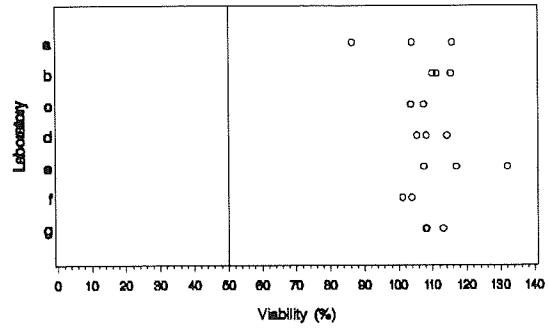


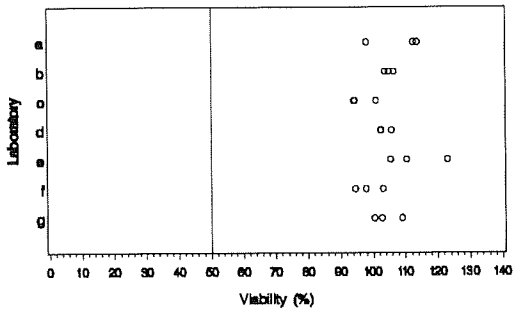
Figure 3. Distribution of the viability for each chemical.



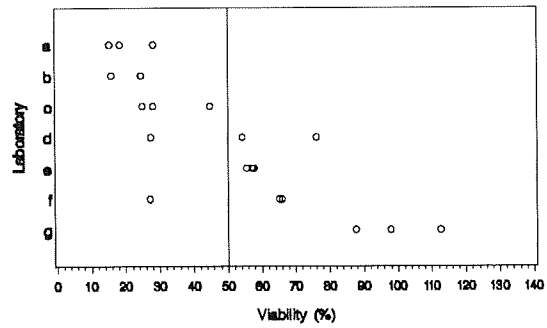
Chemical: 09 Vivo: no (1.7)



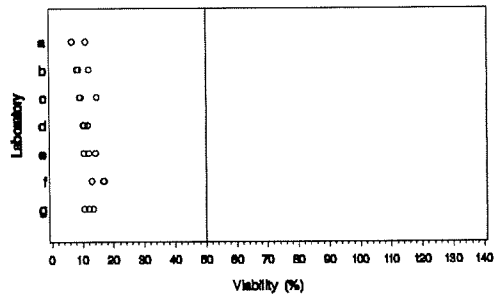
Chemical: 10 Vivo: no (1.7)



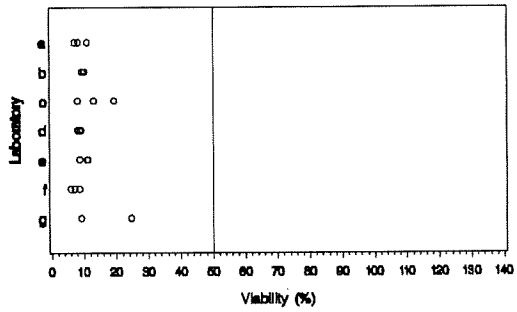
Chemical: 11 Vivo: R38 (2)



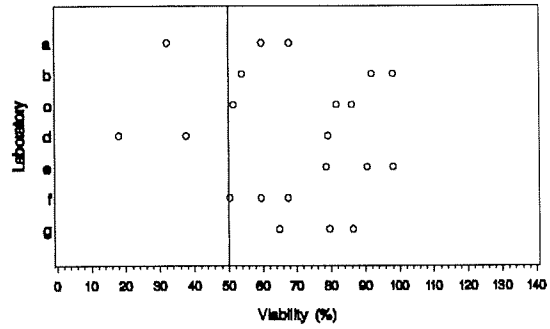
Chemical: 12 Vivo: R38 (2)



Chemical: 14 Vivo: R38 (2.3)



Chemical: 15 Vivo: R38 (2.3)



Chemical: 16 Vivo: R38 (2.7)

Figure 3. continued

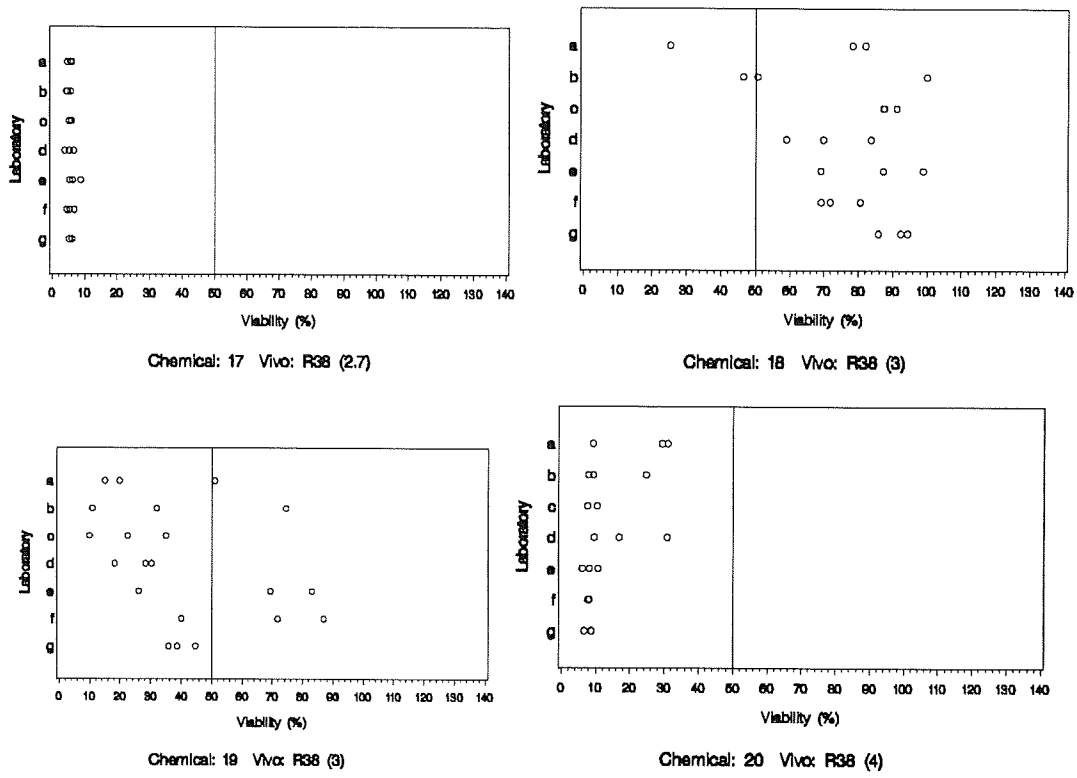


Figure 3. continued.

8-2-5. IL-1α

The results of the LabCyte EPI-MODEL 24 skin irritation test when IL-1α was evaluated as an indicator are summarized in Table 13.

Table 13. IL-1α levels from each laboratory.

				Lab.						
Chem.	Vivo	Score	Exp.	a	b	c	d	e	f	g
01	no	0	1	.	.	.	.	.	.	.
			2	.	.	.	.	.	.	.
			3	.	.	.	.	.	.	.
02	no	0	1	132.8	52.9	59.3	41.2	60.7	61.3	9.4
			2	68.1	56.5	37	89.1	68.4	99.3	9.6
			3	97.6	41.1	76	72.4	46	70.1	12.6
03	no	0	1	12	9.5	15.5	8.6	23.2	12.7	8.1
			2	7.1	8.6	11.7	19.9	10.5	9.2	11.9
			3	10.7	10.3	12.9	9.4	11.3	6.7	15.7
04	no	0	1	10	6	8	11.7	9.5	2.5	6.3
			2	5.3	8	5.5	13.2	15.1	2.6	8.6
			3	6.3	4.7	7.2	7.9	9.7	3.4	6.8
05	no	0.3	1	122	97.6	24.3	81.2	57.7	183.5	15.4
			2	35.7	63.5	35.1	115.3	36.6	.	28.5
			3	44.4	26	31.2	49.4	33	191.6	33.2
06	no	0.3	1	59	85.7	114	85.6	94.4	60.8	112.5
			2	62.9	93.6	104.9	139.5	81.4	48.1	62.1
			3	68.8	85.1	82.9	64.5	52.9	54.8	147.1
07	no	1	1	.	.	.	.	.	.	.
			2	.	.	.	.	.	.	.
			3	.	.	.	.	.	.	.
08	no	1	1	8.2	9.4	84.1	4.1	6.9	21.4	5.3
			2	3.6	6.4	31.6	10.4	8.5	4.9	5.8
			3	6	4.1	33.1	5.2	6.7	2.1	7.2
09	no	1.7	1	10.9	17.1	11.2	42.6	29.5	33	7.4
			2	19.8	8.8	8.8	32.2	6.5	25.3	9.7
			3	31.3	6.8	20.1	21.3	11.2	24.7	10.6
10	no	1.7	1	27.9	7.4	31.3	41.2	46.5	39.3	9.8
			2	17.1	12.7	15	50.4	26.7	26.7	14.5
			3	66.2	12.2	30	42.1	26.3	24.2	13.2

Table 13. continued.

				Lab.						
Chem.	Vivo	Score	Exp.	a	b	c	d	e	f	g
11	R38	2	1	5	31.1	18	15.3	10.4	16.2	6.4
			2	3.3	11.9	15.8	19	9.7	8.1	7.5
			3	18.2	5	8.9	8.7	8.6	12.6	11.9
12	R38	2	1	.	.	.	157.2	120.4	.	34.5
			2	.	.	.	113	118.6	90.2	27.3
			3	.	.	.	.	58.3	66.2	13.6
14	R38	2.3	1	.	.	.	.	.	.	.
			2	.	.	.	.	.	.	.
			3	.	.	.	.	.	.	.
15	R38	2.3	1	.	.	.	.	.	.	.
			2	.	.	.	.	.	.	.
			3	.	.	.	.	.	.	.
16	R38	2.7	1	86.9	68.1	129.4	.	126.8	116.5	90.8
			2	.	100.2	74.4	169.7	76.1	107.5	70.9
			3	121.2	42.5	83.6	.	73.1	87.3	79.2
17	R38	2.7	1	.	.	.	.	.	.	.
			2	.	.	.	.	.	.	.
			3	.	.	.	.	.	.	.
18	R38	3	1	61.5	.	60.6	90.3	86.9	114.5	18
			2	57.7	104.9	45.8	221.3	98.7	76.4	45.1
			3	.	17.2	51.4	138.1	63.9	102.2	22.1
19	R38	3	1	.	57.3	.	.	109.2	.	.
			2	.	.	.	.	.	69.2	.
			3	102.3	.	.	.	68	59.5	.
20	R38	4	1	.	.	.	.	.	.	.
			2	.	.	.	.	.	.	.
			3	.	.	.	.	.	.	.

Cells highlighted in yellow indicate that the classification changed based on the IL-1α data.

8-2-6. Classification of three independent viabilities at each laboratory

The classifications of three independent viabilities only evaluated by the MTT assay are shown in Table 14. Classifications of three independent viabilities evaluated by the MTT and IL-1α assays are shown in Table 13. The IL-1α results changed the classification for only 5 data points. The percent of changed data is 3.0% (12/399).

Table 14. Classification using three independent viabilities.

				Lab.						
Chem.	Vivo	Score	Exp.	a	b	c	d	e	f	g
01	no	0	1	P	P	P	P	P	P	P
			2	P	P	P	P	P	P	P
			3	P	P	P	P	P	P	P
02	no	0	1	N	N	N	N	N	N	N
			2	N	N	N	N	N	N	N
			3	N	N	N	N	N	N	N
03	no	0	1	N	N	N	N	N	N	N
			2	N	N	N	N	N	N	N
			3	N	N	N	N	N	N	N
04	no	0	1	N	N	N	N	N	N	N
			2	N	N	N	N	N	N	N
			3	N	N	N	N	N	N	N
05	no	0.3	1	P	N	N	N	N	N	N
			2	N	N	N	N	N	P	N
			3	N	N	N	N	N	N	N
06	no	0.3	1	N	N	N	N	N	N	N
			2	N	N	N	P	N	N	N
			3	N	N	N	N	N	N	P
07	no	1	1	P	P	P	P	P	P	P
			2	P	P	P	P	P	P	P
			3	P	P	P	P	P	P	P
08	no	1	1	N	N	N	N	N	N	N
			2	N	N	N	N	N	N	N
			3	N	N	N	N	N	N	N
09	no	1.7	1	N	N	N	N	N	N	N
			2	N	N	N	N	N	N	N
			3	N	N	N	N	N	N	N
10	no	1.7	1	N	N	N	N	N	N	N
			2	N	N	N	N	N	N	N
			3	N	N	N	N	N	N	N

P: positive, N: Negative

Table 14. continued.

				Lab.						
Chem.	Vivo	Score	Exp.	a	b	c	d	e	f	g
11	R38	2	1	N	N	N	N	N	N	N
			2	N	N	N	N	N	N	N
			3	N	N	N	N	N	N	N
12	R38	2	1	P	P	P	P	P	P	N
			2	P	P	P	N	N	N	N
			3	P	P	P	P	N	N	N
14	R38	2.3	1	P	P	P	P	P	P	P
			2	P	P	P	P	P	P	P
			3	P	P	P	P	P	P	P
15	R38	2.3	1	P	P	P	P	P	P	P
			2	P	P	P	P	P	P	P
			3	P	P	P	P	P	P	P
16	R38	2.7	1	N	N	P	P	P	N	N
			2	P	N	N	P	N	N	N
			3	N	N	N	P	N	N	N
17	R38	2.7	1	P	P	P	P	P	P	P
			2	P	P	P	P	P	P	P
			3	P	P	P	P	P	P	P
18	R38	3	1	N	P	N	N	N	N	N
			2	N	N	N	N	N	N	N
			3	P	N	N	N	N	N	N
19	R38	3	1	P	N	P	P	N	P	P
			2	P	P	P	P	P	N	P
			3	N	P	P	P	N	N	P
20	R38	4	1	P	P	P	P	P	P	P
			2	P	P	P	P	P	P	P
			3	P	P	P	P	P	P	P

Table 15. Classification using the MTT and IL-1α assay.

				Lab.						
Chem.	Vivo	Score	Exp.	a	b	c	d	e	f	g
01	no	0	1	P	P	P	P	P	P	P
			2	P	P	P	P	P	P	P
			3	P	P	P	P	P	P	P
02	no	0	1	P	N	N	N	N	N	N
			2	N	N	N	N	N	N	N
			3	N	N	N	N	N	N	N
03	no	0	1	N	N	N	N	N	N	N
			2	N	N	N	N	N	N	N
			3	N	N	N	N	N	N	N
04	no	0	1	N	N	N	N	N	N	N
			2	N	N	N	N	N	N	N
			3	N	N	N	N	N	N	N
05	no	0.3	1	P	N	N	N	N	P	N
			2	N	N	N	N	N	P	N
			3	N	N	N	N	N	P	N
06	no	0.3	1	N	N	N	N	N	N	N
			2	N	N	N	P	N	N	N
			3	N	N	N	N	N	N	P
07	no	1	1	P	P	P	P	P	P	P
			2	P	P	P	P	P	P	P
			3	P	P	P	P	P	P	P
08	no	1	1	N	N	N	N	N	N	N
			2	N	N	N	N	N	N	N
			3	N	N	N	N	N	N	N
09	no	1.7	1	N	N	N	N	N	N	N
			2	N	N	N	N	N	N	N
			3	N	N	N	N	N	N	N
10	no	1.7	1	N	N	N	N	N	N	N
			2	N	N	N	N	N	N	N
			3	N	N	N	N	N	N	N

Table 15. continued.

				Lab.						
Chem.	Vivo	Score	Exp.	a	b	c	d	e	f	g
11	R38	2	1	N	N	N	N	N	N	N
			2	N	N	N	N	N	N	N
			3	N	N	N	N	N	N	N
12	R38	2	1	P	P	P	P	P	P	N
			2	P	P	P	N	N	N	N
			3	P	P	P	P	N	N	N
14	R38	2.3	1	P	P	P	P	P	P	P
			2	P	P	P	P	P	P	P
			3	P	P	P	P	P	P	P
15	R38	2.3	1	P	P	P	P	P	P	P
			2	P	P	P	P	P	P	P
			3	P	P	P	P	P	P	P
16	R38	2.7	1	N	N	P	P	P	N	N
			2	P	N	N	P	N	N	N
			3	P	N	N	P	N	N	N
17	R38	2.7	1	P	P	P	P	P	P	P
			2	P	P	P	P	P	P	P
			3	P	P	P	P	P	P	P
18	R38	3	1	N	P	N	N	N	N	N
			2	N	N	N	P	N	N	N
			3	P	N	N	P	N	N	N
19	R38	3	1	P	N	P	P	N	P	P
			2	P	P	P	P	P	N	P
			3	N	P	P	P	N	N	P
20	R38	4	1	P	P	P	P	P	P	P
			2	P	P	P	P	P	P	P
			3	P	P	P	P	P	P	P

Cells highlighted in yellow indicate that the classification changed based on the IL-1α data.

8-2-7. Sensitivity, specificity and accuracy

The final classification was decided by a majority vote at each laboratory. With IL-1α, 4 classifications were changed at Labs. a, d, (2 pieces of data) and f. The sensitivity, specificity and accuracy of each laboratory are shown in Table 16(A), (B) and (C). Total sensitivity, specificity and accuracy are shown in Table 17(A), (B) and (C). In (A), we compared the data from the MTT assay with the EU classification for each chemical. In (B), the data from the MTT assay and IL-1α were compared with the EU classification for each chemical. In (C), we compared the MTT assay data with the GHS-EU classification for each chemical.

Table 16(A). Sensitivity, specificity and accuracy based on the MTT assay vs. the EU classification.

Index	Lab.						
	a	b	c	d	e	f	g
Sensitivity	6/9	6/9	6/9	6/9	4/9	4/9	5/9
	66.7	66.7	66.7	66.7	44.4	44.4	55.6
Specificity	8/10	8/10	8/10	8/10	8/10	8/10	8/10
	80	80	80	80	80	80	80
Accuracy	14/19	'14/1	'14/1	'14/1	12/19	12/19	13/19
	73.7	73.7	73.7	73.7	63.2	63.2	68.4

Table 16(B). Sensitivity, specificity and accuracy based on the MTT assay and IL-1α vs. the EU classification.

Index	Lab.						
	a	b	c	d	e	f	g
Sensitivity	7/9	6/9	6/9	8/9	4/9	4/9	5/9
	77.8	66.7	66.7	88.9	44.4	44.4	55.6
Specificity	8/10	8/10	8/10	8/10	8/10	7/10	8/10
	80	80	80	80	80	70	80
Accuracy	15/19	14/19	14/19	16/19	12/19	11/19	13/19
	78.9	73.7	73.7	84.2	63.2	57.9	68.4

Table 16(C). Sensitivity, specificity and accuracy based on the MTT assay vs. the GHS-EU classification.

Index	Lab.						
	a	b	c	d	e	f	g
Sensitivity	5/7	5/7	5/7	6/7	4/7	4/7	5/7
	71.4	71.4	71.4	85.7	55.6	55.6	71.4
Specificity	9/12	9/12	9/12	10/12	10/12	10/12	10/12
	75	75	75	83.3	83.3	83.3	83.3
Accuracy	14/19	14/19	14/19	15/19	14/19	13/19	15/19
	73.7	73.7	73.7	78.9	73.7	68.4	78.9

Table 17(A). Total sensitivity, specificity and accuracy of the MTT assay vs. the EU classification.

		In vivo classification		
		Irritant	Non-irritant	Total
In vitro prediction	Irritant	37	14	51
	Non-irritant	26	56	82
	Total	63	70	133

Sensitivity (%)	58.7
Specificity (%)	80
Accuracy (%)	69.9

Table 17(B). Total sensitivity, specificity and accuracy of the MTT assay and IL-1α vs. the EU classification.

		In vivo classification		
		Irritant	Non-irritant	Total
In vitro prediction	Irritant	40	15	55
	Non-irritant	23	55	78
	Total	63	70	133

Sensitivity (%)	63.5
Specificity (%)	78.6
Accuracy (%)	71.4

Table 17(C). Sensitivity, specificity and accuracy of the MTT assay vs. the GHS-EU classification.

		In vivo classification		
		Irritant	Non-irritant	Total
In vitro prediction	Irritant	34	17	51
	Non-irritant	15	67	82
	Total	49	84	133

Sensitivity (%)	69.4
Specificity (%)	79.7
Accuracy (%)	75.9