ESAC Peer review of submission of the follow-up validation study of the Modified EpiDerm™ Skin Irritation Test (SIT) for hazard identification and labelling of chemicals according to EU classification system.

The above submission was sent to ECVAM in the Test Submission Template for ECVAM/CORRELATE submissions recently developed by ECVAM and was reviewed by Peer Review Panel :

1. Independent scientific evaluation of the submitted Test Submission Template by three members of the ECVAM Scientific Advisory Committee (Dr M. Dambrova, Dr D. Jirova and Dr C. Westmoreland) was performed.

2. The study was evaluated according to the principles outlined in the ECVAM document 'Performance Standards for Applying Human Skin Models to in-vitro skin irritation testing'1. These performance standards are used to evaluate the reliability and accuracy of the methods which are based on reconstructed human epidermis and which measure or predict the same biological or toxic effect as the fully validated and accepted reference method (see ESAC statement, 2007 and skin irritation validation study [SIVS] report) (2,4). In this context, following discussion of the 3 peer review reports there was a request additional statistical evaluation of the study data using the median test results (performed by ECVAM).

1. Consensus views from scientific evaluation of submission

All procedures are clearly defined, explained and described in satisfactory details. The description of participating units and management structure of the study gives impression of a fully managed and coordinated study which afforded reliable data collection and statistical analysis.

1.1 Data Collection

Are the data collection procedures and selection clearly defined ? YES

The Project Plan is provided in details, the Phases I and II of the study are clearly described, SOP provided. Method Documentation Sheets include uniform sheets for recording the test procedure, for devices and test material verifications, for quality control of the skin samples, for dosing procedures, for MTT plate configuration and spectrophotometrical measurements. Separate sheets for characterization the of test substances are attached.

In agreement with the ECVAM SIVS Performance Standards, 20 commercially available Reference Chemicals (10 non-irritants, 10 irritants) representing an adequate distribution are involved. Data for individual replicate test samples are provided, calculated as percentage cell viability data for each test chemical, means ± standard deviation including positive and negative classification are reported for all laboratories involved in the study.

1.2 Goal of the study

1.2.1 Was the goal of the study clearly understandable? YES

The goal is clearly defined: To evaluate the performance of a Modified EpiDerm™ skin irritation test (SIT) protocol to prove its ability to reliably discriminate R38 skin irritants from non-irritants for the purpose of the EU classification) according to the Dangerous Substances Directive, 67/548/EEC.

1.2.2 Is the scientific rationale given? YES

The rationale is given and documented. The scientific rationale for the use of the EpiDerm™ skin model is based on the accumulated scientific knowledge and practical experience with the EpiDerm™ EPI-200 model as a reconstructed human skin model which is commercially available for more than 13 years. As it is mentioned in submission (reference list included), the EpiDerm™ model has been proved to be usable for skin corrosion testing of chemicals, skin irritation studies of cosmetic products and raw materials, skin penetration, phototoxicity and photogenotoxicity studies.

This study was undertaken following the ESAC statement on the validity of in vitro tests for skin irritation (27 April, 2007) where a previous protocol for the EpiDerm™ SIT was evaluated and the following statement made: 'At this time, due to its high specificity, the EpiDerm™ model reliably identifies skin irritants, but negative results may require further testing (e.g. according to the tiered strategy as described in the OECD TG 404). Improvement of the EpiDerm™ protocol should be made to increase the level of sensitivity'.

The change made to the EpiDerm protocol for the current, follow-up validation study was to extend the exposure time of the tissue to the test agent to 60 minutes (25 minutes at room temperature, followed by 35 minutes at 37°C). Cytotoxicity (assessed by MTT reduction) is then evaluated following an additional 42h incubation once the test agent has been removed. Other aspects of the original EpiDerm protocol (e.g. prediction model etc) remain unchanged.

1.2.3 Is the regulatory rationale given? YES

The regulatory rationale is given, based on an urgent need of validated alternative methods and skin models for skin irritation assessment because of the 7th Amendement of the Cosmetics Directive and the Regulation REACH for chemicals. The regulatory rationale for the modified EpiDerm™ SIT is clearly stated in submission as a full replacement of the in vivo rabbit test (OECD TG 404 & Method B.4 of Annex V to Directive 67/548/ECC). The test could be used for hazard identification and labelling of new and existing chemicals (including cosmetic raw materials and pharmaceuticals) according to the EU classification system (R38 or no label).

1.3. Test definition (Module 1)

1.3.1 Are the test and its purpose well defined? YES

The Modified EpiDerm™ SIT is proposed as a full replacement method to the in vivo rabbit SIT for skin irritation hazard identification and labelling according to the EU classification system (R38 or no label).

1.3.2 Are the proposed standardised protocol and prediction model adequate? YES

The detailed, GLP compliant SOP is provided which explains all aspects of tissue receipt and handling, preincubation, treatment (with test chemical, positive control and negative control), cytotoxicity assessment , assessment of relative cell viability, assay quality controls and subsequent prediction of the skin irritation potential of the test material. Its transferability was proved during training in Phase I of the study, as documented in the Training Report. Even a completely naive laboratory was able to employ the SOP successfully.

The proposed standardised protocol and associated prediction model are adequate for the proposed purpose. Three tissues each are used per test chemical, positive control and negative control; relative cell viability is calculated for each tissue as a percentage of the mean of the negative control tissues and a prediction of irritant (R38) is made if the relative cell viability is below 50%.

The prediction model is fully adequate. The reconstructed human skin model EpiDerm ™ exhibits the structure of human epidermis. Human-derived epidermal keratinoctes have been cultured to form a differentiated model of human epidermis including basal, spinous and granular layers, and a multi-layered stratum corneum with intercellular layers of lamelar lipids. Its general and functional conditions comply with those indicated in the OECD Test Guideline 431, *In vitro Skin Corrosion :* Human Skin Model. The EpiDerm™ EPI-200 model is commercially available for more than 13 years and has been used with good results for skin corrosion testing of chemicals, for skin irritation testing of cosmetics and their ingredients, for skin penetration testing and phototoxicity and photogenotoxicity testing.

1.4 Data quality

The evaluated data were of the required quality, well documented, clearly organized, described and discussed. The data presented appear to be of very high quality. Three of the participating institutions (BASF, IIVS, ZEBET) have either official GLP compliance or have a history of in-house QA procedures, documentation of studies etc. The 4th participant (Zet-LSL) does not have any such quality management procedures. However, the training received and the blinding of the validation study demonstrate the competence of this laboratory. Data of the submission are sufficient to assess the study goal. The study followed ECVAM SIVS Performance Standards and also reference data from ECVAM SIVS were employed.

1.4.1 Are they sufficient to assess the study goal? YES

Although all the raw data from participating laboratories are summarised in Statistical report, and thus enable sufficient evaluation, it is noted that Annex V - Raw data in the form of spreadsheets and Laboratory Reports mentioned in the e-mail of M.Liebsch (of April 23, 2008) and cited in the Test Submission Template were not provided by ECVAM for evaluation as a part of the files sent on the CD in June 2008. Similarly, Annex III (compilation of supporting scientific publications) was not provided by ECVAM to PRP for review on the CD. Raw data in the form of spreadsheets and Annex III were provided by ECVAM additionaly in course of the PR evaluation.

1.4.2 Quality of the reference data

Reference data from ECVAM SIVS were employed. Reference data, i.e. the classification data using the skin irritation effects in the rabbit, were used, as the rabbit represents the regulatory accepted species. That is why the EpiDerm^{TM} SIT had to be modified in order to

improve prediction to the regulatory accepted species. Although the intention is to predict the human health hazard, neither in vivo nor in vitro skin irritation tests are currently calibrated to match human response.

1.5 Test materials

1.5.1 Is the number of evaluated substances sufficient? YES

The selection and number of substances comply with the requirements of the ECVAM SIVS Performance Standards. The selection criteria include among others commercial availability, quality of animal data, the range of irritant responses (from negative to strong positives) and their classification based on both endpoints (MTT and IL-1α release) are available.

The independency in coding and distribution of substances were ensured in line with the study plan.

1.5.2 Are they representative of proposed applicability domain? YES

The selection and number of evaluated substances comply with the requirements of the ECVAM SIVS Performance Standards.

The applicability domain of the Modified EpiDerm™ SIT is the same as for both models (EpiSkin™ , EpiDerm™) validated under the ECVAM SIVS. The selection criteria of the ECVAM SIVS comprised exclusion of rapidly polymerizing and hydrolyzing chemicals, chemical gases, vapours and aerosols. Consequently, the EpiDerm™ EPI-200 model is currently not considered validated for such classes of chemicals. For chemicals that directly reduce MTT, correction techniques were developed. Chemicals that react with the plastic material of the cell culture inserts and quickly evaporating substances may provide higher levels of variability in the Modified EpiDerm™ SIT. At present, other applicability domain restrictions could not be defined for EpiDerm™ SIT (similarly as for EpiSkin™).

1.6 Within-laboratory variability (Module 2) – assessment of reproducibility of the data in the same laboratory

The within-laboratory variability was carefully and properly assessed. The within-laboratory variability of the Modified EpiDerm™ SIT was assessed for each laboratory by means of the assessment of the frequency of non qualified experimental runs, by one-way ANOVA statistics, by analysis of the within-laboratory standard deviation and by a box plot analysis for identification of outliers.

Amongst 240 independent experiments only 10 provided SD >20 . ZET did not report any non-qualified run. Since the frequency of the non-qualified experiments was very low (less than 5%), and the pre-defined 95% confidence interval of acceptable tests was confirmed, the Study Management decided that re-testing of non-qualified runs will not be performed. Thus all test results were included in the bioastatistical analysis, although under regular testing conditions, the non-qualified runs would have to be repeated. Amongst the 720 test results (4 laboratories, 20 chemicals, 3 runs, 3 tissues), only one significantly outlying value has been identified and excluded from the data-set of ZEBET.

1.7 Transferability (Module 3) - how easy is it to transfer the tests to a second laboratory?

Transferability of the Modified EpiDerm™ SIT was assessed during the two-phased training organised at IIVS (US site) and at Zet-LSL and BASF (EU sites). The training was performed with 4 experimental chemicals, NC and PC during October 3 – 26, 2007. Data of the Training Report document very good transferability. Except for chemical #1 tested at Zet-LSL laboratory, all chemicals in all laboratories were classified correctly, providing highly reproducible viability values with low standard deviations. Optical density (OD) values of Negative Control (NC) and Positive Control (PC) were highly reproducible. The Mean OD of NC of four laboratories was 2,166 ±0.166 and the mean OD of PC was 0.166 ± 0.007. Viability of the positive control was in all experiments below 20 %, with the mean value of 5.4 % ± 0.54 % .

The results of 4 participating laboratories document also suitability of the EpiDerm™ skin model for long distance shipment with no effect on the skin model quality and no influence on the assay results. Concordant results of participating labs located in the US and EU support this conclusion.

1.8 Between-laboratory variability (Module 4) - assessment of reproducibility of the data in different laboratories

The data reproducibility was properly assessed. The variability between the four laboratories of the primary MTT endpoint was assessed with three statistical techniques.

Firstly, 1-way ANOVA comparing the data of the four laboratories for each single chemical (significance level of 5% and 1%) was applied, where the mean values of the triplicates were used. Secondly, taking the run mean per laboratory, the standard deviation of these three means was calculated. Differences between laboratories for 7 chemicals were identified. However, the sum of squares and the standard deviation were an indicator that the variability within laboratories resulted in non-significant results, although there are differences between the laboratories.

Thirdly, the proportion of identical run classifications and identical median run classifications over the four laboratories was evaluated. 18 chemicals out of 20 were identically classified by all four laboratories. Since 90% of positive and 90% of negative results have been correctly identified by the Modified EpiDerm™ SIT, it can be concluded, that the test is transferable and produces reliable results amongst different users.

1.9 Predictive capacity (Module 5)

1.9.1 Has the predictive capacity of the methods been properly assessed ? YES – but further statistical anaylsis is required

The predictive capacity of the Modified EpiDerm ™ SIT was assessed by 2 × 2 contingency table statistics. Testing 20 reference chemicals, an overall sensitivity of 80% and a specificity of 77.5 % were identified. The accuracy of 78.8% was found. Compared to the EpiSkin ™ test MTT-results, one additional in vivo irritating chemical (terpinyl acetate) was identified correctly by the Modified EpiDerm™ SIT. It may be recognized as important, that none of

the chemicals known to be irritating to human skin was under-predicted by the modified EpiDerm™ SIT.

A new approach, the calculation of the probability of correct classification, is suggested. In addition to the 2 × 2 contingency table statistics, the linear discrimination analysis may be performed to evaluate the predictive power of the method.

It was recommended that further statistical analysis was performed on the data from this study and the data obtained using these 20 chemicals in the orginal SIVS with EpiSkin ™ to allow direct comparison of specificity, sensitivity and accuracy values generated using identical methodology (see Section 2)

EpiDerm™ SIT submission provided also information on the secondary endpoint IL-1• (Interleukin 1 alpha). On contrary to performance of the EpiSkin™ test in the SIVS (3), the data on IL-1• did not demonstrate an improvement of the predictive capacity of the EpiDerm™ test method. Therefore, only the data for the MTT endpoint were considered with regard to the predictive capacity.

1.9.2 Is the assay relevant for its stated purpose? Yes.

Compared to the EpiSkin™ assay MTT-results, one additional *in vivo* irritating chemical (terpinyl acetate) was identified correctly by the Modified EpiDerm™ SIT. Two over-predictions were obtained for non-irritating chemicals and one underprediction. In general, results obtained with the Modified EpiDerm™ SIT are at least comparable with results of the EpiSkin ™ assay, i.e. they are equivalent or even better.

Of particular relevance are those irritants (R38) that were incorrectly identified by the EpiDerm ™ SIT as non-irritant (no label) [hexyl salicylate and di-n-propyl disulphide] and the non-irritants (no label) that were incorrectly identified by the EipDerm SIT as irritants (R38) [1-bromo-4-chlorobutane and 4-methyl-thio-benzaldehyde]. As mentioned by the authors, hexyl salicylate is a non-irritant to human skin although it causes irritation (R38 labelling) in the rabbit test.

10. Applicability domain (Module 6) - Is the proposed applicability domain well defined ? YES

The applicability domain comprises chemicals as liquids and solids, not gases, vapours or aerosols. Quickly hydrolysing or polymerising chemicals or chemicals with other type of degradation were not included in the ECVAM SIVS. Consequently, for this type of chemicals also the validity of the Modified EpiDerm™ method cannot be considered as assessed. Highly volatile substances and chemicals that react with the plastic material of the cell culture inserts may provide higher levels of variability and may give inconsistent results.

1.11 Performance standards (Module 7) - Have appropriate performance standards been defined for the test ? YES

The ECVAM SIVS Performance Standards Document was exactly followed in this study. However, the appearance of heptanal on the list of standards is surprising due to the fact that during the ECVAM SIVS it was tested in one laboratory only and only during the test optimization

study on EpiSkin™. In addition, a possibly wrong calculation of the dominating rabbit skin irritation median value for heptanal was identified (3.35 instead of 4).

It is suggested, that existing human data should not be ignored, as they provide valuable toxicological information. A considerable number of chemicals classified R38 by the rabbit test do not cause irritation in human skin in vivo. Reconstructed human skin models consist of cells of human origin. They were developed to predict human skin hazard. That is why they classify some of rabbit irritants as human non-irritants (e.g. Hexyl salicylate is
one of the R38 reference chemicals of the PS document which was repeatedly shown to be non-irritating to humans).

Although volatile and rapidly changing/decomposing chemicals should be excluded from the list of standards, some of the standard substances exhibit these characteristics (e.g. 1-bromohexane, 4-methyl-thio-benzaldehyde, a-terpineol).

1.12 Readiness for regulatory purposes

The Modified EpiDerm™ SIT exhibited sufficient sensitivity, specificity and accuracy in a well conducted validation study. It can be suggested as standalone test for classification of skin irritants (R38 or no label).

2. Further statistical analysis requested from ECVAM

Within ECVAM document ‘Performance Standards for Applying Human Skin Models to in-vitro skin irritation testing’ there is a requirement that 'the sensitivity of a 'me too' test must be higher than 70%, and the specificity must be equal to or higher than 80%'. These figures are based on the analysis of the 20 reference chemicals in the SIVS with EpiSkin™ using median test results from the dataset.

It is therefore important when quoting sensitivity and specificity results for a new test that the same statistical methodology is used as was used when defining these requirements within the Performance Standards for Applying Human Skin Models to in-vitro skin irritation testing.

The peer review panel therefore asked ECVAM to recalculate sensitivity, specificity and accuracy figures for the modified Modified EpiDerm™ SIT using exactly the same methodology (i.e. using median test results) to that used for the same 20 chemicals in the SIVS using the EpiSkin™ test. These results are presented in Appendix 1 (EpiSkin ™) and 2 (Modified EpiDerm ™ SIT) and summarised below

	EpiSkin™ (SIVS)	Modified EpiDerm™ SIT
Sensitivity	70%	80%
Specificity	80%	80%
Accuracy	75%	80%

3. Overall Summary

Given the consistently positive comments within the initial scientific peer review of the submission of the follow-up validation study of the modified Modified EpiDerm ™ Skin Irritation Test (SIT) together with the additional statistical analysis of

sensitivity, specificity and accuracy, the combined veiws of the peer review panel can be summarised as:The performance of the Modified EpiDerm™ Skin Irritation Test (SIT) met the criteria outlined to be considered to have sufficient accuracy and reliability for prediction of R38 skin irritating and no-label (non-skin irritating) test substances compared to the validated and accepted method. Limitations associated with the previously validated and accepted in-vitro reference method 1 (EpiSkin™) for skin irritation e.g. applicability domain (ECVAM (2007) Performance Standards for applying human skin models to *in vitro* skin irritation (available under Download study document, at http://ecvam.jrc.ec.europa.eu. Accessed on 27.10.2008.) also apply to the Modified EpiDerm™ Skin Irritation Test (SIT) .

Dr M. Dambrova,
Dr D. Jirova
Dr C. Westmoreland

20. November 2008

References

1. ECVAM (2007) *Performance Standards for Applying Human Skin Models to In Vitro Skin Irritation Testing.* Online: http://ecvam.jrc.ec.europa.eu
2. ECVAM (2007) *Statement of the ECVAM Scientific Advisory Committee (ESAC) on the Validity of In Vitro Tests for Skin Irritation.* Online: http://ecvam.jrc.ec.europa.eu
3. ECVAM (2007) *Skin Irritation Validation Study Phase II: Analysis of the primary endpoint MTT and the secondary endpoint IL-1•* . Online: http://ecvam.jrc.ec.europa.eu.
4. Spielmann, H., Hoffmann, S., Liebsch, M., Botham, P., Fentem, J., Eskes, C., Roguet, R., Cotović, J., Cole, T., Worth, A., Heylings, J., Jones, P., Robles, C., Kandárová, H., Gamer, A., Remmele, M., Curren, R., Raabe, H., Cockshott, A., Gerner, I. and Zuang, V. (2007) *The ECVAM International Validation Study on In Vitro Tests for Acute Skin Irritation: Report on the Validity of the EPISKIN and EpiDerm Assays and on the Skin Integrity Function Test.* ATLA **35,** 559-601.

Appendix 1: Statistical analysis of sensitivity, specificity and accuracy of EpiSkin™ test using 20 reference chemicals tested in the full prospective exposure study

Individual Laboratory Predictions for the 20 Reference Chemicals ONLY. Used for calculating the predictive values (sensitivity, specificity, accuracy)

Nr.	Chemical	EU Class	L'Oréal	Unilever	Sanofi	Median	In Vitro Prediction	
2	**1-bromo-4-chlorobutane**	no label	1	1	1	1	I	FP
22	Diethyl phthalate	no label	0	0	0	0	NI	
24	di-propylene glycol	no label	0	0	0	0	NI	
41	Naphthalene acetic acid	no label	0	0	0	0	NI	
11	Allyl phenoxy-acetate	no label	0	0	0	0	NI	
36	Isopropanol	no label	0	0	0	0	NI	
8	**4-methyl-thio-benzaldehyde**	no label	0	1	1	1	I	FP
39	Methyl stearate	no label	0	0	0	0	NI	
10	Allyl heptanoate	no label	0	0	0	0	NI	
33	Heptyl butyrate	no label	0	0	0	0	NI	
34	**Hexyl salicylate**	R38	0	0	0	0	NI	FN
55	Terpinyl acetate	R38	0	1	0	0	NI	FN
58	Tri-isobutyl phosphate	R38	1	1	1	1	I	
4	1-decanol	R38	1	1	1	1	I	
20	Cyclamen aldehyde	R38	1	1	1	1	I	
3	1-bromohexane	R38	1	1	1	1	I	
15	a-terpineol	R38	1	1	1	1	I	
23	**di-n-propyl disulphide**	R38	0	1	0	0	NI	FN
18	Butyl methacrylate	R38	1	1	1	1	I	
XXX	Heptanal	R38	1	.	.	1	I	

1) CALCULATION ON THE BASIS OF ALL LABORATORY PREDICTIONS

30 individual predictions for NEGATIVES
but only 28 for positives = total of 58 predictions

			absolut	percent
SENSITIVITY				
Sensitivity = TP / (TP+FN)		TP	21	75
Sensitivity	**75**	FN	7	**25**
		Sum	**28**	
SPECIFICITY				
Specificity = TN / (TN + FP)		TN	25	83.3
Specificity	**83.3**	FP	5	**16.7**
		Sum	**30**	
ACCURACY				
Sum (TP + TN) / (TP+TN+FN+FP)				
Accuracy	**79.3**			

1a) CALCULATION ON THE BASIS OF ALL LABORATORY PREDICTIONS

Including 3 concordant laboratory predictions for Heptanal (same weighting as for the other chemicals)

30 individual predictions per class = total of 60 predictions

			absolut	percent
SENSITIVITY				
Sensitivity = TP / (TP+FN)		TP	23	76.7
Sensitivity	**76.7**	FN	7	**23.3**
		Sum	**30**	
SPECIFICITY				
Specificity = TN / (TN + FP)		TN	25	83.3
Specificity	**83.3**	FP	5	**16.7**
		Sum	**30**	
ACCURACY				
Sum (TP + TN) / (TP+TN+FN+FP)				
Accuracy	**80.0**			

2) CALCULATION ON THE BASIS OF THE FINAL DECISION MAKING (MEDIAN)

			10 individual predictions per class		
SENSITIVITY				absolut	percent
Sensitivity = TP / (TP+FN)			TP	7	70
Sensitivity	**70**	***-> led to PS value for me-too's***	FN	3	**30**
			Sum	**10**	
SPECIFICITY					
Specificity = TN / (TN + FP)			TN	8	80
Specificity	**80**	***-> led to PS value for me-too's***	FP	2	**20**
			Sum	**10**	
ACCURACY					
Sum (TP + TN) / (TP+TN+FN+FP)					
Accuracy	**75**				

Appendix 2: Statistical analysis of sensitivity, specificity and accuracy of Modified EpiDerm™ Skin Irritation Test

Individual Laboratory Predictions for the 20 Reference Chemicals used for calculating the predictive values (sensitivity, specificity, accuracy)

No	Chemical	*In vivo* score§	EU label	EU GHS	Lab1	Lab 2	Lab 3	Lab 4	Dominant median	In vitro prediction	
1	1-bromo-4-chlorobutane	0	0	0	1	1	1	1	1	I	FP
2	diethyl phthalate	0	0	0	0	0	0	0	0	NI	
3	di-propylene glycol	0	0	0	0	0	0	0	0	NI	
4	naphthalene acetic acid	0	0	0	0	0	0	0	0	NI	
5	allyl phe0xy-acetate	0.3	0	0	0	0	0	0	0	NI	
6	isopropa0l	0.3	0	0	0	1	0	0	0	NI	
7	4-methyl-thio-benzaldehyde	1	0	0	1	1	1	1	1	I	FP
8	methyl stearate	1	0	0	0	0	0	0	0	NI	
9	allyl hepta0ate	1.7	0	0	0	0	0	0	0	NI	
10	heptyl butyrate	1.7	0	0	0	0	0	0	0	NI	
11	hexyl salicylate	2	1	0	0	0	0	0	0	NI	FN
12	terpinyl acetate	2	1	0	1	1	1	1	1	I	
13	tri-isobutyl phosphate	2	1	0	1	1	1	1	1	I	
14	1-deca0l	2.3	1	1	1	1	1	1	1	I	
15	cyclamen aldehyde	2.3	1	1	1	1	1	1	1	I	
16	1-bromohexane	2.7	1	1	1	1	1	1	1	I	
17	a-terpineol	2.7	1	1	1	1	1	1	1	I	
18	di-n-propyl disulphide	3	1	1	0	0	1	0	0	NI	FN
19	butyl methacrylate	3	1	1	1	1	1	1	1	I	
20	heptanal*	4	1	1	1	1	1	1	1	I	

1) CALCULATION ON THE BASIS OF ALL LABORATORY PREDICTIONS

40 individual predictions per class
Total of 80 prections.

SENSITIVITY

			absolut	percent	
Sensitivity = TP / (TP+FN)		TP	33	82.5	
Sensitivity	**82.5** *ok (• 70%)*	FN	7	**17.5**	*ok (• 30%)*
		Sum	**40**		

SPECIFICITY

Specificity = TN / (TN + FP)		TN	31	77.5	
Specificity	**77.5** *2.5% below PS (• 80%)*	FP	9	**22.5**	*2.5% above PS (• 20*
		Sum	**40**		

ACCURACY

Sum (TP + TN) / (TP+TN+FN+FP)

Accuracy **80** *no values provided in the PS*

2) CALCULATION ON THE BASIS OF THE FINAL DECISION MAKING (MEDIAN)

10 individual predictions per class
Total of 20 Predictions

SENSITIVITY

			absolut	percent	
Sensitivity = TP / (TP+FN)		TP	8	80	
Sensitivity	**80** *ok (• 70%)*	FN	2	**20**	*ok (• 30%)*
		Sum	**10**		

SPECIFICITY

Specificity = TN / (TN + FP)		TN	8	80	
Specificity	**80** *ok (• 80%)*	FP	2	**20**	*ok (• 20%)*
		Sum	**10**		

ACCURACY

Sum (TP + TN) / (TP+TN+FN+FP)

Accuracy **80** *no values provided in the PS*