

Performance of the OptiSafe Ocular Irritation Assay in a Three-Laboratory Validation Study

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Introduction

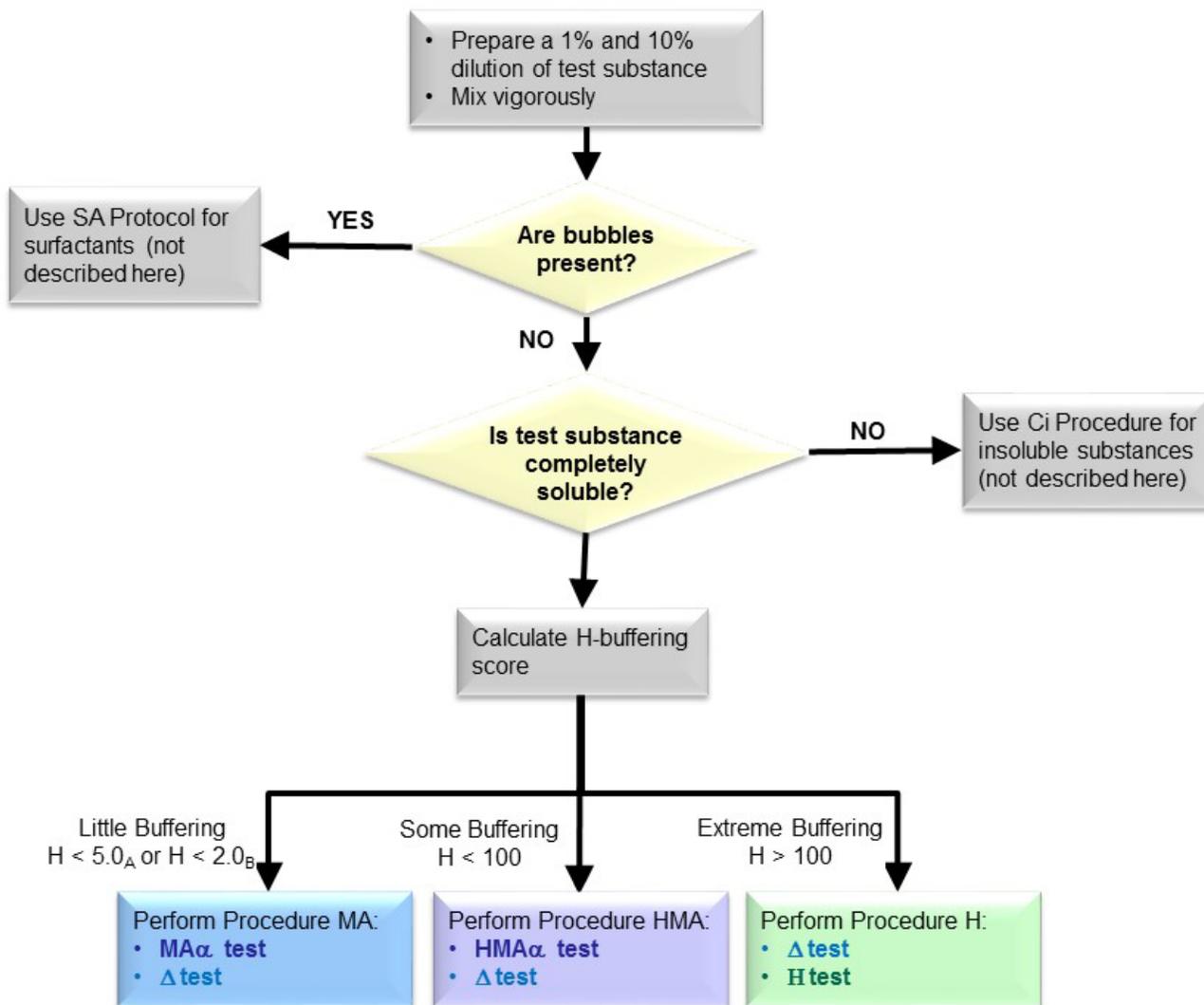
- OptiSafe assesses a test substance's potential to produce eye irritation by measuring damage to macromolecules.
- The test method allows for detection of substances with different mechanisms of ocular injury.
 - Denaturation of water-insoluble polymers in the membrane that model the phospholipid bilayer of cells predicts damage to the corneal epithelium and conjunctiva.
 - Indirect denaturation of molecules and fluid transfer across the membrane by osmotic effects predict damage to the cornea and conjunctiva.
 - Denaturation of macromolecules that model ordered collagen predicts damage to the cornea and conjunctiva.
- OptiSafe has a number of useful features:
 - Sold as a shelf-stable kit
 - Includes step-by-step instructions for easy implementation in a basic laboratory
 - Requires no specialized equipment
 - Can be conducted on a benchtop (no cell culture required)
 - Can be completed in less than 24 hours
 - Includes a pre-test that assesses the test substance's physical and chemical properties to identify the optimal procedure
- NICEATM coordinated a three-laboratory validation study of OptiSafe (**Table 1**) in order to:
 - Assess transferability to naïve laboratories
 - Characterize usefulness and limitations

Table 1 Validation Study Phases

Phase	Activities
Pre-Study Phase	<ul style="list-style-type: none">• Formation of VMT – composed of ICCVAM agency scientists and international representatives• Selection of naïve laboratories• Finalization of documents, reporting forms, and performance criteria
Phase I	<ul style="list-style-type: none">• Qualification and training of naïve laboratories• Testing of all practice chemicals by lead and naïve laboratories
Phase II	<ul style="list-style-type: none">• Testing of 30 chemicals by lead and naïve laboratories
Phase III	<ul style="list-style-type: none">• Testing of 60 chemicals by lead laboratory
Reporting Phase	<ul style="list-style-type: none">• Preparation of validation report

Test Method Protocol

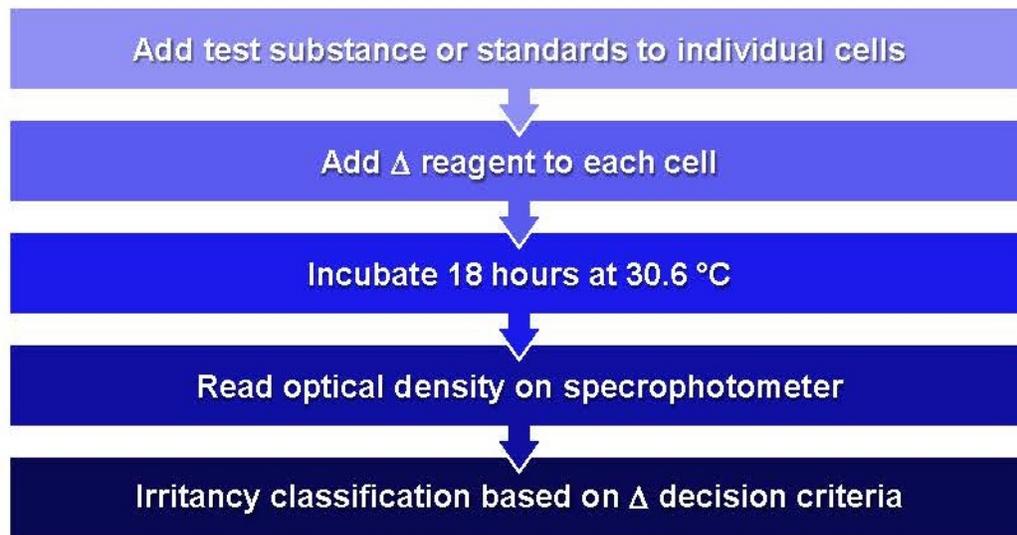
Pre-Test Flow Chart



Main Test Procedure Options

- Procedure Δ – assess water-insoluble denaturation and oxidative damage/excessive reactivity
- Procedure α – assess collagen denaturation and oxidative damage/excessive reactivity
 - MA α – non-surfactants with limited or little buffering capacity
 - HMA α – non-surfactants with moderate buffering capacity
 - Ci – insoluble compounds
- Procedure H – assess ocular pH shift

Option 1. Delta Test Flow Chart



Option 2. Alpha Test Flow Chart

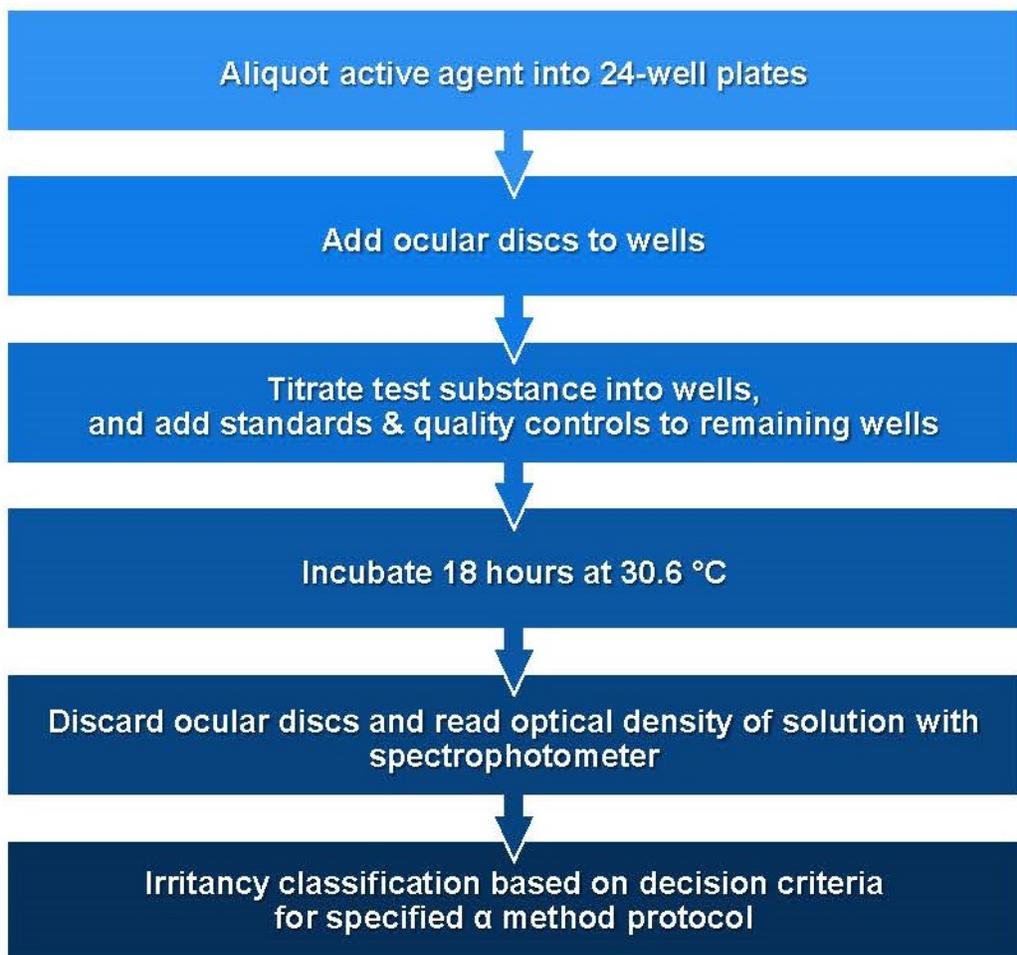
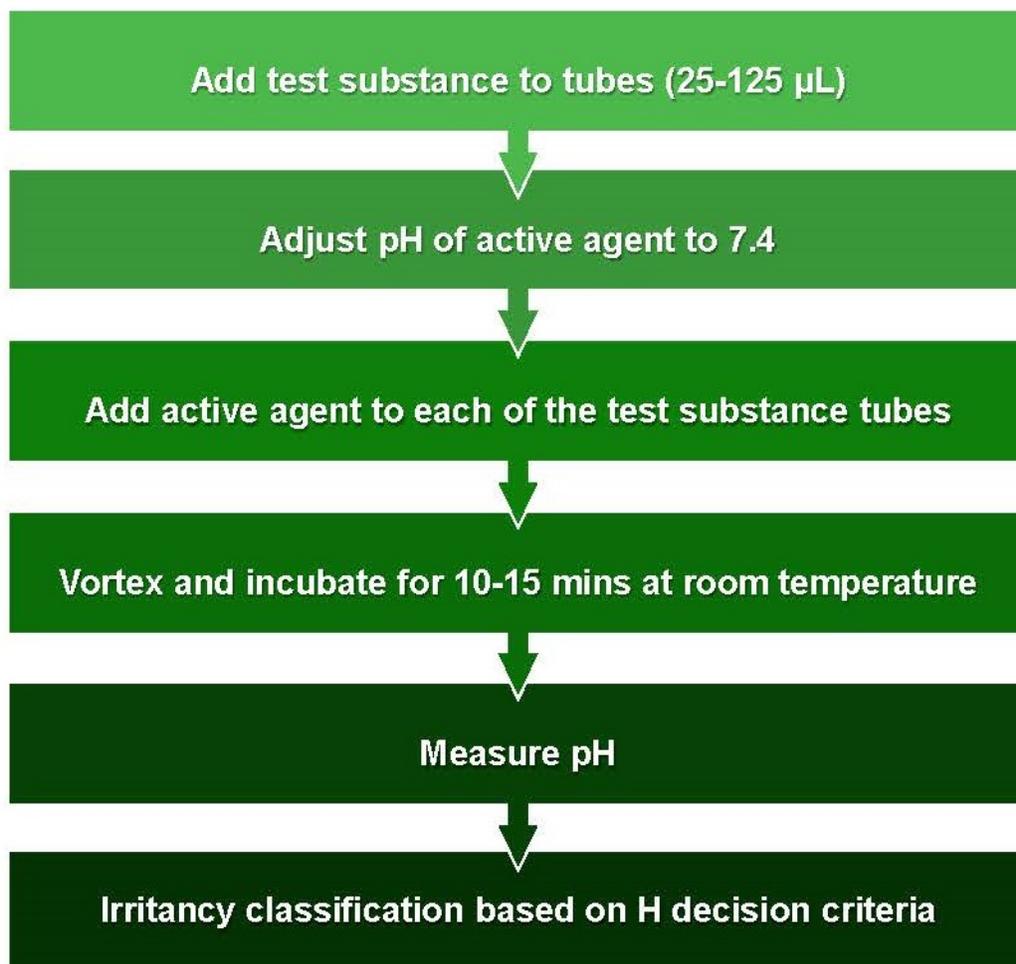


Figure 4. H Test Flow Chart



Chemical Selection

- NICEATM collaborated with the validation study management team (VMT) on chemical selection for all phases of the validation effort.
- Factors used in the chemical selection process included physicochemical properties, ocular irritancy responses, and use in other in vitro validation studies.
- Surfactants were included to assess accuracy of the pre-test procedure.

Phase I Outcomes

- Intralaboratory reproducibility was greater than 90% (n = 15) for all testing laboratories. The VMT concluded Phase II should proceed.
- Minor protocol additions were made to further increase transferability and test method reproducibility in Phase II.
 - Procedures for when an incubation box should be discarded

- Methods to ensure solids were fully in contact with the membrane
- Additional quality control procedures

Phase II Outcomes

- Accuracy statistics for each laboratory under the U.S. Environmental Protection Agency (EPA) and United Nations Globally Harmonized System of Classification and Labelling of Chemicals (GHS) classification systems are shown in **Table 2** and **Table 3**.
- Interlaboratory accuracy statistics for the EPA and GHS classification systems are shown in **Table 4**.
- Intralaboratory reproducibility for the EPA and GHS classification systems was greater than 93% for non-surfactants.
- Interlaboratory reproducibility was 91% for both classification systems.

Table 2. Phase II Test Method Accuracy for Individual Labs: EPA Classification

	Lead Laboratory	Naïve Laboratory 1	Naïve Laboratory 2
Accuracy	88% (22/25 ^a)	81.5% (22/27 ^b)	85.2% (23/27 ^b)
Sensitivity	100% (13/13)	100% (14/14)	92.9% (13/14)
Specificity	75% (9/12)	61.5% (8/13)	76.9% (10/13)
False Positive	25% (3/12)	38.5% (5/13)	23.1% (3/13)
False Negative	0% (0/13)	0% (0/14)	7.1% (1/14)
Negative Predictivity	100% (9/9)	100% (8/8)	90.9% (10/11)
Positive Predictivity	81.3% (13/16)	73.7% (14/19)	81.3% (13/16)

^a Five chemicals were excluded from the analysis: one chemical outside of the applicability domain, one chemical tested using a non-standard test method, and three chemicals identified as surfactants.

^b Three chemicals were excluded from the analysis because they were surfactants.

Table 3. Phase II Test Method Accuracy for Individual Labs: GHS Classification

	Lead Laboratory	Naïve Laboratory 1	Naïve Laboratory 2
Accuracy	88% (23/25 ^a)	81.5% (22/27 ^b)	77.8% (21/27 ^b)
Sensitivity	100% (12/12)	100% (13/13)	84.6% (11/13)
Specificity	76.9% (10/13)	64.3% (9/14)	71.4% (10/14)
False Positive	23.1% (3/13)	35.7% (5/14)	28.6% (4/14)
False Negative	0% (0/12)	0% (0/13)	15.4% (2/13)
Negative Predictivity	100% (10/10)	100% (9/9)	83.3% (10/12)
Positive Predictivity	80% (12/15)	72.2% (13/18)	73.3% (11/15)

^a Five chemicals were excluded from the analysis: one chemical outside of the applicability domain, one chemical tested using a non-standard test method, and three chemicals identified as surfactants.

^b Three chemicals were excluded from the analysis because they were surfactants.

Table 4. Phase II Test Method Accuracy (Summary of All Labs): EPA and GHS Classification

	EPA	GHS
Accuracy	88.5% (23/26 ^a)	88.5% (23/26 ^a)
Sensitivity	100% (14/14)	100% (13/13)
Specificity	75% (9/12)	76.9% (10/13)
False Positive	25% (3/12)	23.1% (3/13)
False Negative	0% (0/14)	0% (0/13)
Negative Predictivity	100% (9/9)	100% (10/10)
Positive Predictivity	82.4% (14/17)	81.3% (13/16)

^a Four chemicals were excluded from the analysis: three chemicals were identified as surfactants, and no overall call could be made for the fourth chemical.

Phase III Outcomes

- To evaluate the applicability domain of OptiSafe, the misclassification rate of structural fragments present in the tested chemicals was evaluated (**Table 5**).
- The Organic Functional Group profiler in OECD Toolbox (v. 4.1), a quantitative structure activity relationship program developed by the Organisation for Economic Co-operation and Development, was used to identify structural fragments in the tested chemicals.
- There were no GHS underpredictions.
- Two chemicals classified as EPA Category III (“mild” irritants) based on in vivo studies (dodecane and 1,4-dibromobutane) were underpredicted by OptiSafe.
 - The Category III classifications are based on mild reactions in only one of three or six animals.
 - Considering the recognized variability of the in vivo test, if tested again these chemicals could be classified as Category IV.

Table 5 Misclassification of Phase III Chemicals by Organic Functional Groups

Structural Feature	EPA Negative	EPA Positive	EPA False Positive	EPA False Negative	EPA False Positive Rate (%)	EPA False Negative Rate (%)	GHS Negative	GHS Positive	GHS False Positive	GHS False Negative	GHS False Positive Rate (%)	GHS False Negative Rate (%)
Acetoxy	0	2	0	0	N/A	0	1	1	1	0	100	0
Alcohol	3	10	1	0	33	0	5	8	3	0	60	0
Aldehyde	2	2	1	0	50	0	2	2	1	0	50	0
Alkane, branched with secondary carbon	3	2	1	0	33	0	3	2	0	0	0	0
Alkane, branched with tertiary carbon	2	3	1	0	50	0	2	3	1	0	50	0
Alkene	2	3	1	0	50	0	4	1	2	0	50	0
Alkenyl (hetero)arenes	0	1	0	0	N/A	0	1	0	1	0	100	N/A
Alkyl	1	1	0	1	0	100	2	0	0	0	0	N/A
Alkyl (hetero)arenes	0	4	0	0	N/A	0	1	3	0	0	0	0
Alkyl halide	1	2	1	1	100	50	2	1	0	0	0	0
Alkyl-, alkenyl- and alkynyl (hetero)arenes	0	5	0	0	N/A	0	2	3	1	0	50	0
Allyl	2	2	1	0	50	0	3	1	1	0	33	0
Aryl	4	9	2	0	50	0	7	6	4	0	57	0
Benzyl	0	1	0	0	N/A	0	1	0	1	0	100	N/A
Carboxylic acid ester	3	6	2	0	67	0	4	5	3	0	75	0

Diketone	0	1	0	0	N/A	0	1	0	1	0	100	N/A
Dihydroxyl derivatives	2	1	1	0	50	0	2	1	1	0	50	0
Ether	4	2	3	0	75	0	6	0	5	0	83	N/A
Guanidine	0	1	0	0	N/A	0	1	0	1	0	100	N/A
Isopropyl	2	2	2	0	100	0	2	2	1	0	50	0
Ketone	0	4	0	0	N/A	0	1	3	1	0	100	0
Methacrylate	1	0	1	0	100	N/A	1	0	1	0	100	N/A
No functional group found	0	2	0	0	N/A	0	1	1	1	0	100	N/A
Phosphite ester	1	0	1	0	100	N/A	1	0	1	0	100	N/A
Sulfide	1	0	1	0	100	N/A	1	0	1	0	100	N/A
Thiol	2	1	1	0	50	0	2	1	1	0	50	0

Conclusions and Future Directions

- OptiSafe may represent a new tool for in vitro assessment of the ocular toxicity potential of chemicals in a tiered-testing system.
- OptiSafe exhibited high transferability and interlaboratory reproducibility in this study.
- High false positive rates for a limited number of substances in certain chemical classes are being further investigated by additional testing.

Acknowledgements

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