NICEATM

National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods

ICCVAM

Interagency Coordinating Committee on the Validation of Alternative Methods

Isolated Chicken Eye (ICE) Test Method

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ILS Inc., Contractor Supporting NICEATM

ICCVAM Workshop Series on Best Practices for Regulatory Safety Testing: Assessing the Potential for Chemically Induced Eye Injuries

January 19, 2011

William H. Natcher Conference Center
National Institutes of Health
Bethesda, MD

National Toxicology Program

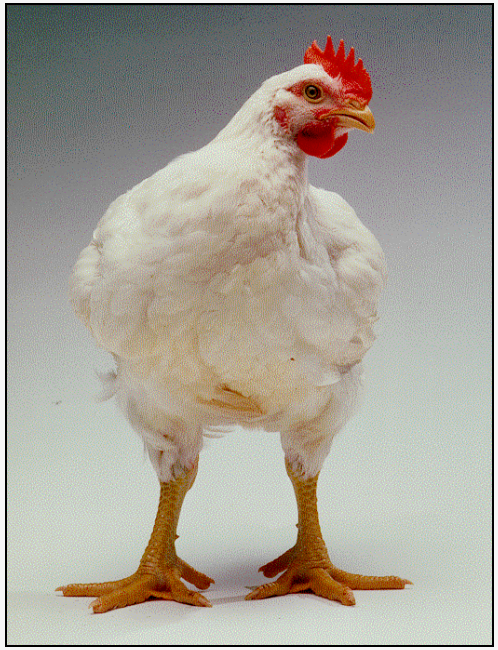
NIEHS

National Institutes of Health

Department of Health & Human Services

History of ICE

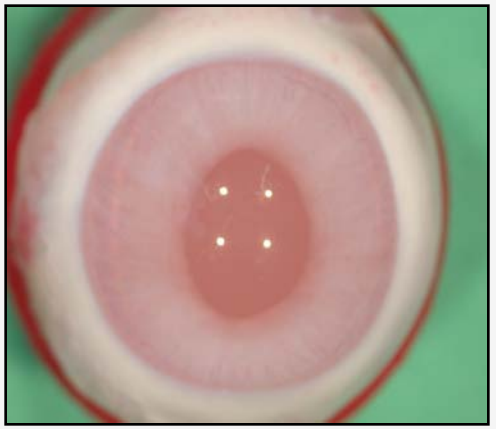
- Developed by Herman Koëter and Menk Prinsen
- Based on method of Burton (1981)
  - Isolated rabbit eyes in superfusion apparatus
  - 10-sec. application
  - Slit-lamp examination cornea (swelling, opacity, fluorescein)

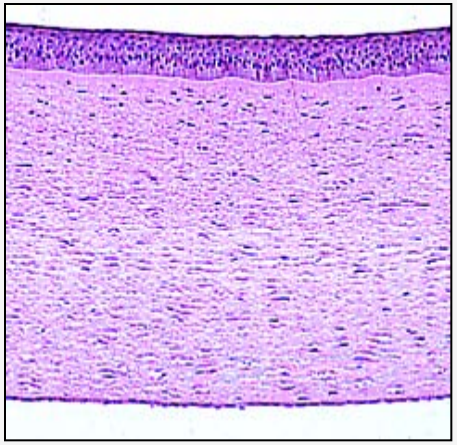


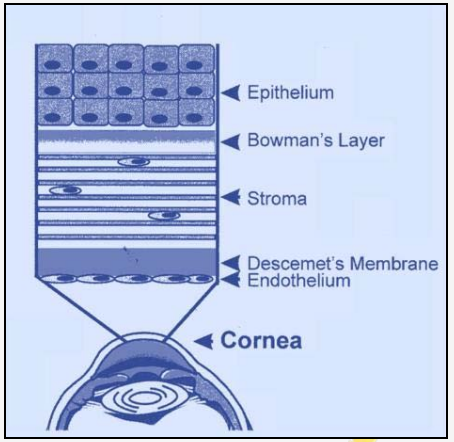
Why Chicken Eyes?

Abattoir species

- Bovine
  - Very large eyes
  - Thick corneas
- Porcine
  - Difficult to collect
  - Thick corneas
- ***Chicken***
  - ***Easy to collect***
  - ***Similar to human***
  - ***Bowman's layer present***
  - ***Very uniform***







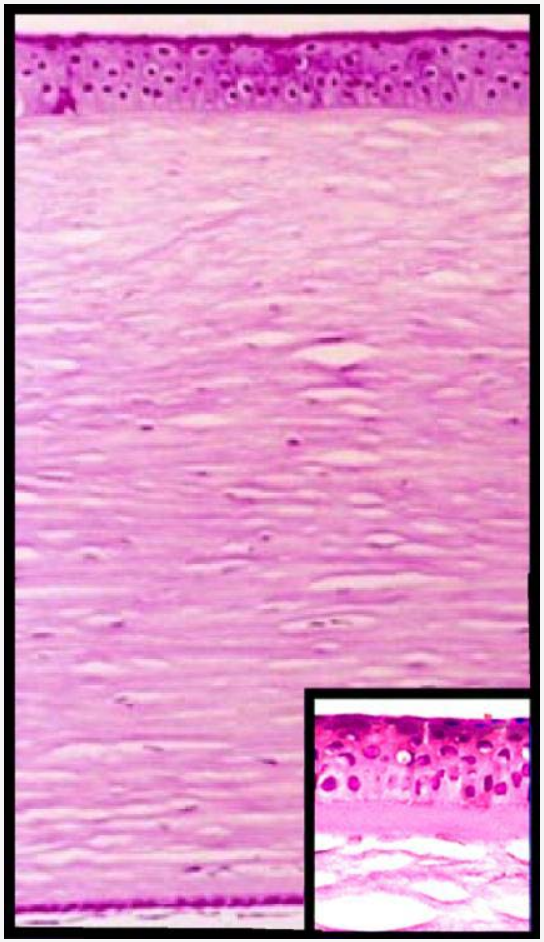
Chicken Corneal Anatomy: Dimensions

	Chicken	**Human***
Diameter (horizontal)	9.08±0.04 mm	11.5 mm
Diameter (vertical)	8.82±0.05 mm	10.5 mm
Thickness (central)	404.7±9.5 µm	520 µm

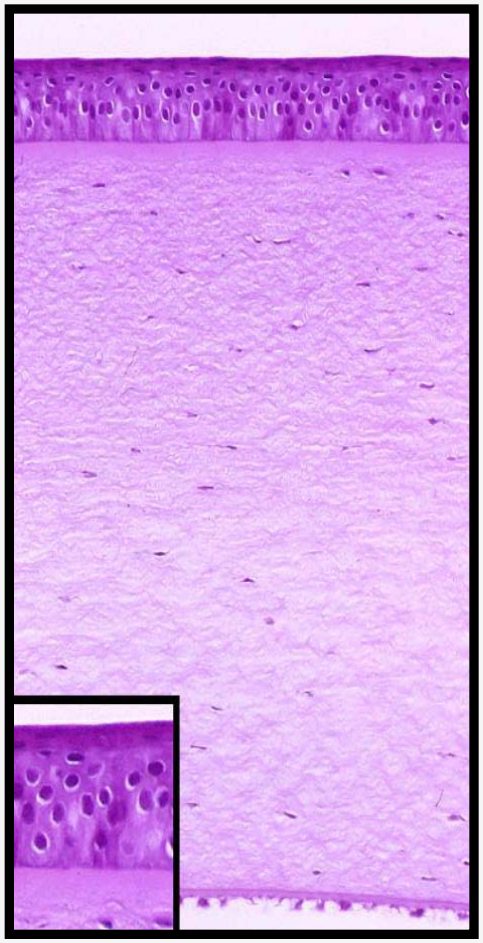
*WH Spencer. “Cornea.” In WH Spencer. *Ophthalmic Pathology*. Philadelphia: W.B. Saunders Company; 1996.
T Nishida. “Cornea.” In JH Krachmer, MJ Mannis, EJ Holland [eds]. *Cornea*. Vol. I. St. Louis:Mosby-Year Book, Inc.; 1997.
Courtesy of Craig Fowler, M.D., UNC-Chapel Hill

Comparative Corneal Anatomy

Human



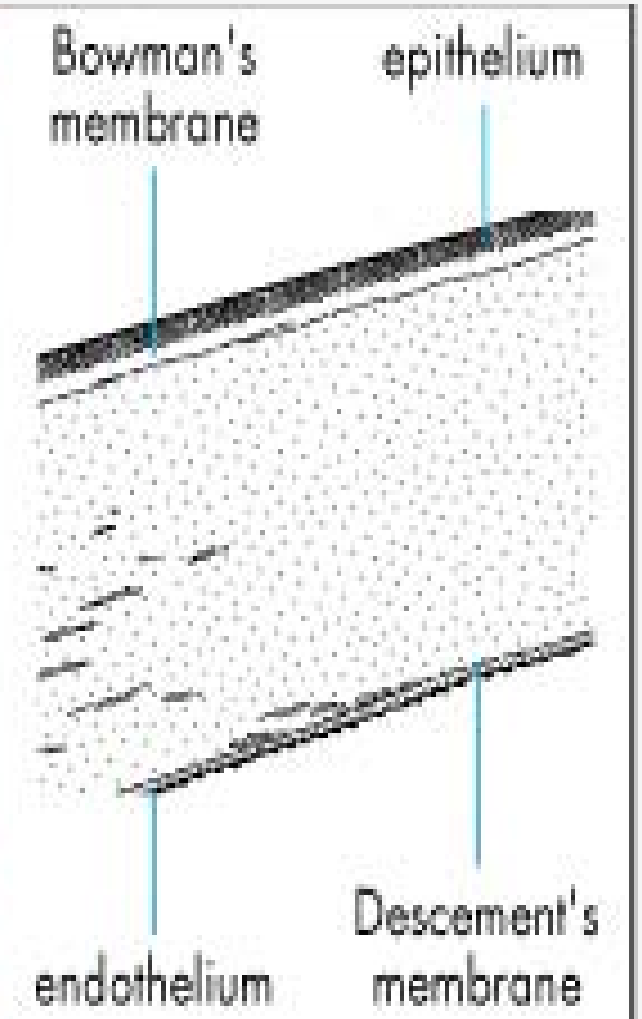
Chicken



Courtesy of Craig Fowler, M.D., UNC-Chapel Hill.

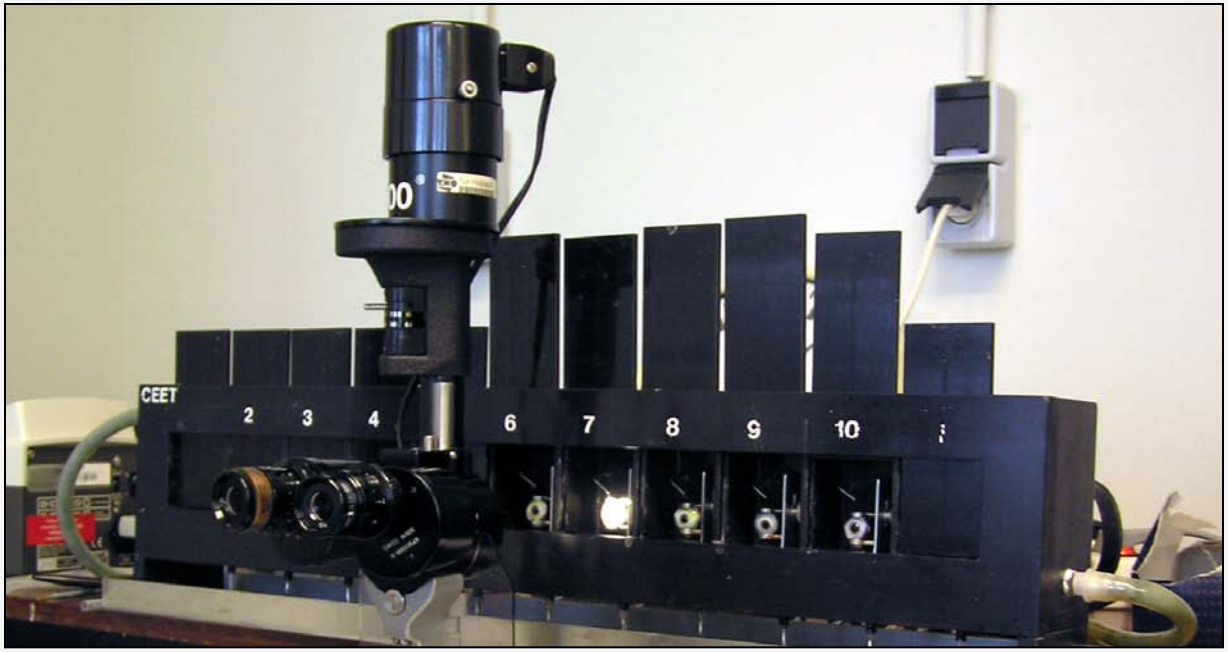
Bowman's Layer

- Light Microscopy
  - Transparent
  - Structureless
- Electron Microscopy
  - Collagenous fibrils & proteoglycans, not a true membrane
- 10 microns thick
  - Considered to be anterior portion of stroma, with smooth anterior surface
  - Acellular
- Damage to Bowman's heals with a scar



Courtesy of Craig Fowler, M.D., UNC-Chapel Hill

ICE Superfusion Apparatus

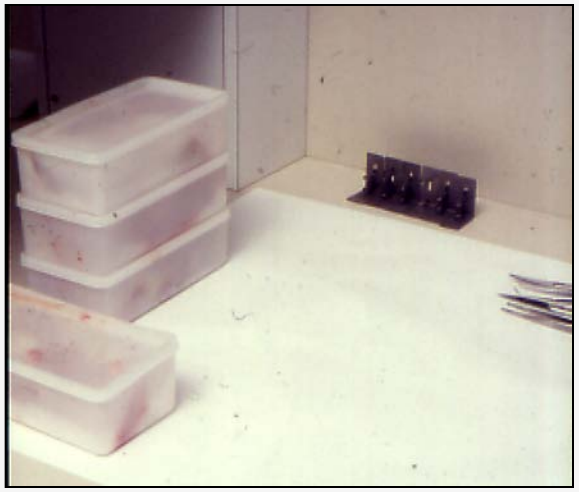


- 11 chambers with sliding doors
- Water mantle
- Saline inlet and outlet

Maintaining an Ambient Environment

- Saline drip
- Channel for each chamber
- Peristaltic pump
- Passes water mantle

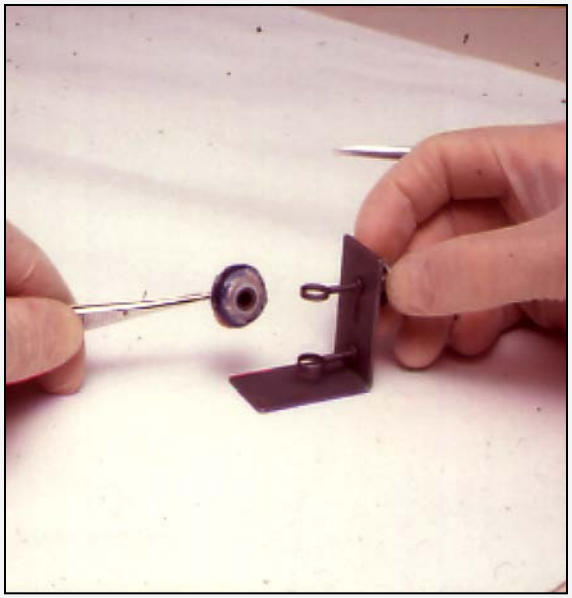
Collection of Eyes

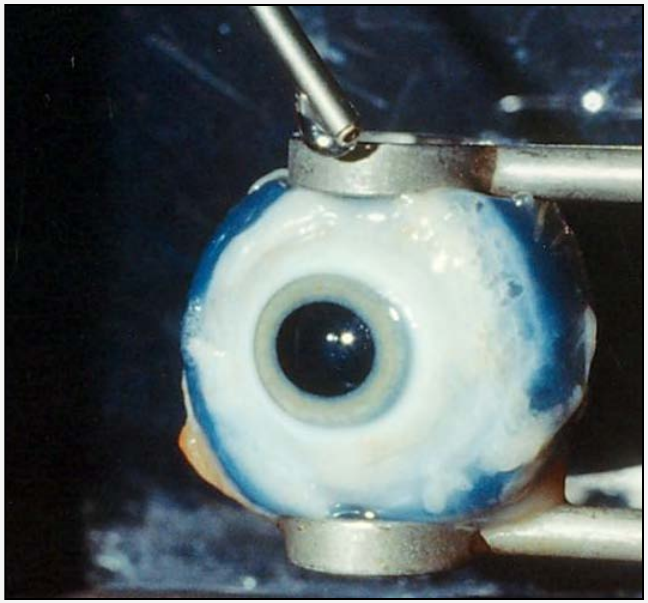


- Transport boxes for heads
- Moistened tissue
- Ambient temperature

- Fluorescein - one drop and immediate rinsing
- Detection of damaged epithelium

Mounting Eyes for Testing

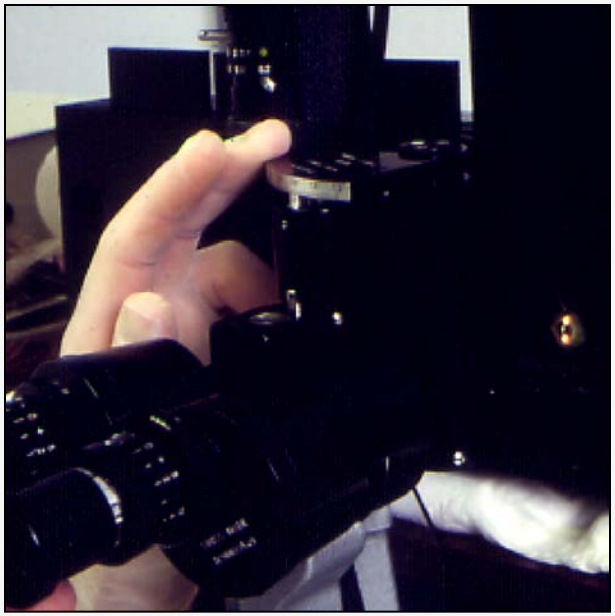


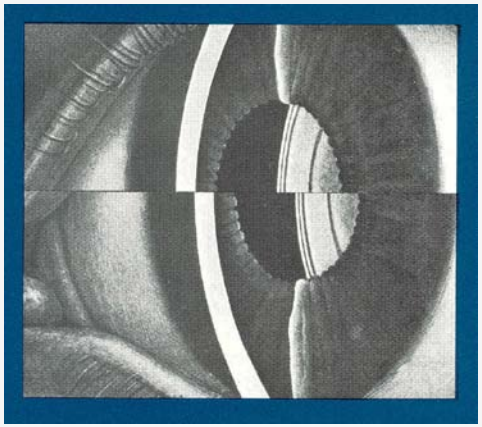


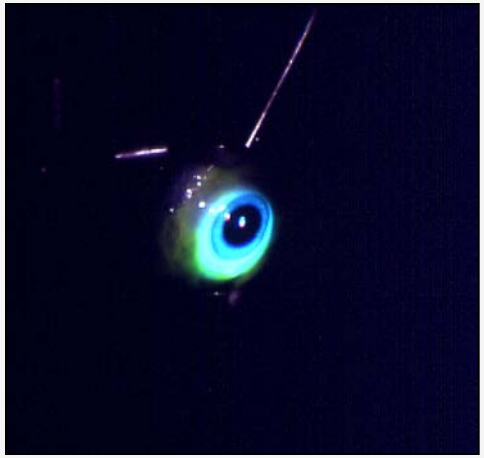
- Enucleation within 10 sec
- Insert eye into holder

- Saline drop every 2-3 sec (32 ^{o}C)
- Acclimatization ca 45 min

Baseline Measurements – 3 Endpoints

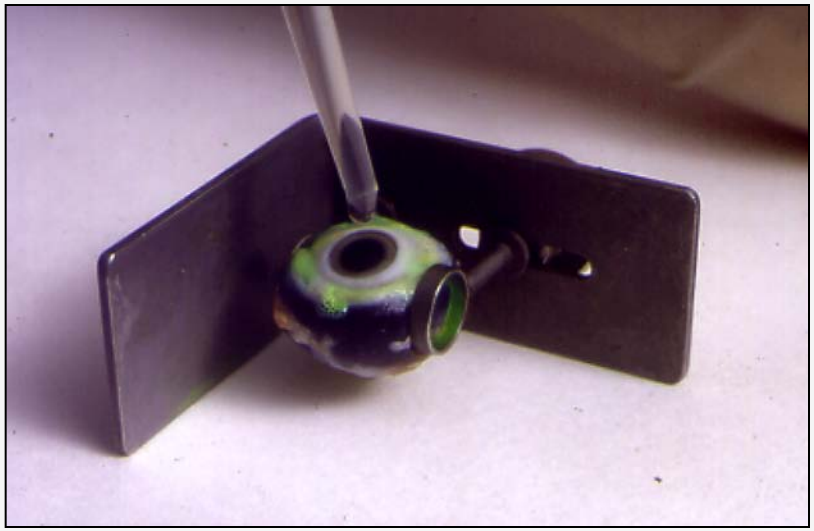




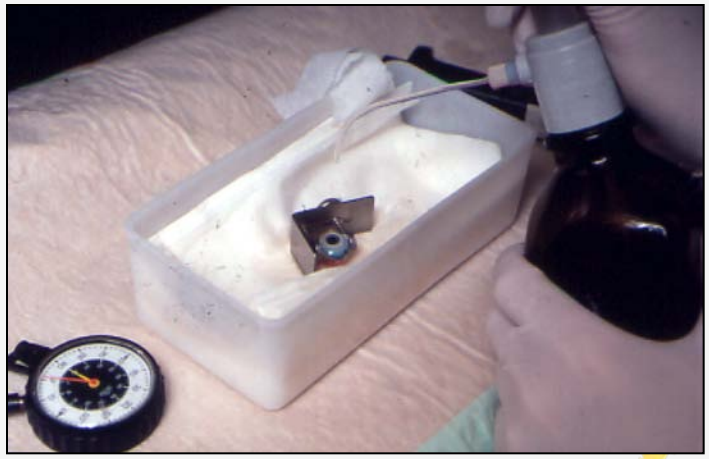


- Corneal opacity
- Corneal thickness
- Fluorescein staining

Test Substance Application



- Liquids 30 µL
- Solids 30 mg
- 10 second application
- Saline rinse (20 mL)
- Endpoints evaluated at 30, 75, 120, 180, and 240 min



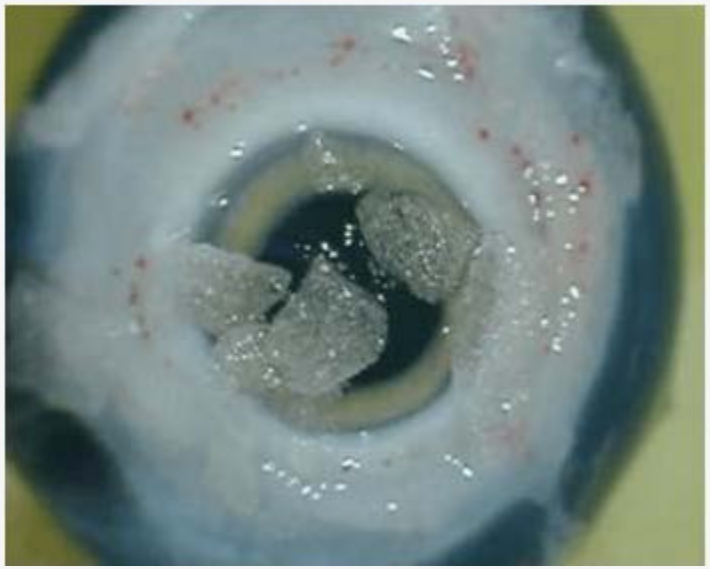
Test Substance Application

Application of Liquid Test Substance

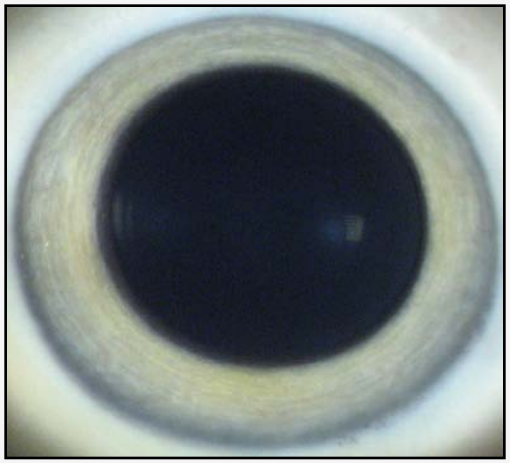
(return to webpage to view video)

Application of Solid Test Substance

(return to webpage to view video)

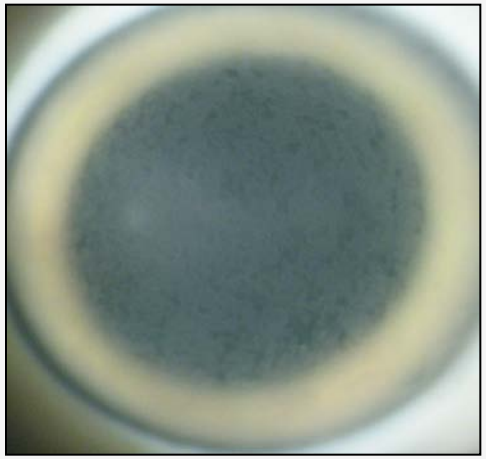


Corneal Opacity



0

1



2

- Opacity evaluated via slit-lamp microscope

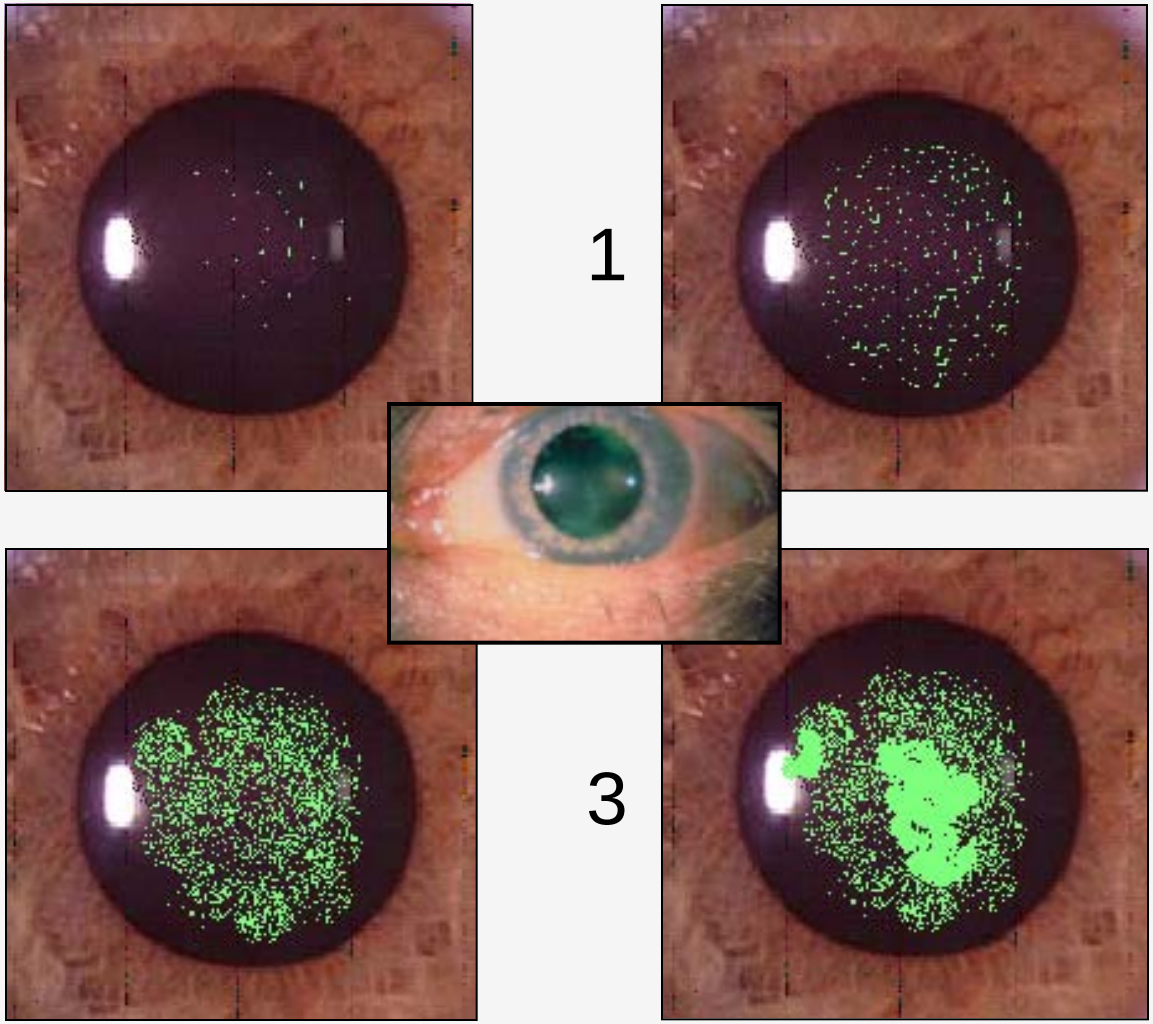
3-4

Corneal Opacity Score

- Opacity score (0-4) assigned to each eye at each time point (30, 75, 120, 180, and 240 min)
- Mean score for 3 eyes calculated for each time point
- Maximum mean score at any time point used for categorization

Mean Max Score	Category
0.0 - 0.5	I
0.6 - 1.5	II
1.6 - 2.5	III
2.6 - 4.0	IV

Fluorescein Staining

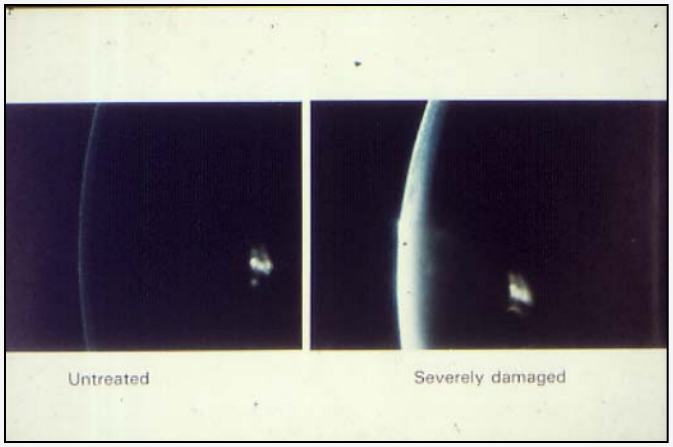


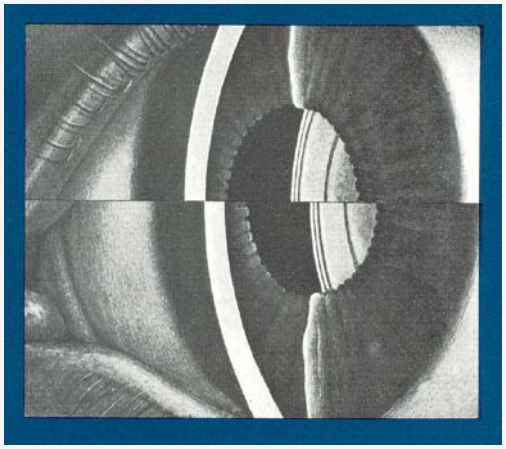
Fluorescein Staining Score

- Score of 0-3 assigned for each eye at 30 min
- Mean score used for categorization

Mean Score	Category
0.0 - 0.5	I
0.6 - 1.5	II
1.6 - 2.5	III
2.6 - 3.0	IV

Corneal Swelling





- Measure corneal thickness by slit-lamp microscope examination
- Calculate corneal swelling (%) based on change in thickness from baseline

Corneal Swelling Score

- Corneal swelling calculated (%) for each eye at each time point (30, 75, 120, 180, and 240 min)
- Mean % for 3 eyes calculated for each time point
- Maximum mean score at any time point used for categorization

Mean Max Score	Category
0% to 5%	I
>5% to 12%	II
>12% to 18% (>75 min after treatment)	II
>12% to 18% (≤75 min after treatment	III
>18% to 26%	III
>26% to 32% (>75 min after treatment)	III
>26% to 32% (≤75 min after treatment	IV
>32%	IV

ICE Test Method Decision Criteria

Combinations of ICE Categories	Ocular Hazard Classification
3 x IV	EPA Category I GHS Category 1
2 x IV, 1 x III	
2 x IV, 1 x II	
2 x IV, 1 x I	
Corneal opacity score ≥ 3 at 30 min	
Corneal opacity score = 4 at any time point	
Severe loosening of the epithelium	

Advantages of Using ICE

- Although chickens are required as a source of eyes, only chickens humanely killed for food or other non-laboratory purposes are used as eye donors (i.e., no live animals are used in this assay)
- Minimal additional training of technical staff relative to the rabbit eye test; could likely be mastered in a short period of time
- Uses the same qualitative assessment of corneal opacity as the rabbit eye test – but greater contrast in chicken eye
- Requires less time to complete than the rabbit eye test (one day vs. up to 21 days)

NICEATM-ICCVAM Evaluation of ICE

- Reviewed available data and information regarding the usefulness and limitations for assessing the ocular hazard potential of chemicals and products
- Determined validation status
  - Accuracy: sensitivity and specificity
  - Reproducibility for identifying ocular corrosives/severe irritants vs. all other hazard categories
  - Scope of substances tested
  - Availability of a standardized test method protocol
- Independent international scientific peer review panel

Validation Database

- 174 substances representing a wide range of chemical classes and product categories.
- Sufficient *in vivo* data to assign an ocular irritancy classification according to the EPA and GHS classification systems
  - EPA : 145 substances
  - GHS: 144 substances
- Reproducibility data from an interlaboratory validation study (4 laboratories, 59 substances)

Chemical Classes Tested in ICE

Chemical Class	# of Substances	Chemical Class	# of Substances
Acetate	1	Inorganic Chloride Cmpd	1
Acid	5	Inorganic Salt	3
Acyl halide	1	Inorganic Silver/Nitrogen Cmpd	1
Alcohol	15	Ketone	4
Aldehyde	2	Lactone	1
Alkali	3	Lipid	1
Amide/Amidine	7	Nitrile	1
Amino Acid	1	Nitro Cmpd	1
Boron Cmpd	1	Not Classified	85
Carbohydrate	2	Onium Cmpd	8
Carboxylic Acid	12	Organic Silicon Cmpd	2
Ester	10	Organic Sulfur Cmpd	3
Ether	1	Organometallic	2
Heterocyclic	9	Organophosphorus Cmpd	1
Hydrocarbon	5	Polycyclic	4
Imide	2	Polyether	3
Inorganic Chemical	1	Urea Cmpd	1

Product Categories Tested in ICE

Product Category	# of Substances	Product Category	# of Substances
Adhesive	2	Fertilizer	1
Antifungal	2	Food Additive	1
Antihistamine	1	Fungicide/Germicide	1
Anti-infective	3	Industrial Chemical, Intermediate or Formulation	20
Antiseptic	2	Not Classified	23
Caustic Agent	4	Optical Resolution Agent	1
Chlorination by-product	1	Paint	4
Cleaner	8	Pesticide/Herbicide	15
Copolymer	3	Preservative	6
Cosmetic Ingredient	1	Pharmaceutical Cmpd	5
Detergent	8	Raw Meterial	9
Developer	1	Reagent	4
Disinfectant	5	Resin	2
Dyes & Stains	10	Silicon Resin	1
Elastomer	2	Soap	9
Enzyme Inhibitor	1	Surfactant	25
Enzyme Solution	3	Solvent	37

ICE Test Method Accuracy: Ocular Corrosives and Severe Irritants

Class. System	Accuracy	Sensitivity	Specificity	False Positive Rate	False Negative Rate
	%	%	%	%	%
EPA	85% (123/145)	53% (16/30)	93% (107/115)	7% (8/115)	47% (14/30)
GHS	83% (120/144)	50% (15/30)	92% (105/114)	8% (9/114)	50% (15/30)

EPA = Cat I vs. Cat II/III/IV, GHS = Cat 1 vs. Cat 2A/2B/NC

ICE Interlaboratory Reproducibility

- Extent of agreement between testing laboratories when identifying ocular corrosives and severe irritants

% Agreement Among 4 Labs*	EPA	GHS
100% (all substances)	75% (44/59)	75% (44/59)
≥75% (all substances)	90% (53/59)	90% (53/59)
100% (only corrosive/severe irritant)	75% (15/20)	72% (16/22)
≥75% (only corrosive/severe irritant)	100% (20/20)	95% (21/22)

*From Balls et al. (1995). Scores for fluorescein retention and corneal swelling were not provided for one severe irritant/corrosive (30% trichloroacetic acid), which was therefore classified based on results from only 3 laboratories

ICCVAM Test Method Recommendations for ICE – Usefulness and Limitations

Usefulness

- Can be used for identification of ocular corrosives and severe irritants (EPA Category I, GHS Category 1) in appropriate circumstances and with certain limitations

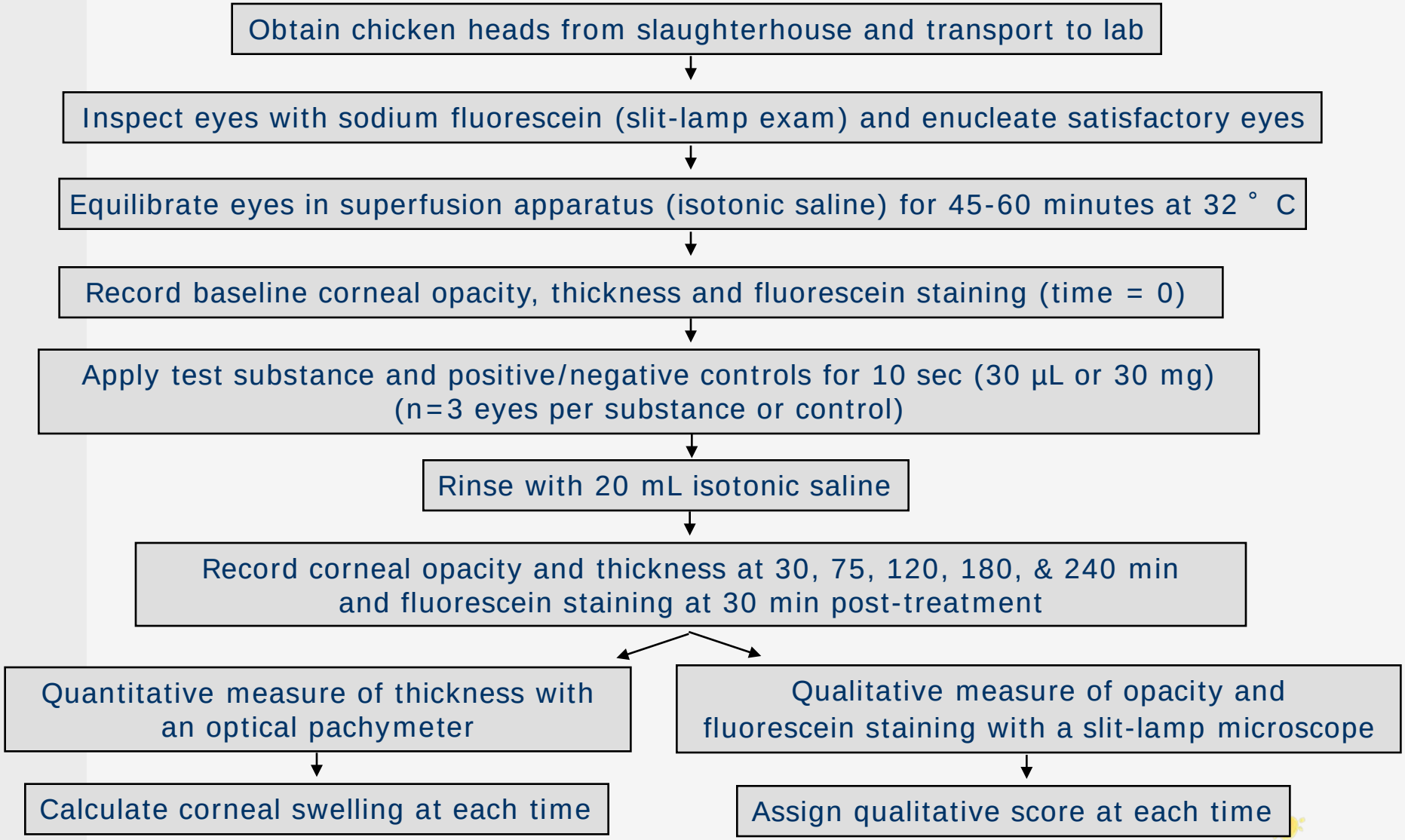
Limitations

- Alcohols: 50% (5/10) false positives
- Surfactants: 57% (4/7) false negatives
- Solid: 70% (7/10) false negatives

ICCVAM Test Method Recommendations: Future Studies

- Additional optimization studies/evaluations to improve the correct classification of mild and moderate ocular irritants and substances not labeled as irritants.
- Further evaluate the usefulness and limitations of using histopathological evaluation of the corneal tissue when ICE is conducted

ICCVAM-Recommended ICE Protocol[1]



[1]Available at http://iccvam.niehs.nih.gov/docs/ocutox_docs/OTeval/appE.pdf

ICE International Acceptance

- OECD TG 438 Isolated Chicken Eye Test Method for Identifying Ocular Corrosives and Severe Irritants
  - Adopted September 7, 2009
  - Available at http://oberon.sourceoecd.org/vl=90797895/cl=28/nw=1/rpsv/ij/oecdjournals/1607310x/v1n4/s55/p1
  - Based on ICCVAM-recommended ICE protocol
  - Expected to result in broader use of ICE, which will further reduce and refine animal use for ocular safety assessments while ensuring human safety

Additional ICE Evaluations by ICCVAM

- In 2010, ICCVAM evaluated ICE for identifying nonsevere irritants and substances not labeled as irritants
- However, ICCVAM concluded that ICE is **not** recommended as a screening test to distinguish substances not labeled as irritants (EPA Category IV; GHS Category Not Classified) from all other hazard categories (EPA Category I, II, III; GHS Category 1, 2A, 2B)
- ICCVAM also concluded that ICE is **not** recommended to identify moderate and mild ocular irritants as defined by the EPA and GHS classification systems

Acknowledgements

- ICCVAM and NICEATM gratefully acknowledge Menk Prinsen, TNO - Netherlands Organisation for Applied Scientific Research, for submitting data to NICEATM for the ICE evaluation

Additional Acknowledgements

- ICCVAM
- ICCVAM Interagency Ocular Toxicity Working Group
- ICCVAM Independent Scientific Peer Review Panel
- NICEATM Staff