



Isolated Chicken Eye (ICE) Test Method

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

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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

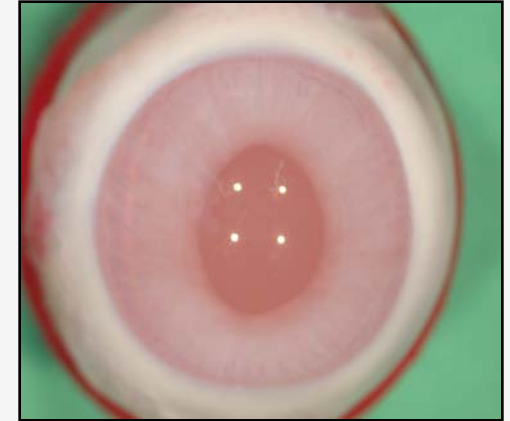
■ Bovine

- Very large eyes
- Thick corneas



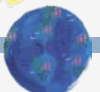
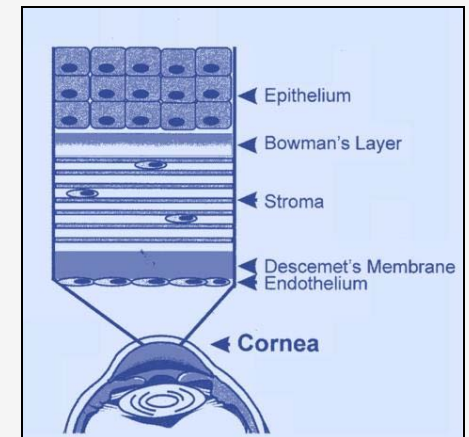
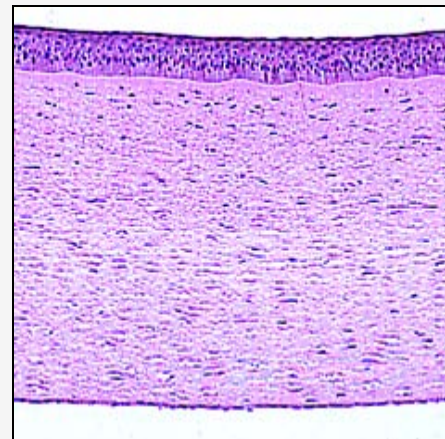
■ Porcine

- 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

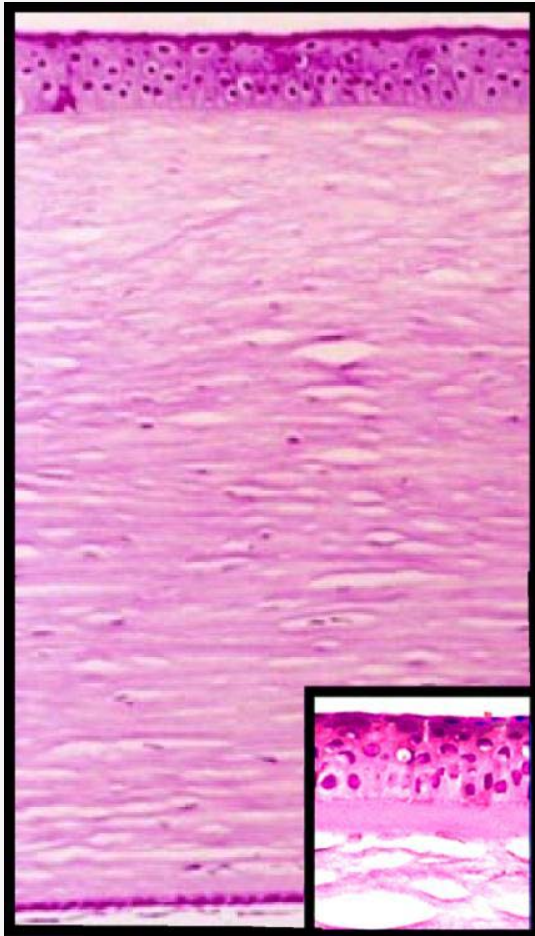
*WH Spencer. "Cornea." In WH Spencer. *Ophthalmic Pathology*. Philadelphia: W.B. Saunders Company; 1996.

T Nishida. "Cornea." In JH Krachmer, MJ Mannis, EJ Holland [eds]. *Cornea*. Vol. I. St. Louis: Mosby-Year Book, Inc.; 1997.

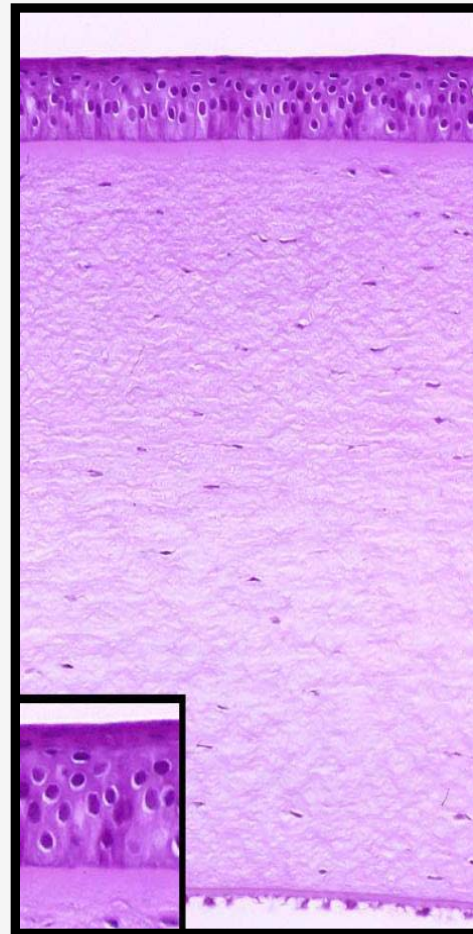
Courtesy of Craig Fowler, M.D., UNC-Chapel Hill

Comparative Corneal Anatomy

Human



Chicken



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

Bowman's Layer



■ Light Microscopy

- Transparent
- Structureless

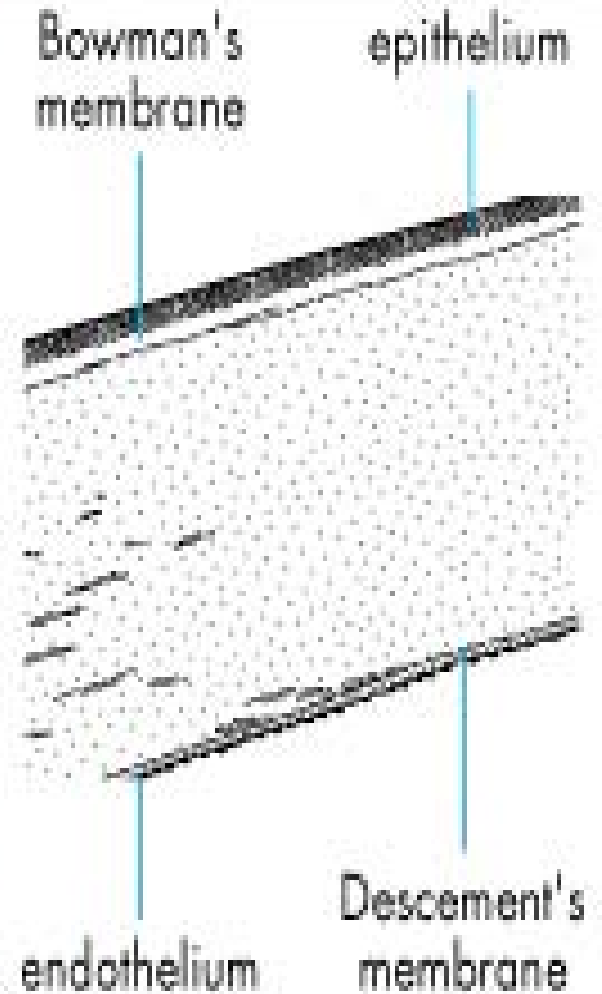
■ Electron Microscopy

- 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

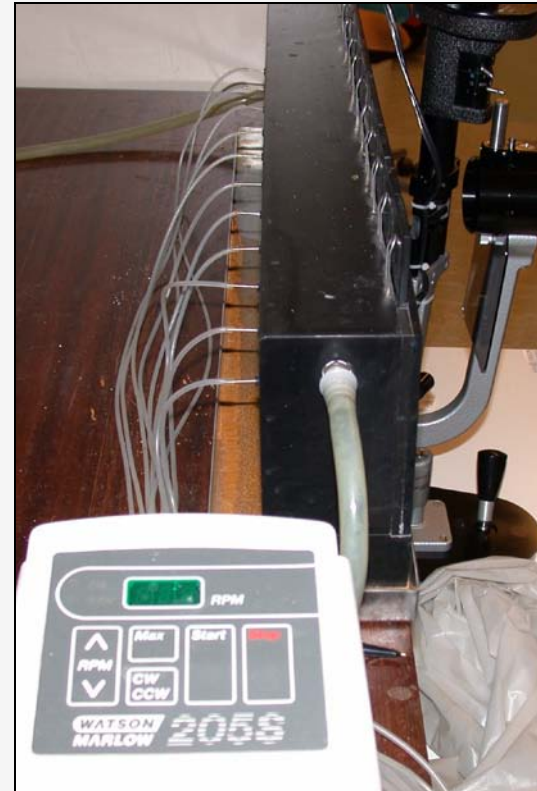


ICE Superfusion Apparatus



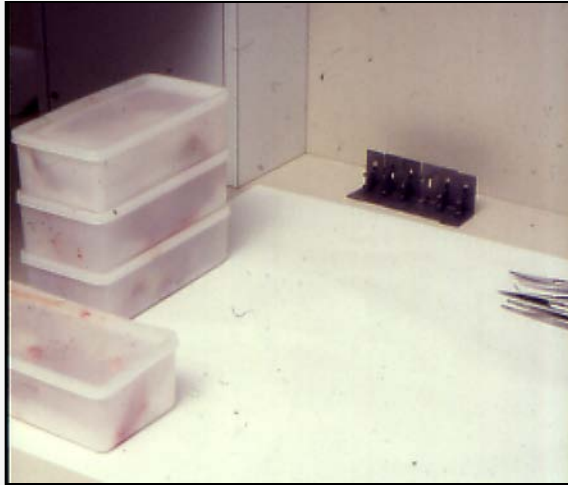
- 11 chambers with sliding doors
- Water mantle
- Saline inlet and outlet

Maintaining an Ambient Environment



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

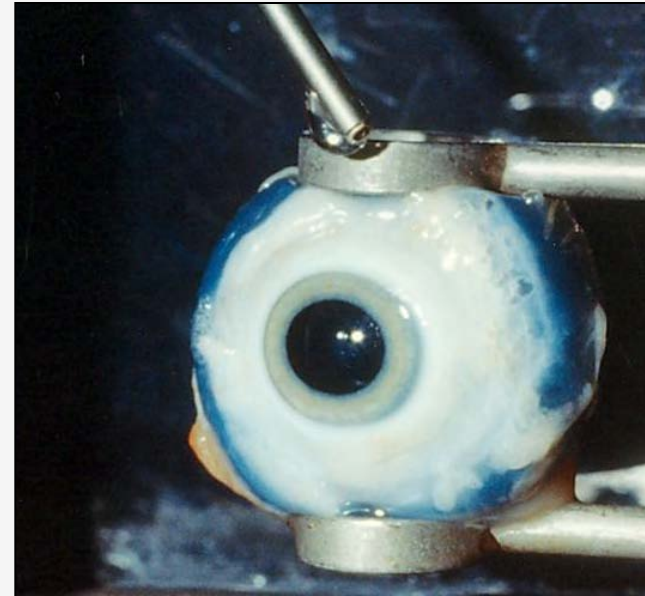
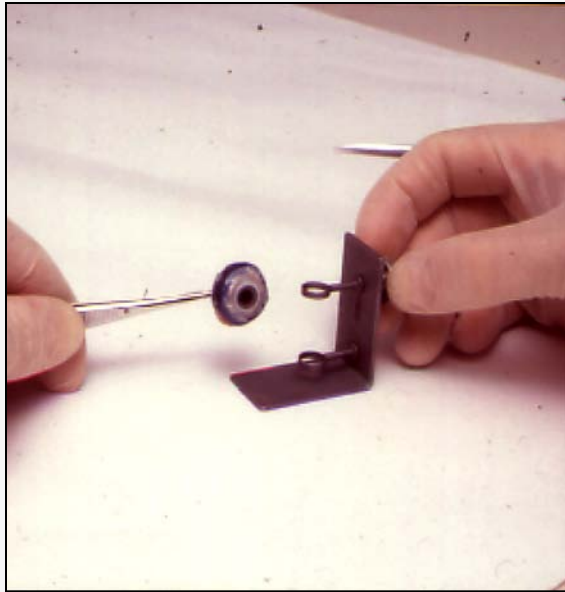
Collection of Eyes



- Transport boxes for heads
- Moistened tissue
- Ambient temperature

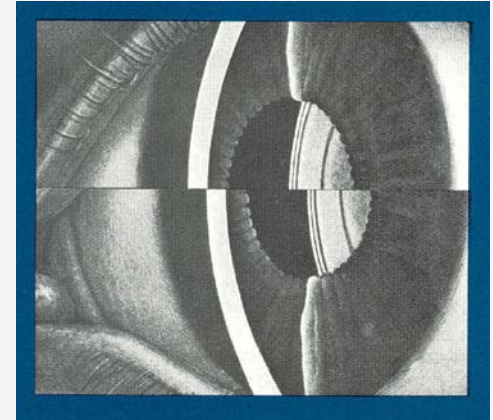
- Fluorescein - one drop and immediate rinsing
- Detection of damaged epithelium

Mounting Eyes for Testing

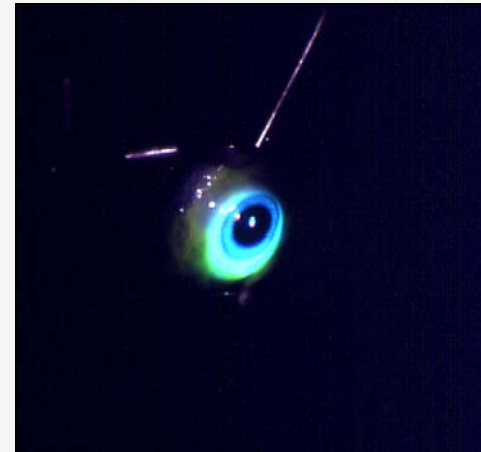


- Enucleation within 10 sec
- Insert eye into holder
- Saline drop every 2-3 sec (32 °C)
- Acclimatization ca 45 min

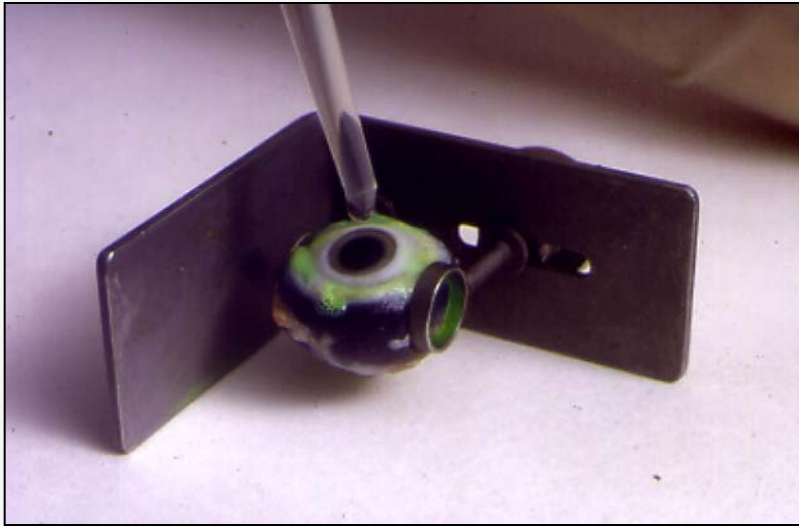
Baseline Measurements – 3 Endpoints



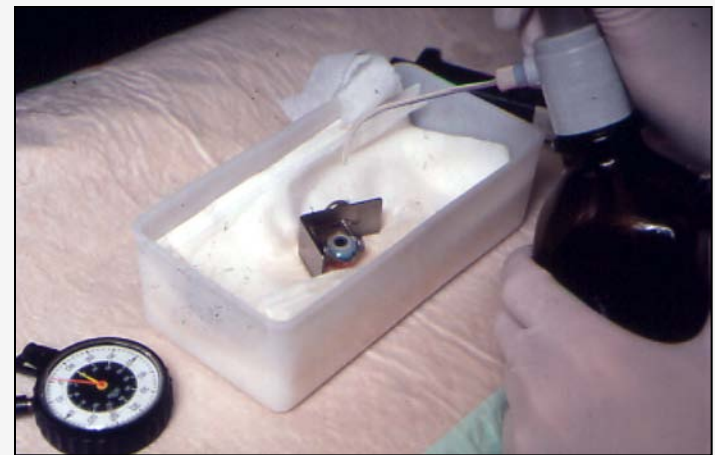
- Corneal opacity
- Corneal thickness
- Fluorescein staining



Test Substance Application



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



Test Substance Application

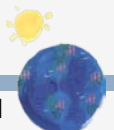
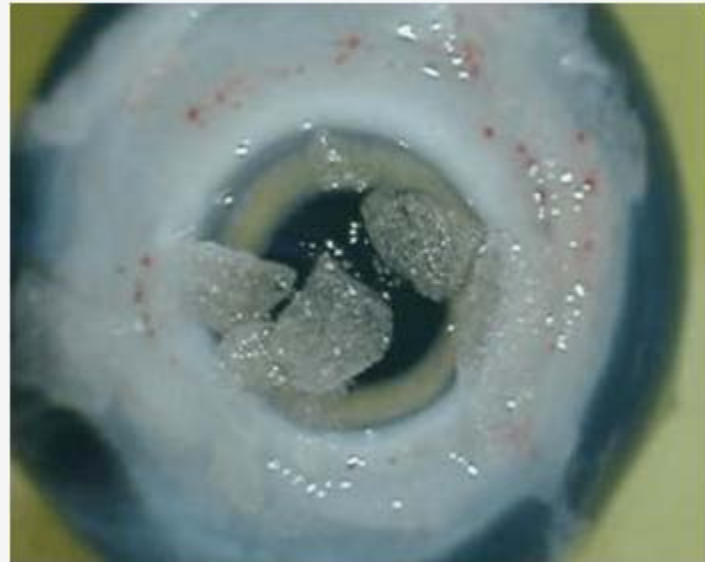
Application of Liquid Test Substance

(return to webpage to view video)

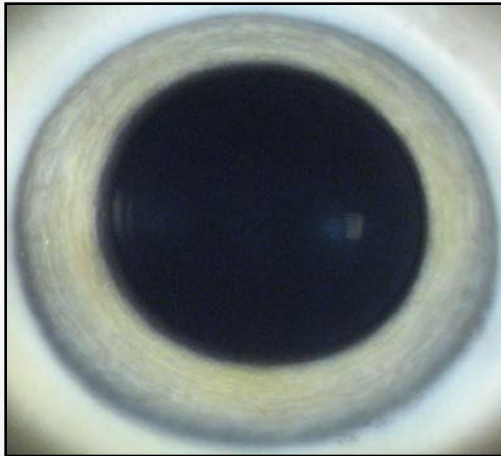


Application of Solid Test Substance

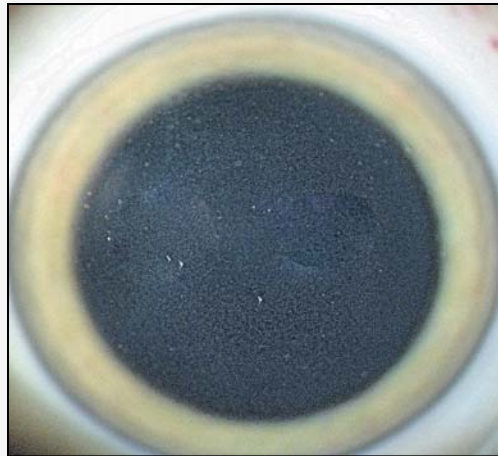
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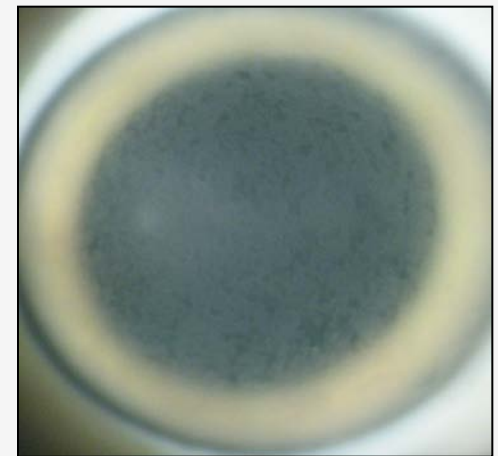
Corneal Opacity



0



1



2

- Opacity evaluated via slit-lamp microscope



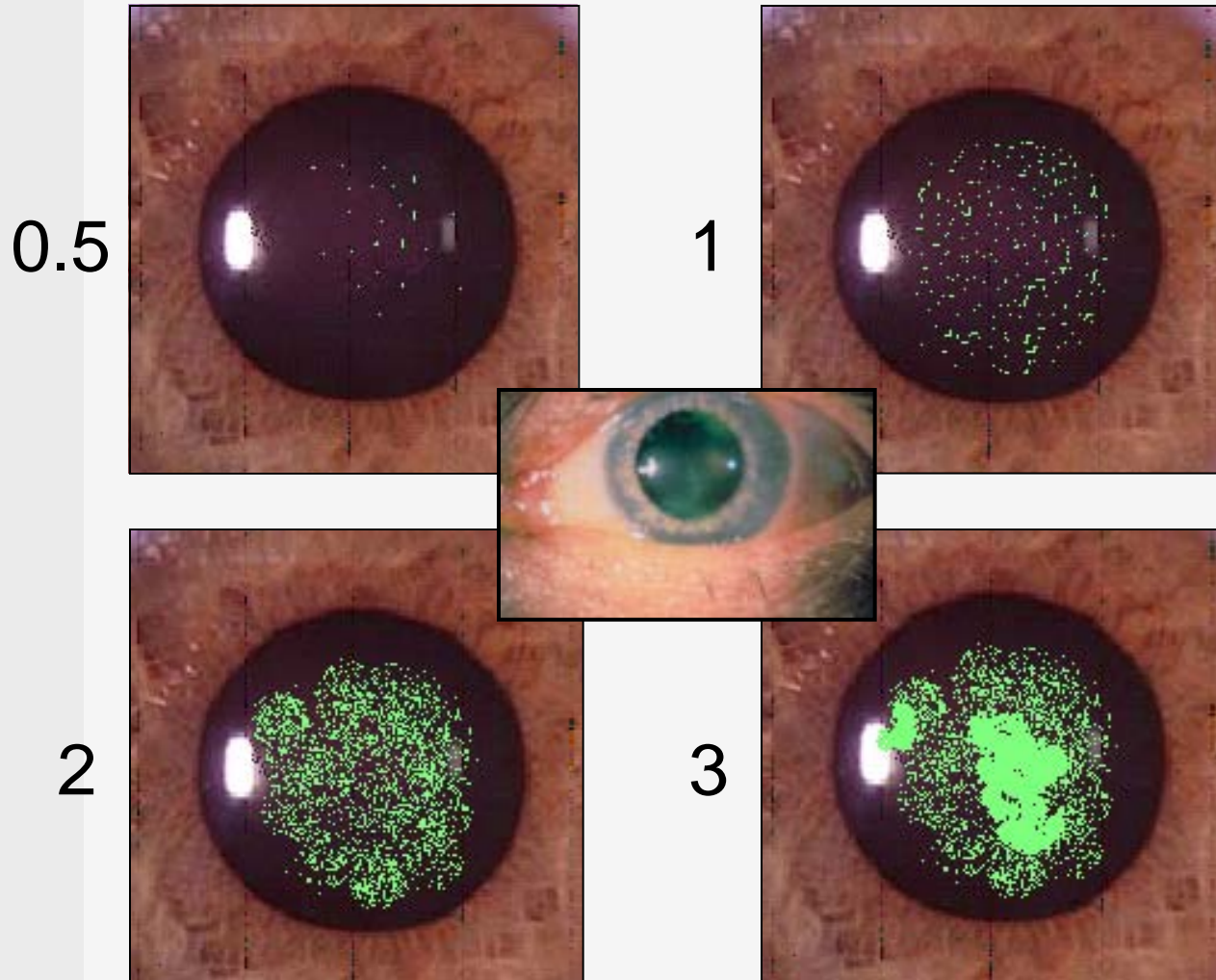
3-4

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

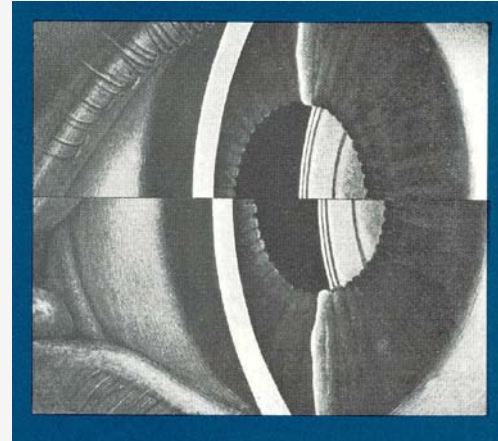
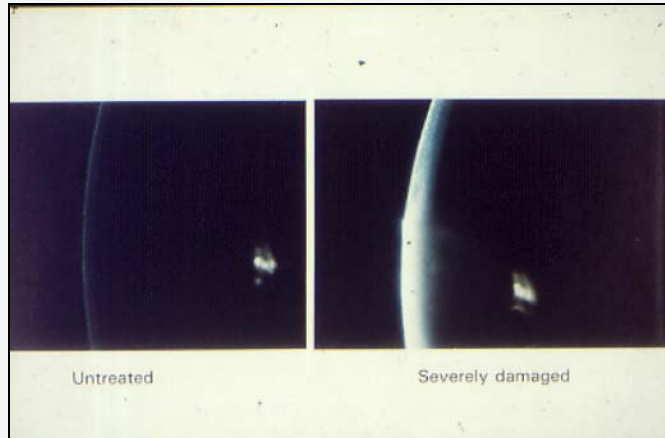


Fluorescein Staining Score

- 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

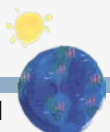
- Corneal swelling calculated (%) for each eye at each time point (30, 75, 120, 180, and 240 min)
- Mean % for 3 eyes calculated for each time point
- Maximum mean score at any time point used for categorization

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	<p data-bbox="1120 756 1584 985">EPA Category I GHS Category 1</p>
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)

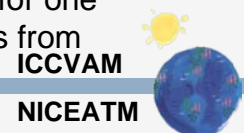
EPA = Cat I vs. Cat II/III/IV, GHS = Cat 1 vs. Cat 2A/2B/NC

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 Protocol¹

Obtain chicken heads from slaughterhouse and transport to lab

Inspect eyes with sodium fluorescein (slit-lamp exam) and enucleate satisfactory eyes

Equilibrate eyes in superfusion apparatus (isotonic saline) for 45-60 minutes at 32 ° C

Record baseline corneal opacity, thickness and fluorescein staining (time = 0)

Apply test substance and positive/negative controls for 10 sec (30 µL or 30 mg)
(n=3 eyes per substance or control)

Rinse with 20 mL isotonic saline

Record corneal opacity and thickness at 30, 75, 120, 180, & 240 min
and fluorescein staining at 30 min post-treatment

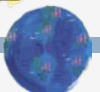
Quantitative measure of thickness with
an optical pachymeter

Calculate corneal swelling at each time

Qualitative measure of opacity and
fluorescein staining with a slit-lamp microscope

Assign qualitative score at each time

¹Available 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

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Additional Acknowledgements

- ICCVAM
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- ICCVAM Independent Scientific Peer Review Panel
- NICEATM Staff