

Project to improve the Bovine Corneal Opacity and Permeability (BCOP) assay: Development a new opacitometer prototype

Humberto de Mello Brandão

Brazilian Agricultural Research Corporation
Embrapa Dairy Cattle, Nanotechnology Lab.

humberto.brandao@embrapa.br

+55(32) 33117460

2nd Pan-American Conference for Alternative Methods

The aim of our Laboratory

Innovation in veterinary drug development



24
NANOTECNOLOGIA
Novidade! Será usada para controlar a mastite, evitando o desperdício de medicamentos

NOVEMBRO 2013 | GLOBO RURAL 45



Pharmaceutical formulation and Pharmacokinetics/pharmacodynamics



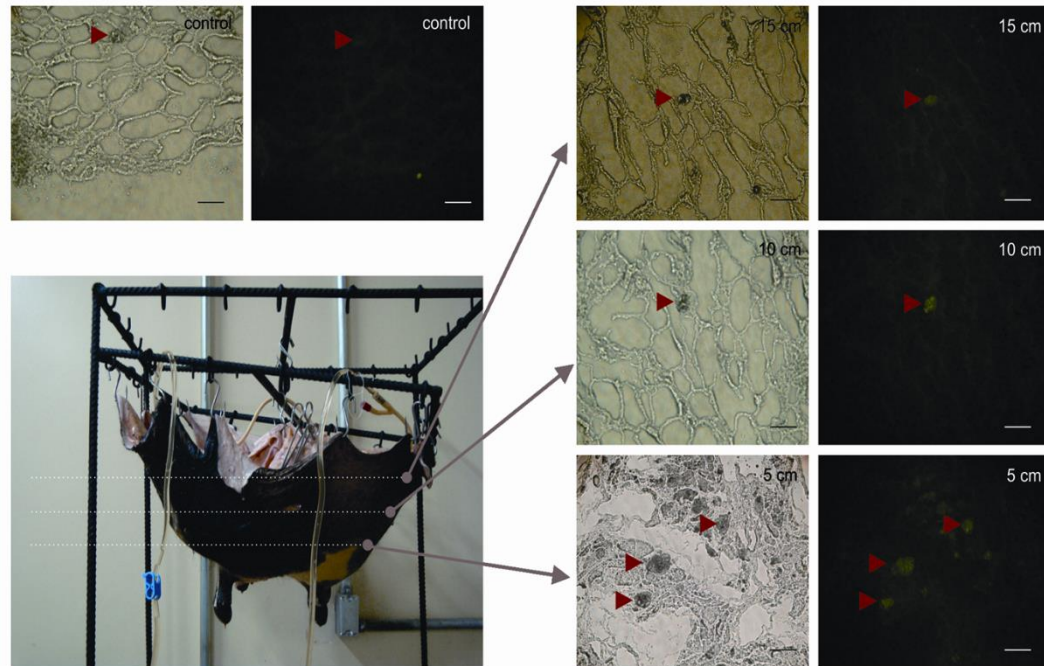
Preclinical Assays

Evaluation of perfused bovine udder for gene expression studies in dairy cows

I.S.B. Pinto¹, I. Fonseca², H.M. Brandão³, J.C. Gern³, A.S. Guimarães³,
W.A. Carvalho³, M.A.V.P. Brito³, L.F. Vicini¹ and M.F. Martins³

Genetics and Molecular Research 16 (1): gmr16019637

Ex vivo



In vitro



Nanomedicine: Nanotechnology, Biology, and Medicine
12 (2016) 1151–1159

nanomedicine
Nanotechnology, Biology, and Medicine

nanomedjournal.com

Biocompatibility assessment of fibrous nanomaterials in mammalian embryos

Michele Munk, PhD^{a,*}, Luiz S.A. Camargo, PhD^b, Carolina C.R. Quintão, MSc^b,
Saulo R. Silva, MSc^b, Eliza D. Souza, MSc^b, Nádia R.B. Raposo, PhD^a,
Jose M. Marconcini, PhD^c, Ado Jorio, PhD^d,
Luiz O. Ladeira, PhD^d, Humberto M. Brandão, PhD^b



RESEARCH ARTICLE

Encapsulated *Brucella ovis* Lacking a Putative ATP-Binding Cassette Transporter ($\Delta abcBA$) Protects against Wild Type *Brucella ovis* in Rams

Ana Patrícia C. Silva¹, Auricélio A. Macêdo¹, Luciana F. Costa², Cláudia E. Rocha², Luíze N. N. Garcia¹, Jade R. D. Farias¹, Priscilla P. R. Gomes¹, Gustavo C. Teixeira¹, Kessler W. J. Fonseca¹, Andréa R. F. Maia¹, Gabriela G. Neves¹, Everton L. Romão¹, Teane M. A. Silva¹, Juliana P. S. Moí¹, Renata M. Oliveira³, Márcio S. S. Araújo⁴, Ernane F. Nascimento¹, Olindo A. Martins-Filho⁴, Humberto M. Brandão⁵, Tatiane A. Paixão², Renato L. Santos^{1*}

Vaccine clinical trial



Improvement of the BCOP test: the challenge

ATLA 39, 37–53, 2011

The Bovine Corneal Opacity and Permeability Test in Routine Ocular Irritation Testing and Its Improvement Within the Limits of OECD Test Guideline 437

published BCOP and *in vivo* data. Our results matched the published *in vitro* data very well, but with some intentionally selected **false negatives (FNs) and false positives (FPs), the concordance was 77% (24/31)**, with FN and FP rates of 20% (2/10) and 24% (5/21), respectively. In addition, we tested 21 in-house materials,

Toxicology in Vitro 44 (2017) 122–133



ELSEVIER

Contents lists available at [ScienceDirect](#)

Toxicology in Vitro

journal homepage: www.elsevier.com/locate/toxinvit



CON4EI: Bovine Corneal Opacity and Permeability (BCOP) test for hazard identification and labelling of eye irritating chemicals



Sandra Verstraelen^{a,*}, Gareth Maglennon^b, Karen Hollanders^a, Francis Boonen^a, Els Adriaens^c,

The results show that the accuracy of the **BCOP OP-KIT in identifying Cat 1 chemicals was 73.8% while the accuracy was 86.3% for No Cat chemicals**. BCOP OP-KIT false negative results were often related to an *in vivo*

Improvement of the BCOP test: the challenge

Limitations in corneal shelf-life



Project to improve BCOP assay: objectives

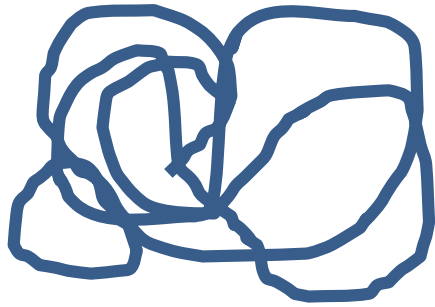
1°) Development the prototype:

- Proof of concept of the new sensors response
- **Optimize the instrumentation for BCOP use**
- Expand the number of evaluating substances
- Make an Interlaboratory Comparison Test

2°) Improve the corneal shelf-life:

- 3th Pan-American Conference for Alternative Methods → Canada

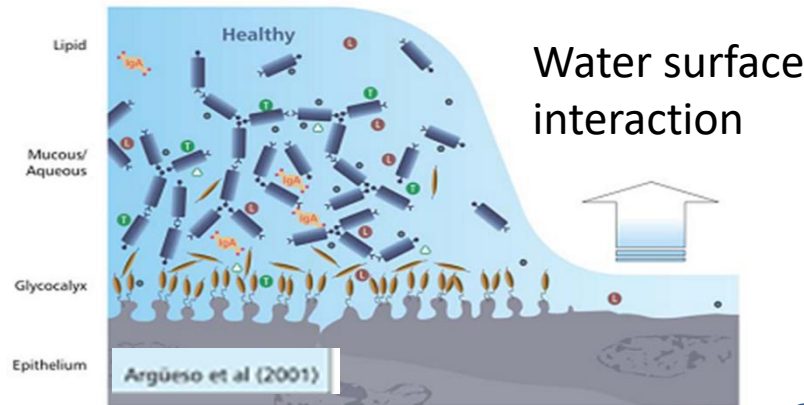
The BCOP assay basis



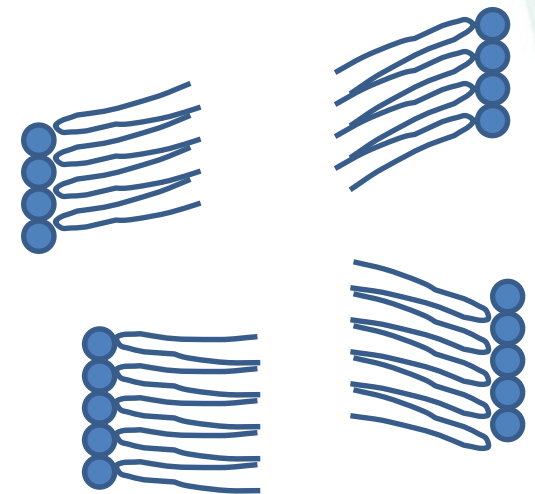
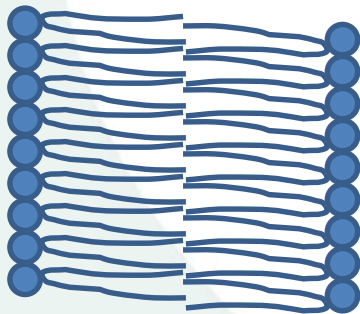
Macromolecules



- Denaturation
- PPT
- ↑ absorbance



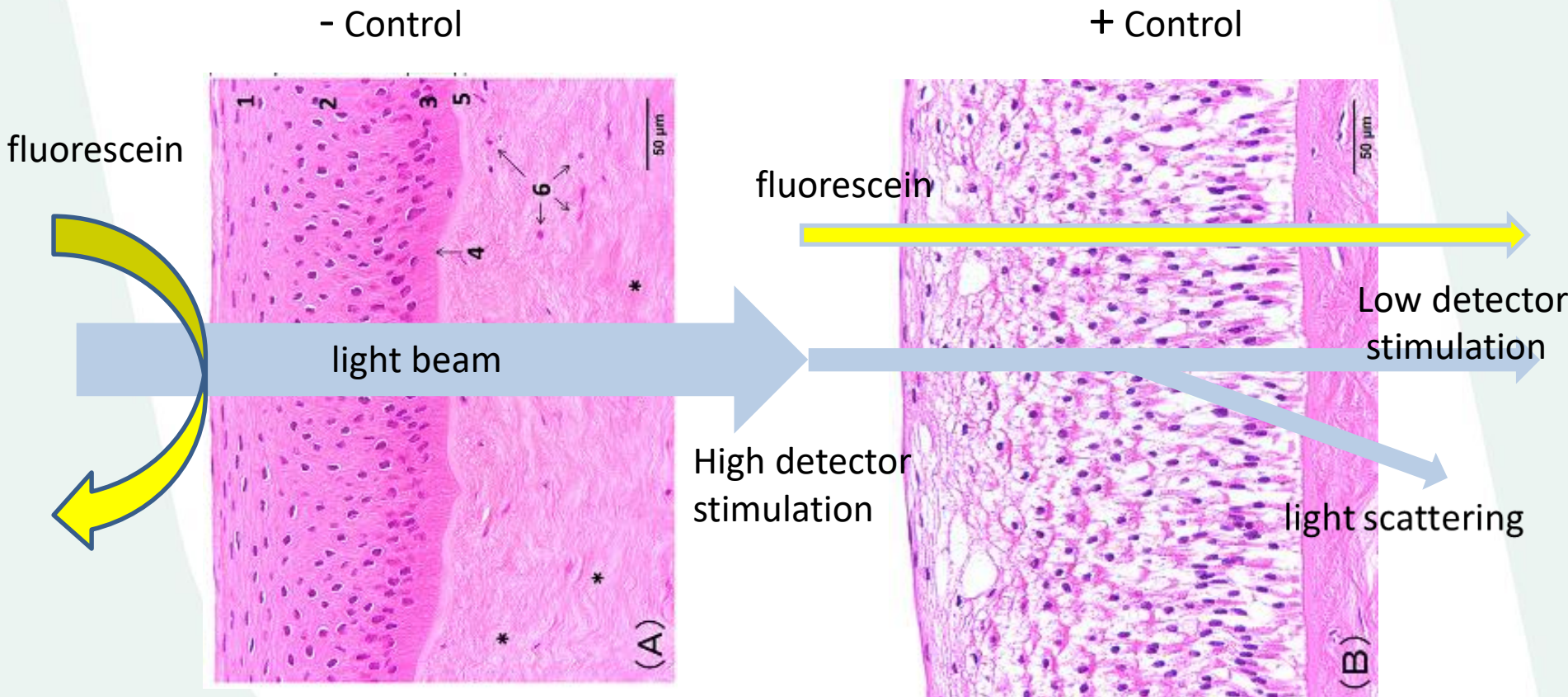
Bilayer membrane damage



Histopathological evaluation of the ocular-irritation potential of shampoos, make-up removers and cleansing foams in the bovine corneal opacity and permeability assay

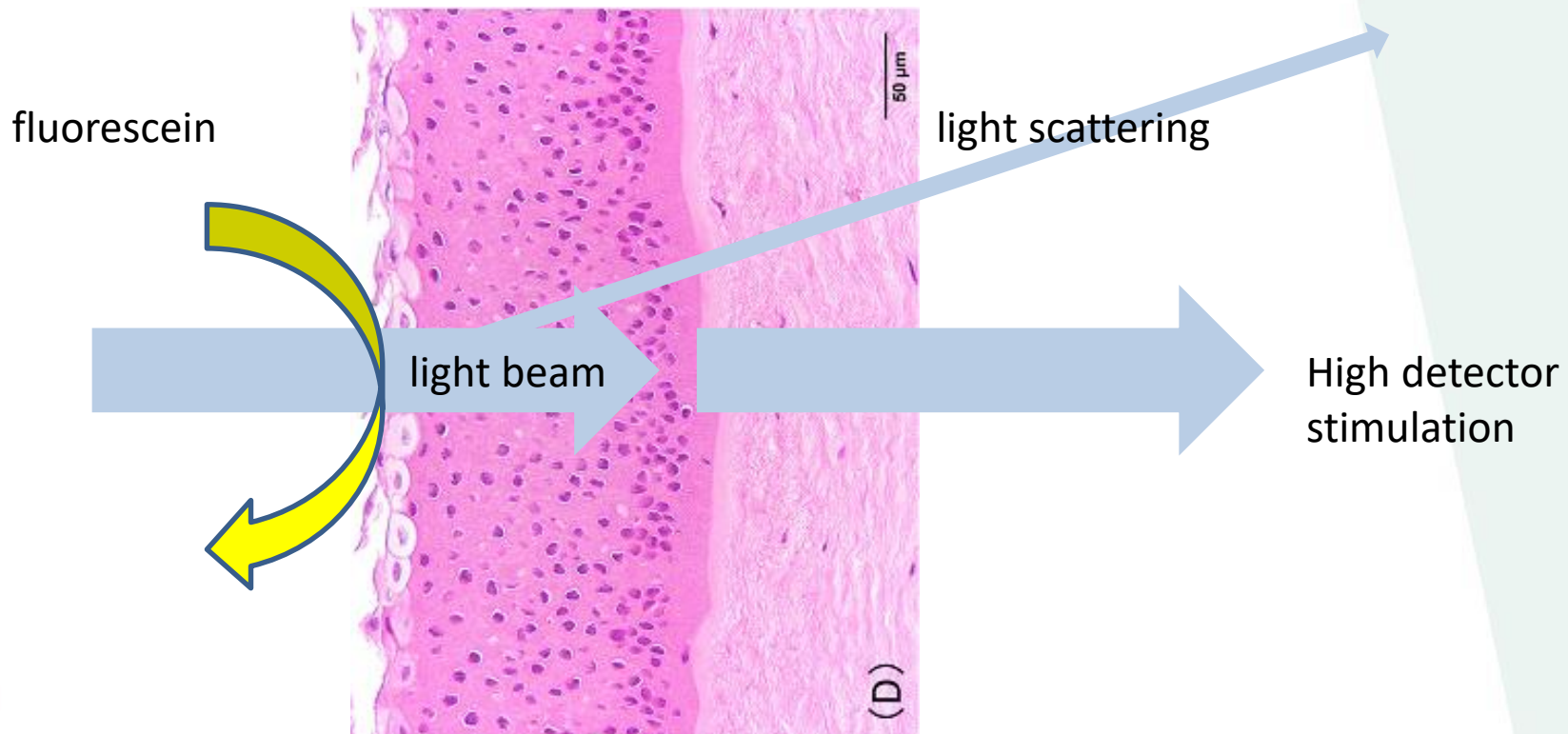
J Toxicol Pathol 2015; 28: 243–248

Masatoshi Furukawa¹, Takashi Sakakibara¹, Kouta Itoh¹, Kohtaro Kawamura¹, Satoshi Sasaki^{1*}, and Masao Matsuura¹



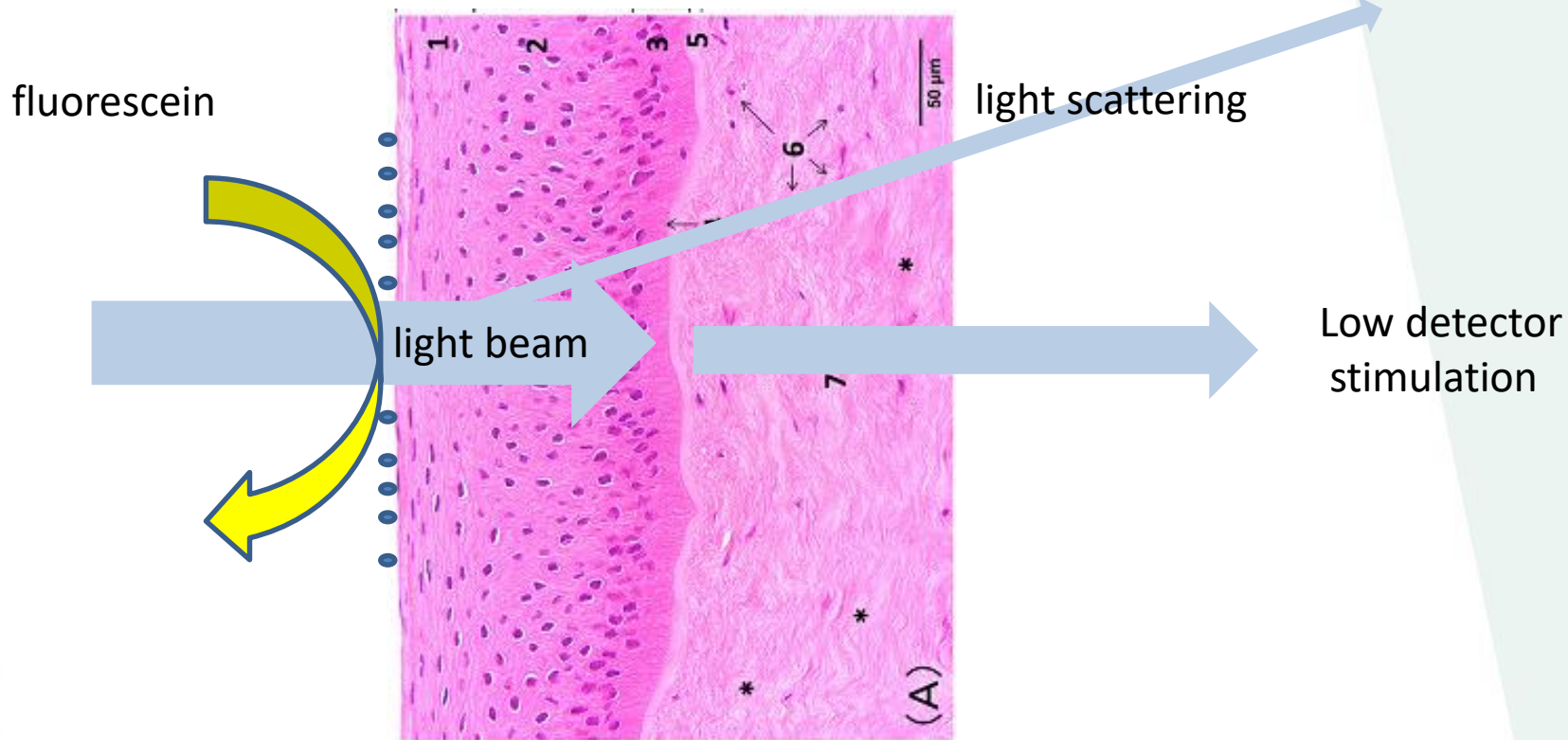
Only histological image from Furukawa et al., 2015

False Negative



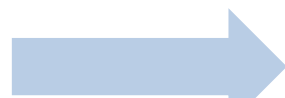
Soft bilayer damage with poor or no
macromolecules denaturation

False Positive, mucoadhesive substances



Commercial Opacitometer

Light Source



Holder



Optical diffuser glass
with Lambertian surface



Optical Sensor



Prototype

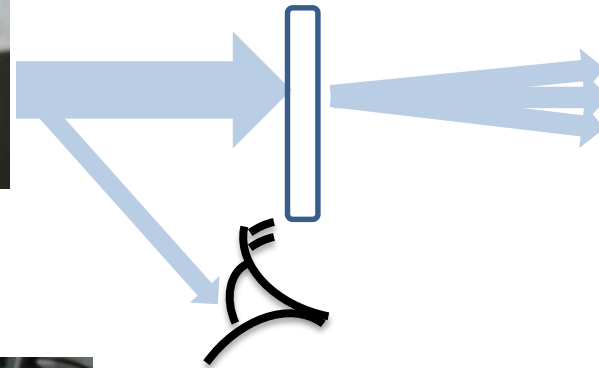
Light Source



Holder



Optical diffuser glass
with Lambertian surface



New Sensor



Optical Sensor

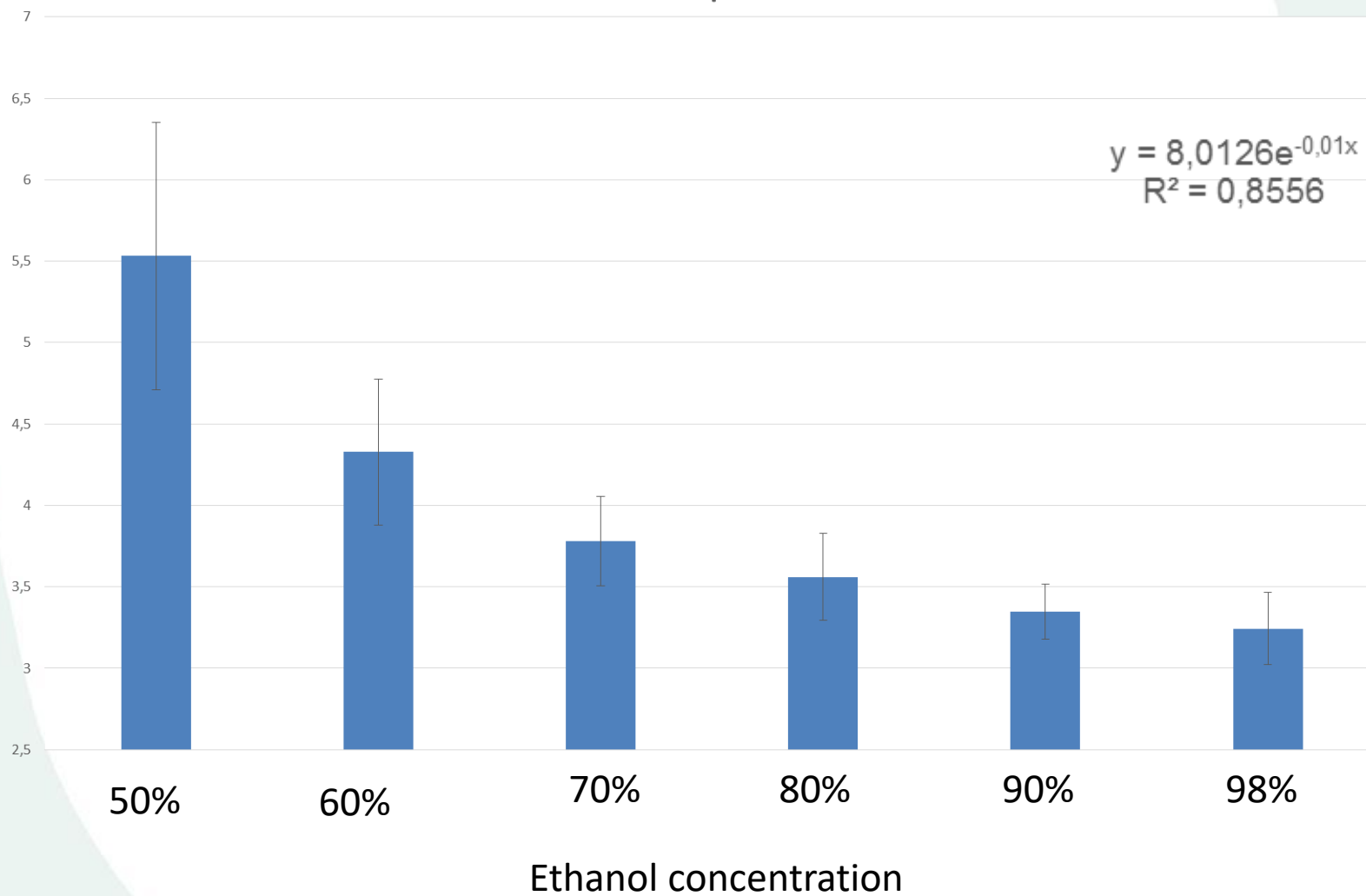
Optical Sensor



- We don't change optical path
- An algorithm correct the light scattering distortions (corneal manipulation)

Proof of concept

Sensor Response



Proof of concept

SAMPLE	Light	Light
	IVIS OP-KIT	IVIS PROTOTYPE
CT -	1.5 ± 1.1	1.2 ± 2.2
CT +	53.7 ± 2.6	89.4 ± 8.5
SDS	41.1 ± 2.6	45.9 ± 6.6
Ethyl 2-methylacetoacetate	11.2 ± 1.9	22.6 ± 3.8
Trichloroacetic acid	25.83 ± 1.4	51.6 ± 2.41

CT- NaCl 0,9%; CT+ Ethanol 98% ; Globally Harmonized System → E-2MACT (Irritant, 2B)

ATLA 39, 37-53, 2011

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In vivo
TG 437
BRD06
C&L

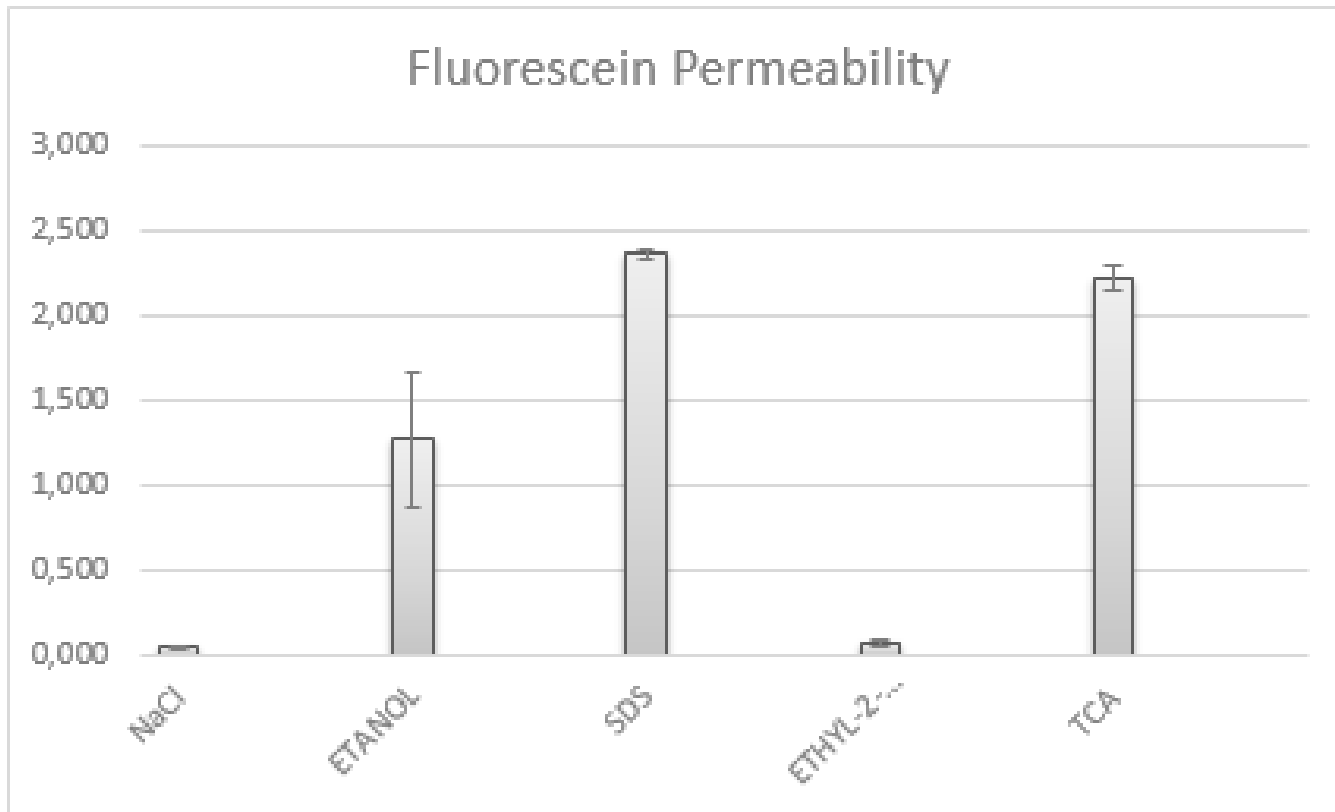
IVIS

Cons. class. C&L Correlation

2,6-Dichlorobenzoyl chloride (l)	4659-45-4	2A	mild	not 1	RN
Ethyl-2-methylacetoacetate (l)	609-14-3	2B	mild	not 1	RN
Ammonium nitrate (s)	6484-52-2	2A/2E	mild	not 1	RN

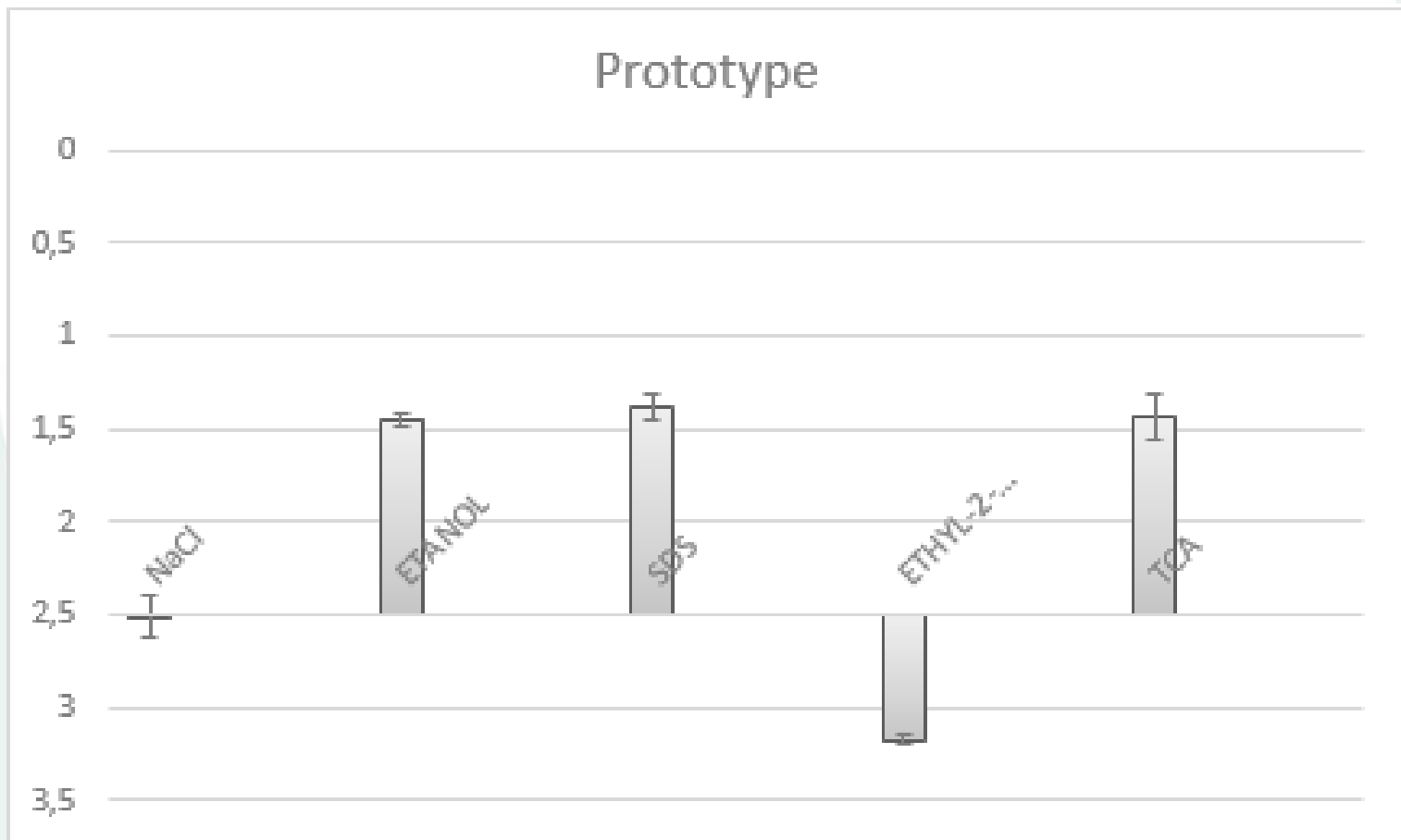
OECD/OCDE

IVIS	UN GHS
≤ 3	No Category
> 3; ≤ 55	No prediction can be made
> 55	Category 1



Was similar for both equipments

New sensors



Final results

SAMPLE	Light	Light	Fluorescein Permeability	BCOP CLASSIFICATION		
	IVIS OP-KIT	IVIS PROTOTYPE		OP-KIT	PROTOTYPE	New sensors
CT -	1.5 ±1.1	1.2 ±2.2		NC	NC	-
CT +	53.7 ±2.6	89.4 ±8.5	+	Cat 1	Cat 1	Cat 1
SDS	41.1 ±2.6	45.9 ±6.6	+	Cat 1	Cat 1	Cat 1
Ethyl 2-methylacetoacetate	11.2 ±1.9	22.6 ±3.8	-	NA	NA	Cat 2
Trichloroacetic acid	25.83 ±1.4	51.6 ±2.41	+	Cat 1	Cat 1	Cat 1

Globally Harmonized System: Category 1, corrosive; Category 2, irritant

Optimize the instrumentation for BCOP evaluation

- Expand the number of evaluating substances

The image displays a software interface for BCOP evaluation, divided into two main panels: 'TESTE DE OPACIDADE' (Opacity Test) and 'TESTE DE PERMEABILIDADE' (Permeability Test).

TESTE DE OPACIDADE:

- Three channels (Canal 1, Canal 2, Canal 3) are shown, each with a semi-circular scale from 0 to 10 and a yellow needle.
- Below each channel is a small input box containing the number '0'.
- A 'Registra' button with a green checkmark icon is located below the channels.
- A 'Linha N°' input box contains the number '0'.
- Summary statistics for three elements are provided: Soma Elem 1, Soma Elem 2, Soma Elem 3, Núm de elem 1, Núm de elem 2, Núm de elem 3, Média 1, Média 2, Média 3, Desvio Pad 1, Desvio Pad 2, and Desvio Pad 3. Each has an input box containing '0'.
- A 'Salvar em...' input box with a folder icon is present.
- A 'Finaliza' button with a red square icon is at the bottom right.

TESTE DE PERMEABILIDADE:

- A single circular scale from 0 to 10 with a yellow needle is shown.
- Below the scale is a small input box containing the number '0'.
- A 'Registra' button with a green checkmark icon is located below the scale.
- A 'Linha N° 2' input box contains the number '0'.
- Summary statistics for five elements are provided: Soma Elem 4, Soma Elem 5, Núm de elem 4, Núm de elem 5, Média 4, Média 5, Desvio Pad 4, and Desvio Pad 5. Each has an input box containing '0'.
- A 'Salvar em...' input box with a folder icon is present.

Our Team

Hideraldo B De Filippo; Alexandre Balbinot (Universidade Federal do Rio Grande do Sul-PPGEE);

Luciene B L Balottin; José M Granjeiro ; Muriel M Carlo; Jaqueline F de Oliveira (INMETRO);

Michele Munk (Universidade Federal de Juiz de Fora);

Juliana Gern; Humberto M Brandão (Embrapa).

Thank you!!!!!!

humberto.brandao@embrapa.br

+55(32) 33117460 ; +55(32)991253450