Considerations for Demonstrating the Inter-Laboratory Reliability of Chorioallantoic Membrane Vascular Assay (CAMVA) and the Bovine Corneal Opacity and Permeability Assay (BCOP)

G. Mun[1]; N. Wilt[1]; D. A. Donahue[2]; J. Avalos[2]; K. Norman[1]; A. Hilberer[1]; F. A. Simion[2]; H. Raabe[1]

[1]Institute for In Vitro Sciences, Inc., Gaithersburg, MD, [2]Kao Brands Company, Cincinnati, OH

Abstract

In vitro assays evaluating ocular irritation potential are routinely used by personal care companies. Two of these *in vitro* assays include the Chorioallantoic Membrane Vascular Assay (CAMVA) and the Bovine Corneal Opacity and Permeability Assay (BCOP). These assays do not require the use of live animals, provide reliable predictive data and are rapid to conduct. The BCOP uses excised bovine corneas to predict ocular irritation. The CAMVA uses the vascular network of fertilized chicken eggs as a conjunctival model to predict eye irritation. Both BCOP and CAMVA have been used for over fifteen years for product development, worker safety, and safety claims substantiation.

This poster describes procedures and considerations for demonstrating the inter-laboratory reliability of the BCOP and CAMVA. It is important to have a valid assay that can be implemented consistently at several different laboratories. For Kao Brands Company, a large BCOP and CAMVA database exists that covers multiple consumer product categories such as hair shampoos, skin cleansers, and hair styling sprays (containing ethanol). Therefore, a proper review of candidate laboratories is important for seamlessly generating consistent results that can be used for assessing potential ocular irritation of new products. First, a candidate laboratory should be audited for proper facility operation and personnel training. Second, the laboratory's use of Good Laboratory Practices (GLPs) should be reviewed. Third, reference materials with known BCOP and CAMVA data (one irritant and two non-irritants for initial assessment) should be tested at each new laboratory for verification of proper assay performance.

Introduction

Prior to using IIVS as a contract testing facility for *in vitro* assays such as BCOP and CAMVA, Kao Brands Company had visited the facility to conduct an audit. The audit included visual verification of proper assay performance and GLP compliance of the facility and the assays. Items such as technician training records, reagent batch records, and study workbooks were examined for technical qualifications and proper documentation. The use of appropriate assay controls was also examined. IIVS incorporates ethanol and 20% imidazole as the positive controls for the BCOP and 0.01M SLS as the positive control for CAMVA. Negative controls and solvent controls are also tested concurrently. For an assay to be considered valid, the results of the positive control must fall within two standard deviations of the historical mean for the BCOP assay, or exhibit an acceptable positive response in the CAM of the tested eggs for the CAMVA. The use of controls ensures that the test systems and conduct of these assays are consistent.

Next, reference materials with known BCOP and CAMVA responses (with the overall conclusion being: one irritant and two non-irritants) were blind coded and tested at IIVS. After the results of reference materials were assessed and determined to be acceptable, IIVS was confirmed as a qualified contract laboratory for testing Kao Brands Company's materials.

Materials and Methods

BCOP

The assay should be conducted under GLPs and the most current Organization for Economic Cooperation and Development (OECD) guideline. The critical steps of this assay include cutting and mounting corneas, dosing and rinsing of the test articles, use of the opacitometer, fluorescein preparation and treatment, and use of the plate reader (see Figure 1).

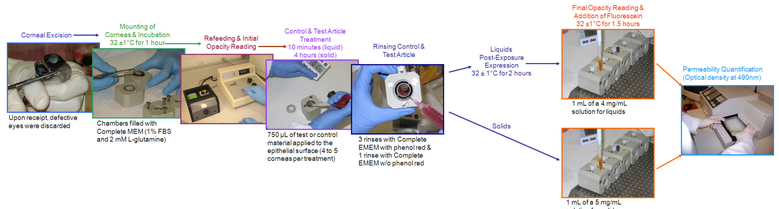

Figure 1 Critical Steps for the BCOP assay

CAMVA

The assay should be conducted under GLPs. The critical steps for this assay include receipt and incubation of the eggs, cutting of the eggs, determining daily survival, placement of O-rings onto the vascular membrane, treatment and scoring of the eggs, and calculation of the results (see Figure 2).

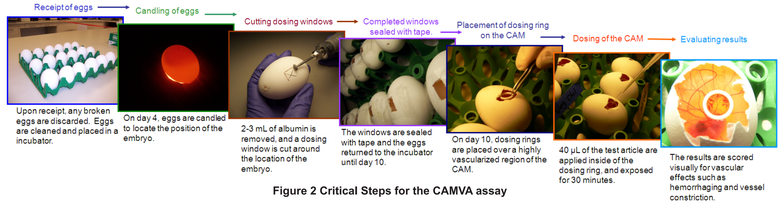

Figure 2 Critical Steps for the CAMVA assay

Assay Controls

BCOP

IIVS uses ethanol as the positive control for liquid test materials and 20% solution of Imidazole for solid test materials in the BCOP assay. The negative control for the assay is sterile, deionized water or other solvents such as saline. The positive control results are collected and historical ranges are updated every three months to provide acceptance criteria for a valid assay (see Figures 3 and 4) and monitor assay performance. Those assays with positive control results that fall outside the acceptance range are considered invalid and are repeated.

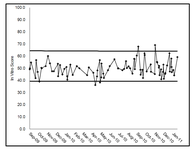

Figure 3 Historical Data Range for Ethanol

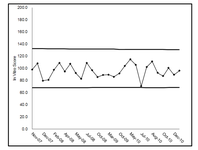

Figure 4 Historical Data Range for Imidazole

CAMVA

The positive control for the assay is 0.01M SLS. In order for an assay to be considered valid, the positive control must exhibit a positive response in the CAM of all tested eggs. A negative control (sterile deionized water) and any applicable solvent controls are tested in the assay, and must exhibit no responses in the CAM of all tested eggs.

Results

Initial testing – Reference Materials

Kao Brands Company initially tested 3 materials with known ocular irritation for evaluation in the CAMVA and BCOP. These materials were blind-coded and represented typical formulations being evaluated in these assays. The assay results were within the expected historical ranges (see Tables 1 and 2), and IIVS was validated as an acceptable contract lab for conducting the BCOP and CAMVA for Kao Brands Company.

Table 1 IIVS – Comparison of CAMVA Data

Reference Material	Category	IIVS CAMVA RC_{50} (%)	KAO Brands Historical Data: Irritant* (Y/N)	KAO Brands Historical Data: Mean RC_{50} (%)	KAO Brands Historical Data: Historical Confidence Interval
A	Shampoo	0.24	Yes	0.86	0.0021-16
B	Conditioner	2.36	Indeterminate	62.0	1.6-111
C	Skin Lotion	> 50	No	86.1	16-110

RC_{50} – Concentration of test material that causes positive responses in 50% of CAM.
*** - Determined by criteria in Table 5 and the flow chart in Figure 5.**

Table 2 IIVS – Comparison of BCOP Data

Reference Material	Category	IIVS BCOP IVS	KAO Brands Historical Data: Irritant* (Y/N)	KAO Brands Historical Data: Mean IVS	KAO Brands Historical Data: Historical Confidence Interval
A	Shampoo	-0.4	No	7.63	-0.4 – 48.31
B	Conditioner	-1.2	No	3.53	-1.49 – 43.96
C	Skin Lotion	-1.5	No	1.30	-0.62 – 3.96

IVS – In Vitro Score
*** - Determined by criteria in Table 5 and the flow chart in Figure 5.**

For materials containing known irritants or untested ingredients, Kao Brands Company performs the BCOP and CAMVA to make their potential ocular irritancy determinations as shown in Table 3. Kao Brands has maintained historical ranges for different product categories using data from 15 years of testing. Kao Brands Company developed a flow chart for determining irritancy based upon their chemical composition (as shown in Figure 5) and past performance of similar materials in the BCOP and CAMVA.

Figure 5 Flow chart for predicting potential irritancy.

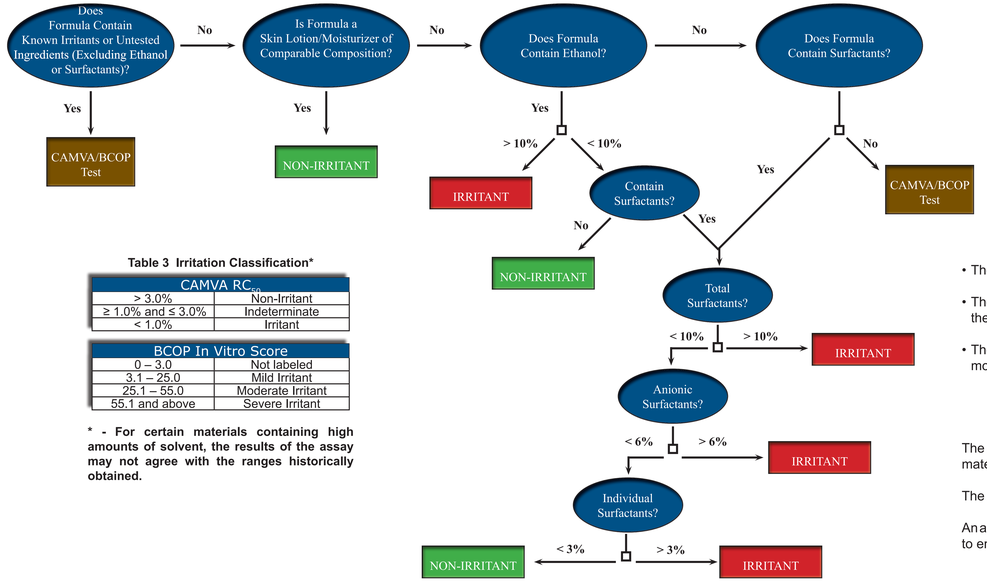

Table 3 Irritation Classification*

CAMVA RC_{50}	
> 3.0%	Non-Irritant
≥ 1.0% and ≤ 3.0%	Indeterminate
< 1.0%	Irritant

BCOP In Vitro Score	
0 – 3.0	Not labeled
3.1 – 25.0	Mild Irritant
25.1 – 55.0	Moderate Irritant
55.1 and above	Severe Irritant

*** - For certain materials containing high amounts of solvent, the results of the assay may not agree with the ranges historically obtained.**

Testing Update

Tables 4 and 5 lists additional test materials tested at IIVS in the BCOP and CAMVA since initial qualification. The same flow chart and irritancy classification criteria were utilized.

Table 4 IIVS – Comparison of CAMVA Data

Test Article	Category	IIVS CAMVA RC_{50} (%)	KAO Brands Historical Data: Irritant* (Y/N)	KAO Brands Historical Data: Mean RC_{50} (%)	KAO Brands Historical Data: Historical Confidence Interval
10-034-IVS	Deodorant	>50	No	86.8	62 - >100
10-212-IVS		>50	No		
10-214-IVS		>50	No		
10-100-IVS	Hair Styling Mousse	>50	No	26.93	1.1 - >100
10-037-IVS	Conditioner	2.0	Indeterminate	62.0	1.6 – 111
10-103-IVS		>50	No		
10-208-IVS		>50	No		
10-210-IVS		>50	No		
10-228-IVS		>50	No		
10-028-IVS	Skin Lotion	>50	No	86.1	16 – 110
10-031-IVS		>50	No		
10-105-IVS	Hair Styling Spray	>50	No	28.54	0.78-100
10-116-IVS		>50	No		
10-096-IVS		17.3	No		
10-098-IVS		31.9	No		

RC_{50} – Concentration of test material that causes positive responses in 50% of CAM.
*** - Determined by criteria in Table 3 and the flow chart in Figure 5.**

Table 5 IIVS – Comparison of BCOP Data

Test Article	Category	IIVS BCOP IVS	KAO Brands Historical Data: Irritant* (Y/N)	KAO Brands Historical Data: Mean IVS	KAO Brands Historical Data: Historical Confidence Interval
10-034-IVS	Deodorant	0.5	No	2.01	-0.40 – 11.93
10-212-IVS		0.4	No		
10-214-IVS		0.2	No		
10-233-IVS		1.2	No		
10-100-IVS	Hair Styling Mousse	0.6	No	3.96	-0.56 – 12.75
10-037-IVS	Conditioner	2.9	No	3.53	-1.49 – 43.96
10-103-IVS		2.0	No		
10-208-IVS		3.0	No		
10-210-IVS		0.0	No		
10-228-IVS		0.6	No		
10-028-IVS	Skin Lotion	-0.7	No	1.3	-0.62 – 3.96
10-031-IVS		0.9	No		
10-105-IVS	Hair Styling Spray	0.3	No	10.68	-1.44 – 43.96
10-116-IVS		0.5	No		
10-096-IVS		3.9	Yes (mild)		
10-098-IVS		38	Yes (moderate)		

IVS – In Vitro Score
*** - Determined by criteria in Table 3 and the flow chart in Figure 5.**

Observations

- The materials identified as deodorant/antiperspirant (n > 4) consistently fell in non-irritant category.
- The materials identified as hair styling sprays (n > 4) showed a wide range of responses. For this product category, the volatile organic content may be the main driver of the assay responses.
- The two materials identified as skin lotions were classified as non-irritants at IIVS, which supports the prediction model flow chart.

Conclusions

The results of the reference materials showed similar responses at the new testing facility. Subsequent testing of materials at the new testing facility showed expected responses and resulted in expected irritancy categories.

The use of the assay controls such as ethanol and SLS 0.01M is needed to monitor assay conditions and performance.

An audit of a testing facility and inter-laboratory results comparison of reference materials are important considerations to ensure high quality of data and proper irritation assessment.