

# *In vitro* Cytotoxicity of Soft Contact Lens Care Products With and Without PHMB

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## Abstract

**Purpose:** The cytotoxicity potential of poly-hexa-methyl-biguanide (PHMB) was determined using colorimetric *in vitro* assays with murine fibroblast cells (L929) and an immortalized human corneal epithelial cell line (HCE-T). These colorimetric assays are useful for the quantitative factor-induced cytotoxicity within a 24 to 72 hour period of cell culture. Soft contact lens care products containing PHMB corresponding soft contact lens care product excipient solutions, without PHMB, and PHMB in PBS were tested.

**Methods:** Lens care solutions with and without PHMB were diluted 1:3 with growth medium. PHMB in PBS was diluted at 10, 5, 2.5, 1.25 and 0.625 ppm in growth medium. Tests used were the cell viability assays, MTS/PES (MTS/PES) and Alamar Blue (AB), and the cell membrane integrity assay using neutral red uptake and release, (NRUR). The endpoint was spectrophotometric measurement using a microplate reader for MTS/PES at 490 nm and NRUR at 540 nm and a cytofluorometer for AB at 530/580 excitation/emission. The data were expressed as the ratio of the test solution's absorbance value to the absorbance value of the negative control and the final percentage calculated. These percentage values were evaluated by their IC50 (inhibition of control by 50%) and compared using ANOVA/Tukey HSD test and t - test for statistical significance at a p<0.05 to the negative control and between samples.

**Results:** Based on these studies, the following lens care solutions were noncytotoxic at 48 hours exposure using the MTS/PES, AB and NRUR: SOLOcare® PLUS (CIBA Vision) and the corresponding excipient solution without PHMB and AQuify™ MPS (SOLOcare®AQUA) (CIBA Vision) and the corresponding excipient solution without PHMB, and PHMB solutions of 5, 2.5, 1.25 and 0.625 ppm. The following solutions were cytotoxic using the same assays: ReNu MultiPlus® (Bausch & Lomb), ReNu MultiPlus® (Bausch & Lomb) excipient solution without PHMB and PHMB at 10 ppm.

**Discussion:** The PHMB concentration of the marketed contact lens solutions is approximately 1 ppm. PHMB at 1ppm was noncytotoxic using the three *in vitro* assays, MTS/PES, AB and NRUR with L929 and HCE-T cells. The presence of PHMB in the ReNu solution did not increase the cytotoxicity indicating that the excipients are responsible for the cytotoxicity observed with ReNu MultiPlus®. SOLOcare® PLUS and AQuify™ MPS were both noncytotoxic. These results suggest that not all PHMB-containing solutions are equal.

## Materials and Methods

Plate HCE-T cells by adding 100 µl cell suspension (1.5 X 10<sup>5</sup>cells/ml) to each well of a 96 well flat bottomed plate. One column of 3-4 replicates was allowed for each sample of PHMB or control to be tested. The plates were incubated overnight or for 36 hours at 37°C, 5 % CO<sub>2</sub> in a humidified incubator. Confluency of the wells was checked before proceeding. The medium was aspirated and replaced with a fresh 100 µl of media. Neat solutions of the six soft contact lenses solutions and PHMB dissolved in was Dulbecco's Phosphate Buffered Saline (DPBS) were serially diluted on the 96 well plates. Solutions of the six soft contact lens/media were tested at 25%. Positive control of PHMB/media was tested at final concentrations of 10 ppm (0.001%), 5 ppm (0.0005%), 2.5 ppm (0.00025%), 1.25 ppm (0.000125%) and 0.625ppm (0.0000625%), by serial dilution on 96 well plates. The negative control for these experiments was Dulbecco's Phosphate Buffered Saline (DPBS) at the same dilution as used for the soft contact lens products. HCE-T cells were grown in UltraCulture™ media without antibiotics.

The endpoints used to determine the cytotoxicity of PHMB and the test solutions were the cell viability assay using Alamar Blue™ (AB) and cell membrane integrity assay using Neutral Red uptake and release (NRUR). Statistical analysis provided by STATISTICA® 6 software.

## Method 1: Alamar Blue™ [AB]

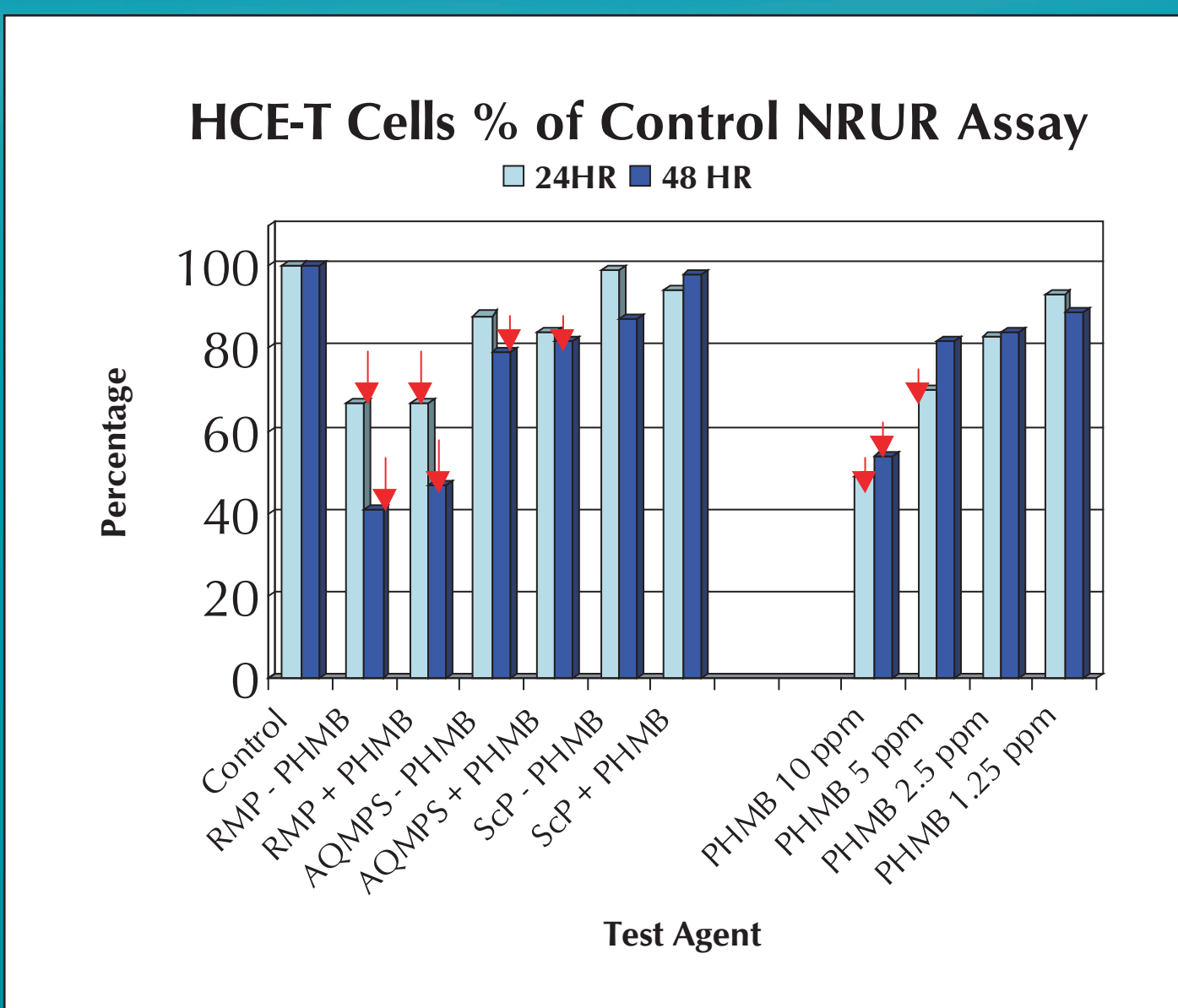
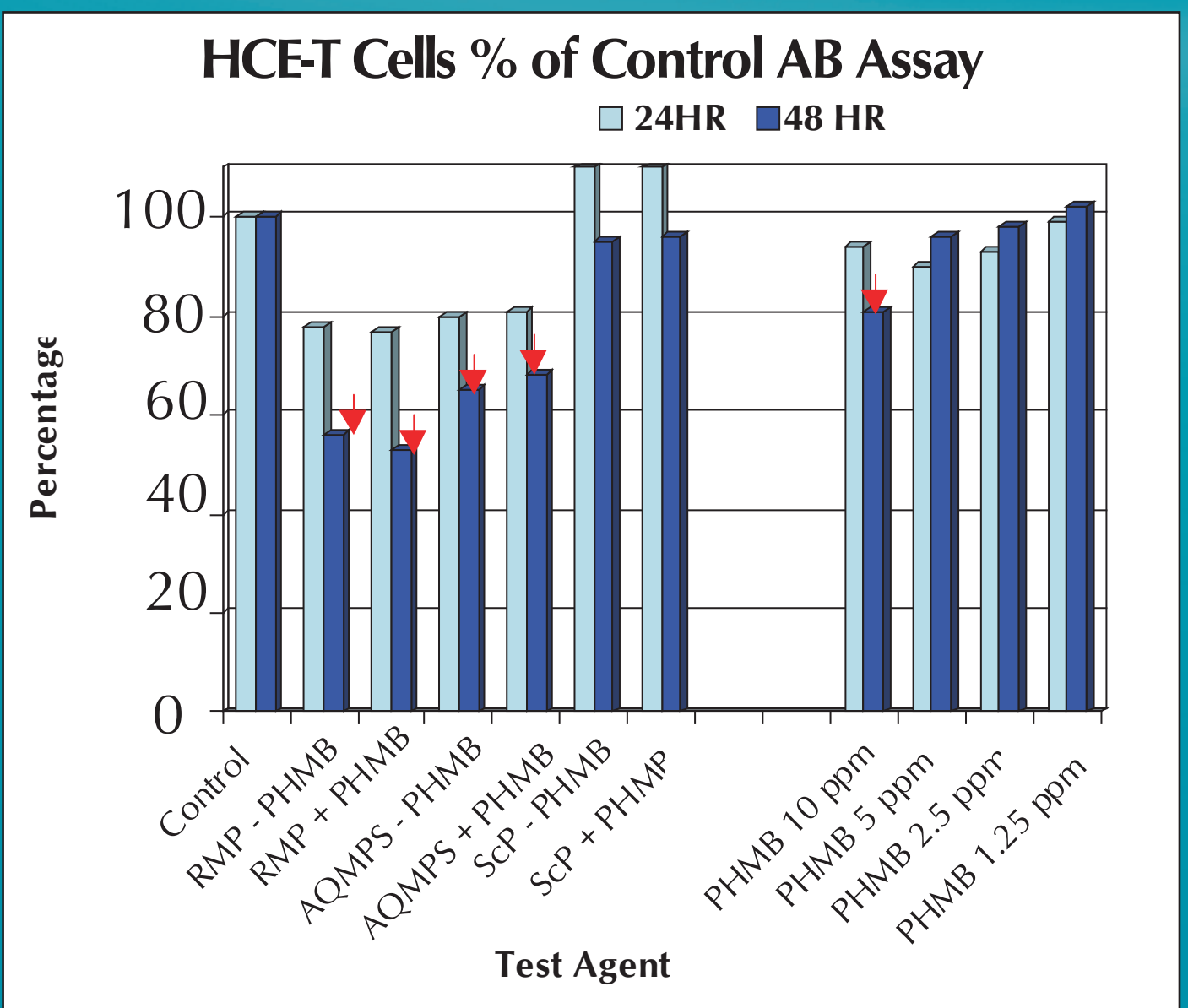
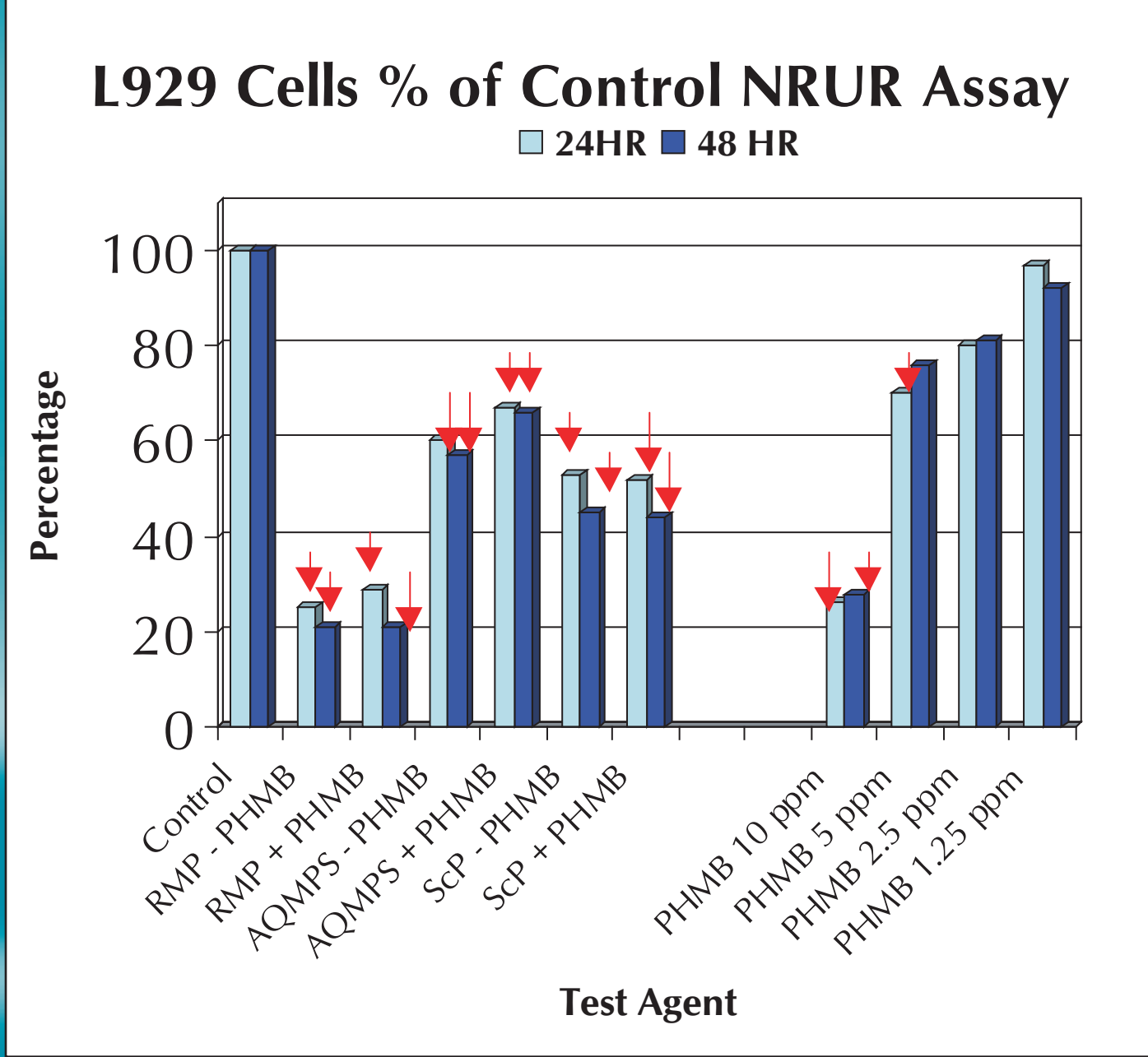
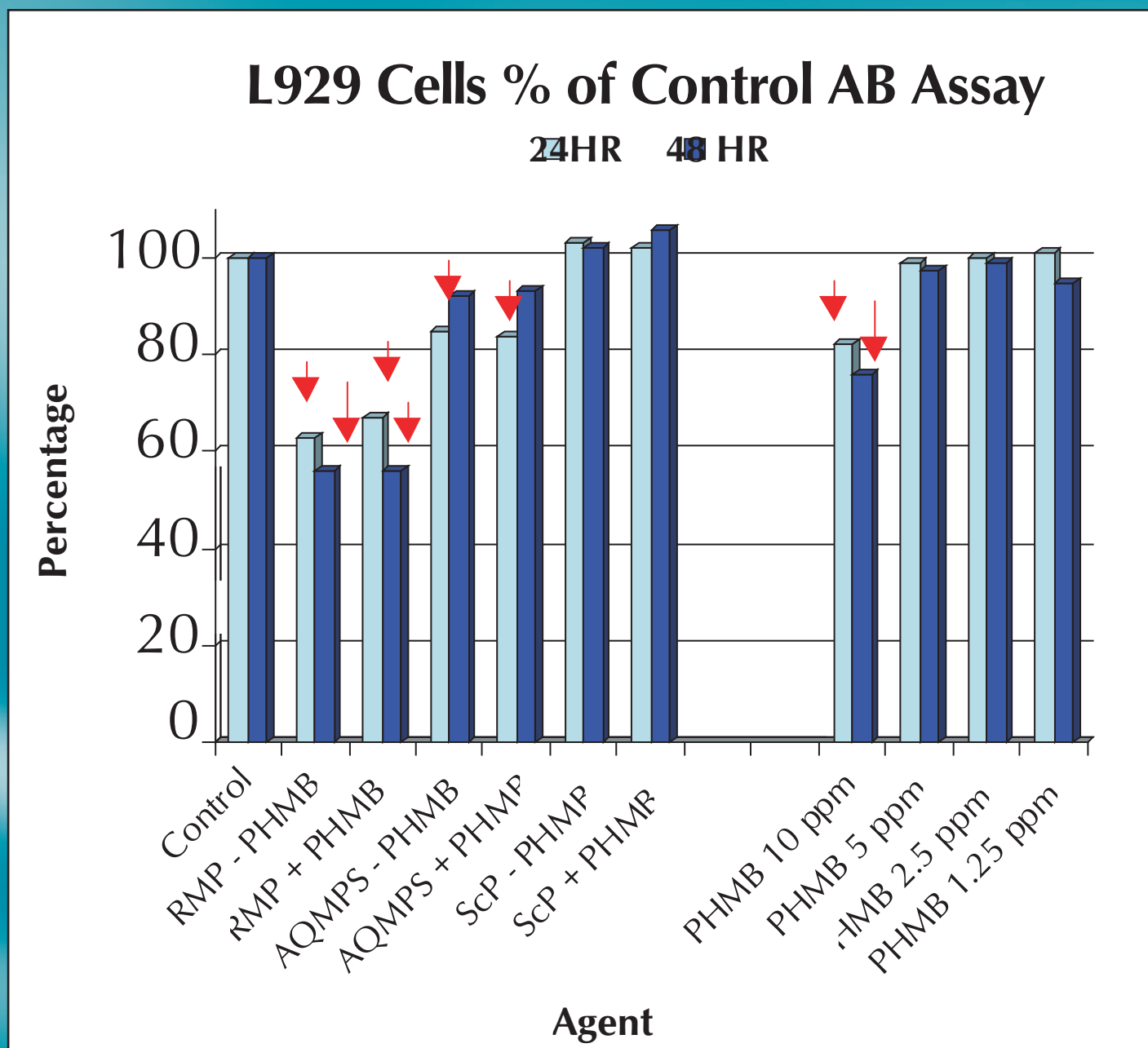
The wells were rinsed with DPBS and 10% AB in DPBS was added to each test well after 24 hours and 48 hours of test solutions and PHMB exposure. The plate was returned to the incubator for 3 hours and then read at an  $\lambda_{exc}$  =530nm,  $\lambda_{em}$  =580 nm of the oxidized and reduced AB using a CytoFluor®, (PerSeptive Biosystems). Cell viability was determined by comparing the control absorbance to the test solution absorbance.

## Method 2: Neutral Red Uptake and Release [NRUR]

20 µl of a 0.3% NR solution was added to each test well after 24 hours and 48 hours of test solution and PHMB exposure and the plate was returned to the incubator for 2 hours. The plate was removed from the incubator and the NR solution was aspirated from the plate. The plate was rinsed with 100 µl of DBPS. The plate was fixed with 100 µl of a formaldehyde/Ca solution that was quickly removed. The NR solution retained by the viable cells was then eluted with 100 µl of an ethanol/ acetic acid solution. The plate was read on a microplate reader at 570 nm. The resulting absorbance values for each test solution and PHMB concentration were compared to the control absorbance.

Test Agent	Abbreviation
ReNu MultiPlus®	RMP
AQuify™ MPS	AQMPS
SOLOcare® PLUS	ScP
w and w/o PHMB	+ PHMB / - PHMB

*Note: Red arrow denotes significance with Tukey ad hoc and student t-test at a p<0.05 to the negative control and between samples*



## Results

• Based on an ED50 value, PHMB was NONCYTOTOXIC for both cell lines and both test methods = 5 ppm. The SOLOcare family of products concentration of PHMB is well below at 1 ppm and did not show any cytotoxicity at 24 or 48 hours exposure.

• Each pairing of soft contact lens solution, with AND without PHMB exhibited similar results within cell lines and between testing methods and were not found significant to one another using statistical analysis.

• The L929 cell line was more sensitive to the test and PHMB solutions than the HCE-T cells using the NRUR assay. This sensitivity could be due to differing membrane permeability potential to solution excipients from that of the HCE-T cell line.

• L929 mean values were found within 24 hours of exposure and did not increase up to 48 hours exposure. HCE-T cells did show a change in mean values from 24 to 48 hours exposure for only RMP - PHMB and RMP + PHMB

## Conclusions

• PHMB at or below 5 ppm is NONCYTOTOXIC to both L929 and HCE-T cell lines following 24 or 48 hours of exposure using both the AB and NRUR assays.

• Rank order of soft contact lens solutions with and without PHMB from the least cytotoxic to the most cytotoxic is: SOLOcare® PLUS ≤ AQuify™ MPS < ReNu MultiPlus®.

• No ED50 cytotoxic effects were evident for SOLOcare® PLUS and AQuify™ MPS with HCE-T and L929 cells exposed to the solutions for 24 in both the AB and NRUR methods.

• ReNu MultiPlus® at 24 hours did exhibit a cytotoxic response below the ED50 level for the NRUR assay with L929 cells and was considered cytotoxic below the ED50 value using both cell lines for the NRUR test at 48 hours.

## References

- Araki-Sasaki, K. Y. Ohashi, et al. (1995) Immortalized Human Corneal Epithelial Cell Line and Its Characteristics." IOVS 36(3): 614
- Borenfreund, E; Shopsis,C. "Toxicity monitored with a correlated set of cell culture assays." Xenobiotica (1985),15(8-9) 705-711.
- Mowery-McKee, Mary and Alicia Sills and Ann Wright. (2002) "Comparative Cytotoxicity Potential of Soft Contact Lens Care Regimens" CLAO Journal 28 (3) 160-164
- O'Brien,J,Wilson,L,Orton,T,Pognan,F. "Investigation of the Alamar Blue (resazurin) Fluorescent dye for the assessment of mammalian cell cytotoxicity." Eur.J. Biochem. (2000) 267, 5421-5426.
- Petrou-Binder, S. "PHMB-containing antiseptics 'may offer alternative' to iodine perioperative agents, say researchers." <http://www.esrs.org/eurotimes/April2003/phmb.asp>
- Pham, X and J W Huff. (1999) "Cytotoxicity evaluation of multipurpose contact lens solutions using an in vitro test battery" CLAO Journal 25(1) 28-35
- Rat, Patrice and Sebastien Perrot and Jean Michael Warnet (2002) " Cell Tolerance Investigation of Four Commercial Multipurpose Contact Lens Solutions", 4th World Congress on Alternatives and Animal Use in the Life Sciences, New Orleans, E26, pg 270
- Tchao, Ruy, David McCanna and Michael Miller. (2002) "Comparison of Contact Lens Multipurpose Solutions by In Vitro Sodium Fluorescein Permeability Assay" CLAO Journal 28 (3) 151-156