EVALUATION OF CYTOTOXICITY FOR THE FAMILY OF REVITAL-OX™ ENZYMATIC DETERGENTS

PURPOSE

Although all reprocessing of medical devices involves a rinsing step, there is still the risk that residues may remain on the surfaces of medical devices which might cause complications. To ease the concern associated with the risk of poor rinsing, it is important to demonstrate that detergent residues which might remain are innocuous to contacted tissue. This can give an extra level of confidence when using medical devices where chemical residues are of greatest concern. Two different experiments were performed to demonstrate the non-cytotoxic nature of the family of Revital-Ox Enzymatic Detergents.

The products covered by this testing are:

- Revital-Ox™ 2X Concentrate Enzymatic Detergent (2D99)
- Revital-Ox™ Dye and Fragrance Free Enzymatic Detergent (2D97)
- Revital-Ox™ Enzymatic Detergent with Color and Fragrance (2D96)
- Revital-Ox™ PowerLift Cleaning Technology Enzymatic Detergent (2D98)

METHODS

Surgical (416) grade stainless-steel coupons with a smooth finish were used to simulate the surface of a medical device. Each test coupon was cleaned and dried prior to being dipped in the Revital-Ox Enzymatic Detergent use dilution of either 1/8 oz./gal. (1 mL/L) or the highest recommended label concentration of 1/2 oz./gal. (4 mL/L). The unrinsed coupons were allowed to air dry before being placed in peel pouches and autoclaved at 250°F (121°C) for 21 minutes. The coupons were then submitted to an independent contract laboratory for cytotoxicity testing using Good Laboratory Practices (GLP). Tests were performed using mouse fibroblast L929 cells to meet the requirements of ISO 10993-5, 1999, Biological Evaluation of Medical Devices – Part 5: Tests for In Vitro Cytotoxicity.

Controls

- Negative control consisted of a portion of High Density Polyethylene (HDPE).
- Positive control consisted of a portion of latex.
- Additional coupons that had been immersed in deionized water were submitted for testing with the Direct Contact Method to demonstrate that the stainless-steel coupons were not cytotoxic.

Cytotoxicity Tests

- MEM Elution Method – the coupon was extracted at 99°F (37°C) for 24 hours and the mammalian cells were grown in the extraction. The positive control was plasticized vinyl containing 10,10'-Oxybisphenoxarsine.
- Direct Contact Method – the coupons were placed directly in the culture medium of the cell monolayer. The positive control was latex.

RESULTS

MEM Elution Method Results

After exposure to coupons treated with the deionized water, 1/8 oz./gal. (1 mL/L) or 1/2 oz./gal. (4 mL/L) Revital-Ox Enzymatic Detergents, no biological reactivity (Grade 0, Table 1) was observed in any of the three replicates for the L929 MEM Elution Method for cells exposed for 48 hours. The observed cellular response obtained from the positive control articles (Grade 4) and negative control articles (Grade 0) confirmed the suitability of the test systems.

Direct Contact Method Results

After exposure to coupons treated with the deionized water or 1/8 oz./gal. (1 mL/L) Revital-Ox Enzymatic Detergents, slight reactivity (Grade 1, Table 2) was observed in the Direct Contact Method with L929 mammalian cells at 24 hours for both sets of test coupons. A grade of 2 or lower is considered non-cytotoxic. This is a typical value observed in other studies using stainless-steel coupons.

After exposure to coupons treated with 1/2 oz./gal. (4 mL/L) of the Revital-Ox Enzymatic Detergents, mild reactivity (Grade 2, Table 2) was observed at 24 hours. A grade of 2 or lower is considered non-cytotoxic. The observed cellular response obtained from the positive control articles (Grade 3) and negative control articles (Grade 0) confirmed the suitability of the test systems.
Based on criteria of the test protocols, the metal coupons treated with 1/8 to 1/2 oz./gal. (1 to 4 mL/L) of the Revital-Ox Enzymatic Detergents are considered non-cytotoxic across the range of label recommended use-dilutions. These results should address the concern associated with the risk of poor rinsing during the use of the family of Revital-Ox Enzymatic Detergents on medical devices.

**CONCLUSION**

The range of recommended use dilutions for the family of Revital-Ox Enzymatic Detergents have been demonstrated to be non-cytotoxic in studies using mouse fibroblast L929 cells.

**REFERENCES**

Data on file.