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Handling Cytotoxicity Failure: Why it is not the end

Cytotoxicity is one of the most common assays performed as a part of a medical device biocompatibility assessment. Described in ISO 10993-5, Cytotoxicity is one of the "big three" assays used in testing during the biological evaluation stage of medical device development.

There are several methods used to perform Cytotoxicity testing; the main methods are direct contact and extraction. The direct contact method involves placing the medical device (of the test item) directly on a cell monolayer. The extraction method involves extracting the constituents of the medical device using appropriate extraction media (e.g., culture medium), time (e.g., 24 or 72 hours) and temperature (e.g., 37°C). Both the qualitative evaluation and the quantitative assessment are performed at the end of the selected contact time. Qualitative evaluation involves a scoring system based on the condition of the cell monolayer. Typically, these are scored from zero (no cytotoxicity) to four (severe cytotoxicity), with a cutoff at grade two. The quantitative assessment objectively quantifies cell viability, with a viability loss over 30% considered to be cytotoxic. Also in this case, several methods are available with MTT, XTT and Neutral Red Uptake (NRU) being most commonly used.

A cytotoxic response suggests that a substance released from the test article impacts cultured cells, but it does not necessarily mean the device is unsafe for clinical use. Investigating the reasons behind cytotoxicity is crucial and may include examining device materials, manufacturing residues, cleaning processes, or any other aspect that may have altered material chemistry or left residue. Positive test responses require thorough investigation, including checking in with the testing lab, assessing controls, examining sample preparation, and considering the constituents of the device. Chemical characterization may also be necessary to identify leached constituents and assess potential risks to patients.

There are commonly used materials that induce cytotoxicity (e.g., many medical adhesives) and do not pass cytotoxicity tests but are nonetheless safe when used as intended. In these situations, one should be careful to isolate the source of cytotoxicity to provide assurance to regulators that all cytotoxic materials have been identified. Once the cytotoxic materials are identified, a safety case can be made. Useful points to consider in making a safety case include an understanding of the cytotoxic mechanism, and whether or not this mechanism presents a risk in the intended application. For example, a material may contain a detergent that causes cell lysis in culture but could be safe in an intact skin contacting application. Other considerations include a history of use in similar applications and other testing data to support the safety of the device. The final safety case should be documented and submitted as part of your overall biological evaluation report.

Even negative cytotoxicity results (i.e., non-cytotoxic) require scrutiny as changes in device appearance or media observations (cloudiness, particulate, color change) can require explanation. Analytical chemical tests can be helpful at this stage to assist a manufacturer in identifying what materials any observed particulates are comprised of.

In conclusion, cytotoxicity by itself does not necessarily mean that a given device will be unsafe. It is the discovery of the root cause of the cytotoxicity that is important to either make the case that the device is safe, despite the cytotoxic result, or to assess if changes to the device or manufacturing process need to be made to ensure device safety.