

Development, Validation and Regulatory Approval of a Cytokine Release Test for Contaminants

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In the early 90ties several countries reported unusually large numbers of adverse drug reactions associated with Deferoxamin treatment. The occurrence of the complaints was linked to a number of specific batches of Deferoxamin, since the reaction usually did not recur when a different batch was used, even when given in high concentrations. It was postulated that the local and systemic adverse reactions were possibly caused by an unknown substance contained in the vials. It was presumed that this as yet unknown substance stimulates cytokine release, which in turn, acts on the hypothalamus and triggers increased heat production, i.e. fever or inflammation.

No chemical or physical analytical method showed quality indicating differences between non-reactive and reactive batches. Even with the standard methods for the detection of pyrogens or endotoxins no contamination could be detected in clinical reactive batches.

Novartis Biological Analytics started consequently with the development of a Cytokine Release Test, based on a publication of Pharmeuropa from the year 1989. The design of the test is very simple: Three different, rather high concentrations of the sample are mixed with freshly prepared peripheral blood mononuclear cells (PBMC). The set of these mixtures is incubated over night and then the concentration of IL-6 is measured in the supernatant by ELISA technique. After a series of tests it was observed, that the variability of donors is too large to describe a testing method that can be validated according to cGMP. It was necessary to implement a similar but known clean batch as a reference batch for the normalization of the individual reactivity of the blood donors. Once having this standardized method, it was possible to set specifications. The definition of these specs was based on clinical evidence and outcome of the cytokine release test. In a next step it was possible to define several validation points and acceptance criteria e.g. linearity, intermediate precision, accuracy and interference of the ELISA. This validation was succesful and the test was finally accepted and approved by FDA as a release test.

Thanks to the cytokine release test it was possible to improve the downstream process and to reduce complaints due to contamination, to zero.

Today it is presumed to be true that the nature of the contamination are PAMPs from the fermentation process and that they can be eliminated by comprehensive cleaning steps.