



Baxter

July 7, 2011

Department of Health and Human Services
NICEATM, National Institute of Environmental Health Sciences (NIEHS)
Dr. Warren Casey, Deputy Director
Post Office Box 12233
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Research Triangle Park, North Carolina 27709
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RE: FR Docket No. 2011-12627: Nomination of In Vitro Test Methods for Detection and Quantification of Botulinum Neurotoxins and Detection of Non-Endotoxin Pyrogens; Data Request for Substances Evaluated by These Test Methods.

Dear Dr. Casey,

Baxter Healthcare Corporation (Baxter) is pleased to have this opportunity to submit comments to Docket No. 2011-12627: Nomination of In Vitro Test Methods for Detection and Quantification of Botulinum Neurotoxins and Detection of Non-Endotoxin Pyrogens; Data Request for Substances Evaluated by These Test Methods. Baxter is a globally diversified healthcare company that assists healthcare professionals and their patients with treatment of complex medical conditions including, but not limited to hemophilia, immune disorders, kidney diseases, cancer, trauma and other conditions. Given an 80-year history of innovation with approximately 45,000 current employees, Baxter applies its expertise in biologics, medical devices, pharmaceuticals and combination products to make a meaningful difference in patients' lives.

While Baxter is fully supportive of any alternative assay development which could potentially replace the *in vivo* test for pyrogens in rabbits, it is essential that the adopted assay be validated for its proficiency in detecting non-endotoxin pyrogens, particularly those arising in pharmaceutical and medical device manufacturing settings, and to show that it is at least equivalent, if not better than the rabbit test in such regard. One of the major hurdles in this respect has been the lack of an internationally recognized non-endotoxin pyrogen standard for validation purposes. Indeed due to the huge diversity of known non-endotoxin pyrogen substances it would be highly recommended and advantageous to have multiple standards of different origin to truly evaluate and validate potential alternative *in vitro* assays.

Although Baxter's experience with using the Biotest assay is rather limited, we have used the combination of whole blood (fresh) with an IL-1 β readout along with a number of other assays as potential trouble shooting tools in investigation where non-endotoxin pyrogens were suspected. Unfortunately, the whole blood/IL-1 β assay proved to be of little value in these investigations as it could not distinguish between issue and non-issue lots. Many of the reasons for this are clearly common to all methods that attempt to detect non-endotoxin pyrogens, namely, their variable sensitivity in detecting different substances among the vast array of potential agents which could give rise to the non-endotoxin reactogenicity responses. This variability in sensitivity appears to stem from the extremely variable response of individual donors to specific non-endotoxin pyrogens. While the use of pooled blood from multiple donors in the case of the Biotest assay would broaden the potential specificity of the assay, it could nevertheless reduce the sensitivity, as the response of strong responders could be diluted among those of poor or non-responders. The fact that the assay also only uses a single and relative insensitive pro-inflammatory readout is also rather limiting, given the known variability of immune cell response.

Furthermore, with the use of cryopreserved pooled blood from multiple donors, as is the case in the Biotest assay, it is essential to verify not only the viability but also the functional viability of the thawed cells prior to their use in the test and to quality assure the reproducibility of results obtained from different batches of pooled blood from the same donors as well as those from different donor sets. Although the use of cryopreserved blood in the Biotest assay

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Baxter Healthcare

July 6, 2011

does provide convenience and potential for lab-to-lab consistency, we are not convinced that at this point in time cryopreserved blood has been fully assessed enabling this assay to begin a second validation phase.

From direct experience with the use of several Monocyte Activation Tests (MAT) (including the Biotest assay) in various investigational efforts, we have found great utility, as well as significant impediments in the use of such tests. For the Biotest assay, we are furthermore not convinced that the proposed use of lipoteichoic acid as a positive control for non-endotoxin pyrogens is ideal and adequately represents a broad enough range of potential non-endotoxin pyrogens found in the pharmaceutical manufacturing environment. Hence, for non-endotoxin pyrogens in particular, it has become increasingly evident that there is currently no MAT assay universal enough to be considered ready for validation as a quality control release test. Based on our experience, we are of the opinion that further development work needs to be done before ICCVAM and collaborating institutions invest time, money and effort into a rigorous validation exercise of non-endotoxin pyrogens.

Baxter remains absolutely committed and supportive of identifying and validating an alternative *in vitro* release test system that demonstrates its proficiency in detecting a number of different non-endotoxin pyrogens and is at least equivalent to the rabbit pyrogen test in doing so. We will, however, continue to employ MAT assays, including the Biotest MAT assay where appropriate, along with a number of other tests as part of root cause investigations where non-endotoxin pyrogens are suspected and where it is found to be the most appropriate assay on an individual case by case basis.

Based on these comments, Baxter reiterates its support to the agency and would welcome any questions you may have by contacting the undersigned.

Thank you for the opportunity to provide comments to this notification.

Respectfully Submitted,

(signature redacted)

Christian Supina
Research Scientist

(signature redacted)

Winston R. Brown
Director, Global Compliance