AUT-F0476	FORM
VERSION 1	EFFECTIVE DATE 25 NOVEMBER 2014

EVALATUION RECORD FOR REVIEW OF RETROSPECTIVE ASSESSMENT

PROJECT AUTHORISATION NUMBER	[redacted]
PROJECT TITLE	**MOUSE PROTECTION ASSAY: DETERMINATION OF THE ANTIBODY TITRE AGAINST BOTULINUM TOXIN TYPE [redacted] IN HUMAN SERUM SAMPLES**
PROJECT MANAGER	[redacted]

A. ANIMAL NUMBERS AND SEVERITY

1. Comment on whether the animal numbers differed from the original project authorisation and the reasons for this.

7500 mice are authorised for use, however only 552 mice have been used to date under this project authorisation. Fewer animals have been used to date than anticipated as the number of animals required is based the number of positive sera samples identified during the RIPA assay; it is not possible to accurately determine the number of samples that will test positive and subsequently require testing in the Mouse Protection Assay (MPA).

2. What was the actual overall severity of the project and was this as predicted in the original project evaluation? If so, can you identify any reasons for this difference?

The actual severity to date has been:
- Severe for 207 animals
- Moderate for 207 animals
- Mild for 138 animals

It was originally expected that a large proportion of the animals would experience severe suffering, therefore this is as predicted in the original project evaluation. However since the project authorisation was originally granted by the HPRA, the humane endpoints have been refined to avoid death as an endpoint (details below). Therefore it is expected that for the remainder of the project, animals that experience severe suffering will experience this for a shorter duration.

B. IMPLEMENTATION OF THE 3RS

Detail any elements identified that may contribute to the further implementation of the 3Rs should similar work be conducted in future.

An in vitro method exists (RIPA), however this is an initial screen and where positive results are obtained using this method, these samples are then tested by the MPA assay. The MPA assay is recognised as the gold standard for this type of testing. Of note, when this project was originally

approved by the HPRA, it was as an extension of ongoing work taking place under a Department of Health project license. This included [redacted] approval for the the associated human clinical trial to include the MPA assay. To disrupt the human clinical trial mid-study could compromise the study as a whole and the results of animals already used as part of the trial could potentially go to waste, which would not be in keeping with the principles of the 3Rs.

However since the project authorisation was originally granted by the HPRA, the humane endpoints have been refined. [redacted]

C. WELFARE CONCERNS, UNEXPECTED ADVERSE EVENTS AND DEVIATIONS

Comment on any welfare concerns, unexpected adverse events and deviations which may have arisen during the course of the project, and describe how these were dealt with.

An invalid result was obtained for one study, which was due to an operational error during sample preparation. This resulted in 5 additional assays being conducted, however this was conducted within the terms and conditions of the project authorisation.

D. ACCURACY OF ORIGINAL HARM-BENEFIT ANALYSIS AND ACHIEVEMENT OF OBJECTIVES

Were the proposed objectives of the project actually achieved? If not, outline the reasons for this.

Yes. The project is taking place as part of a human clinical trial in order to identify patients that have developed neutralising antibodies to botulinum toxin. [redacted] has achieved their scientific objectives in relation to this.

In retrospect, was the original harm-benefit analysis accurate and in line with the actual harms that occurred and the benefits that were achieved? If not, outline the key areas in which this differed and any possible reasons for this.

Yes, the original harm-benefit analysis was accurate. The benefits are being achieved as predicted and the harms have been reduced through the implimentation of more humane endpoints.

E. FEEDBACK PROVIDED TO PROJECT MANAGER

Enter the feedback to be given to the project manager

Thank you for providing the completed retrospective assessment report for the above project authorisation. We have reviewed your assessment and are satisfied with the outcome of this project.

In particular, we are satisfied that humane endpoints are being used and that a commitment has been made to increase monitoring of the animals to minimise suffering.

We would like to remind the project manager of the specific condition of this project authorisation at this time:

"During the lifetime of this project authorisation, every effort must be made to ensure that the project is refined to avoid, as far as possible, death as an end-point. Where possible, more humane end-points should be used so that the animals may be euthanised without any further suffering."

Please do not hesitate to contact us if you need any clarification on the content of this e-mail or the retrospective assessment process

F. FEEDBACK FOR SCIENTIFIC ANIMAL PROTECTION ASSESSORS

Outline the key points of the feedback for scientific animal protection project assessors below.

This is a severe project and was originally approved with death as an endpoint for the Mouse Protection Assay, but with the following specific condition:
"During the lifetime of this project authorisation, every effort must be made to ensure that the project is refined to avoid, as far as possible, death as an end-point. Where possible, more humane end-points should be used so that the animals may be euthanised without any further suffering."

This project authorisation was renewed in August 2015 and at that time it was outlined that the following additional monitoring time points were implemented: 12h ± 1 hour, 17h ± 1 hour, 21h ± 1 hour, 24h ± 1 hour, 36h ± 1 hour, 41h ± 1 and 48h ± 1 hour post dosing. This was reviewed by the HPRA and deemed acceptable to avoid death as an endpoint.

The project manager has recently submitted an update in relation to this project (see correspondence from 12/02/16 uploaded to [redacted] which confirms that humane endpoints have been included in all assays since August 2015.

G. KEY FINDINGS FOR THE NATIONAL COMMITTEE

Outline the key findings from this retrospective assessment which can be provided in the future to the National Committee if required.

This is a regulatory project involving botulinum toxins and the Mouse Protection Assay. Humane endpoints have been introduced for a procedure that previously required death as an endpoint. This involved increasing the monitoring of the animals to humanely euthanise the animals prior to death.

ASSESSOR SIGNATURE: [redacted]

DATE: **29/02/16**