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Regulatory Toxicology and Pharmacology 45 (2006) 97–103
Regulatory Toxicology and Pharmacology
www.elsevier.com/locate/yrtph

Ensuring quality of in vitro alternative test methods: Current practice ☆

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Received 23 August 2004
Available online 27 April 2006

Abstract

In Vitro toxicology methods are being validated and adopted by regulatory agencies for use as alternatives to animal testing. Such methods may use ex vivo tissues or bioconstructs, some of which may be proprietary. Users of the data from these methods need to be reassured that the assays or assay components used in their studies provide consistent, good quality data over time, matching the standards achieved during the validation process. This paper presents an overview of approaches currently used by representatives of a manufacturer and a contract testing laboratory to ensure that the results from in vitro alternative methods are reproducible and of high quality over time. These approaches include full characterization of cells or tissues, sampling of each lot of manufactured bioconstructs for performance, and regular use of controls and benchmark chemicals to provide assurance of consistency of assay performance.
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Keywords: Toxicology; In vitro; Alternative; Methods; Quality; Assurance; Control; Testing kits; Dermal; Ocular

1. Introduction

In vitro toxicology assays are being developed, validated, and approved for regulatory use as alternatives to animal testing (ECVAM, 2002). Such assays may also serve to reduce the cost of testing, the quantity of test materials needed and increase the predictive ability of testing by focusing more closely on relevant endpoints or adding mechanistic information. In vitro assays may be designed using cultured cells or engineered bioconstructs as the target tissue. Bioconstructs, some of which are produced from normal human cells, have become essential target tissues for many types of toxicology studies. They provide useful models, where the tissue's differentiated state and/or physical structure are required to replicate in vivo exposure and response. Ex vivo tissues can also serve as essential target tissues in in vitro assays. Current experience indicates that bioconstructs and ex vivo tissues are often fragile and care must be taken to ensure they are functioning as expected to ensure that the results of the assay are meaningful and can be compared with data from previous studies and/or across laboratories. This paper describes, and provides examples of, quality control processes and sets forth key terms.

When studies using in vitro systems are performed to fulfill regulatory testing needs, Good Laboratory Practice principles and regulations (OECD, 1981; USEPA, 2002; USFDA, 2003) place the ultimate responsibility with the test sponsor (the company submitting data to regulatory authorities) to verify that the assay performs appropriately. The test sponsor depends on the laboratory performing the test to help ensure that quality control measures are adequate. Under Good Laboratory Practice,

☆ This article is a companion to "Ensuring quality of in vitro alternative test methods: Issues and answers", by Kailash Gupta, Amy Rispin, Katherine Stitzel, Sandra Coecke, John Harbell [Regul. Tox. Pharmacol. 43 (2005) 219–224].

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0273-2300/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.yrtph.2005.03.005

responsibilities of the user laboratory to assure the quality of the data produced in any study include: using a defined study protocol which describes how the study is executed; training the technical staff; documenting each stage in the study's execution; employing concurrent controls (as called for in the protocol); and establishing and following defined acceptance criteria (OECD, 1981).

2. Data needs

2.1. Test system quality control information

When an in vitro system using biocontructs or ex vivo tissues is proprietary, quality control of the manufacturing process prior to distribution should be conducted by the manufacturer of the assay system to ensure that test systems maintain consistency of performance. The data demonstrating test system performance for each batch of proprietary test methods should be available as part of the study record. If not, the testing laboratory should be able to request such quality control documentation from the manufacturer. In addition, the testing laboratory should also have its own procedures to verify that performance of the test system did not degrade during shipping.

2.2. Controls

Controls should address each endpoint reported in the assay and provide a measure of the performance of the assay for each run. Controls help to establish whether a valid trial was performed when data for the unknown chemical being tested are submitted to regulatory agencies. In addition, results from control trials can be compared with historical data and used for trend analysis so that any drift in the assay system can be detected.

For in vitro studies, the negative control responses are often used to set the baseline of cell or tissue viability against which the responses of the cells or tissues treated with the test article or positive control is compared. Positive control response(s) are critical to demonstrating the functional integrity of the target tissue, proper execution of the treatment of the cells or tissue, and proper functioning of the endpoint(s) assessment method. Specific types of performance information, such as ET_{50} values and their historical ranges depend on the type of in vitro system. Study directors or system developers recommendations of the appropriate performance measures should be scientifically supported.

The use of concurrent positive and negative controls allows the user laboratory to establish acceptance criteria for each assay system. In this context, the criteria refer to acceptable limits set for the endpoint values obtained from the control(s) and are used to assure that the assay is performing within set limits over time. The acceptance criteria are predetermined and are quantitative standards for the proper functioning of the assay. The term acceptance criteria used in this context has a different meaning than the same term when it refers to the criteria used for determining the utility of an assay for a specific regulatory purpose. Test materials (unknowns) would be tested in parallel with the positive and negative controls as a single trial of the assay. A trial in which the control values fell outside the acceptable limits would be repeated and the data from such a trial would not be included in the overall evaluation of the test material(s). This avoids the inclusion of spurious data from a trial that is outside the normal limits of the assay (Curren et al., 1995). In some types of assays (e.g., cytotoxicity studies), the negative control is used to normalize the measure of cell viability (e.g., dye uptake) and so the acceptance criteria focus on the performance of the positive control. The positive control should be able to detect over *and* under response. The acceptance criteria are developed once the assay design is finalized, often during a pre-validation phase (Harbell and Curren, 2001). Multiple, independent trials are performed using the positive control to establish the mean and confidence intervals for the endpoint measured. Independent trials are defined as those involving different lots of tissues tested on different days. A common practice is to use the 95% confidence interval (approximately 2 standard deviations) around the mean as the acceptable range for the positive control. Establishment of the mean and confidence intervals should precede the generation of test material data in a validation study. Table 1 shows the

Table 1
Mean response values, standard deviations, and acceptable ranges for those response values for positive controls for EpiDerm, EpiOcular, Corrositex, and Bovine Corneal Opacity and Permeability

Assay	Mean value[a]	SD	Acceptable range mean ± 2 SD	Positive control material
EpiDerm	5.40 h	0.67 h	4.06–6.75 h	1% Triton X-100
EpiOcular	27.1 min	6.0 min	15.1–39.0 min	0.3% Triton X-100
Corrositex	11.55 min	1.25 min	9.06–14.45 min	NaOH (pellet)
Bovine Corneal Opacity and Permeability (BCOP)	105.1	16.6	71.9–138.4	Imidazole

[a] Mean value for the end point(s) measured in each of the assay. For the EpiDerm and EpiOcular assays, it is the time required to reduce tissue viability to 50% of the negative control-treated tissue. For the Corrositex assay, it is the time required for the material to penetrate to biobarrier and interact with the chemical detection system below. For the BCOP assay, it is the In Vitro Score that is the combined value for the opacity and permeability (fluorescein passage) measurements.

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