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Critical Aspects and Challenges Associated With Auditing of Direct Peptide Reactivity Assay (DPRA) and KeratinoSens Assay

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ABSTRACT

The hazard assessment based on data derived from in-vitro studies, to a large extent, is gaining prominence as alternative or supplement to *in-vivo* safety testing. OECD has published advisory for in-vitro Studies to clarify the application of the GLP principles. As GLP Principles require QA to inspect especially the critical phases of a study, it is important that QA should be well aware of what constitute critical phases (and critical aspects) of the in-vitro studies. This poster will discuss and describes the challenges and QA approach applied at GLP facilities for auditing two highly specialized in-vitro studies i.e., DPRA and KeratinoSens assays.

INTRODUCTION

- The DPRA (OECD 442C) is an In chemico assay that models the first key event in the skin sensitization Adverse Outcome Pathway (AOP) - skin, protein reactivity. Compounds implicated in causing Allergic Contact Dermatitis (ACD) are generally electrophilic in nature. This assay identifies dermal sensitizers based on their reactivity with synthetic peptides containing the nucleophilic amino acid residues lysine and cysteine.
- The KeratinoSens assay is a cell-based reporter gene assay that models the second key event in the Adverse Outcome Pathway for Dermal Sensitization, namely keratinocyte activation. This approach measures the induction of luciferase under the control of the antioxidant response element (ARE) derived from the human AKR1C2 gene, and can be used in a Weight of Evidence approach as an alternative to animal testing methods for the purpose of hazard identification.

CRITICAL PHASES IDENTIFIED FOR

DPRA Study	KeratinoSens Assay
Stock solution preparation of peptide solution (test system)	Preparation of cell culture (thawing, maintenance, freezing and cell seeding)
Solubility check of test item	Solubility check of test item
Preparation of HPLC calibration curve	Sample dilution and treatment of cells in plates
Weighing of test item and incubation	Incubation of treated plates
HPLC analysis batch file verification	Luciferase activity measurement
Preparation of co-elution control	Cytotoxicity measurement

CHALLENGES ASSOCIATED TO QA FOR MONITORING OF IN VITRO STUDIES

The monitoring of in-vitro GLP studies requires the auditors to interpret regulations and guidance documents. Emphasis in auditing should be placed on those areas of the in vitro laboratory and assay systems that are critical to control when working with in-vitro systems.

- Test system supplier, receipt, storage, maintenance, sub culturing documentation and other relevant information to identify test system
- Retrieval of the cells from the frozen condition and documentation
- Culture media preparation, lot number/batch number documentation, storage condition and expiration date
- Labelling of test system during storage and use
- Cleaning and decontamination of the facilities and equipment
- Specialized equipment properly maintained and calibration status of the equipment
- Procedure/aspects undertaken to minimize the source of the contamination
- Sterility of the materials/supplies for the cell/media
- Validation status of the software/excel to be used for calculation
- Adequate separation between studies and test system

QA AUDIT APPROACHES

- General documents
  SOP's, Guidelines, Validation protocol, Instrument
- Specific documents
- Study plan, facilities inspection observation, decontamination record of the facilities, test system documentation
- Training and education
- Study Director CV and training, Study Person training, On-job training status
- Checklist
- Study specific check list, auditor designed check list,
- Sponsor specific checklist, risk focused checklist, SOP controlled checklist
- Critical phase audit

ASSAY EVALUATION CRITERIA

It is the role of QA to ensure acceptance criteria for the study and criteria for repetition of the experiment have been met.

DPRA Study

- For SST, the calibration curve should have $r^2 > 0.99$
- Mean peptide depletion for PC should be between 60.8 to 100% (cysteine) and 40.2 to 69% (lysine)
- Mean peptide concentration for reference control A should be 0.50 ± 0.05mM

KeratinoSens Assay

- Gene induction with PC should be above the threshold of 1.5 in at least one of the tested concentration.
- Average gene induction in the three replicates for PC at 64 µM should be between 2 and 8.
- CV of the luminescence reading for the NC should be below 20% in each experiment.

CONCLUSION

QA Auditor should give close attention to

- Correct mathematics in the dose preparation calculations
- Changes in the experimental procedure correctly addressed with proper justification
- Proper documentation of all test item usageage including multiple repeat experiments
- Proper adherence to the study plan with special emphasis to cell exposure and incubation times
- Correct temperature ranges for the incubators, refrigerators, or freezers used in the study
- Qualifications/training of the study personnel performing critical data gathering phases
- Audit trail status for computerized system and correct performance and interpretation of the statistical analysis
- Criteria for a valid test (i.e., positive and solvent controls within the historical ranges stated in the study plan/SOPs), colony counters, image analyzers)