【S-11】

GLP-Application to Cell Culture-Based Toxicity Tests

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Compare to the toxicity tests using experimental animals, the GLP application and compliance in toxicity studies using cell culture systems may be less straightforward and difficult. The efforts to concretize the GLP in cell culture-based studies have been elucidated in the two documents published by the OECD Working Group on GLP "The Application of the GLP Principles to Short Term Studies (1999)" and "The Application of the Principles of GLP to in vitro Studies (2004)". The object of this presentation is to show how to interpret the GLP principles and to apply with actual performances in a well known toxicity test using cell culture, chromosome aberration study. The presentation will cover test substance, test system (cell line), study environment management, documentation, quality assurance, and study protocol and report.

2006 KSOT/KIT Sponsored Symposium

GLP Application to Cell Culture-Based Toxicity Testings

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Toxicity Studies Using Cell Culture

- Genotoxicity Study (Ames test, chromosome aberration test)
- Immunotoxicity Study (Lymphoproliferation)
- Phototoxicity Study (TG432)
- Bioanalytical Efficacy Assay (Cytokine activity measurement)

Key Words: GLP, cell (line), culture conditions, test substance and reagents, 96-well plate

Chromosome Aberration Test Using Cultured Chinese Hamster Lung Cells

in the Template Protocol of FDA

GOOD LABORATORY PRACTICE

A. Is a GLP compliance statement included?

B. Is a quality assurance statement included?

C. Availability and location of original data/test substance

in the Template Protocol of FDA

TEST CELLS IN CULTURE

A. Type of Cells

B. Maintenance of Cell Cultures: If an established cell line or strain was used,
- Was it checked for stability in modal chromosome number?
- Periodically checked for Mycoplasma contamination?

C. Cell Culture Media

in the Template Protocol of FDA

TEST SYSTEM:
Identify the cells used with respect to type, tissue of origin, source, **ATCC designation if available and any other pertinent information** provided. If human lymphocytes were used, describe the donor's sex, health, smoking status, whether whole blood cultures or isolated lymphocytes were used, the mitogen used and any other information provided that helps characterize the cell.

AUTHENTICATION:
Instances of cross-contamination are more common than generally appreciated. Estimates suggest that up to one in five experiments in fields such as cancer and microbiology employ the wrong cells.
Doubling time, karyotype (modal number), *in vitro* age of a cell culture (passage number, population doubling time)

TESTING FOR MICROBIAL CONTAMINATION:
Microplasma, bacteria, fungi, virus

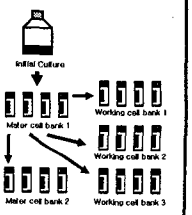
Introduction of Cell Banking System

- Risk of microbial contamination
- Risk of cross-contamination with other cell lines increased
- Loss of characteristics of interest
- Unwanted genetic drift
- Loss of cell line due to exceeding finite life-span

Cell Bank

(ICH guideline Q5D, 1998)

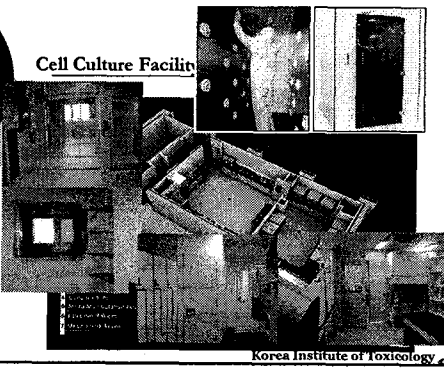
- **Master Cell Bank (MCB)**
  - Single pool of cells which has been prepared from a selected cell clone under defined conditions, dispensed into *multiple containers and stored under* defined conditions.
  - Usually 100 vials (10^7 cells per vial)
- **Working Cell Bank (WCB)**
  - A homogenous suspension of cells obtained from culturing the MCB under defined culture conditions.
  - Usually 100 - 500 vials



Component of Cell Banking System

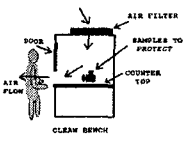
- Cell Culture Facility
- Cell Storage Facility
- Cell Banking Program

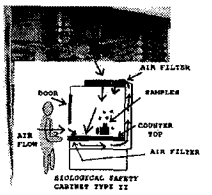
Cell Culture Facility



Cell Culture Facility

- CO_2 incubator
- Clean bench or biological safety cabinet
- Centrifuge
- Microscope



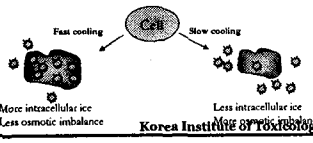


Cell Storage Facility

- Cell line preservation
- Reasons for freezing cells:
  - Genotypic drift due to genetic instability and morphological changes
  - Microbial-contamination
  - Work conducted using cells at a consistent passage
  - Reduced costs (consumables and staff time)

Cryopreservation

Stabilizing cells at cryogenic temperature (storage below -100°C)
Used method for the long-term storage of cells
Critical areas
- Proper handling and gentle harvesting of the cultures
- Correct use of the cryoprotective agent (DMSO, glycerol..)
- A controlled rate of freezing (1°C /min) and thawing
- Storage under proper cryogenic conditions (LN_2 tank, mechanical freezer)



Cell Banking Program

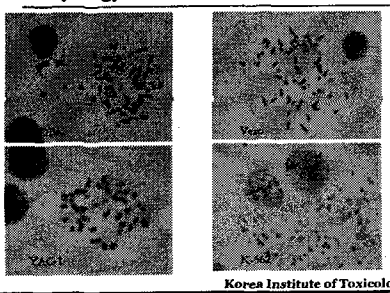
Quality control
- Tests for identity, stability, purity
- Cell environment (including types of culture medium)
- Release of cell banks

Documentation
- Protocols, SOPs, regulations

Periodic controls of the instrumentation

Training of the laboratory staff

Karyology



in the Study Protocol of KIT

4.2 Culture Conditions

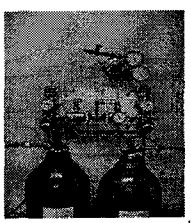
Cells are cultured in Minimum Essential Medium supplemented with sodium bicarbonate (2.2 g/L) , L-glutamine, streptomycin sulfate (100 μg/L), penicillin G (100 units/L) and 10% (v/v) Fetal Bovine Serum (FBS). For routine culture maintenance, the cells are grown as monolayers in T-75 culture flasks and incubated in a humidified atmosphere of 5% CO_2 in air, at 37℃. Cells are subcultured every 2-3 days.

- Preparation and Use Record of the Culture Medium
- Record of CO_2 Concentration and Temperature during the Culture Period

in the Study Protocol of KIT

7. Metabolic Activation System: S-9 Mix

- S9 fraction: Inducer, Animal induced (strain, sex, age), Tissue
- Cofactor I
- COA



in the Template Protocol of FDA

TEST and CONTROL SUBSTANCES

TEST SUBSTANCE
- Chemical name:
- CAS registry name:
- CAS registry number:
- Structure
- Molecular weight:
- Source/batch/lot no:
- Purity:
- Impurities:
- Physical description:
- Stability:
- Storage conditions:

TEST SUBSTANCE as administered
- Vehicle used:
- Tested adequately for concentration?
- Tested for stability?
- Tested for homogeneity?
- Problems with storage?

in the Template Protocol of FDA

Dose Levels (Test Substance):

- Was the highest concentration tested based on solubility characteristics or was it the limit concentration for this test method (e.g., 5000 μg/mL or 10 mM)?

-Any test substance induced pH and/or osmolality changes of treatment medium?

- Summary:

Preliminary Cytotoxicity Test	Cytogenetic Test
Without activation: Concentration(s)	Without activation: Concentration
With activation: Concentration(s)	With activation: Concentration

-Comments: Summarize the results if test material solutions were analyzed for stability, homogeneity, concentration, etc. For example "Test material solutions (give concentrations) were analyzed using HPLC with UV detection and found to be within 5% of the nominal values". "Test material solutions were stable for the duration of the study".

Actual GLP-Compliant Study

Study Protocol

7.2. Preparation of Test Substance
The mixture of the test substance with vehicle will be prepared daily and used within 4 hours
7.3. Administration of Test Substance
The vehicle control and test substance will be added into the culture medium.
7.4. Dose Levels: 100, 200, 400, 800 μg/mL

Storage → Balance → Preparation → Administration

Actual GLP-Compliant Study

Study Protocol

시험계획서에 따라 시험계에 100, 200, 400, 800 μg/mL로 투여되었음을 증명

시험물질 특성 (시험책임자)
- 시험물질 유효기간: 시험계획서 작성시 보관조건(냉장)에서 시험물질 마지막 조제시까지 안정

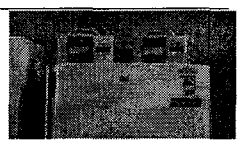
- 시험물질의 순도 (또는 농도): 순도가 80%라면 100%로 보정하여 처리 → 125, 250, 500, 1000 μg/mL로 처리

Storage → Balance → Preparation → Administration

Actual GLP-Compliant Study

경보시스템의 Validation

온도범위 이탈시 경보시스템 → 경보시의 대처방안



보관기간 동안 온도(2-6℃) 이탈여부 (온도측정기록) → 온도계 → 온도계의 정확성여부 → 정기적으로 표준온도계와 비교 후 보증

Storage → Balance → Preparation → Administration

Actual GLP-Compliant Study

표준분동을 사용한 저울의 정확성 보증

자율 작동의 정확성여부 → 분동 → 표준저울을 사용한 보증

Storage → Balance → Preparation → Administration

Actual GLP-Compliant Study

Valid 한 분석법이 있는 어느 곳에서나 분석가능;
다만 반드시 조제시험물질 transfer의 기록이 필요

분석법 Develop 및 Validation ← 조제물의 함량, 균질성 및 안정성 분석

100 μg/mL

시험물질의 특성에 따른 조제법 선정 → 조제량 및 조제시간 등 조제기록

Storage → Balance → Preparation → Administration

Actual GLP-Compliant Study

Quality Assurance

투여 전/후
조제 시험
물질량 측정

투여량 및
투여시간
기록

투여량계산

계획서이탈사항은
반드시 시험책임자의
지시사항에 따라 조치

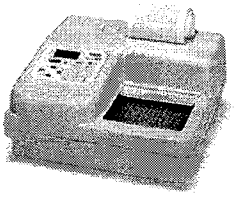
실시간 입력 및
변경사유 명기

Culture volume

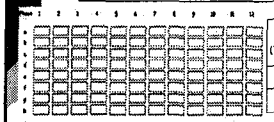
Storage → Balance → Preparation → Administration

Actual GLP-Compliant Study

96-Well Plate



Administration of Test Substance



Administration (Test Substance)	Time (00:00)	Performer	Study Director
Start			
End			

A1-A3: Blank A4-A6: " A7-A9: A10-A12:
B1-B3: Substance 1 B4-B6: " B7-B9: B10-B12:
C1-C3: Substance 2 C4-C6: " C7-C9: C10-C12:
D1-D3: " 3 D4-D6: " D7-D9: D10-D12:
E1-E3: " 4 E4-E6: " E7-E9: E10-E12:
F1-F3: " 5 F4-F6: " F7-F9: F10-F12:
G1-G3: " 6 G4-G6: " G7-G9: G10-G12:
H1-H3: " 7 H4-H6: " H7-H9: H10-H12:

MINIMUM REQUIREMENT FOR GLP-COMPLIANT STUDY

- SOP
- Study Protocol
- Quality Assurance Department

PREPARATION OF STUDY PROTOCOL & REPORT

TO BE DETERMINED

- Test System
- Administration Route
- Administration Period
- Dose Level

Amount of Test Article needed

- Test Article Preparation
- Analytical Method

***Analytical* Laboratory Schedule**

- Examination Items
- *Guidance To Comply With*

Animal Purchase
Animal Room
Necropsy
Pathology
Ophthalmoscopy

REPORT SCHEDULE

IN STUDY PERIOD

- STUDY BRIEFING: Animal Room, Pathology Lab, Formulation Lab (Before or During Acclimation)
- CHECK EVERY PART OF STUDY: Test Article, Test System, Housing, Acclimation, Formulation, Dosing, Observation, Necropsy, Hematology, Chemistry, Pathology, Archiving, QC (Path/Tox System or In Person)
- PROTOCOL AMENDMENT & DEVIATIONS

IN STUDY PERIOD

- WRITING DRAFT REPORT: Collecting Information and Documents, Tables and Appendices, Pathology Report, Analysis Report, Review by Other Study Director
- QAU EXAMINATION
- SUBMISSION OF DRAFT REPORT to Sponsor
- SUBMISSION OF FINAL REPORT to Sponsor

POST STUDY PERIOD

- Maintenance of Archive
- Raw Data Amendment
- Study Report Amendment
- Study Audit by Regulatory Authorities
- Termination of Archiving

CONCLUSIONS:

OBJECTIVES OF PRECLINICAL TOXICITY STUDIES

- Identify Adverse Effects and Target Organs
- Reversibility
- Dose Response and Mechanism
- Safety Margin
- Determination of Dose Level for Clinical Trials

감사합니다.