

# The Presence of Drug in Control Samples During Toxicokinetic Investigations – A Novartis Perspective

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# The Presence of Drug in Control Samples During Toxicokinetic Investigations – A Novartis Approach

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# Background Information

What is the problem?



Blood or tissue samples from control animals in toxicology studies show a sporadic incidence of test item concentration

## Background Information

- Animals in treated groups are dosed with high concentrations of test item in the same animal room which houses the control group
- Analytical methods have considerably improved >1000 fold over the last ten years
- Sample quality is a complex and multidisciplinary issue

# Working Groups

## Novartis approach:

- Two teams were launched (one in US, one in Europe)
- Teamleaders: QA heads
- Team members from Test Article Formulations, Toxicology, Pathology, Bioanalytics
- Teams met regularly
- Common team goal

# Working Groups

## Main activities:

- Definition of preventive measures against cross-contaminations
- Contacts and knowledge transfer with CROs and other pharmaceutical companies
- Literature search
- Listing of all studies with TK analysis including any contamination issues
- Decision tree in case of contaminations
- Study with the aim to assess the new insights
- Regular information to management

# Working Groups

Preventive measures, a few examples:

## General issues

- Cleaning procedure for equipment and material enforced
- Protective clothing changed when control materials or animals handled after treated materials or animals
- Separate equipment for control animals (e.g. balances, pipettes, necropsy instruments, anaesthesia boxes)
- Color coding for dosing formulation containers/syringes, glassware, and animal room material

# Working Groups

Preventive measures:

## Test article formulation:

- Complete separation of control and test item formulations at all stages (separate rooms, equipment, materials)
- Control and test item formulation samples taken for analysis on the same day as TK blood collection



# Working Groups

Preventive measures:

## Toxicology:

- All study activities performed first for controls and then in ascending dose group order
- Control samples processed at a separate dedicated workstation from treated samples
- Dedicated areas within freezers for control samples

# Working Groups

Preventive measures:

Pathology (for collection of tissue samples):

- Separate area identified for isolation of control animal necropsies
- Separate containers for storage and shipment of control samples to the analytical laboratory

# Working Groups

Preventive measures:

## Bioanalytics and pharmacokinetics:

- Control samples processed separately
- Only one control sample opened at any time during processing
- Separate analysis of control samples: one blank placed before and after control samples in the analysis equipment
- Criteria for the relevance of contamination developed

# Study

2-week in feed (powdered diet) methodological study in male mice with AFY861

- Study design with three rooms
  - One control group alone in a separate room (1)
  - One control group in the same room as treated animals but on different racks (2)
  - One control group in the same room and on the same battery as treated animals (3)
  
- In room 1 and 2 the new procedures were applied
- In room 3 the traditional procedures were applied



# Study

## Results

- No contamination in plasma and tissue samples from control animals in room 1
- Plasma samples were also essentially free of contaminations in rooms 2 and 3
- Tissue samples taken from control animals were contaminated to a minimal degree in room 2 and to a moderate degree in room 3
- Fur samples from control animals were not contaminated in room 1 and 2 and to a moderate degree in room 3

# Study

## Swab analyses:

- Swab samples were taken from cleaned equipment and material in the test article formulation area, animal room and necropsy laboratory

# Study

## AFY861 concentrations in swab samples

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Area/equipment (after cleaning if appropriate)	Mean concentration in two samples (µg/swab)
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### ***Test article formulation***

Spatula	0.0099
Balance – inside	0.874
Worktable	0.167
Mortar and pestle	0.525

### ***Animal room***

Table (animal room)	0.113
Table (blood sampling room)	0.000
Balance	0.0065
Centrifuge inside	0.0069
Blood pot (outside)	0.0177
Treated cage food container	0.0408
Overall <sup>a</sup>	0.486
Shoes <sup>a</sup>	0.213
Gloves <sup>a</sup>	2.31
Hands after blood sampling	0.0085
Face after weighing animals <sup>a</sup>	0.171
Air filter <sup>a</sup>	2.96

### ***Necropsy***

Scissors	0.063
Scalpel	0.000
Anesthesia box	0.055
Necropsy table	0.000

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LLOQ: 0.0100 µg/swab

<sup>a</sup> Not cleaned

# Study

## Conclusions from analyses of swab samples:

- Many areas were free of contaminations
- In test article formulation laboratory relatively large amounts of substance detected
- Overalls, shoes and gloves after working in animal room contained significant amounts

This emphasizes the necessity of exchanging clothes

- The amount of substance on the filters shows that the potential for air-borne contamination could be clearly demonstrated



# The Role of Good Laboratory Practice

Key question:

Can a study with positive control samples still be a  
GLP study?

# The Role of Good Laboratory Practice

What is GLP?

A quality system concerned with the organizational process and the conditions under which studies are planned, performed, monitored, recorded, archived, and reported (OECD 1998)

# The Role of Good Laboratory Practice

GLP compliance does not assure:

- that the scientific design of a study is sound
- that SOPs or analytical methods are scientifically adequate
- Analyses are appropriate
- cannot decide whether the occurrence of positive control samples invalidates a study

→ this is a scientific judgment

# The Role of Good Laboratory Practice

Can compliance with GLP prevent contamination of control samples?

Not necessarily!

But GLP provides a set of principles that may reduce the potential for contaminations and may help to find reasons for any occurrences during study conduct.

# The Role of Good Laboratory Practice

GLP principles that may help to reduce contaminations:

- SOPs for all major activities that are followed
- Training records for all involved individuals
- Direct and prompt records
- Records are maintained (archives)
- Quality Assurance Unit

# The Role of Good Laboratory Practice

Records are necessary for the reconstruction of the study  
Sometimes they can identify:

- Mix-up of animals
- Mis-dosing of animals
- Mis-calculations of test item

→ In vivo contamination (not very often the case)

# The Role of Good Laboratory Practice

What about ex vivo contamination?

Can happen everywhere:

- Handling of animals
- Blood collection
- Processing of samples
- Storage and shipment of samples
- Analyses
- Due to specific substance properties

Not easy to reconstruct with the help of records  
(SOPs, methods, Quality Assurance may help)

# The Role of Good Laboratory Practice

Role of Study Director and/or Principal Investigator if contamination occurs:

- Evaluate records, search for reasons
- Report any issue in final report and address the impact on study integrity



# EMA Guideline on Positive Controls

## Scope

- Guidance how to assess the level of test item in samples from control animals, how to report such findings and how to assess the impact in the validity of the studies

## Study Types

- All pivotal studies with TK evaluation need to be analysed (irrespective of the route of administration)
- In non-rodent studies: control samples should be collected and analysed the same way as treated samples
- In rodent studies: in at least the proximity of T<sub>max</sub> of the test item

# EMA Guideline on Positive Controls

## Consequences of contaminations

- Trace levels **below the LLOQ!!** may be considered as nonrelevant
- Invalidation of study depends on extent of contamination, impact on the validity of statistical analysis, definition of safety margins and reliability of animal exposure
- **The sources of contamination should be investigated and identified**
- Clarify whether contamination occurred *in vivo* or *ex-vivo* (i.e. check control tissues for test item, identify metabolite in control plasma, detect antibodies against test item for biotechnology products)
- Contaminations in control samples should be reported and discussed in final reports and in summaries for regulatory authorities (frequency, pattern and magnitude of contaminations)

## Summary

- Positive controls are frequently observed in toxicokinetic investigations
- Analyses have shown that strict procedures for the handling of test items, animals and samples may prevent or at least diminish contaminations of control samples
- GLP may help to reduce the occurrence of positive control samples, but cannot totally avoid it
- The EMEA guideline provides information how to proceed, but adherence can only partly be achieved