

# TT24: Tissue analysis

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## Background and Aim

- Background – tissue (homogenate) analysis is/has:**
- Hot topic in many BA labs
  - Continued scientific challenge to perform in correct/smart/efficient
  - Ambiguous regulatory requirements (GLP or not?)
  - Risk of over-validation

EBF community committed to share experience, engage with HA and provide recommendation on scientific/process

## The survey

- 57 Questions divided into following chapters
- General questions
  - Qs around quality and GLP status of analysis
  - Qs on origin and types of tissue and reason for analysis
  - Qs related to tissue sampling
  - General Qs related to Method Establishment (ME)
  - Method specific Qs related to ME
  - Qs related to protocol and reporting

## Team Members

- Nathalie Mokrzycki (MSD)
- Irene Lentheric (Harlan)
- Pascal Delrat (Servier)
- Pawel Dzygiel Roche)
- Morna McIntosh (CRL)
- Marc De Meulder (Janssen R&D)
- Eva Erbach (Bayer)
- Philip Timmerman (Janssen R&D) – team sponsor

## Survey results

### Introduction/general questions

**Q1: Does your lab perform tissue analysis?**

Answer Options	Response
Yes, go to Q2	16
No or rarely but I will give my opinion or share previous experience, go to Q2	7
No and I cannot give my opinion. In this case the survey is finished for you.	7
<b>Total responders</b>	<b>30</b>

**Q2: How often does your lab perform tissue analysis?**

Answer Options	Response
Weekly	5
Monthly	2
Sporadically	14
Other (please specify)	2

**Q3: Does your lab analyze small molecules or large molecules?**

Answer Options	Response
Small molecules using LC-MS/MS	21
Large molecules using LBAs	5
Large molecules using LC-MS/MS	4

### Question around quality and GLP status of analysis

Our interpretation of GLP in the context of the continuation of this survey: "Any analysis of study samples which is performed using an a priori developed and validated method, with acceptance criteria predefined in an SOP (preferably in line with the BMV, but can be different from acceptance criteria for plasma as mentioned in BMV) and for which the process from sampling to analysis and reporting has been performed in compliance with OECD-1, 8, 13 and 15 principles (including QA oversight).

**Q4: We agree with the above definition :**

Answer Options	Response
Yes, and we apply it like this	14
Yes, but currently we apply it differently	5
Other (please specify)	4
<b>Total responders</b>	<b>23</b>

**Q5: In our lab, we apply following regulations when performing tissue analysis**

Answer Options	Response
Fully GLP	4
According to GLP principles i.e. same quality but non-GLP	3
Using the principles of qualified assays	9
A mixture of above 3 depending on stage of development	4
Other (please specify)	3

**Q6: If you give GLP status, is it the same for all tissues in a particular study?**

Answer Options	Response Percent
Yes	71%
No	29%

**Q7: What is the driver to perform analysis under GLP conditions?**

Answer Options	Response Percent
The data are generated as part of a GLP study	73%
At customer request	60%
The data are used on pivotal decision other than safety	7%
Driven by the Drug Development stage	7%

**Q8: Does your company have experience with filings of studies including tissue analysis to authorities**

Answer Options	Response Percent
Yes	38%
No	62%

**Q9: n case of compound filing, are all tissue analysis done under GLP status**

Answer Options	Response Percent
Yes	23%
No	77%

**Q10: Did you get questions from the agency on the status of study sample analysis and the method validation (GLP or Non-GLP or others)**

Answer Options	Response Percent
No	94%
Yes, because the authorities didn't agree with the non-GLP status of tissue analysis performed	0,0%
Yes, because the authorities didn't understand the details of tissue analysis performed	6%

**Q11: What are the 3 most abundant type of tissue analyzed?**

Answer Options	Drug Discovery phase	Drug development	Response
Liver	11	12	15
Brain	16	14	18
Lung	6	6	9
Kidney	3	2	4
Heart	4	4	5
Muscle	1	0	1
Tumor	5	2	5
Other (please specify)			7

**Q12: What are the 3 main reasons for tissue analysis?**

Answer Options	Drug Discovery phase	Drug Development phase	Response
PK (Distribution or Tissue ratios	15	13	19
PD (for PK/PD assessment)	14	9	14
Tox/TK (target tissue, TK/TD assessment)	11	16	19
I don't know	1	1	1
Other (please specify)			0

**Q13: From which species do you analyze tissues, indicate 3 most frequent**

Answer Options	Drug Discovery phase	Drug Development phase	Response
Mouse	16	12	18
Rat	17	17	21
Dog	6	12	12
Non human primates (NHP)	2	7	7
Human	0	3	3
Other (please specify)			3

### Questions related to tissue sampling

**Q14: For small animals (mice, rat), how do you manage to take a homogenous sample from the tissue you need to analyze?**

Answer Options	Response Percent
We prefer to take the whole tissue	73%
We combine subsections from the tissue prior to homogenization	5%
We take subsections from the tissue and do not bother on potential regional concentration differences in 1 tissue	9%
Other (please specify)	14%

**Q15: For larger animals (dog, NHP, other), how do you manage to take a homogenous sample from the tissue you need to analyze?**

Answer Options	Response Percent
We prefer to take the whole tissue	22%
We combine subsections from the tissue prior to homogenization	28%
We take subsections from the tissue and do not bother on potential regional concentration differences in 1 tissue	33%
Other (please specify)	17%

### Questions on origin and types of tissue and reason for analysis

**Q16: Do you prefer to have the tissues homogenized at the in-life facility?**

Answer Options	Response Percent
Yes	14%
Yes, and we recommend/guide the tissue sampling procedure to the laboratory where the in-life part of the study is performed	14%
No, we make sure tissues are homogenized by the BA lab staff	64%
Other (please specify)	9%

**Q17: In your lab (or the lab you work with), do you use automated tissue homogenization equipment**

Answer Options	Response Percent
Yes	32%
No, each tissue is homogenized individually and manually	59%
Other (please specify)	9%

**Q18: Do you prefer to have the tissue homogenized immediately after harvesting?**

Answer Options	Response
Yes, for convenience	3
Yes, we feel this is a scientific requirement	2
Yes, but we have difficulties in managing the logistics of this preferred process	3
Yes, but only for soft tissue. Less a requirement for hard tissues	0
No, for convenience, we prefer to freeze the tissue prior to homogenization	11
No, for scientific reasons, we prefer to freeze the tissue prior to homogenization	2
Other (please specify)	1

**Q19: In case fresh tissue is frozen prior to homogenization, what is your preferred process of harvesting/freezing?**

Answer Options	Response Percent
After harvesting (or biopsy), tissue is washed, dried and stored in the freezer (normal speed)	32%
After harvesting (or biopsy), tissue is washed, dried, snap frozen (e.g. -80, liquid N2,...) and stored in the freezer	26%
After harvesting (or biopsy), tissue is washed (e.g. PBS) and stored in the freezer normal speed	16%
After harvesting (or biopsy), tissue is washed (e.g. PBS) snap frozen (e.g. -80, liquid N2,...) and stored in the freezer	16%
Other (please specify)	11%

**Q20: What is your preferred technique for tissue homogenization (large animal / large tissues)?**

Answer Options	soft tissue	hard tissue	Response
Mechanical (e.g. grinding, blending, Sonication, Bead-beating...)	19	17	21
Chemical (Saponification...)	0	1	1
Biochemical (Enzymatic treatment...)	0	1	1
Other (please specify)			0

**Q21: What is your preferred technique for tissue homogenization (small animal)?**

Answer Options	soft tissue	hard tissue	Response
Mechanical (e.g. grinding, blending, Sonication, Bead-beating...)	20	17	22
Chemical (Saponification...)	0	1	1
Biochemical (Enzymatic treatment...)	0	1	1
Other (please specify)			0
<b>Total responders</b>			<b>22</b>

**Q22: What is your preference for the sample shipment?**

Answer Options	Response Percent
Tissue samples (non-homogenized) shipped on dry ice	68%
Tissue homogenates shipped on dry ice	27%
Other (please specify)	5%

**Q23: How do you store your tissue samples or tissue homogenates?**

Answer Options	Response Percent
-20°C	23%
-80°C	46%
One of the above depending on compound or tissue	32%
Other (please specify)	0%

**Q24: Do you have different procedures for harvesting/homogenization of small or large molecules?**

Answer Options	Response Percent
No, we have the same procedures	88,2%
Yes, we have different procedures depending on compound or tissue. Please describe the differences.	11,8%

**Q25: In case you analyze large molecules in tissues, do you add protease inhibitors during sample preparation?**

Answer Options	Response Percent
Yes	54,5%
No	45,5%

### General Questions related to Method Establishment (ME = the combination of method development followed by method qualification or method validation)

**Q26: Do you use a generic approach to analyze tissue samples?**

Answer Options	Response Percent
Yes - go to question 27	85%
No - go to question 28	15%

**Q27: In Drug Discovery (pre-GLP), what does your generic approach look like?**

Answer Options	Response Percent
I can not share	11%
We use the method which is in place for plasma and just apply it for tissues, by making only small changes to the sample preparation	72%
We use a generic process which looks like this: please try to summarize approach in 4 lines in the box below for sample homogenization and extraction and for Chromatography (if LC-based) and/or detection	17%
Sample homogenization and extraction / Chromatography and or detection	

**Q28: In Drug Discovery (pre-GLP), if you don't use a generic approach, how do you establish your assays?**

Answer Options	Response Percent	Response
A different assay is established per tissue and per compound	25%	1
A different assay is established per compound, but we don't consider different tissue types when developing the assay	75%	3
Other (please specify)	0%	0
<b>Total responders</b>		<b>4</b>

**Q29: In Drug Development, do you use a generic approach to analyze tissue samples?**

Answer Options	Response Percent	Response
Yes - go to question 30	45%	10
No - go to question 31	55%	12
<b>Total responders</b>		<b>22</b>

SURVEY CONTINUES ON ADJACENT POSTER