Olink®
Accelerating proteomics together

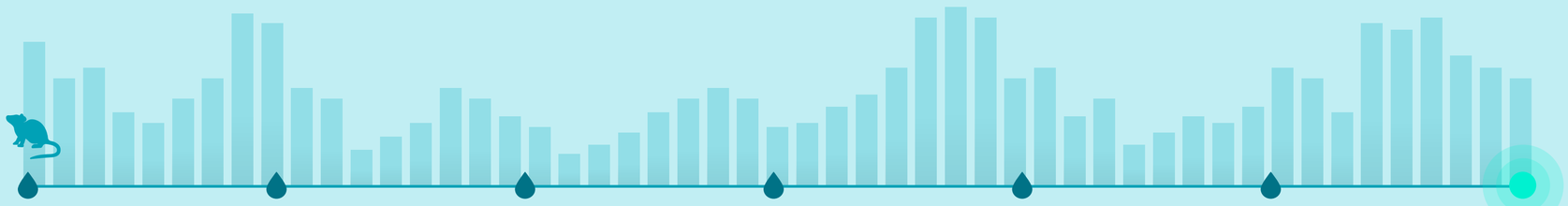
Designing Non-Terminal Longitudinal Mouse Studies

A 7-step checklist to track circulating biomarkers using only 1 µL of sample volume

Deeper insights from true longitudinal studies

Using just 1 µL of sample for comprehensive cytokine profiling significantly advances biomarker analysis, enhancing consistency in longitudinal mouse studies and adhering to ethical standards for minimal animal use. This method offers researchers flexibility in selecting specific time points, allowing for more frequent and nuanced observations of cytokine changes. This small volume profiling technique is a transformative tool for preclinical research, combining technical innovation with ethical consideration.

To harness the potential of Olink's groundbreaking technology, we've outlined a 7-step checklist.



From Research Question to Data Analysis

Step 1
Define Research Question

1. Clearly define research question to inform all subsequent steps of experimental design and study execution.
2. Consult the 3Rs (reduce, replace, refine) for humane animal research during study planning.
3. Select mouse model based on literature, repositories, or by creating a new *in vivo* model tailored to the disease or biological process of interest.
4. Determine relevant measurement timepoints and the study's endpoint.

Step 2
Select Cohort and Sample Size

1. Consider mouse strain, genetic background, age, and sex for cohort selection and consistently use the same sub-strain to ensure genetic uniformity and data reproducibility.
2. For mutant mouse strains, select suitable controls, including both sexes in each group to enhance disease modeling, explore sex differences, and minimize breeding costs.
3. Perform power analysis for sample size determination and assess effect size via literature review or pilot studies.
4. Implement randomization at all stages of experimental design to evenly distribute remaining variations among study groups.

Step 3
Sampling Method and Baseline Measurements

1. Report each study group's baseline characteristics (weight, microbiological status) pre-treatment.
2. Choose non-terminal, minimally invasive sampling methods to preserve the longitudinal design.
3. Use non-surgical blood methods like tail or saphenous vein sampling. For frequent, small samples, consider microsampling.
4. Document adverse events, mitigation measures, and any bias introduced during treatment or sampling.

Step 4
Determine Timepoints

1. Consult timepoints proposed through literature review and study conceptualization, and run pilot studies to gauge optimal intervals.
2. Consider mouse lifespan, circadian rhythm and disease progression dynamics.
3. Opt for frequent intervals to capture rapid fluctuations and rich data detail; use longer intervals for overarching trends.
4. If appropriate, use counterbalancing to prevent sequence or time-dependent bias.

Step 5
Biomarker Analysis

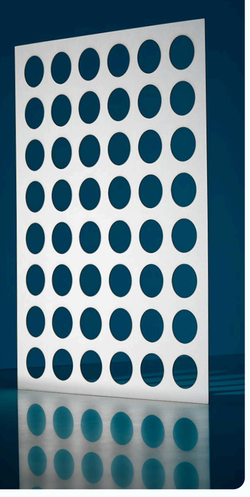
1. Select biomarkers that reflect relevant pathways.
2. Opt for platforms that require minimal sample volumes yet deliver comprehensive insights with optimal performance.
3. Identify factors affecting biomarker levels, considering the impact of time, temperature, haemolysis, anticoagulant type and minimum sample volume.
4. Apply standardized protocols of sample collection, storage and processing.

Step 6
Interim Data Analysis

1. Analyze the data at interim periods to adjust, if needed, or to make preliminary interpretations.
2. Identify potential issues with methodology and, if needed, correct protocols.
3. Adjust timepoints and sample size if needed.
4. Check model assumptions such as linearity, normality, and homoscedasticity. Use diagnostic plots and tests to assess model fit.

Step 7
Data Analysis

1. Choose statistical models based on research objectives, data characteristics, and assumptions. Mixed effect models are a good choice to capture group-level trends and individual variations in longitudinal data.
2. Identify the nature of eventual missing data (random, completely random, or not) to select the right imputation method.
3. Determine random effects and specify fixed covariates, including time-varying predictors.
4. Ensure errors arising from multiple comparisons are controlled (Tukey, Bonferroni, or Holm's procedures.)



Challenges and Opportunities

Challenges of longitudinal studies	Considerations	Proposed Solution
Time and Cost	Balance between getting comprehensive data and resources.	Use only 1 µL of sample volume to track the same mouse over various time points, aiming to decrease the required number of animals for your studies and achieve significant cost savings.
Inter-subject Variability	Even genetically similar mice can have individual differences.	Reduce effect of inter-individual variability by focusing on the same animal over time.
Ethical Concerns	Secure ethical committee approvals, monitor animal welfare and work towards the 3Rs (Reduce, Refine, Replace).	Use just 1 µL of sample to adopt minimally invasive sampling methods to reduce animal stress and reduce need for animals.
Operational Consistency	Set up SOPs, periodic training, use reliable method with minimal hands-on operation.	Employ extensively proven and validated assays.
Reproducibility	Ensure study designs and results are reproducible.	Tracking the same mouse over time provides more consistent data. Opt for robust assays that are accurate and precise

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