Methodology for Health Monitoring of Mice Maintained in IVCs

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Tasks of the Working Group (WG)

- Give recommendations for the health monitoring (HM) of mice maintained in IVCs

Recommend **practical definitions of microbiological units** for IVC husbandry

Review sampling and detection methods

Evaluate different strategies with special regard to the **3R's and research validity**

Discuss advantages and disadvantages of different samples and detection methods

Support persons responsible for HM programs to establish and adapt their existing HM

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Members of the WG

- Convenor
  Isabelle Goncalves da Cruz (AFSTAL)
- Members
  Marion Berard (AFSTAL)
  Ferdinando Scavizzi (AISAL)
  Stephanie Buchheister (GV-SOLAS)
  Arthur Humbert (SGV)
- Corresponding member
  Lorna Cleverley

AISAL

afstal

SGV swiss laboratory animal science association

GV-SOLAS
Gesellschaft für Versuchstierkunde
Society for Laboratory Animal Science

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https://www.afstal.com/ https://sgv.org/de https://www.aisal.org/ https://www.gv-solas.de/

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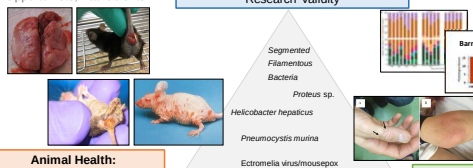
Health Monitoring (HM)

Pathogens/Subclinical Infections
Opportunists, Pathobionts

Standardization:
Definition of the Hygienic Status, Research Validity

Individual Study Confounders
Microbiome



Animal Health:
Exclusion of Pathogens/Clinical Disease

Biosafety:
Exclusion of Zoonotic Agents

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Held et al. 2011, Laboratory Animals 2011; 45: 276–279 DOI: 10.1258/la.2010.010150
The Laboratory mouse, Hans J. Hedrich, Second Edition,
Open Forum Infect Dis. 2017 Mar 1;4(2):ofx038. doi: 10.1093/ofid/ofx038. eCollection 2017 Spring.
Mähler et al. 2002. Inflamm Bowel Dis. 2002 Sep;8(5):347-55.
Slack et al. 2020; Front Microbiol. 2020 Jan 10;10:2999. doi: 10.3389/fmicb.2019.02999.

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Definition of the agents

Panel of agents tested have to be individually defined, based on:

- Existing FELASA recommendations (which is not an exclusion list!)
- Risk of agent introduction and relevance of agents: Individual considerations
  - Facility management/purpose of units/immune status of animals
  - Improvement of the microbiological quality during the last decades
  - Relevance of commensal bacteria and the microbiome
- Recent Updates
  - Changes in nomenclature/reclassifications (e.g. *Pasteurellaceae*)
  - Novel agents (e.g. Mouse Kidney Parvovirus, Murine Astrovirus)

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Mähler et al., 2014, *Laboratory Animals*. 2014;48(3):178-192

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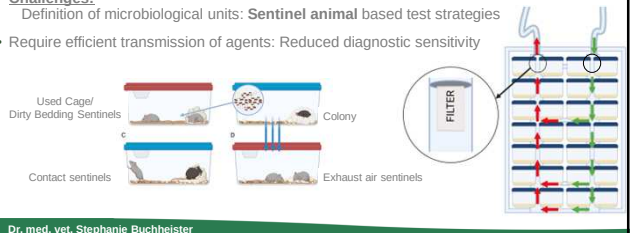
Individually Ventilated Cages (IVCs)

- Equipment is designed to prevent (reduce) the spreading of infectious agents
- **Challenges:**
  Definition of microbiological units: **Sentinel animal** based test strategies
- Require efficient transmission of agents: Reduced diagnostic sensitivity



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Doctoral Thesis Lena Brix, TiHo Hannover, 2023
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Environmental Sampling Strategies

- Molecular methodology enables the use of environmental sampling strategies

e.g. cage feces, cage swabs, bedding/nesting material, **exhaust air dust (EAD)**

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Comparison of Strategies

100% animals sentinels + colony animals	Mix of animals and environmental	100% environmental
use of a large number of dedicated animals		no animals used
questions of suitability of strains (outbred vs. inbred vs. immunodeficient)	Good compromise? Complex strategies required!	no questions about strains
various diagnostic methodology		molecular methodology only
diagnostic sensitivity varies		improved diagnostic sensitivity
all known agents can be tested		not validated for all agents, yet
control for false negatives		control for false positives

Always perform follow up diagnostic of sick animals

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Choice of the best method (for each agent)

Agent	Animal/Sentinels	Method	Environmental	Method
Murine Norovirus	Yes, bedding sentinels, Dubelko 2018 Yes, bedding sentinels, Hanson 2021 No, bedding sentinels, Miller 2018 Yes, bedding sentinels, O'Connell 2021 Less efficient, bedding sentinels, Zorn 2016	Serology PCR	Yes, filters, Dubelko 2018 Yes, media in soiled bedding, Hanson 2021 Yes, filters, O'Connell 2021 No, exhaust debris, Bauer 2016 Yes, EAD, Pettan-Brewer 2020 Yes, EAD, Zorn 2016	PCR (NGS)
Rodentibacter sp.	Yes, bedding sentinels, Dubelko 2018 No, bedding sentinels, Miller 2018 Less efficient, bedding sentinels, Miller 2016 Yes, bedding sentinels, Roegener 2018	Culture PCR	Yes, filters, Dubelko 2018 Yes, exhaust debris, Bauer 2016 Yes, EAD, Mahabir 2019 Yes, EAD, Miller 2016	PCR (NGS)
Ectoparasites	Yes, bedding sentinels, Gerwin 2017 No, bedding sentinels, Hanson 2021 Yes, bedding sentinels, De Bruin 2016 No, bedding sentinels, Miller 2018 Yes/No, bedding sentinels, Korner 2019	Microscopy PCR	Yes, Filter top, Gerwin 2017 Yes, Media in soiled bedding, Hanson 2021 Yes, EAD, Körner 2019 Yes, exhaust debris, Bauer 2016	PCR (NGS)

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Optimizing sampling

- **False positive** and **false negative** results
- **Quality** of samples, **number** of samples
  - When **pooling**, keep in mind:
  - Pathogen dilution might cause false negative results
  - Only reasonable for animals that are representative from the same microbiological unit
  - Seek advice from the testing lab to match the requirement of diagnostic methods
  - **= balance between cost and quality**
- **Reduction** or **Replacement** of animals; **Refinement** and welfare of sentinels
- **Storage and transport** of samples

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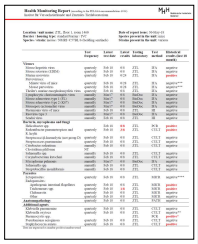
About the results

- What to do when there is a positive?
  - a) Confirm result – re-testing
    infectious agents vs. residual nucleic acids
  - b) Isolate the contaminated microbiological unit quarantine measures
  - b) Decide about the fate of the colony
    re-derivation vs. termination
- Communication of the results!
  - Proper description of HM concepts and methodology in health reports
  - Suspicious results should be reported, comments on measures taken



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Summary

- **HM** of mice maintained **in IVC** is **challenging**
- **Microbiological units** have to be defined based on **husbandry practices** and **sampling approaches**
- **Environmental sampling strategies** are used to **improve animal welfare** and diagnostic **sensitivity**
- Environmental sampling strategies **have limitations** and require **critical result interpretation**
- Optimize sampling procedures and do "not forget the animals"!
- Timeline: Currently writing of the paper, **publication expected early 2024**

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Thank you!
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