

Institutional protocols for the oral administration (gavage) of chemicals and microscopic microbial communities to mice: Analytical consensus

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Impact statement

Institutional protocols designed for the oral administration of live microbial communities, either complex or microscopic (microcosmic), to mice do not exist. However, this approach is increasingly employed by investigators focusing on the gut microbiome in experimental research. Herein, we propose two analytically Kappa-based consensus protocols to promote reproducibility and standardization in research practices and describe biologically relevant factors in achieving optimal microbial engraftment of communities in germ-free mice.

Abstract

Although there are numerous Institutional Animal Care and Use Committee (IACUC) protocols aimed at standardizing the oral administration (gavage) of liquid chemicals to laboratory rodents, there is ample variability across protocols, and there are no protocols intended for the gavage of microbial communities to mice or rats. The objective of this study was to conduct a scoping review of publically available IACUC protocols from institutions in the United States, identify protocol criteria and deficiencies, and generate an 'analytical consensus' to unify such criteria into two revised protocols: one for chemicals, and another for microbes in mice. Eighteen ($n = 18$) written institutional protocols from prominent universities, and 26 demonstrations videos from various sources (accounting for >155,000 views) were identified in the World Wide Web. Although written protocols listed up to five criteria for consideration (dosing/volume, pregnancy, animal weight), collectively there was

major variability and poor statistical agreement across methods (0% Kappa, $p = 0.98$). Because protocols also lacked details relevant for the bypassing and survival of live microorganisms in the gastric (antimicrobial) environment, we compiled two 'analytical (Kappa-based) consensus' protocols from available and new criteria, for the 'gavage of chemicals' and for the 'gavage of delicate microscopic microbial communities' to mice. A major difference lies in the volume of administration, (i.e., 20 mL/kg, without restrictions, after 4–6 h of fasting, for microbes), which was graphically illustrated simulating the predicted impact of administering large and small volumes on microbial distribution of simple microscopic communities. In conclusion, publicly available IACUC gavage protocols are highly variable, and do not reflect the need to adjust the dose volumes to ensure the rapid bypassing of the gastric environment which would impact the survival of microbes, especially if composing delicate microscopic communities. An 'analytical consensus' of IACUC-approved protocols is herein presented as a unifying baseline protocol for consideration.

Keywords: Gavage, gnotobiotic, mouse models, microbiome, inflammatory bowel disease, fecal matter transplant

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Introduction

Because confounding factors often render study conclusions false or invalid,^{1,2} great interest exists on improving research methodologies to control for confounding factors

in murine research, which is critically important for the discovery of biological features mediated by the gut microbiome in human diseases.³ Although well-controlled forms of studying environmental heterogeneity can be

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