In vivo bioluminescence imaging in Galleria mellonella to non-invasively quantify fungal infection over time

Commonly used acronym: *Greater Wax Moth, BLI, caterpillar*

Created on: 28-05-2025 - Last modified on: 28-05-2025

Contact person

Eliane Vanhoffelen

Organisation

Name of the organisation Katholieke Universiteit Leuven (KUL)

Department Imaging & Pathology

Specific Research Group or Service Biomedical MRI

Country Belgium

Geographical Area Flemish Region

SCOPE OF THE METHOD

The Method relates to	Animal health, Human health
The Method is situated in	Basic Research, Translational - Applied Research
Type of method	In vivo
Used species	Galleria mellonella larvae (Greater wax moth)
Targeted organ system or type of research	Drug screening, virulence assays, fungal infection modeling

DESCRIPTION

Method keywords

Galleria mellonella
in vivo imaging
In Vivo Imaging System (IVIS)
Bioluminescence
fungal infection
longitudinal
non-invasive
drug screening
Virulence assays

Scientific area keywords

antimicrobial resistance
antifungals
aspergillosis
cryptococcosis
fungal infections
Aspergillus
Cryptococcus

Method description

Longitudinal imaging of bioluminescent fungi in Galleria mellonella can complement standard survival and health scoring data by quantifying the fungal burden over time and monitoring early infection before clinical symptoms arise, and by more sensitively and more early detection of treatment effects. By using bioluminescence flux as a readout of fungal burden instead of relying only on secondary health effects, drug doses can be linked to a quantitative reduction in fungal burden which is valuable towards further experimenting in e.g. mice. It also provides a more standardized readout that is less prone to inter-lab variability in e.g. rearing of larvae, possibly leading to intrinsic differences in health. We validated and published this method for aspergillosis (azole-resistant and susceptible) and cryptococcosis, and have shown translatability of this method to Galleria mellonella models of Candida, Fusarium and Mucorales spp. In short, larvae are injected with the bioluminescent fungus of interest, and after injection of 40 µg/g D-luciferin, they are

placed individually in a black 12-well plate inside of the IVIS Spectrum (or other detection system) and photon flux from each individual larva can be quantified. Daily imaging was performed. Longitudinal follow-up has been tested up to 1 week, depending on the virulence and inoculum size of the infectious agent. Treatment was injected daily starting from 1h post-infection. Between imaging timepoints, larvae were kept individually in 12-well plates at 37°C in the dark with food during the whole experiment.

Lab equipment

- Hamilton syringe;
- Incubator 37°C;
- Luciferase-producing fungus;
- D-luciferin;
- IVIS Spectrum or other Bioluminescence Imaging equipment (e.g. plate reader).

Method status

Internally validated

Published in peer reviewed journal

PROS, CONS & FUTURE POTENTIAL

Advantages

- Quantitative, non-invasive readout of fungal burden over time,
- Sensitive detection of treatment efficacy over time,
- More informative results compared to only health and survival readouts,
- Cheap, easy and time-efficient,
- No ethical requirements,
- Can be kept at human temperature, 37°C,
- Innate immune system present,
- Easy and cheap to rear the larvae in-house.

Challenges

- Luciferase-producing fungal strain is required,
- No adaptive immune system,
- *In vivo* model, so prone to variability,

- Limited follow-up time due to life cycle of caterpillar (pupating),
- No commercially available research-grade Galleria mellonella larvae in Europe.

Modifications

We developed this method using an IVIS Spectrum imaging device, but other detectors of bioluminescence flux could also work (e.g. plate reader) given the possibility to keep the larvae in a 12-well plate.

Future & Other applications

This method can also be translated to bacterial infections (luciferase-producing species or presence of lux-operon).

REFERENCES, ASSOCIATED DOCUMENTS AND OTHER INFORMATION

References

Vanhoffelen E, Michiels L, Brock M, Lagrou K, Reséndiz-Sharpe A, Vande Velde G. Powerful and Real-Time Quantification of Antifungal Efficacy against Triazole-Resistant and -Susceptible Aspergillus fumigatus Infections in Galleria mellonella by Longitudinal Bioluminescence Imaging. Microbiol Spectr. 2023 Aug 17;11(4):e0082523. doi: 10.1128/spectrum.00825-23. Epub 2023 Jul 19. PMID: 37466453; PMCID: PMC10433797.

Vanhoffelen E, Vermoesen L, Michiels L, Lagrou K, Reséndiz-Sharpe A, Vande Velde G. Sensitive bioluminescence imaging of cryptococcosis in Galleria mellonella improves antifungal screening under in vivo conditions. Virulence. 2024 Dec;15(1):2327883. doi: 10.1080/21505594.2024.2327883. Epub 2024 Mar 13. PMID: 38465639; PMCID: PMC10939141.

Vanhoffelen E, Van Win T, Van Braeckel E, Reséndiz-Sharpe A, Cammue BPA, Lagrou K, Thevissen K, Vande Velde G. Combinations of posaconazole and tacrolimus are effective against infections with azole-resistant Aspergillus fumigatus. Front Cell Infect Microbiol. 2025 Apr 25;15:1550457. doi: 10.3389/fcimb.2025.1550457. PMID: 40353221; PMCID: PMC12062170.

Coordinated by

sciensano VUB

Financed by

Vlaanderen verbeelding werkt

bruxelles environnement leefmilieu brussel .brussels