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BACKGROUND/ PURPOSE: Honeybee populations, vital pollinators, are severely threatened by *Paenibacillus* larvae, the spore-forming bacteria causing American Foulbrood (AFB). Antibiotics are not a viable option, as they are either forbidden or discouraged in honey production, depending on the country, and they are ineffective against spores. This highly contagious disease spreads via resilient spores that germinate in larval midgut, degrading the host into a viscous mass. Incineration of infected colonies is mandatory, disrupting ecosystems and beekeeping economy.

PROBLEM/ OBJECTIVE: AFB clinical symptoms appear only after an outbreak, leaving beekeepers without timely intervention options, while current diagnostics are slow and labor-intensive. What solutions can enable early detection of *P. larvae* spores in hives, facilitating proactive disease management and reducing hive losses?

METHODOLOGY: We present a novel method for rapid and early detection and quantification of *P. larvae* spores in adult honeybees through light emission. This approach combines a highly efficient magnetic spore capture system—using magnetic beads coated with a keratin-binding protein D-crystallin (MB-Cryst)—with a highly specific recognition protein for *P. larvae*, a cell wall binding domain (PlyPI23CBD) fused to NanLuc (NLuc-CBD).

RESULTS AND CONCLUSION: We have developed a rapid method for specific detection and quantification of *P. larvae* spores by measuring light emission. We found that D-crystallin binds to *P. larvae* spores, MB-Cryst achieves over 90% spore capture efficiency from bee guts, and NLuc-CBD enables highly specific luminescence-based detection after a 3-hour germination step. This sequence of procedures forms a new protocol with a total processing time of about 4.5 hours, significantly improving existing diagnostics. Additionally, the method is designed for future adaptation into a user-friendly, on-site detection system using a portable luminometer or a mobile phone camera.

IMPLICATIONS: Timely detection of *P. larvae* spores in asymptomatic hives enables beekeepers to take preventive or corrective measures, preventing disease outbreaks and hive loss. This supports beekeepers through cost savings and increased honey production while enhancing agricultural pollination, boosting crop yields, and contributing to biodiversity conservation.



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A Rapid and Early Detection Method for *Paenibacillus larvae* Spores: Advancing AFB Diagnosis and Hive Protection



Purpose of the research

- **AFB is a worldwide distributed and highly contagious disease**
- **Incineration of infected colonies is mandatory, disrupting ecosystems and beekeeping economy.**

AFB spreads via resilient SPORES that germinate in larval midgut leading to larvae death



	Real hive health status	Apparent hive health status
<i>P. larvae</i> spores are carried by adult bees. <u>No symptoms.</u>	SUBCLINICAL INFECTION	HEALTHY 
Transmission during feeding. <u>Syntoms: larvae degradation into a viscous mass</u>	INFECTION	INFECTION 

**The early detection of spores in adult bees is essential to prevent/
reduce AFB impact**

Agent: *Paenibacillus larvae*



Spores are the infectious forms, carried by honeybees



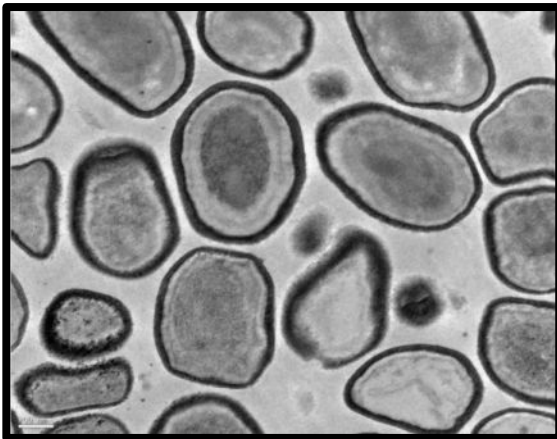
Highly resistant spores can easily propagate



Gram+ spore-forming bacterium



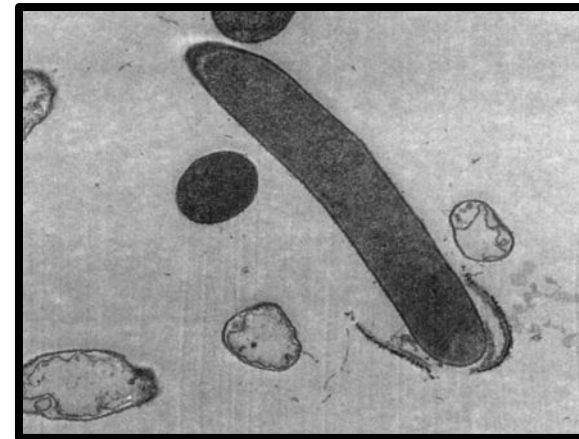
Germinating inside into a vegetative cell



Dormant Spores



MYPGP
L-Tyrosine+uric acid



Vegetative cells



Current solutions

PRODUCT features	Microbiology cultures	Molecular methods (PCR)	Diagnostic kits
Detect spores /early phases	✓	✓	✗
Fast response	✗	✓	✓
High Simplicity and accuracy	✓	✗	✓
One-site analysis	✗	✗	✓
Affordable	✓	✗	✓



The question...

Which method can **early detect *P. larvae* spores**, enabling proactive disease management and hive losses reduction?

Objectives



Specific method for **rapid** and early detection/ quantification of *P. larvae* **spores** in adult bees through **light emission**.

Spore capture
system

Specific
recognition
of *P. larvae*

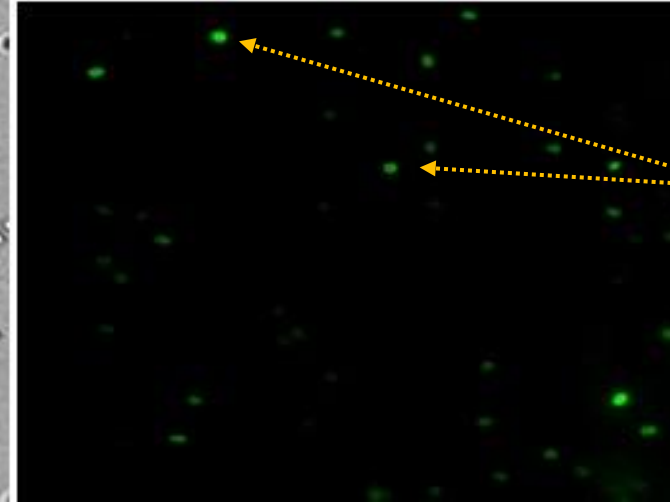
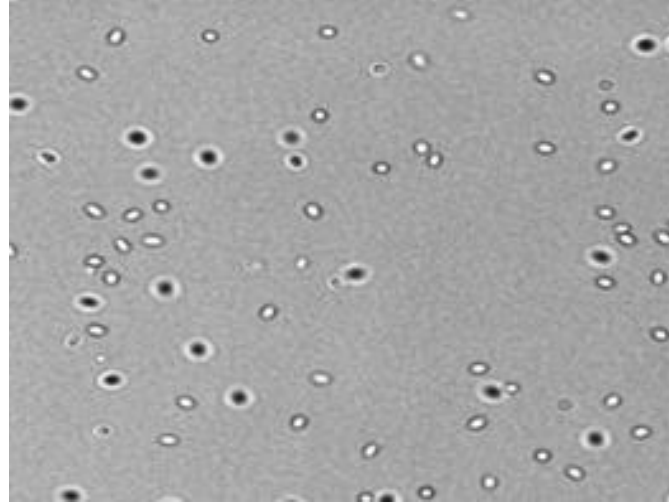
Challenges

- 1-Capture spores from bee matrix (guts) to allow washing and concentration
- 2- Detect *P. larvae* spores rapidly and in a specific manner.
- 3- Explore na easy way to assess *P. larvae* spores in the field with a favourable limit of detection

Challenge #1-Capture spores from bee matrix (guts) to allow washing and concentration

Achievements:

A spore binding protein - γ D-crystallin - binds to *P. larvae* spores (non-specifically)

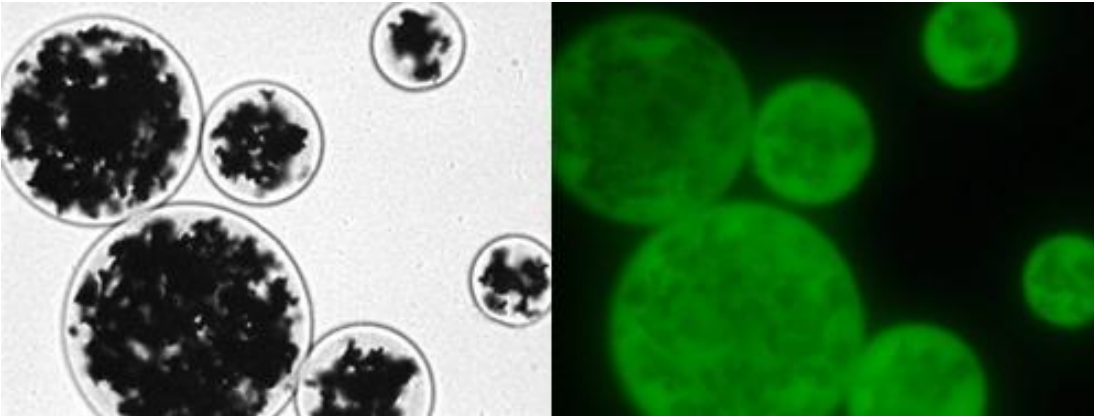


peptide tagged
with a
fluorescent
protein

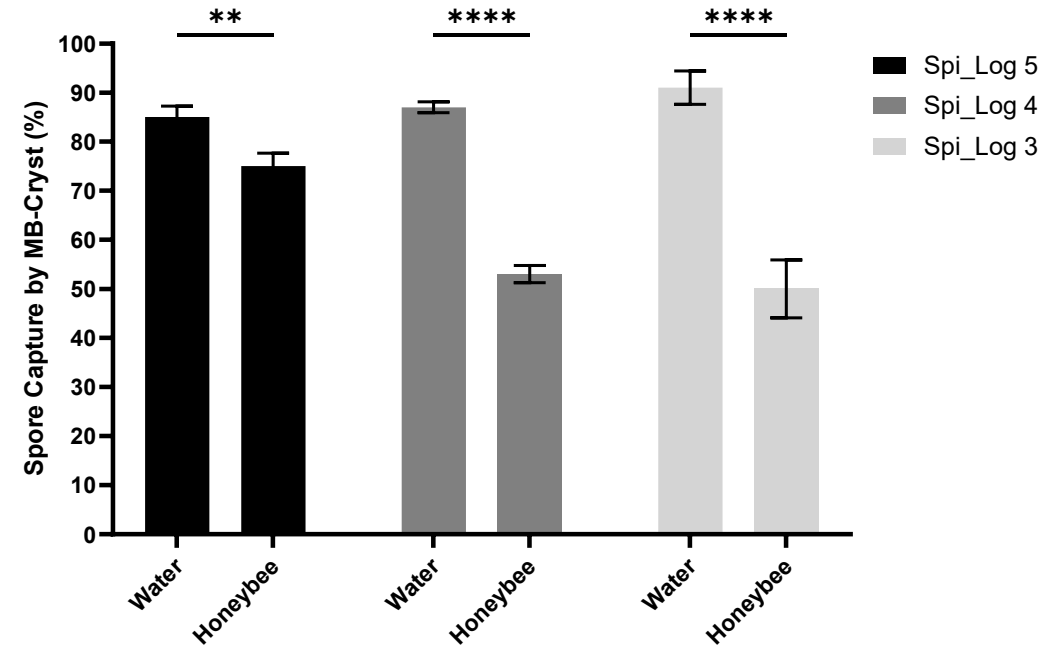
Challenge #1-Capture spores from bee matrix (guts) to allow washing and concentration

Achievements:

Magnetic beads coated with γ D-crystallin allows spore capture for washing and concentration



Magnetic beads covered with γ D-crystallin tagged with FITC

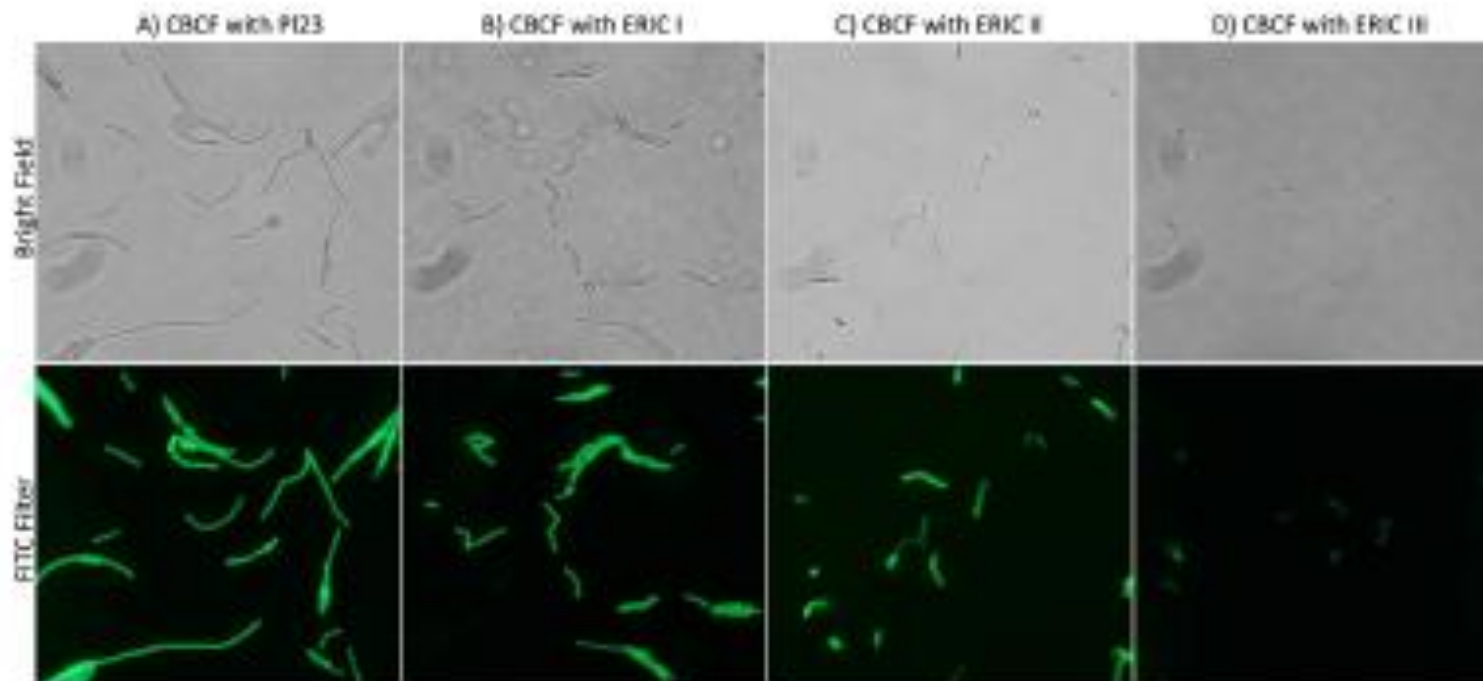


	Spi_Log 5			Spi_Log 4			Spi_Log 3		
	Mean	SD	N	Mean	SD	N	Mean	SD	N
Water	85.0	2.23	3	87	1.10	3	91	3.40	3
Honeybee	75.0	2.64	3	53	1.76	3	50	5.89	3

Challenge #2- Detect *P. larve* spores rapidly and in a specific manner.

Achievements:

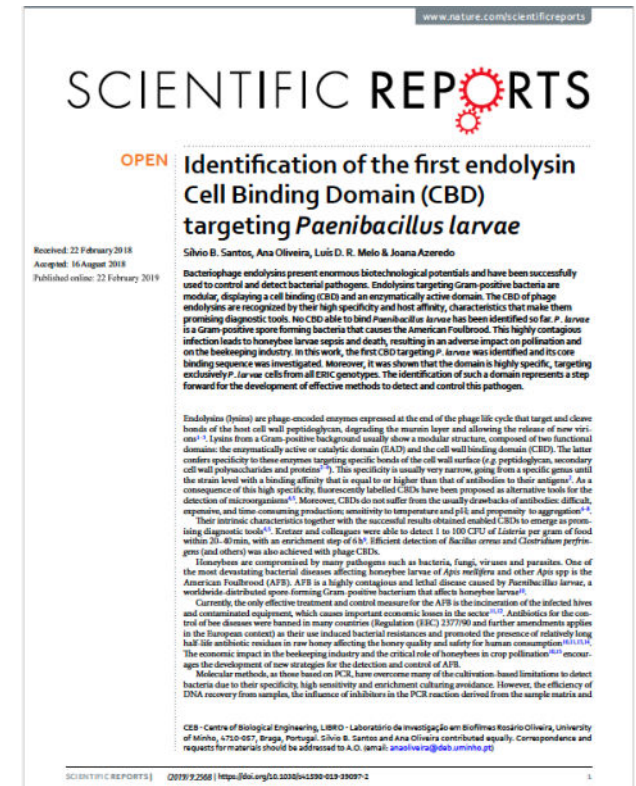
PlyPl23 specifically binds to *P. larvae*



Peptide PlyPl23CBD-GFP green-decorates (= binds) *P. larvae* vegetative cells (ERIC I-IV)



PlyPl23CBD-GFP does not bind to dormant **spores**.



Challenge #2- Detect *P.larve* spores rapidly and in a specific manner.

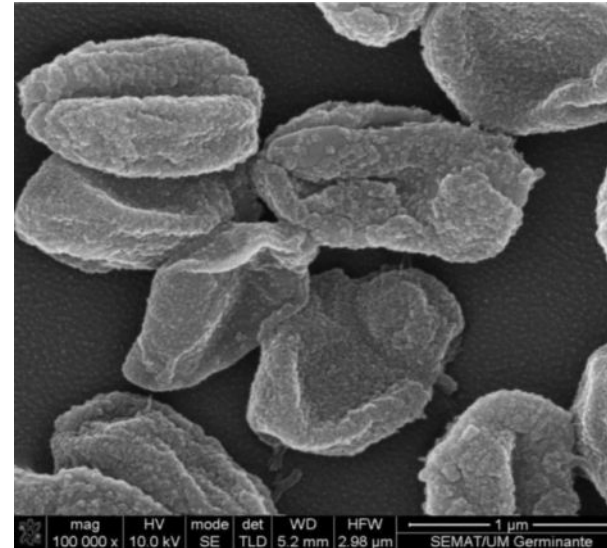
Achievements:

PlyPl23CBD-GFP green-decorates (=binds) to germinant *P. larvae* spores



Dormant spores

MYPGP+ 3 mM UA+3mMLtyr

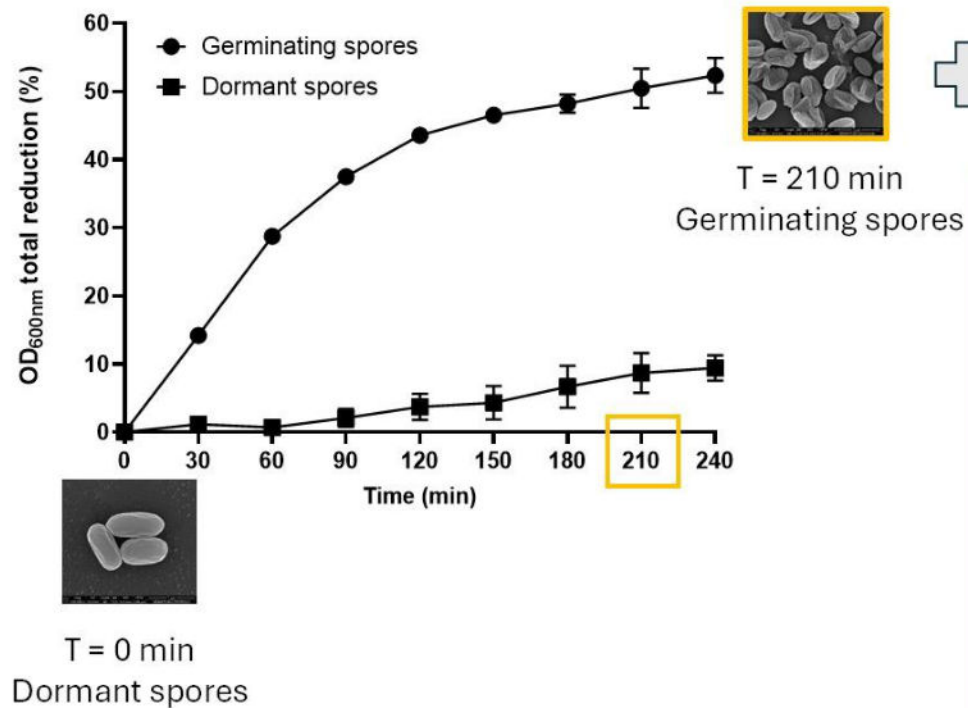


Germinating spores

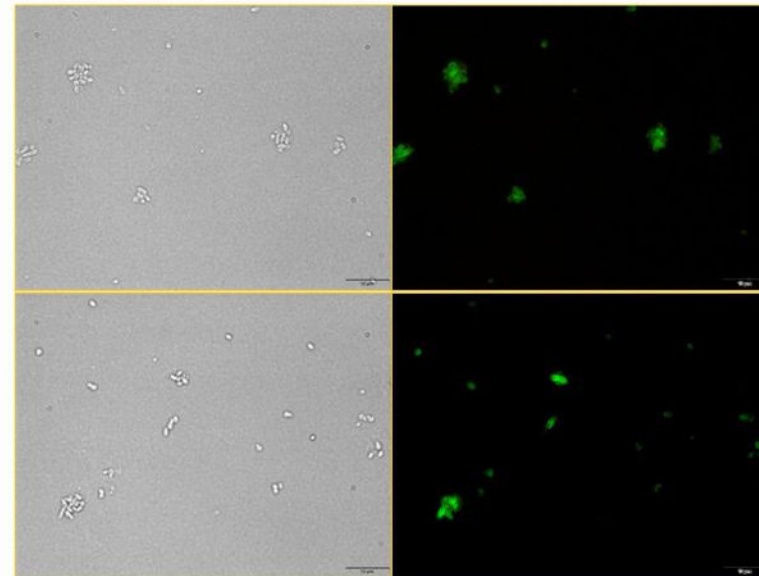
Challenge #2- Detect *P.larve* spores rapidly and in a specific manner.

Achievements:

PlyPl23CBD-GFP green-decorates (=binds) to germinant *P. larvae* spores



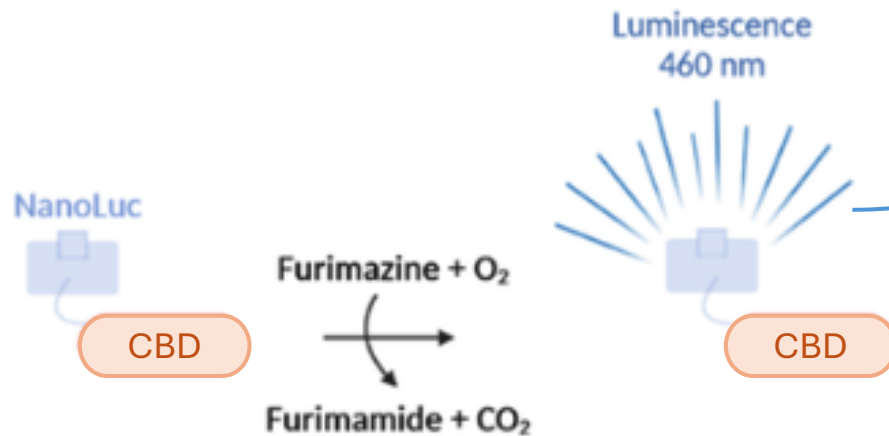
⊕ GFP-PlyP23_CBD



Challenge #3- i) increase the limit of detection ;
ii) explore na easy away to detect/ quantify *P. larvae* spores

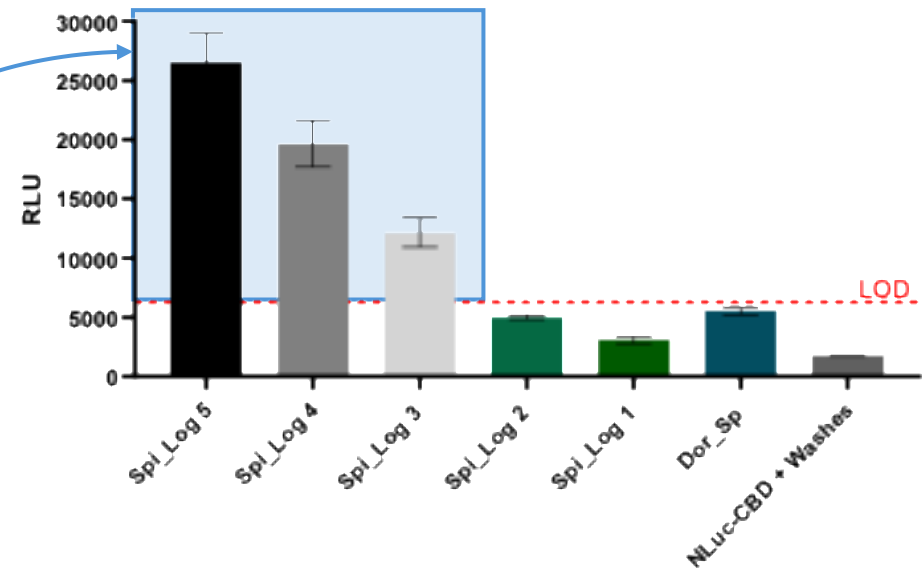
Achievements:

Fusing luciferase to the binding protein allows a light signal to be obtained



Source: adapted from <https://www.mdpi.com/2076-2607/10/2/260>

Luciferase, fused to a protein of interest (CBD) emits light (460 nm) upon addition of its substrate Furimazine in an O₂-dependent manner.

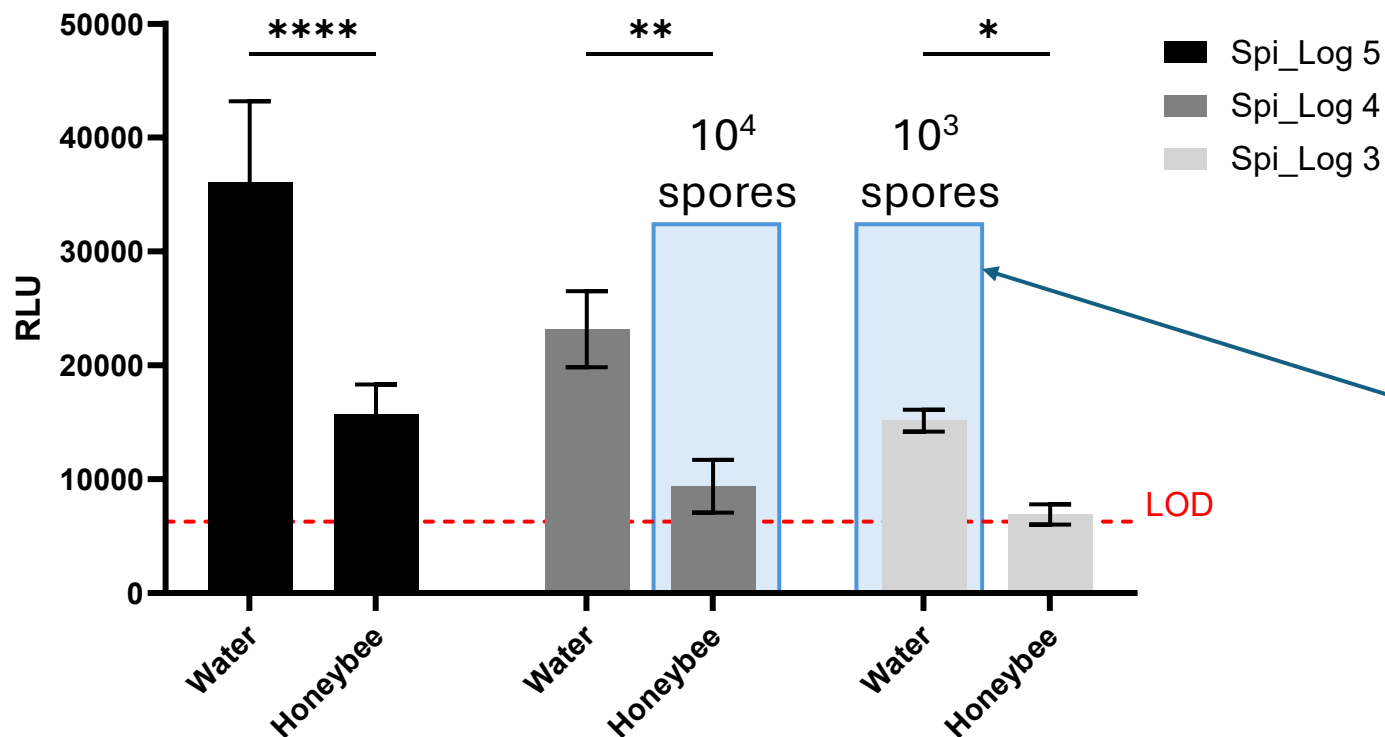


Spi_Log –Germinating spore concentration
Dor_Sp- Dormant spore concentration
LOD= Limit of detection

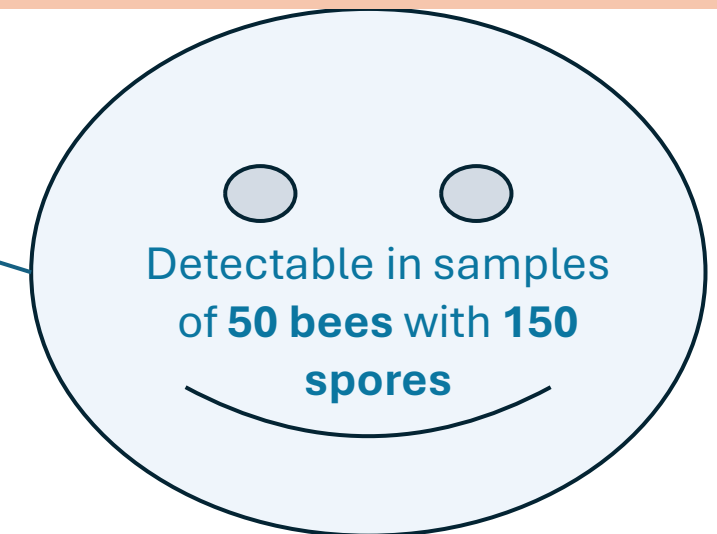
Challenge #3- i) increase the limit of detection ;
ii) explore na easy away to detect/ quantify *P. larvae* spores

Achievements:

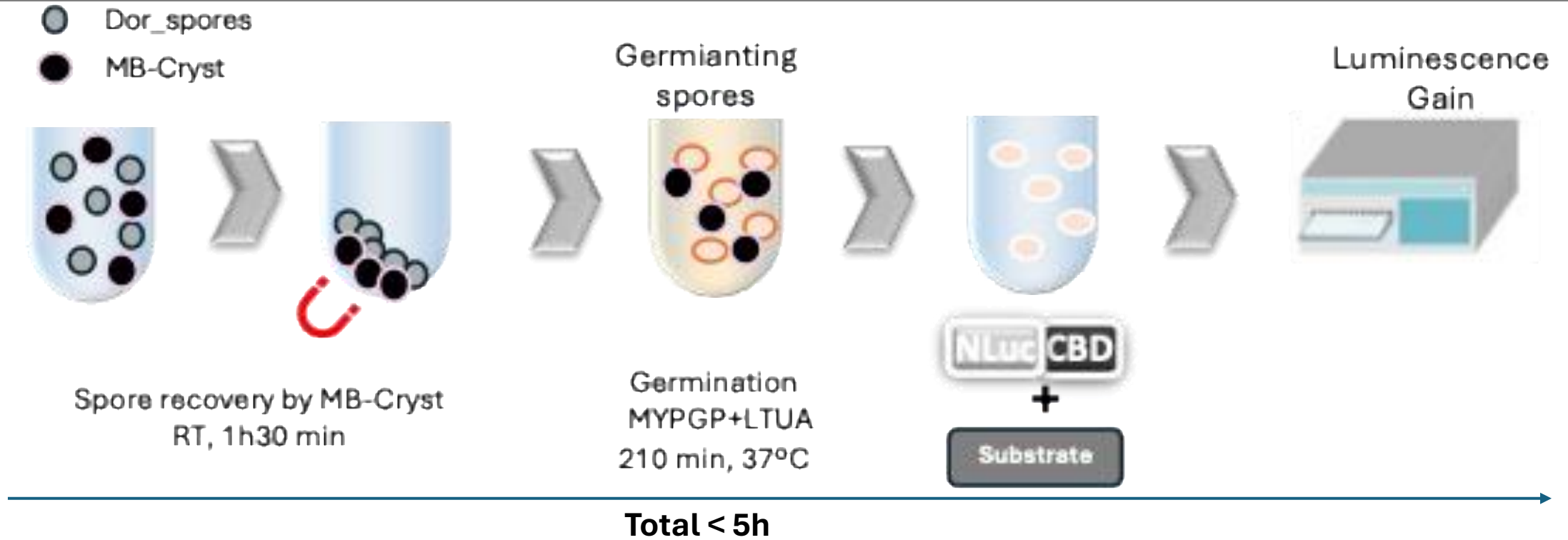
Capture and Detection of spores by luminometry



Thresholds for clinical symptoms in a colony, range from **150** (Stephan *et al.*, 2020) to **3 000** spores/bee (Gende *et al.*, 2011)



The Protocol



FOR FIELD ANALYSIS...



Portable
luminometer



Mobile
camera

Take home messages

- Improvement of existing AFB diagnosis: this is a rapid method for specific detection and quantification of *P. larvae* spores (< 5 hours) by measuring light emission
- A sample of up to 50 adult bees will indicate whether the hive is asymptotically infected.
- The method was designed for future adaptation into an easy-to-use, on-site detection system using a portable luminometer or a cell phone camera.

Acknowledgements

Team:



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