# Beeosensor

## Early detection of the American foulbrood

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### Introduction

The American foulbrood (AFB) is a fatal, highly infectious bacterial disease of the honey bee brood caused by the spore-forming bacterium *Paenibacillus larvae*.

Because the diagnosis process takes weeks, disease outbreaks can often not be prevented. In this case, beekeepers are obliged to report the outbreak to the authorities.

Next, a vet needs to officially confirm the AFB outbreak, which if positive, requires the bee garden and a 3 km surrounding area to be under quarantine for at least a month. This will lead to a massive loss of honey products for the beekeeper.

### Project

We developed an impedance measuring biosensor that will enable beekeepers to quickly screen for *P. larvae* contaminations with high sensitivity.

*P. larvae* spores are often present in beehives, but if a certain threshold is reached it may lead to the outbreak of the devastating AFB[1].

Therefore, we aimed for a low CFU/mL detection limit, in order to enable beekeepers to react early to a AFB-threat. The outbreak can be prevented by non-radical methods, to "clean" a potentially sick hive. Thus, beekeepers won't be facing public shaming or financial crisis.

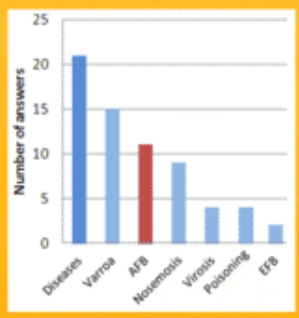


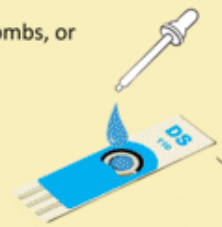

**Figure 1:** Main causes of colony mortality, in 2010[2]. Diseases: non-specified diseases, AFB: American foulbrood, EFB: European foulbrood

### 1. Proposed sampling

- Washing of honey bees with washing solution to detach *P. larvae* spores, or
- Diluting honey from feed combs, or
- Dissolving bee debris

### 2. Measuring

- Applying spore sample on the electrode
- Incubation 15 min (RT)
- Adding redox couple solution
- Measurement




### 3. Data Output

- Different types of data output: disease status or spore concentration


### Human Practice

- Interviews and discussions with beekeepers, beekeeping institutions (South Tyrol and Styria) and other beekeeping experts about problems, possibilities and advice of AFB testing. They helped a lot with developing the idea of a user-friendly method and motivated us.
- Survey among beekeepers about their interest in *Beeosensor*, to learn about their preferences and to formulate requirement specifications for the measuring device.
- First considerations of the environmental impact e.g. sustainability of used resources.

### Conclusion

- Phage HB10c2 recognizes *P. larvae* and *P. larvae* spores specifically and is suited for EIS measurements.
- Our sensing device performs nearly as good as expensive lab devices.
- Beekeepers are highly interested in this diagnostic method.

### Modeling

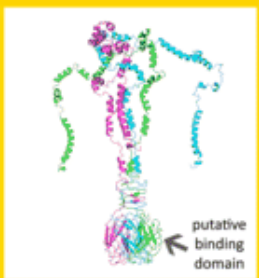



**Figure 2**: Modeling of the putative receptor binding protein (RBP) of the phage with GalaxyHomomer. The distal part of the protein resembles known RBPs of other phages. The Identification of the RBPs is important to understand the mechanism of the phage-spore binding to improve the detection accuracy.

### Electrode setup

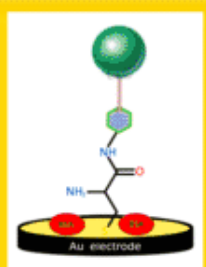



**Figure 4**: Schematic representation of the *Siphoviridae*-bacteriophage biosensor composed of:

- Gold electrode
- L-cysteine
- *Paenibacillus* phage HB10c2
- Bovine serum albumine (BSA) blocks free sites
- *P. larvae* spore captured by phage

### Results

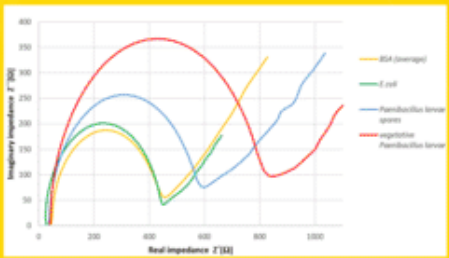


**Figure 3:** Nyquist impedimetric diagram of the phage-based sensor device with different samples. Measurements were performed in PBS (pH 6.8) containing 5 mM $Fe(CN)_6^{3-/4-}$. Concentration of the spores is 10^6 CFU/mL.

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- Business plan scaffold from Team UNSW
- Designin circuit print Team Madrid

### Literature:

1. Gende, L. et al. Searching for an American foulbrood early detection threshold by the determination of *Paenibacillus larvae* spore load in worker honey bees. Bulletin of Insectology 64, 229-233 (2011)

2. Chuazat, M. P. et al. Demographics of the European Apicultural Industry. PLOS ONE 8 e79018 (2013).