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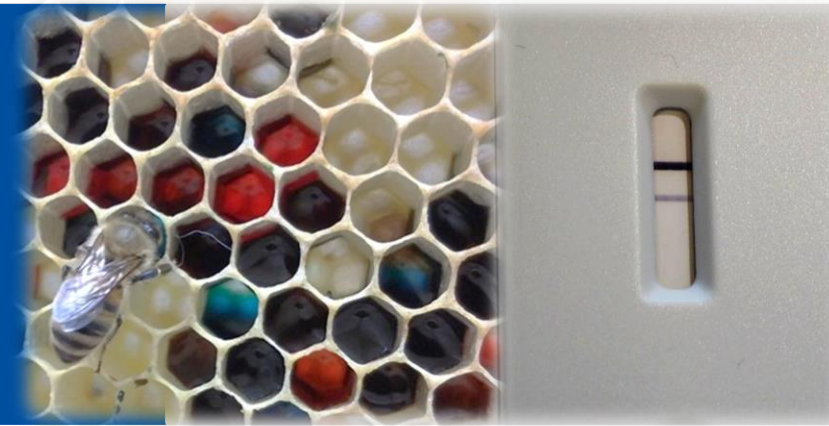
American (AFB) and European foulbrood (EFB) are devastating honey bee brood diseases that can cause high numbers of colony losses after infection. AFB is caused by the bacterial agent *Paenibacillus larvae* that can be categorized to different ERIC-genotypes. These ERIC-genotypes can differ in virulence. EFB is caused by *Melissococcus plutonius* and is considered less harmful compared to AFB. For the prevention of disease spread and the conduction of suitable and fast control measures, a rapid, reliable and affordable diagnostic tool is needed.

Therefore, we developed an antibody based lateral flow assay (LFA) for detection of EFB, AFB and the distinction of the two main occurring ERIC-genotypes of the AFB causative agent. Monoclonal antibodies were generated in mice, tested for their suitability using the target bacteria and other bee associated bacteria solutions. After testing the mAbs in a sandwich ELISA the LFAs were produced for each disease separately and tested with bacteria solutions and honey bee larvae. Certain additional features have been developed using the AFB-LFA such as an easy and save sample application tool for the LFA and a tool for objective evaluation of the result using a smartphone App. Furthermore, the tests for EFB, AFB and ERIC-genotyping have been combined on one test strip to finally have an all-in-one test kit for the main bacterial honey bee brood diseases. Here we present an affordable, rapid and reliable tool for the diagnosis of EFB, AFB and the differentiation of the two main occurring ERIC-genotypes.

A rapid diagnosis of American and European foulbrood in one test kit

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People involved at the FLI & partners

Friedrich-Loeffler-Institut

Robert Kammerer
Marc Schäfer

FRIEDRICH-LOEFFLER-INSTITUT



FLI

Bundesforschungsinstitut für Tiergesundheit
Federal Research Institute for Animal Health



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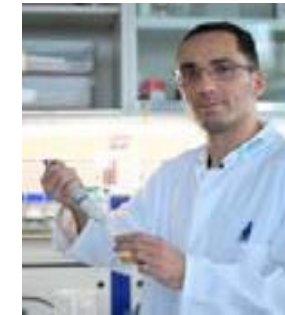
FLI

Bundesforschungsinstitut für Tiergesundheit
Federal Research Institute for Animal Health



Senova

Lukas Rüttinger
Andreas Gütter



Phaeosynt

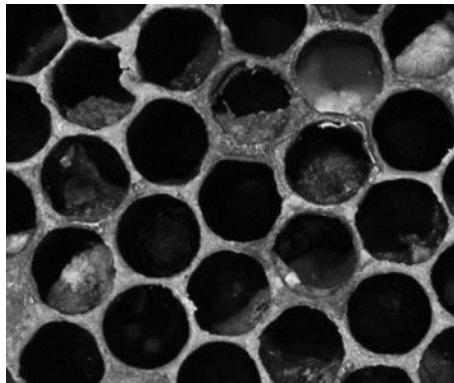
Alina Eilers
Stas Hans



European foulbrood & American foulbrood

European foulbrood (EFB)

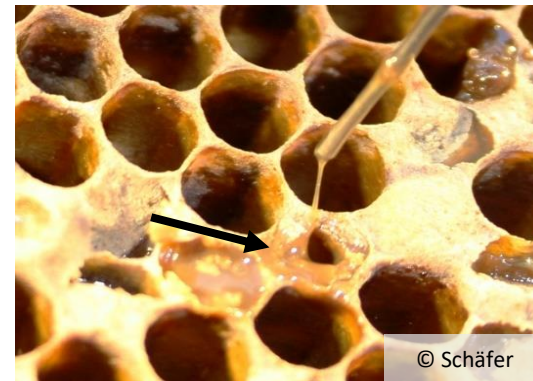
- *Melissococcus plutonius*
- associated with secondary invaders



Forsgren, 2009

American foulbrood (AFB)

- *Paenibacillus larvae*
- spore-forming
- genotypes differ in virulence



Genersch et al., 2010; Forsgren et al., 2018

Genotypes of *P. larvae*

ERIC I



larvae die after ~12 days

ERIC II



larvae die after ~7 days

Ashiralieva & Genersch, 2006; Forsgren et al., 2018

Genotypes of *P. larvae*

ERIC I



larvae die after ~12 days

More clinical symptoms:

- Holes in capping
- Sunken/darkened caps
- Often ropy larvae

ERIC II



larvae die after ~7 days

Less clinical symptoms:

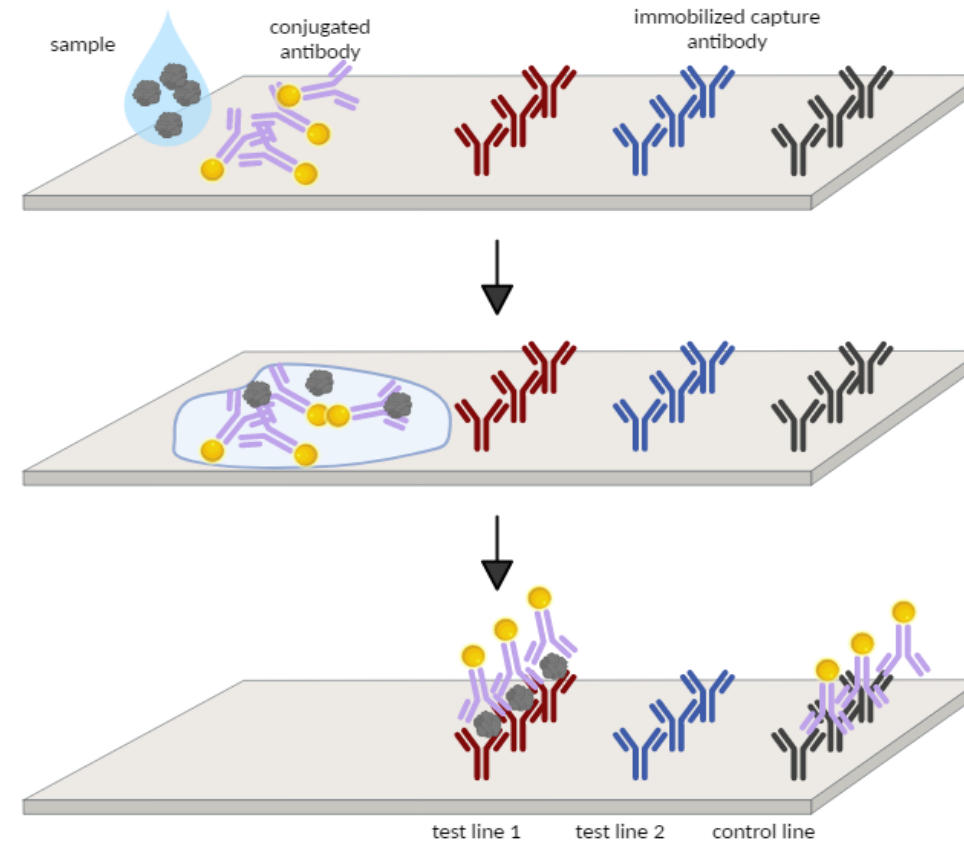
- Patchy brood pattern
- Rare ropy larvae

Ashiralieva & Genersch, 2006; Forsgren et al., 2018

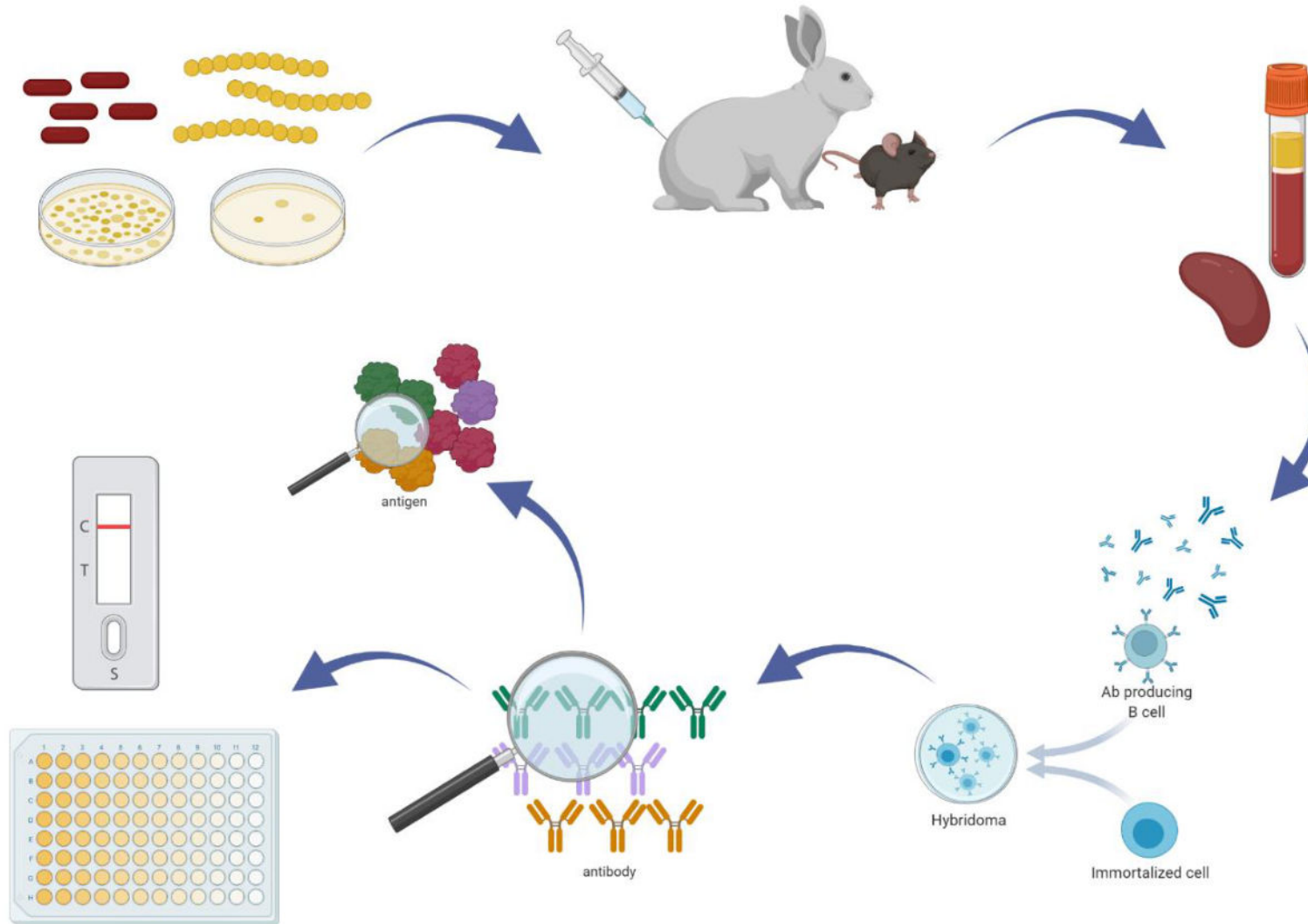
Healthy vs diseased brood



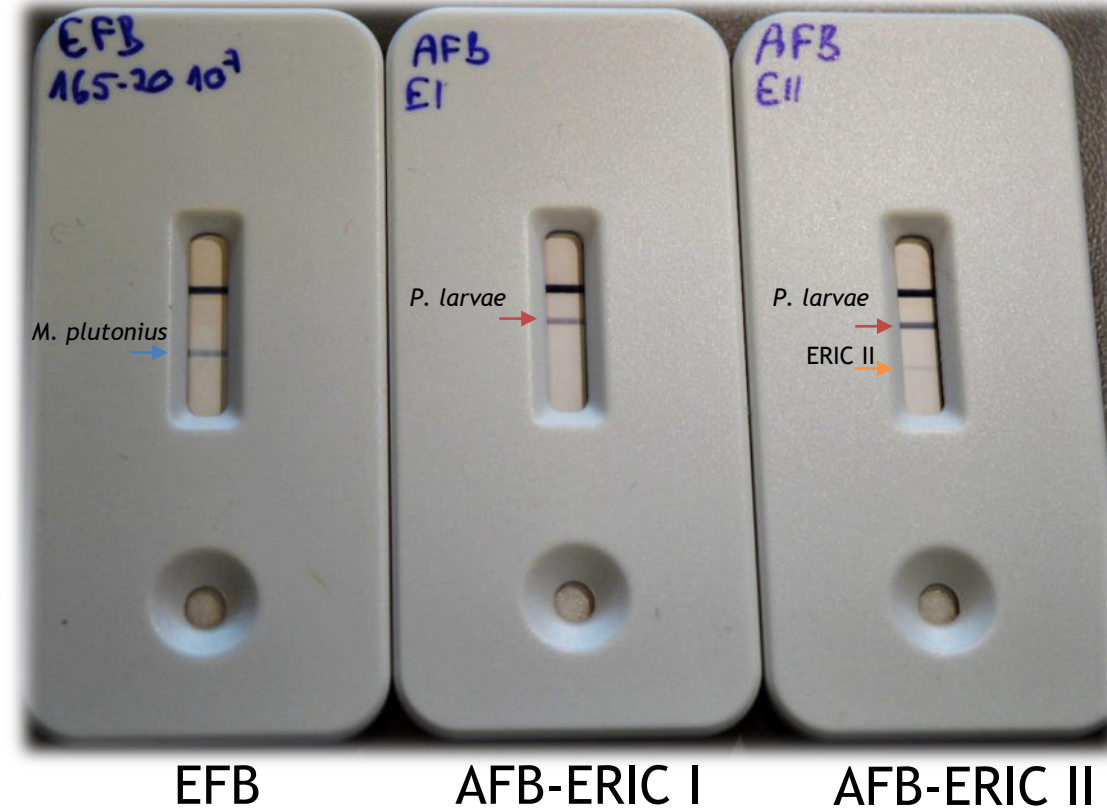
Lateral Flow Assay (LFA)



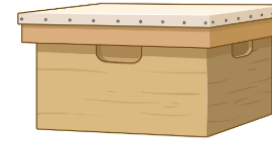
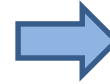
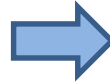
Former projects dealing with the topic: FLOWERIC & DEAD



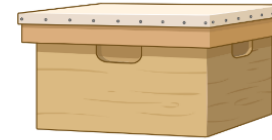
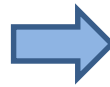
1st LFA prototypes



LFA and honey bee colonies



LFA and honey bee colonies



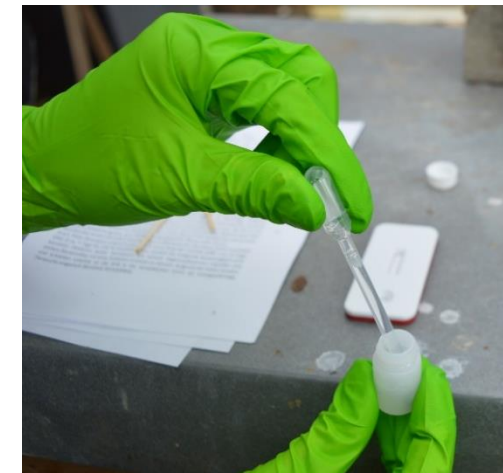
Sampling



Transfer of sample to lysis



Lysis of the sample

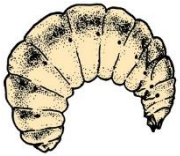


Transfer of lysed sample



Sample application

AFB-ERICII-LFA with field samples



larvae



bees



All in one test: AFB-ERICII-EFB-LFA



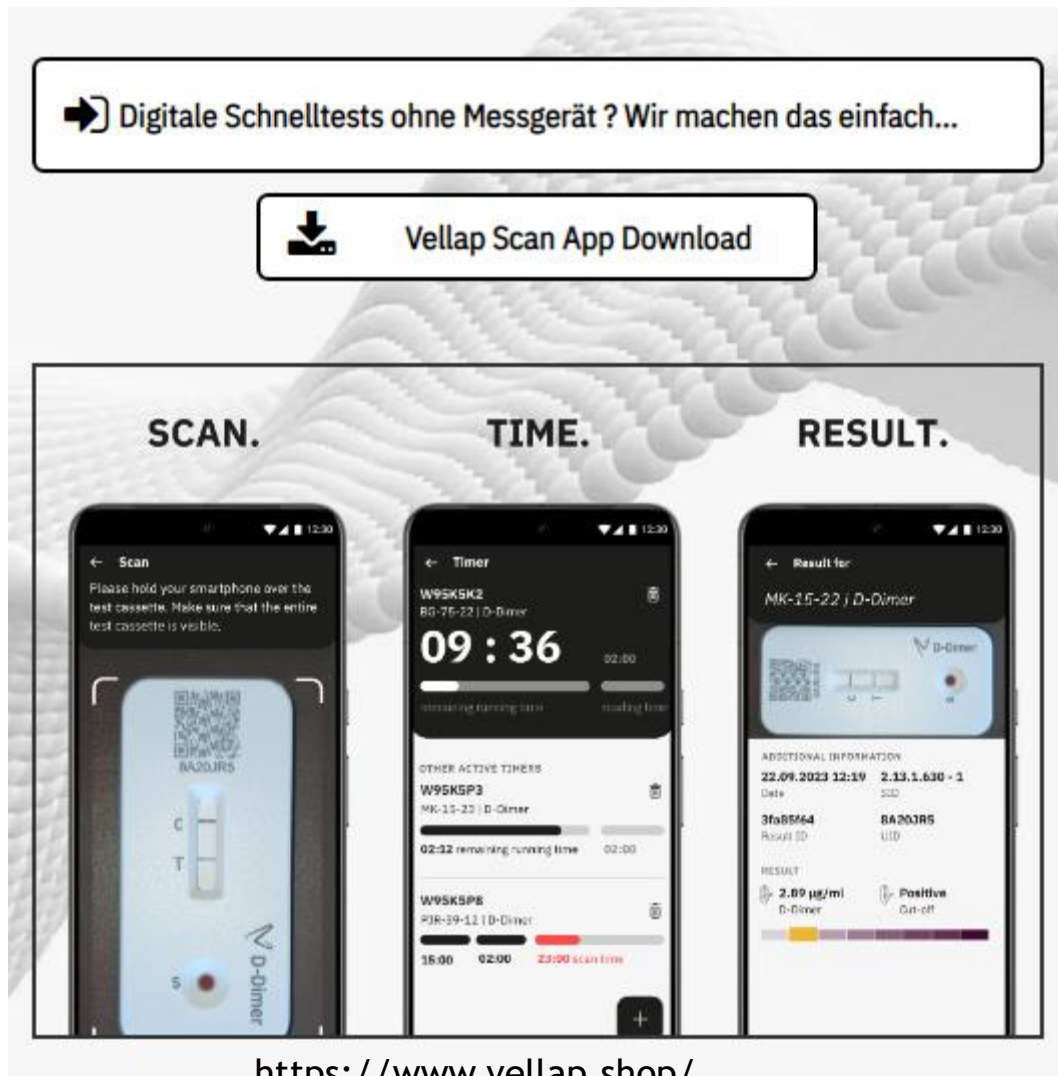
Control

ERIC I

ERIC II

M. plutonius

LFA analysis with Vellap-App

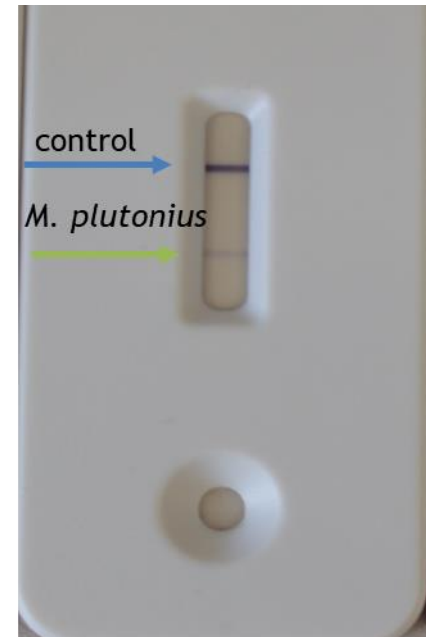
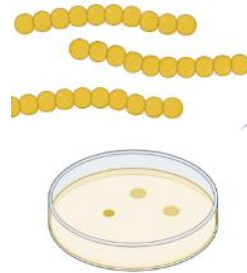


LFA = lateral flow assay

Summary

1. EFB-LFA established

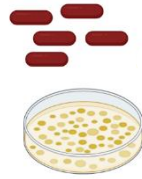
- LFA produced
- Tests in progress...



Summary

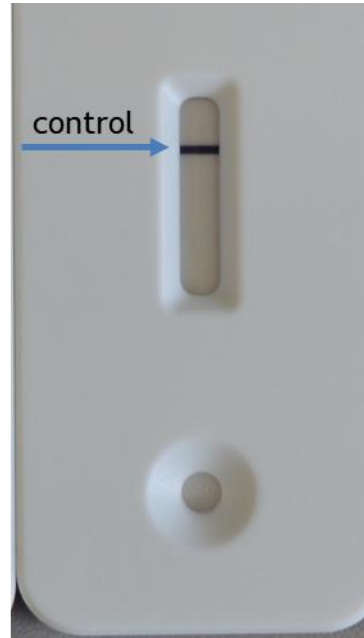
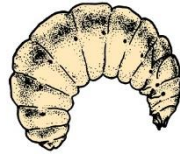
1. EFB-LFA established

- LFA produced
- Tests in progress...

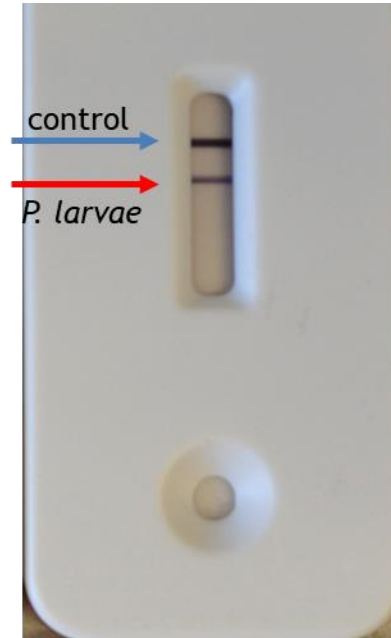


2. AFB-LFA established

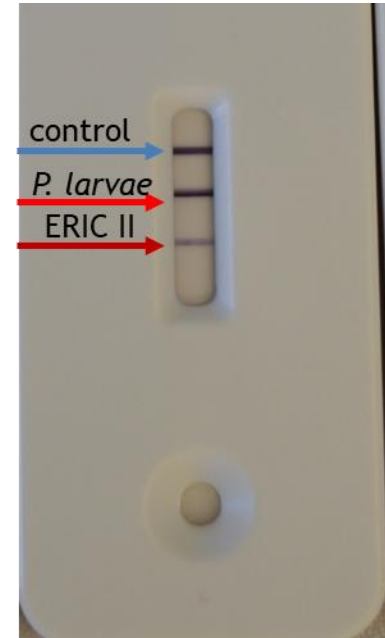
- Field tests



negative control



ERIC I



ERIC II

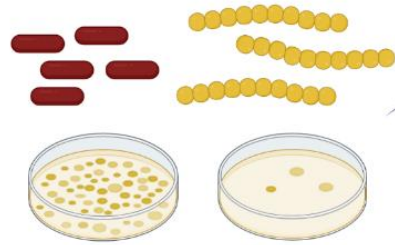
Summary

1. EFB-LFA established

- LFA produced
- Tests in progress...

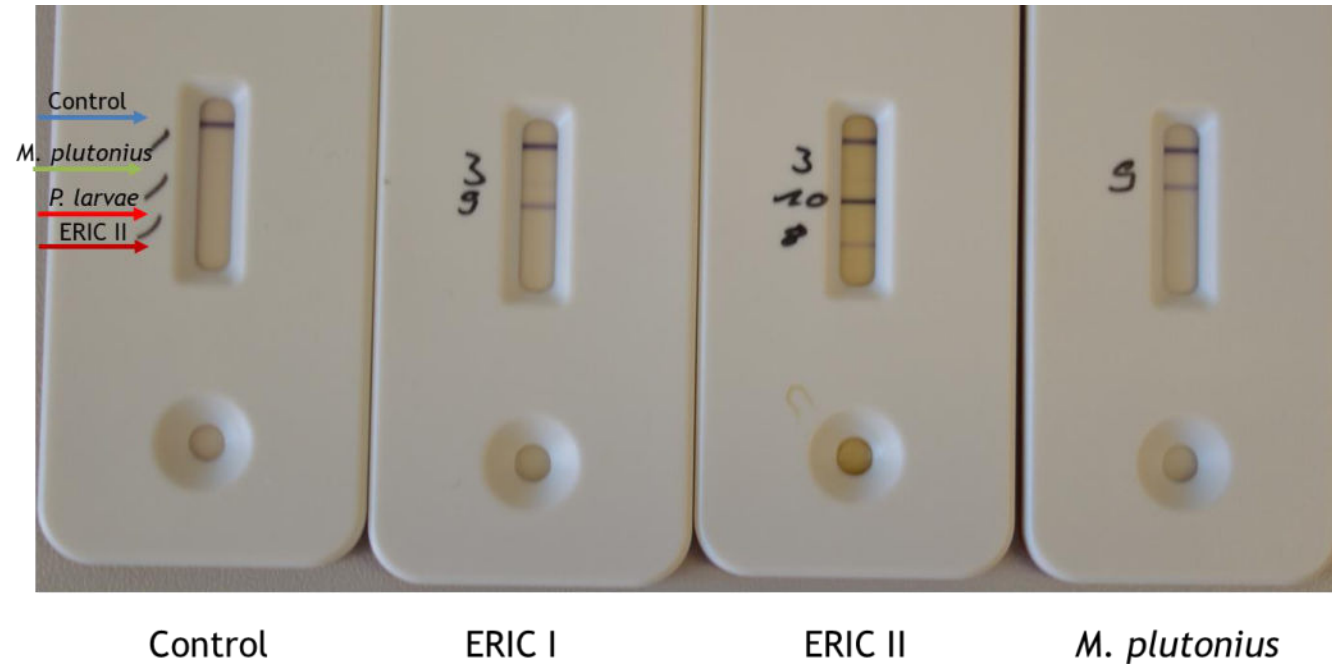
2. AFB-LFA established

- Field tests



3. AFB-ERICII-EFB-LFA

- Production
- Optimization in progress...

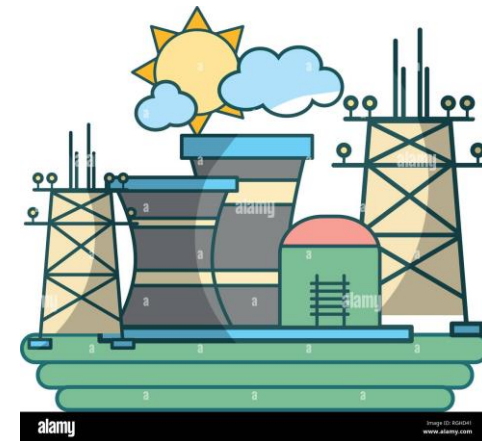
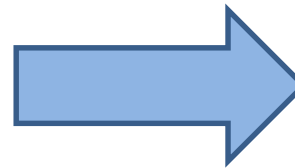


Outlook: Transfer from research to industry:

- 1) Antibody production in large scale in diatoms
- 2) Quality check of new mAbs from diatoms
- 3) Validation of LFAs with mAbs from diatoms



<https://www.vecteezy.com/free-vector/research>



Thank you for your attention!

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