Development of prototypes of rapid molecular diagnostic tests for pathogens of honeybees (*Apis mellifera* L.) on a chromatographic NALF platform (Nucleic Acid Lateral Flow)

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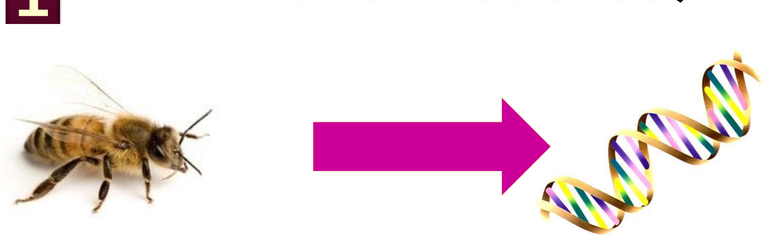
Honeybee pathogens investigated:
Deformed Wing Virus (DWV), Israeli Acute Paralysis Virus (IAPV) and the microsporidian Nosema (*Nosema ceranae*)

We developed a rapid and innovative POC (Point of care) diagnostic tool based on isothermal amplification (LAMP) and NALF (Nucleic Acid Lateral Flow) detection. The core of the test is a set of specific primers suitably modified in order to obtain an amplification product that is linked to both biotin and Gold Nanoparticles (GNP). The oligonucleotide-GNP conjugates that we used are designed to resist both high temperature and ionic strength of the amplification reaction.

TEST procedure

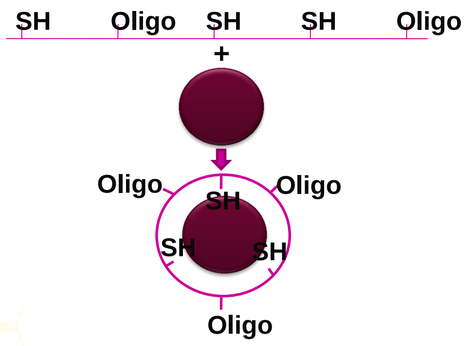
1 Extraction of nucleic acids



We used a rapid extraction method for RNA according to Wei et al, 2012 with some modifications:
manually homogenize abdomens of bees in a solution of 0.5 M NaOH (500 µl of NaOH / bee abdomen) for at least 2 minutes. Collect 250 µl of homogenate and transfer to 6 ml of TRIS-HCl pH 8.

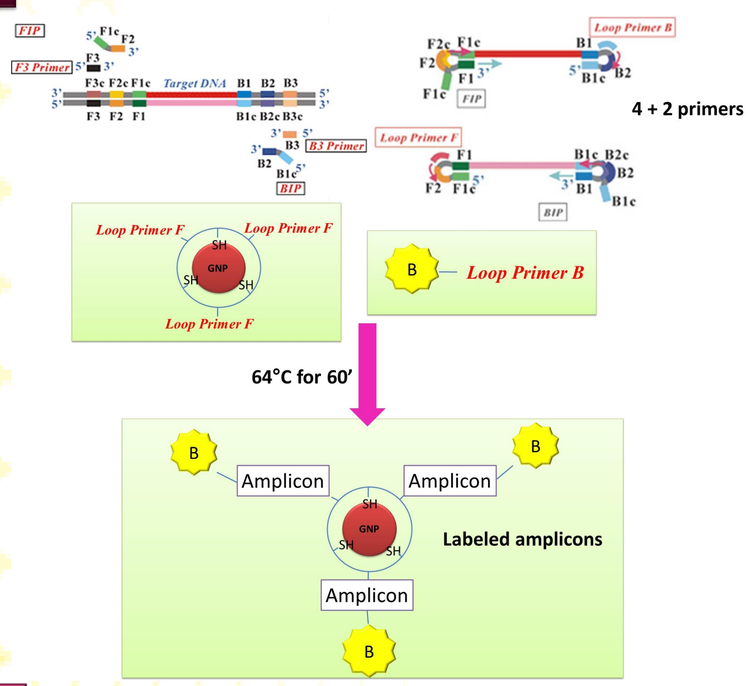
We applied a rapid extraction method for DNA according to Xin et al, 2003 with some modifications:
manually homogenize abdomens of bees in a solution containing 100 mM NaOH and 2% Tween 20 for at least 2 minutes. Transfer 100 µl to a clean eppendorf tube. Boil for 10 minutes at 95 °C. Then add 100 µl buffer: 100 mM Tris-HCl, 2 mM EDTA.

2 Conjugation of GNP with oligonucleotides



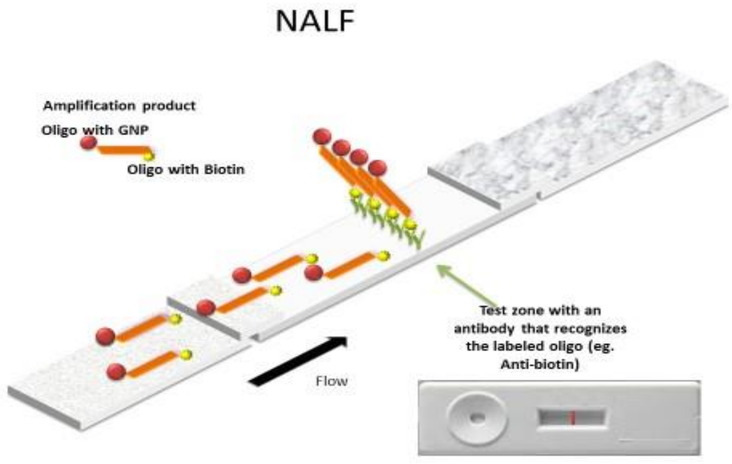
Our conjugation strategy is based on a double modification of the aminodextran molecule in order to obtain a polidextran containing a high number of thiol groups and at least 2-4 oligos. Multiple thioles allow a high stability of the link between modified polidextran and GNPs. The polidextran layer also confers to the GNPs resistance to high temperatures and actions of chemicals.

3 LAMP reaction with two labeled oligos



Loop-mediated Isothermal Amplification (LAMP) is a very sensitive, easy and time efficient method for detection of infectious diseases (Notomi et al., 2000; Mori and Notomi, 2009). The LAMP reaction proceeds spontaneously at a constant temperature using a DNA polymerase with strand displacement activity and exploiting the formation of single stranded DNA loops by means of four specifically designed primers that recognize six distinct regions of the target locus. The use of two additional oligos called Loop Primers can further enhance the speed and sensitivity of the reaction.

4 Detection of amplification products of LAMP using lateral flow (NALF)



Technical points of strength

- NO expensive technical devices or equipment needed ("In field use" - POC)
- User friendly: the test can be performed by ordinary technical personnel
- Rapid results: the whole test procedure can take less than 2 hours
- Sensitivity and specificity comparable to a PCR based essay
- Eco-friendly

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