

# Molecular based identification of insect ingredients in animal feeds

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## ANIMAL FARMING FOR A HEALTHY WORLD

GHENT - BELGIUM

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# Innovative Ingredients

EU [Regulation 2017/893](#) authorized the use of seven breeding insect species in feed for aquaculture animals.

✓ *Hermetia illucens*



✓ *Tenebrio molitor*



✓ *Alphitobius diaperinus*



✓ *Musca domestica*



✓ *Acheta domesticus*



✓ *Gryllus assimilis*



✓ *Gryllodes sigillatus*



# Species for aqua-feed purposes

✓ *Hermetia illucens*



✓ *Tenebrio molitor*

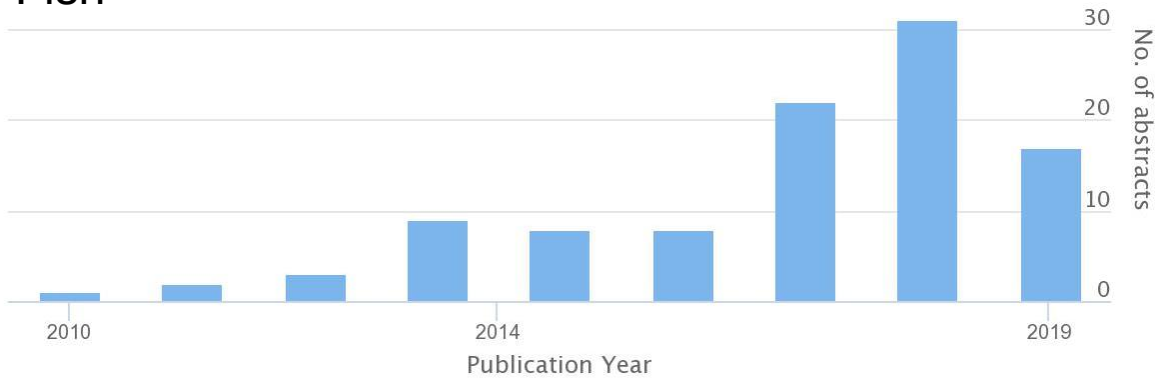


*Tenebrio molitor* (TM) and *Hermetia illucens* (HI) are the most attractive species that could be suitably used in aquafeeds for their availability and market price.

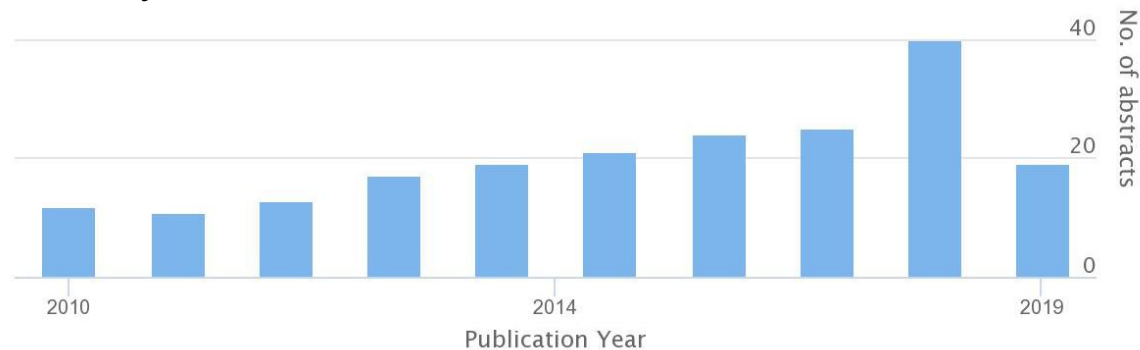
# Publications

These innovative ingredients have been largely tested to evaluate their effects and properties

## Fish



## Poultry



(CabDirect access on 19/08/2019)

# Feed/Food control

The development of assays is needed for

- **labeling** of products containing insects
- identification of **fraud** and adulteration in industrial insect products



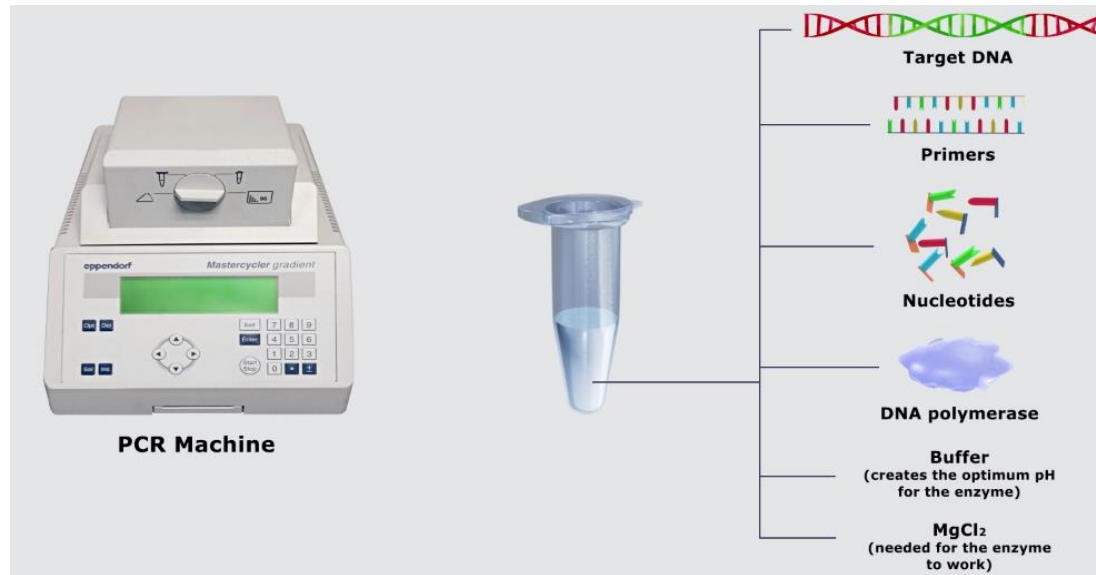
The image shows a hand holding a magnifying glass over a nutrition label. The label is titled 'Nutrition Typical values' and lists various nutrients with their values per 100g, per 1/4 pack, and as a percentage of adult GDA. The label also includes a column for GDA for children (0-4 years) and a note at the bottom stating 'GDAs = Adult Guideline Daily Amounts are based on a diet of average energy intake for a 70kg adult male. GDAs are guidelines and personal requirements vary depending on age, gender, weight and activity level.'

Nutrition Typical values (cooked as per instructions)	per 100g	per 1/4 pack	% adult GDA, 1/4 pack	GDA children 0-4 yrs
Energy kJ	1007	2014		
Energy kcal	241	482		
Protein	8.4g	16.8g	24.1%	1800
Carbohydrate	20.6g	41.2g	37.3%	24g
of which sugars	1.8g	3.6g	17.9%	220g
of which starch	18.8g	37.6g	4.0%	85g
Fat	13.7g	27.4g	39.1%	70g
of which saturates	5.7g	11.4g	57.0%	20g
mono-unsaturates	5.9g	11.8g		
polyunsaturates	1.5g	3.0g		
Fibre	0.9g	1.8g		
Salt	0.50g	1.00g		
of which sodium	0.20g	0.40g		

GDAs = Adult Guideline Daily Amounts are based on a diet of average energy intake for a 70kg adult male. GDAs are guidelines and personal requirements vary depending on age, gender, weight and activity level.

# Use of the qPCR

The qPCR is still the election method for the detection of contaminants and to check for the presence or absence of a target in a biological matrix.



Species specific primers were designed for the detection of the 7 allowed insect species.

# Primers specificity

Primers	Hermetia	Tenebrio	Musca	Gryllodes	Gryllus	Alphitobius	Acheta
DNA	illucens	molitor	domestica	sigillatus	assimilis	diaperinus	domesticus
Hermetia illucens	23,2 *	N/A	N/A	N/A	N/A	N/A	N/A
Tenebrio molitor	N/A	20,2*	N/A	N/A	N/A	N/A	N/A
Musca domestica	N/A	N/A	24,1*	N/A	N/A	N/A	38,7^
Gryllodes sigillatus	N/A	N/A	N/A	18,9*	N/A	N/A	N/A
Gryllus assimilis	N/A	N/A	N/A	N/A	21,1*	N/A	N/A
Alphitobius diaperinus	N/A	N/A	N/A	N/A	N/A	26,5*	N/A
Acheta domesticus	N/A	N/A	N/A	N/A	N/A	N/A	19,2*

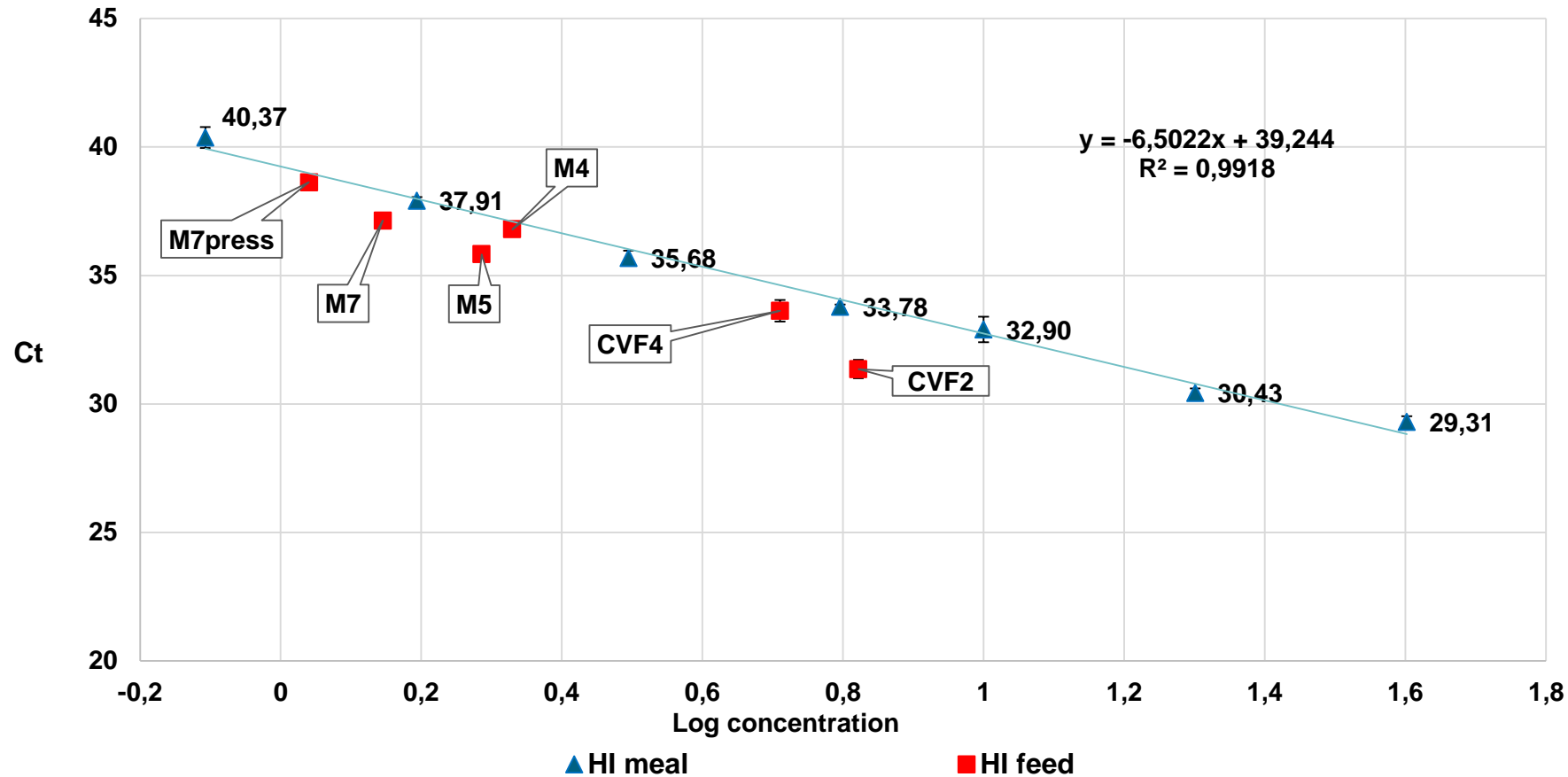


Perfection  
doesn't  
exist

Cross reactivity table

# Test with feed matrix - HI

Six different diets (■) including graded levels of *H. illucens* meal (0,5 – 5,0 ng/ul) and a negative control provided from CycleFarms (F) were tested. Results were compared with pure HI meal (▲) dilution curve.

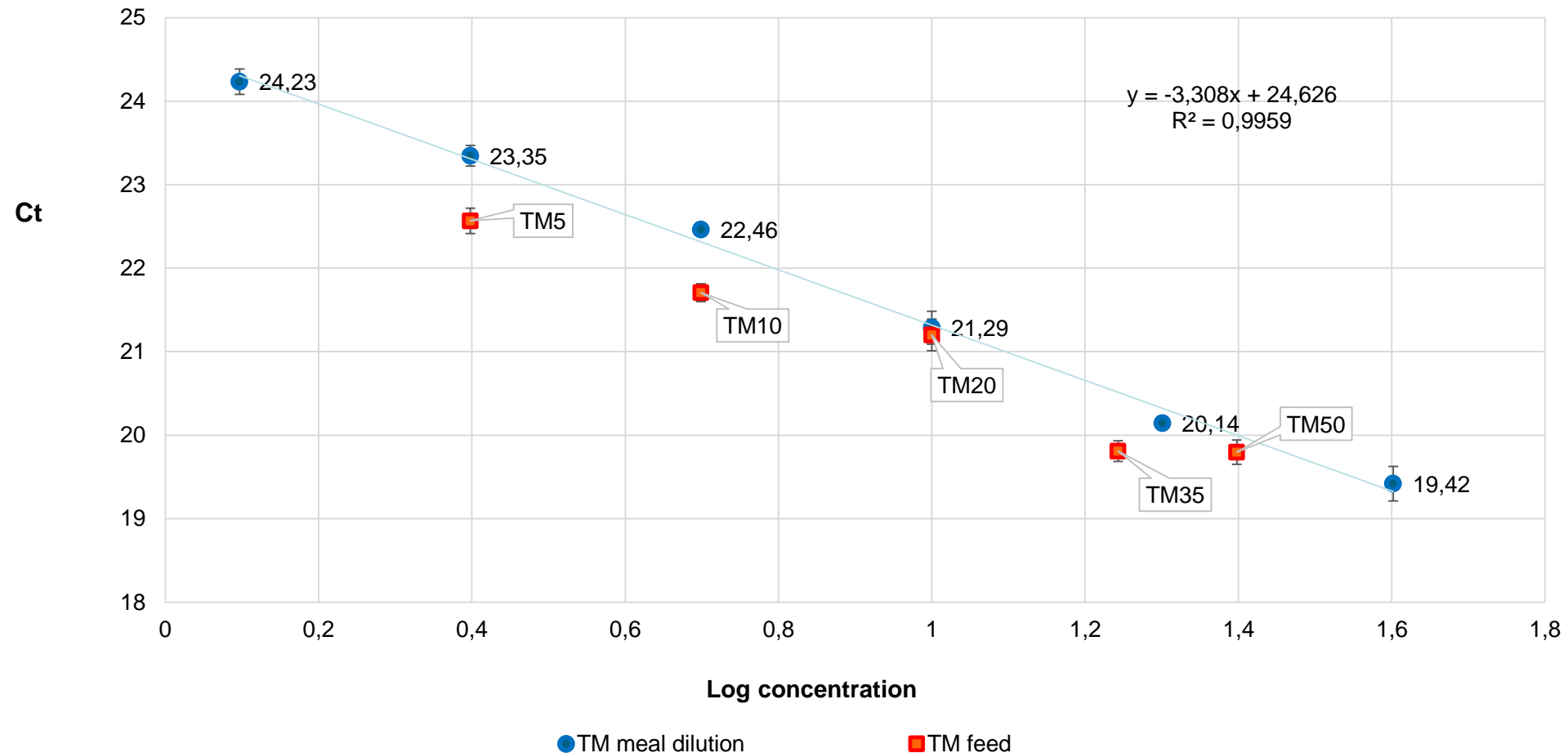




# Test with feed matrix - TM

Five different experimental diets including graded levels of *T. molitor* meal (0,5 – 5,0 ng/ul)

(■) and a negative (TM0) control were produced and tested. Results were compared with TM meal (●) dilution curve.



# From qPCR to Bio-Sensor

PCR Primers sequence previously tested were used to design DNA probes.

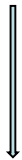
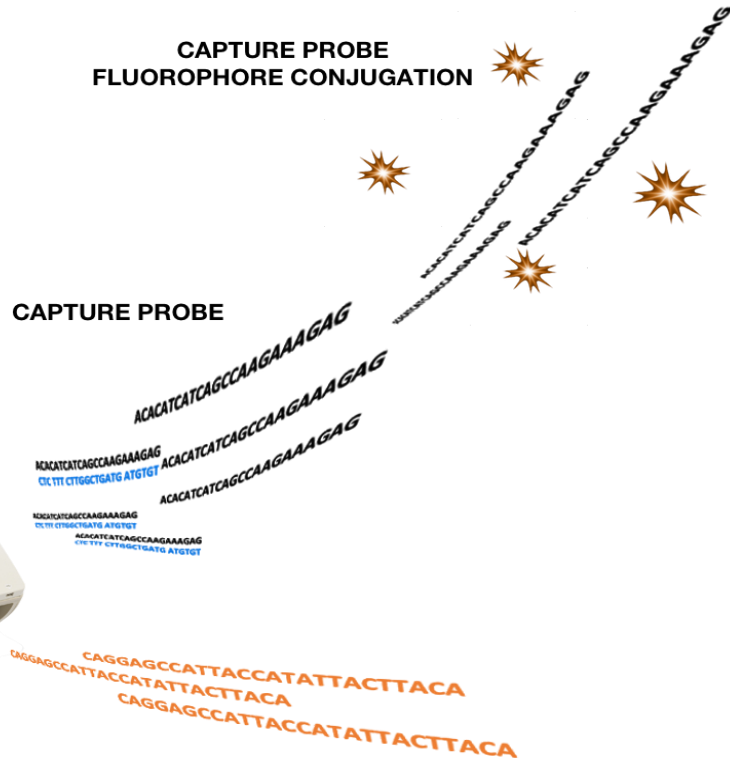


Diagram showing PCR primers sequences:  
CAGGAGCCATTACCATATTACTTACA  
CTCTTTCTTGGCTGATGATGTGT  
CTCTTTCTTGGCTGATGATGTGT  
CAGGAGCCATTACCATATTACTTACA  
CTCTTTCTTGGCTGATGATGTGT  
CAGGAGCCATTACCATATTACTTACA  
CTCTTTCTTGGCTGATGATGTGT

PRIMERS



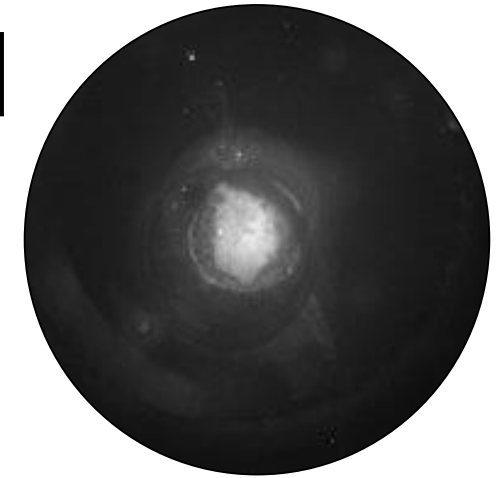
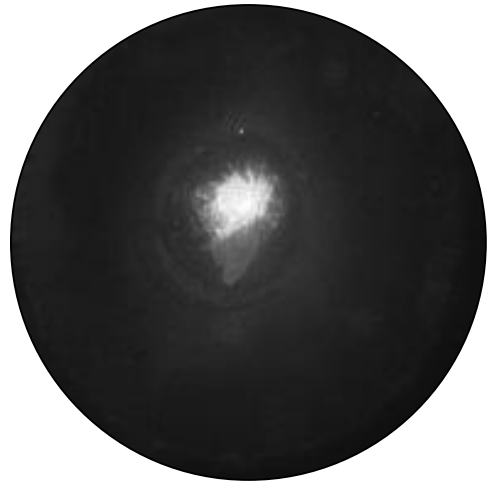
1CGAACATCAATACACACTTTTCTGGCTGATGATGTTTATTATTATTATTCCTGCGAGATTAATGGCTCTGTAAGTAATATGGTAATGGCTCTCG  
ACACATCATGCCCAAGAAAGAG  
CAGGAGCCATTACCATATTACTTACA  
CAATATTACTTACA

Bio-Sensor assay

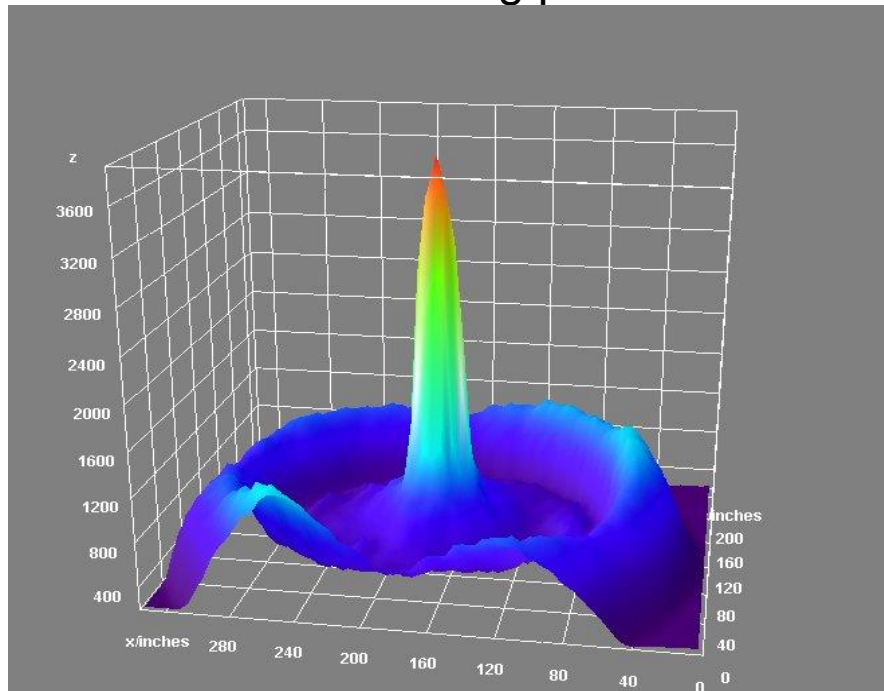
The probes have been applied to develop an innovative Point Of Care (POC) method of detection based on fluorescence excitation by Oled. This technology use a disposable cartridge as Bio-Sensor

# Detection with Oled based Bio-Sensor

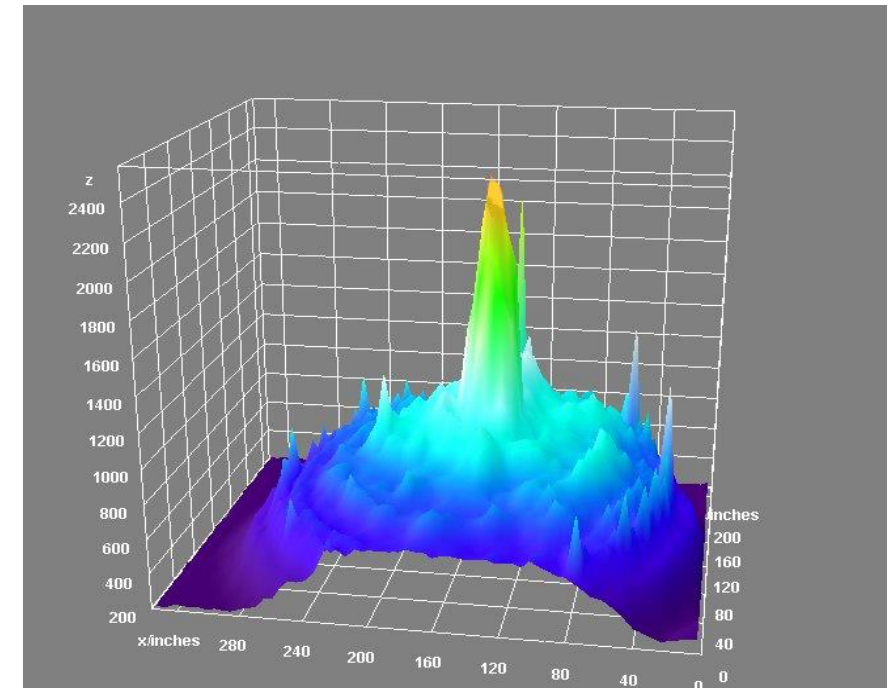
A relationship between the fluorescent signal and the DNA target concentration was observed.



25 ng/ $\mu$ l



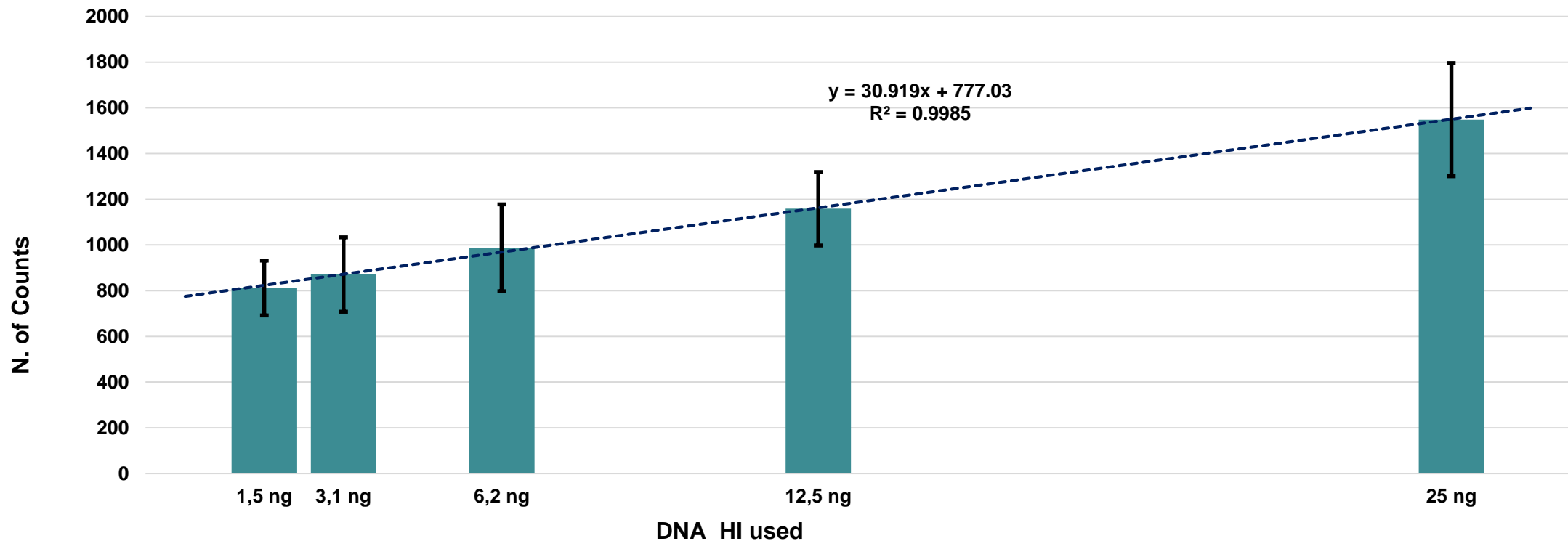
3,1 ng/ $\mu$ l



# Bio-Sensor Data

- The images acquired by the Bio-Sensor were analysed with a Photo images program.
- Measured fluorescence intensity is proportional to the number of counts per pixel with a grey scale range from **0** (complete black) to **4095** (white/saturation)
- The signal is the average of the number of counts for each pixel in the area considered

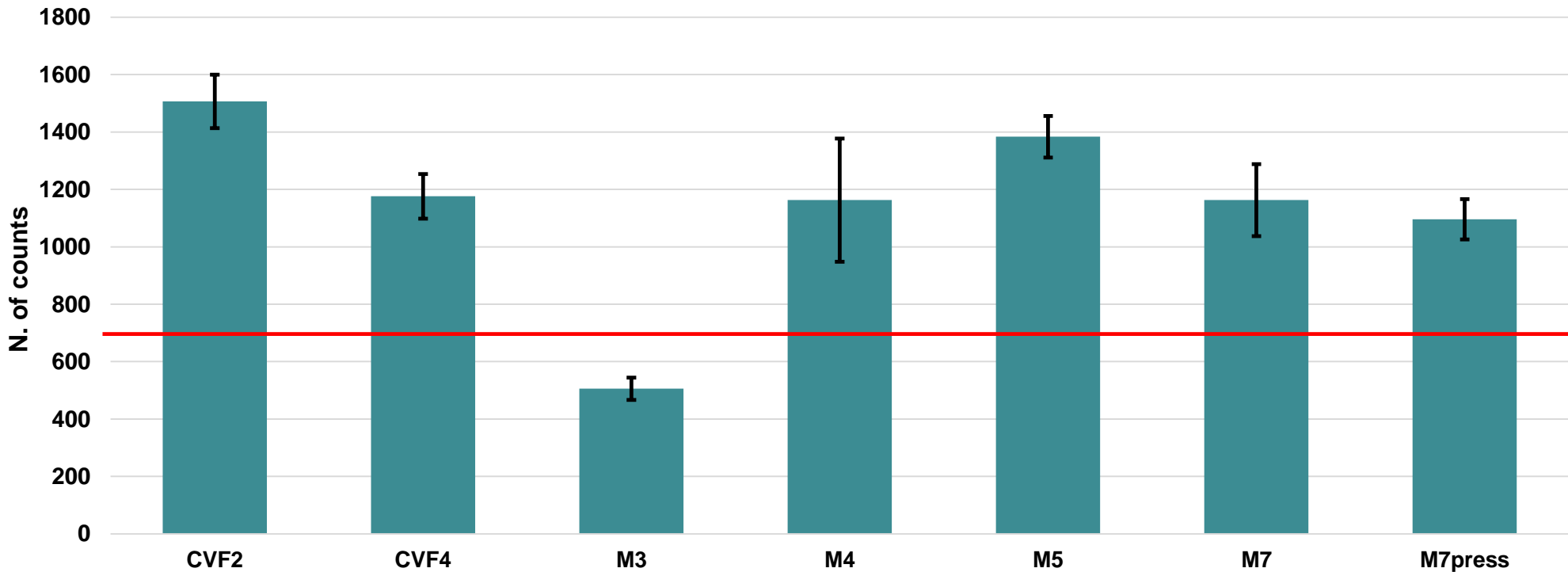
## H. illucens DNA dilutions



# Test with feed matrix - HI

Six different diets including graded levels of *H. illucens* meal (0,5 – 5,0 ng/ul) and a negative control (M3) were provided from CycleFarms and tested with the Bio-Sensor system. The signal was considered as negative if below 700 counts.

HI detection in Aqua-feeds by Bio-Sensor



# Conclusions

- ✓ Results of the analysis confirm the accuracy of the qPCR method for insect detection in complex matrix like practical feeds.
- ✓ DNA primers result in **high target specificity**.
- ✓ Both primers allowed the use of the assay also in feeds subjected to heat-temperature processing.
- ✓ Bio-Sensor based results are **coherent** with the ones obtained using qPCR.



Thanks for your attention ...



In collaboration with



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