

The Foster AFB Method: rapid detection of clinical American Foulbrood through non-invasive hive sampling and DNA quantification via qPCR

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American Foulbrood (AFB) is a devastating bacterial disease of honeybees and subject to careful inspection measures in New Zealand. Despite a low prevalence of approximately 0.5% infection in New Zealand colonies, it has been estimated to cost New Zealand beekeepers more than 12 million euros per year, mostly in costs to inspect colonies for the disease. Diagnosis is centred on the beekeeper's skill in recognising visual symptoms – symptoms that may be confused with other brood issues such as viruses or damage from varroa.

Nearly all current AFB diagnostic testing methods rely on inspecting/sampling colonies that necessitate dismantling the colony and/or require multiple visits to retrieve passive samples. The Foster AFB method uses a novel environmental DNA (eDNA) sampling method using colony entrance swabs together with a quantitative DNA test (qPCR) for *Paenibacillus* larvae: the causative bacteria of AFB disease. DNA is extracted from the swabs using beadbeating and a column or precipitation extraction methods before detection and quantification is performed using a commercially available multiplex qPCR test (two targets for *P. larvae* plus an internal control). The quantification data generated is a critical element as it can be used to differentiate hives with clinically significant infections, even before visual symptoms are apparent (or are otherwise missed by the beekeeper). Sample pooling is used to increase testing throughput while reducing costs per colony to identify infected (and potentially infectious) colonies within an apiary, while individual affected colonies can still be identified through this strategy. Since the first report of the Foster Method in 2021, other studies have used the same sampling method for other honeybee pests and pathogens further confirming the use of the hive entrance as a rapid and non-invasive means to monitor colony health.

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New Zealand



American Foulbrood

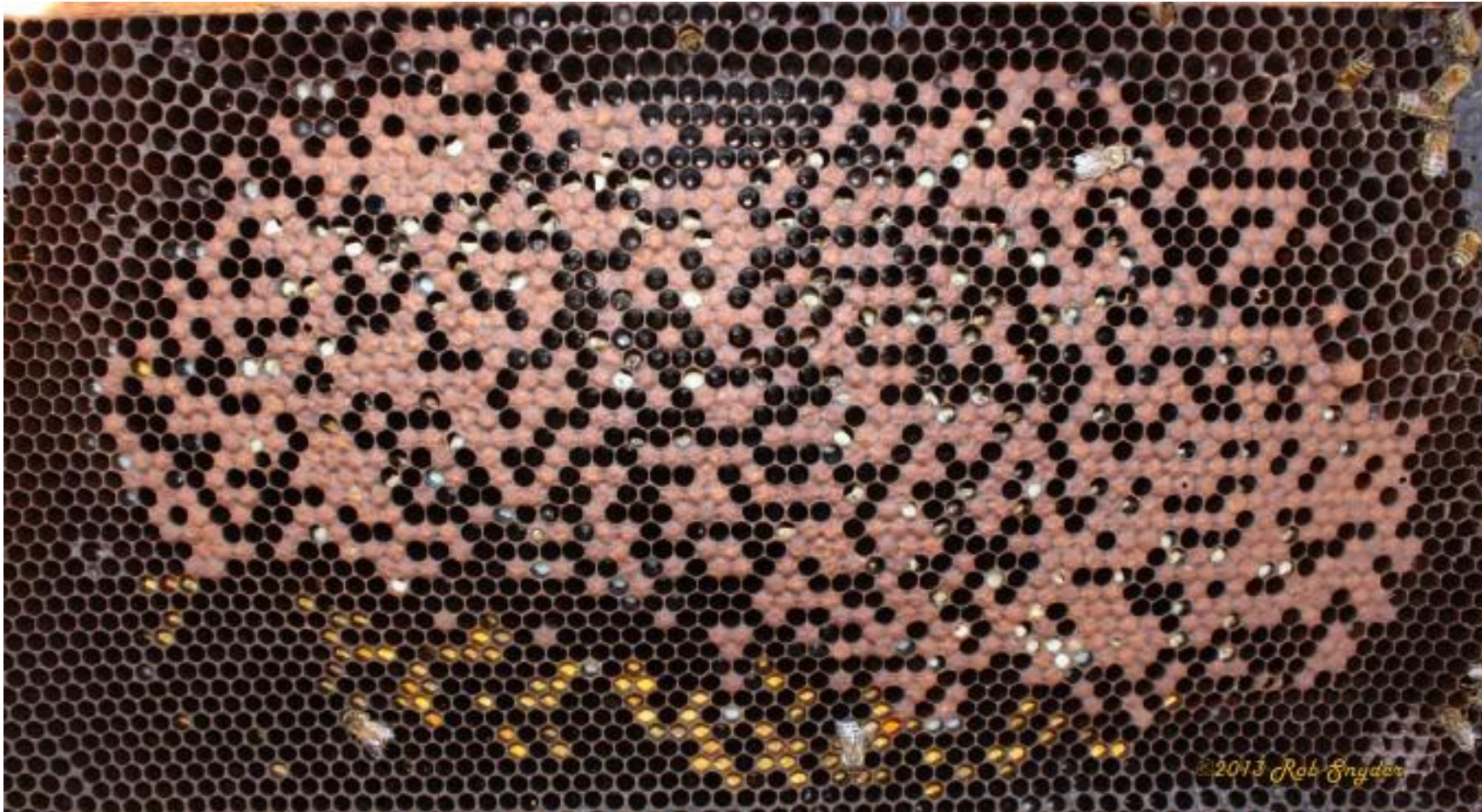
2022

estimated to cost New Zealand
beekeepers € 12 million
(US \$13.7 million) per year



American Foulbrood

- Disease being missed by beekeepers
- Other diseases can mimic symptoms



Parasitic
Mite
Syndrome

AFFB



dnature[®]

detect • discover



American Foulbrood

Laborious to
get samples

- open hives
- multiple visits

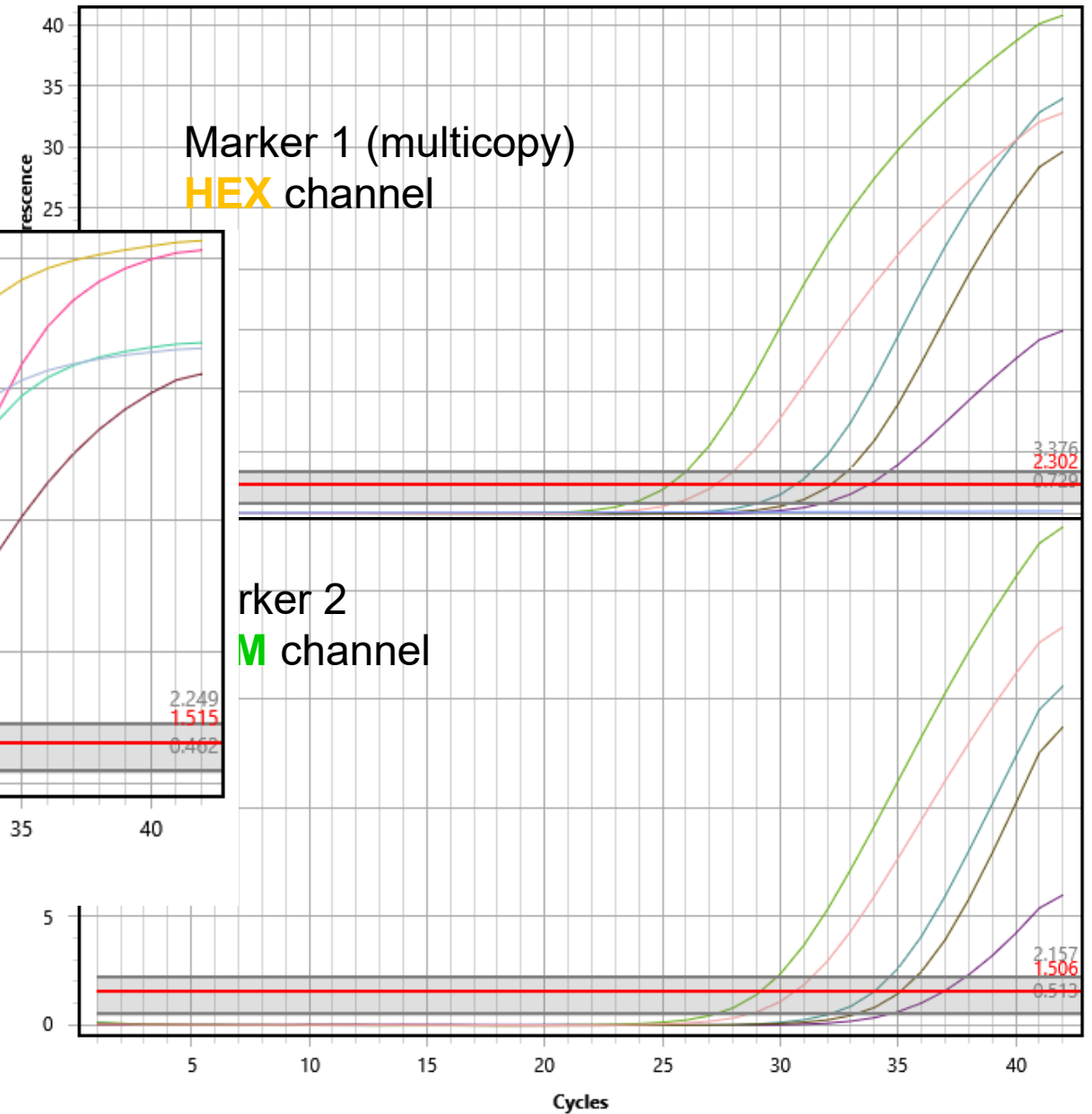
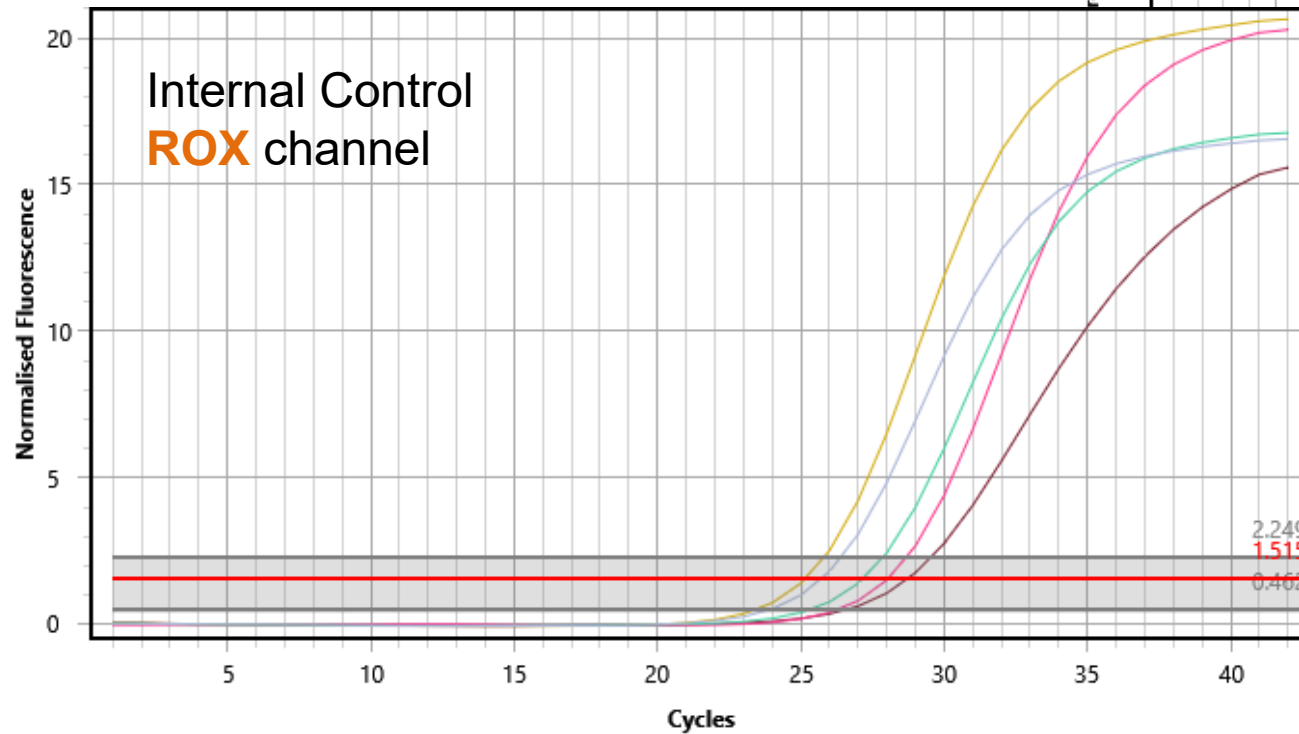


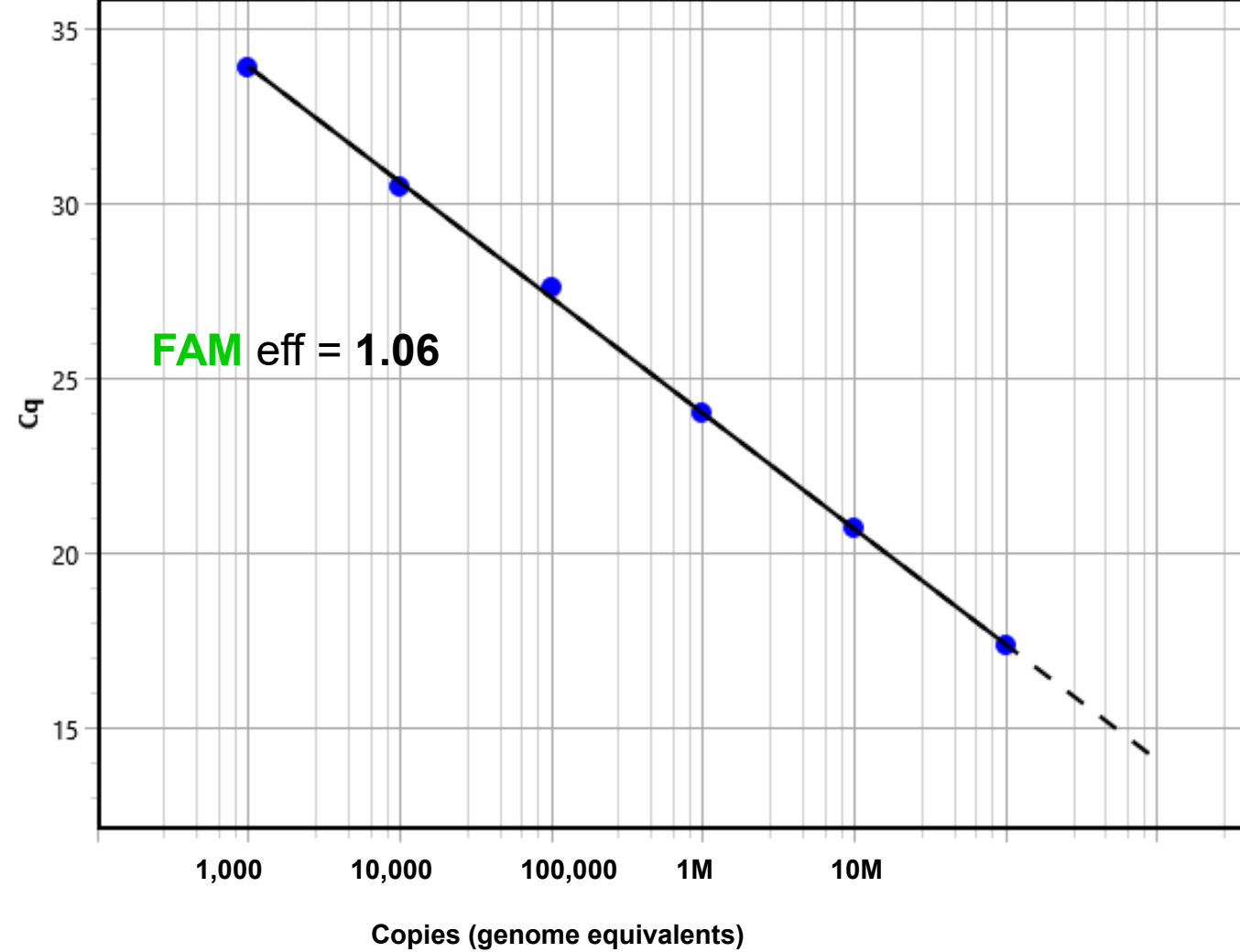
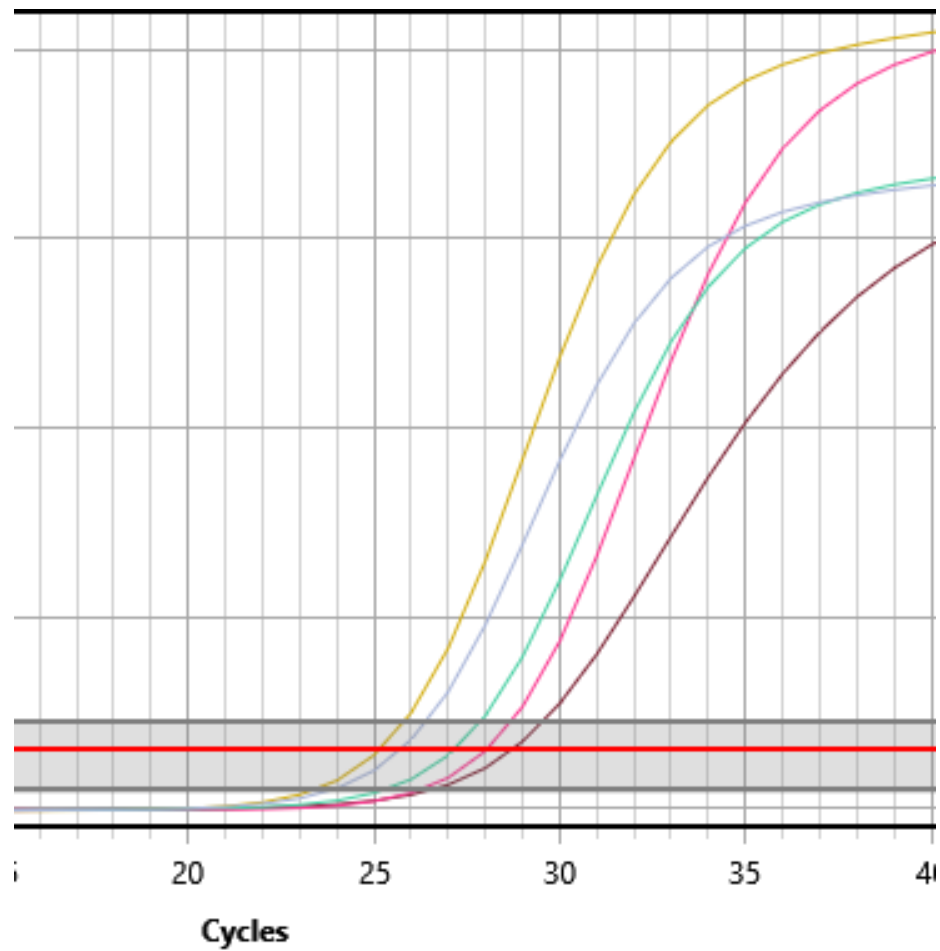
2017 thought:

is there an easier way to detect this disease?

American Foulbrood

Already developed: AFBduo

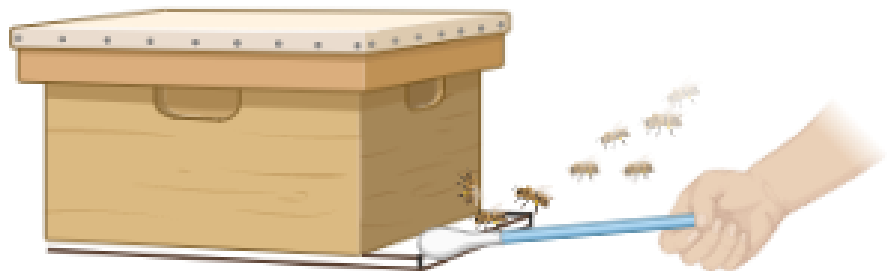




Cq to cell numbers

American Foulbrood

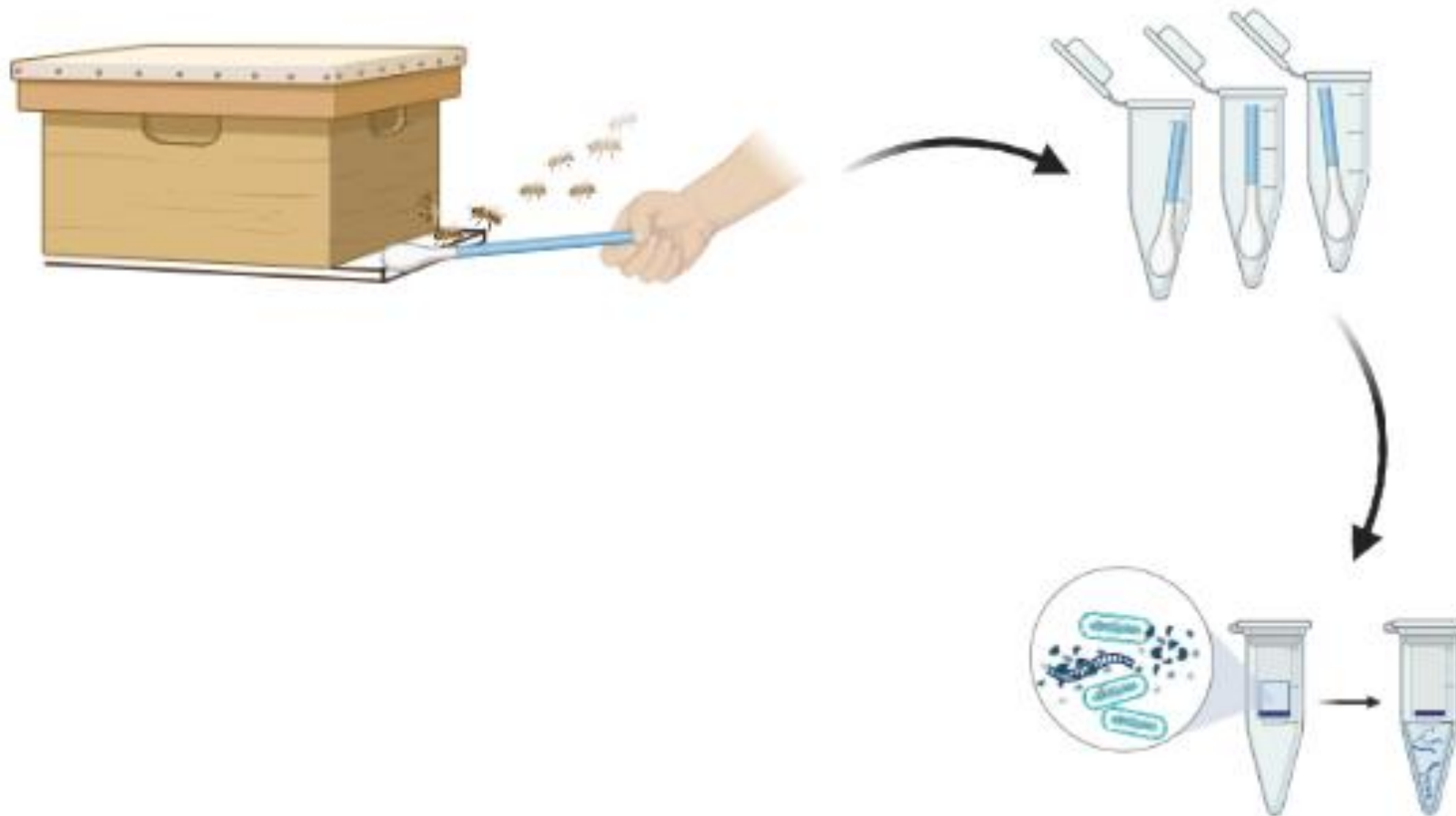
- Swabs - Infectious disease - qPCR
- 2018 !
- **Could we sample hives through the entrance to determine clinical status of hive?**
- Project began in 2018
 - Compared bees vs entire baseboard vs entrance
 - Followed quarantine apiaries from 6 beekeepers
 - 4 different regions in New Zealand



Swab entrance

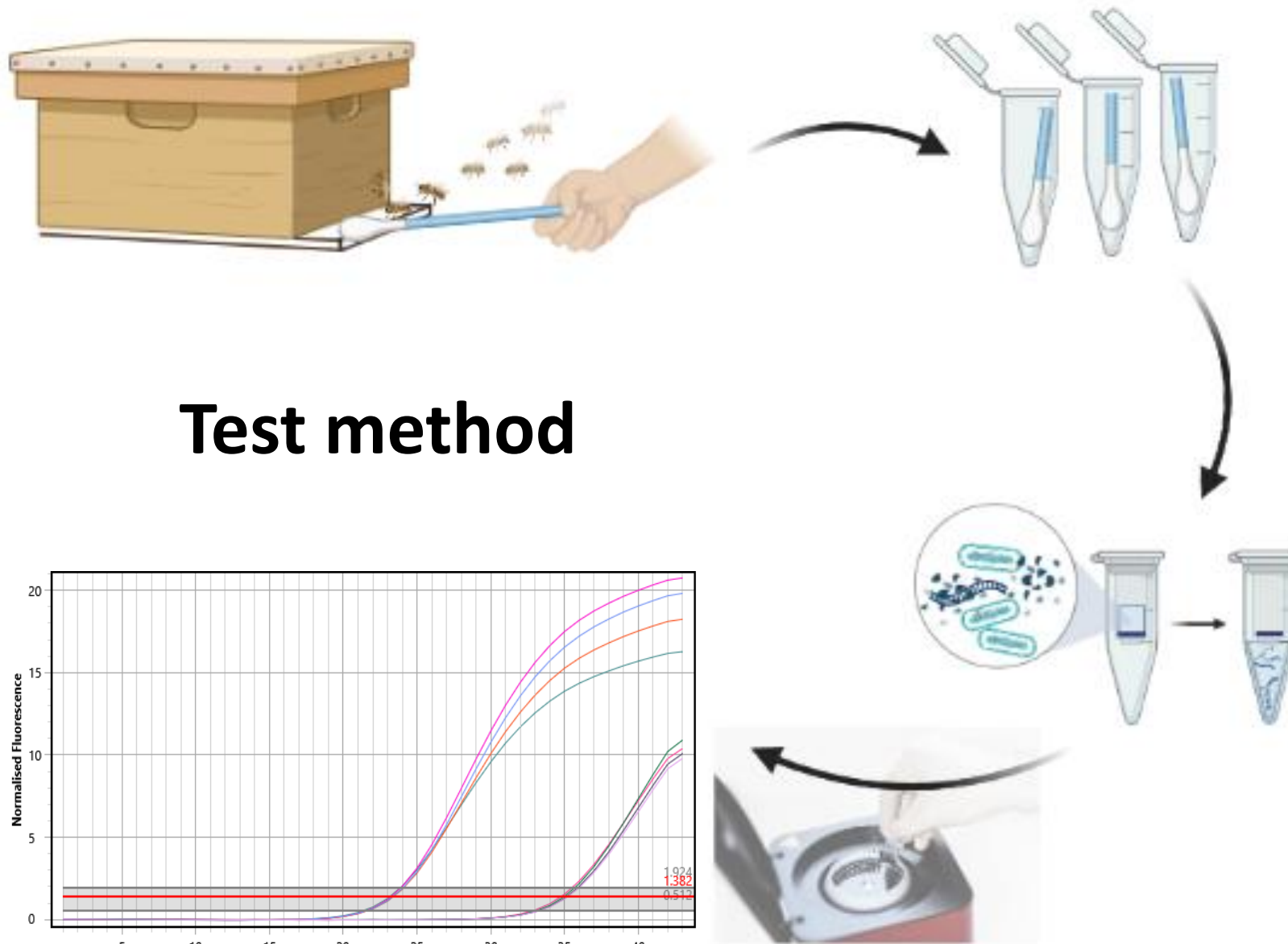


Snapped into tubes

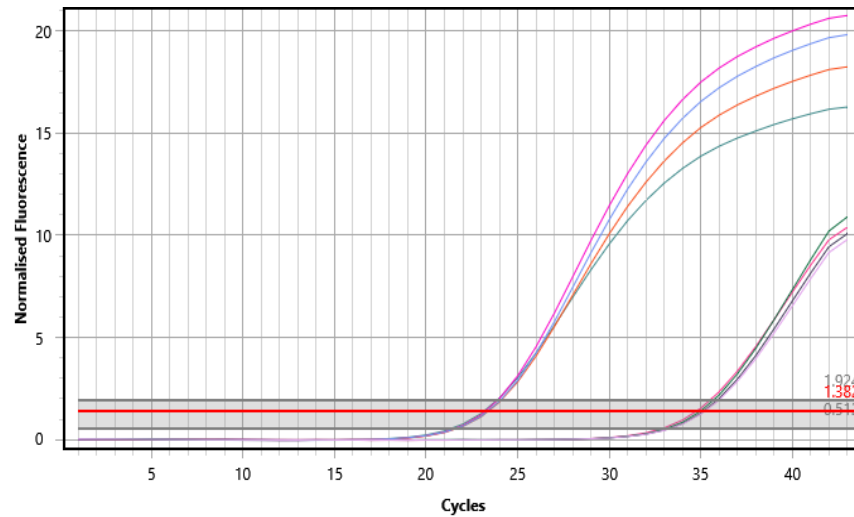


Lab: Extract DNA

Mackay et al. 2024



Test method



Run qPCR and estimate spores (based on Cq)

Do we need the whole baseboard ?

Do we need the whole baseboard ?

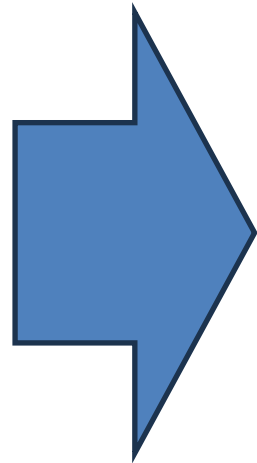
Hive	Location	Estimated Spore level (ftsZ target)	Hive status
ML Shearer	Baseboard	52 million	Clinical signs of AFB
	Entrance	25 million	
ML Ellis	Baseboard	3.6 million	Clinical signs of AFB
	Entrance	3.4 million	
FL 10	Baseboard	61	No AFB seen
	Entrance	335	

Apiary screening

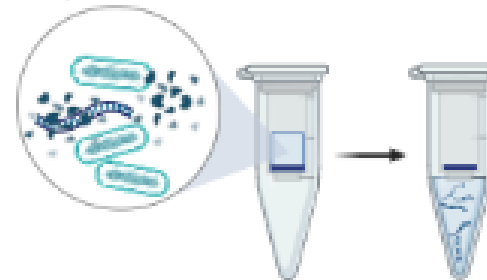
	Clinical signs of AFB?	Bees Spores/10 bees	Entrance Swab Spores/swab
MLHive 1	Yes	37,097,589	3,138,994
MLHive 2	Yes	514,061	24,008
MLHive 3	No	ND	ND
MLHive 4	No	387	1,107
MLHive 5	No	539	377
MLHive 6	No	ND	ND
MLHive 7	No	80	ND
MLHive 8	No	210	ND
MLHive 9	No	205	ND
MLHive 10	Yes	55,866,177	962,621
MLHive 11	No**	9,518,751	ND*
MLHive 12	Yes	12,561,091	890*
MLHive 13	No	<10	<10
MLHive 14	No	ND	<10
MLHive 15	No	2049	1,318
MLHive 16	No	ND	861
MLHive 17	No	ND	326
MLHive 18	No	ND	1,092
MLHive 19	No	97	207
MLHive 20	No	ND	11,235
MLHive 21	No	ND	326
MLHive 22	No	ND	201
MLHive 23	No	943,739	2,365

Major benefit of swabs ?

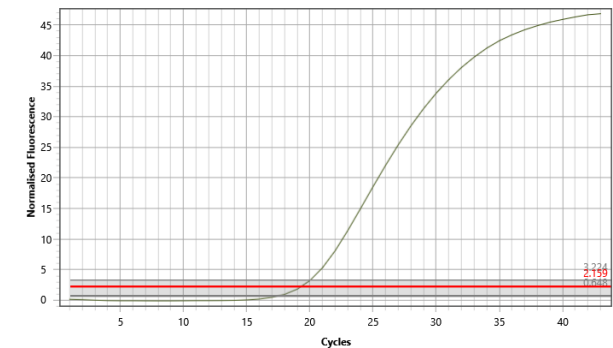
Ability to composite samples (pooling)



Single DNA extraction



Single qPCR reaction



	Pooled result (12) ftsZ spore levels	Individual Swab Result ftsZ spore levels (per swab)	Clinical Signs of AFB (following testing)
Hive SB216	15,630	Not detected	No
Hive SB217		Not detected	No
Hive SB218		Not detected	No
Hive SB219		165,103	Yes
Hive SB220		Not detected	No
Hive SB221		Not detected	No
Hive SB222		Not detected	No
Hive SB250		Not detected	No
Hive SB251		Not detected	No
Hive SB252		Not detected	No
Hive SB253		Not detected	No
Hive SB254		Not detected	No
Hive SB255	Not Detected	-	No
Hive SB256		-	No
Hive SB257		-	No
Hive SB258		-	No
Hive SB259		-	No
Hive SB260		-	No
Hive SB261		-	No
Hive SB262		-	No
Hive SB263		-	No
Hive SB264		-	No
Hive SB265		-	No

Swabbing hive ware (supers)

Same swab can be used for multiple boxes

Swabbing in 'W' pattern across middle of frames (top or bottom)



Lab testing

Old hive ware suspected of being contaminated by AFB

Swab #	284		290		291		293		294	
dnature #	19676		19677		19678		19679		19680	
AFB	Result	Cq	Result	Cq	Result	Cq	Result	Cq	Result	Cq
Marker 1	Pos	20.56	Pos	27.92	Pos	9.68	Pos	19.49	Pos	8.19
Marker 2	Pos	25.43	Pos	31.62	Pos	13.51	Pos	23.51	Pos	11.84
Spore est.	389,554		6,537		1,020,889,196		1,384,153		3,075,390,843	



> 3 billion spores

Swab #	295		296		297		298		300	
dnature #	19681		19682		19683		19684		19685	
AFB	Result	Cq	Result	Cq	Result	Cq	Result	Cq	Result	Cq
Marker 1	Pos	26.38	Pos	17.68	Pos	21.42	Pos	27.42	Pos	22.73
Marker 2	Pos	30.41	Pos	21.32	Pos	25.44	Pos	31.27	Pos	26.64
Spore est.	14,535		5,878,019		386,990		8,237		175,212	

Swabbing 'game-changer'

By **Shawn McAvinue**



2021

Rural life > Other News

A new technology helping fight against a bee-killing disease is a “massive breakthrough”, an Otago apiarist says.

New Zealand Alpine Honey owner and Project CleanHive chairman Peter Ward, of Hawea, said he ran about 5000 hives across Otago, Southland and the West Coast.



Bee smart: Covid detection method inspires apiarists

Publication (2024) after 2021 preprint

JOURNAL OF APICULTURAL RESEARCH
<https://doi.org/10.1080/00218839.2024.2306445>



ORIGINAL RESEARCH ARTICLE



The Foster method: rapid and non-invasive detection of clinically significant American Foulbrood disease levels using eDNA sampling and a dual-target qPCR assay, with its potential for other hive pathogens

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with its potential for other hive pathogens

Since 2021 preprint release, entrance swabs used to test for:

Small hive beetle, EFB (Boardman et al, 2023)

Viruses (DWV) (Mackay et al. 2021, 2024). Others – hive debris

Varroa (Roberts et al. 2025, Boardman et al, 2023)



Barry Foster

Foster Method

AFB Board member 9 years

AFB recognition course trainer

AFB identifier (AP2)

11 years on national board

Apiculture NZ Life Member

Early adopter of method

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