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Digital PCR (QIAcuity) as a Next-Generation Diagnostic Method

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Abstract: Cardiopulmonary dirofilariosis, caused by *Dirofilaria immitis*, is a mosquito-borne zoonosis with an increasing distribution across Europe. Molecular diagnosis of the infection in vectors requires methods with high analytical sensitivity, particularly when the genomic load in entomological samples is extremely low.
This study compared the diagnostic performance of digital PCR on the QIAcuity platform (QIAGEN) with quantitative PCR (qPCR) by analyzing four series of serial dilutions (up to D7) of DNA samples derived from mosquitoes confirmed to be positive for Dirofilaria immitis. Four dilution series (T29, T75, T9, T10) were prepared with initial concentrations ranging from 1.075 cp/µL (T9) to 8977.8 cp/µL (T10) in the reaction, tested on a Nanoplate 26K with 24 wells (total volume 40 µL, template volume 7 µL). Each well generated approximately 25,000 valid partitions. The negative control (NTC) confirmed the absence of contamination. The results showed that dPCR detected Dirofilaria immitis DNA at concentrations of 0.054 cp/µL (T75-D5, 1 positive partition out of 25,470 valid partitions) and 0.157 cp/µL (T10-D6, 3 positive partitions). The experimentally determined limit of detection (LOD) was approximately 0.054–0.108 cp/µL in the reaction.The T29 and T9 series reached the negativity threshold at D5 and D2, respectively, corresponding to concentrations below 0.1 cp/µL in the reaction. The NTC showed 0 positive partitions for both targets. The QIAcuity dPCR platform demonstrates superior analytical sensitivity compared with conventional qPCR, being capable of detecting individual DNA molecules in samples with extremely low genomic loads. It is therefore recommended as a reference method for the molecular surveillance of vectors involved in dirofilariasis transmission.
Keywords: ***Dirofilaria immitis; serial dilutions; limit of detection; analytical sensitivity.***

Materials and Methods

Between April and October 2025, mosquito surveillance for *Dirofilaria immitis* transmission risk in Tulcea County was performed using CDC Light Traps baited with dry ice, followed by morphological identification, pooling of mosquito specimens, DNA extraction, and multiplex qPCR analysis based on TaqMan chemistry, using the CFX96™ Real-Time Detection System for simultaneous pathogen detection and melting curve analysis [Turcitu, 2024; Sandu, 2025].

To evaluate the analytical sensitivity of digital PCR, four D. immitis-positive samples with different DNA concentrations were subjected to serial dilution and analyzed on the QIAcuity One dPCR platform, enabling absolute quantification through Poisson-based partition analysis, determination of the limit of detection, and theoretical comparison with qPCR performance at very low DNA concentrations where conventional qPCR approaches its detection threshold.

- DNA Extraction→ PureLink Genomic DNA Mini Kit
- **Testing qPCR**

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- **CFX96 Real-Time**
- **Principle TaqMan**
- **Channels: FAM, HEX, Cy5**

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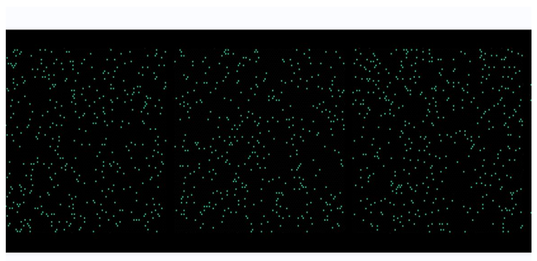
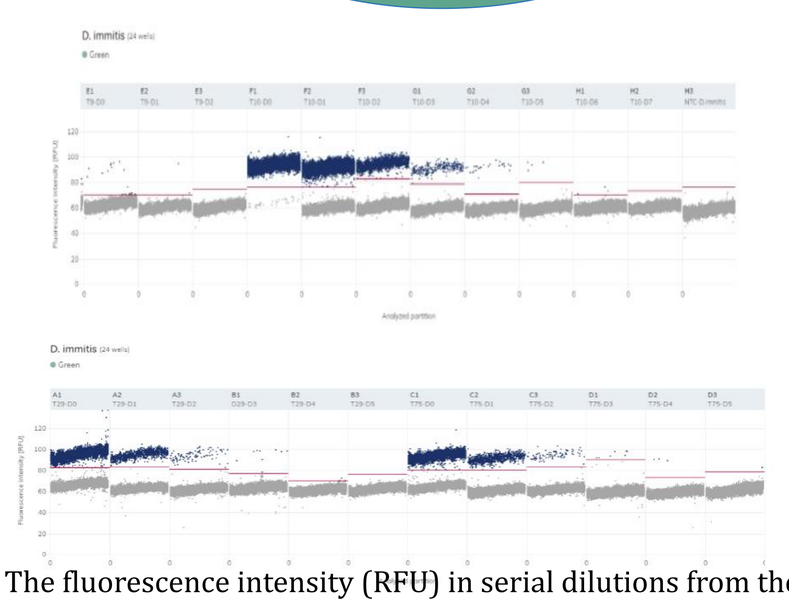
- **dPCR -serial dilution analyzed on the QIAcuity One dPCR platform**



Positive partitions



The fluorescence intensity (RFU) in serial dilutions from the undiluted sample to dilution the highest dilution

Results

All analyzed wells passed VPF validation, while the stable amplification of the internal control and the absence of positive partitions in the negative control confirmed both the quality of DNA extraction and the lack of contamination, demonstrating the reliability of the dPCR assay for Dirofilaria immitis detection.

Serial dilution analysis showed that dPCR maintained detectable signals at extremely low DNA concentrations (as low as 0.054–0.157 cp/µL), corresponding to theoretical qPCR values beyond the conventional detection threshold (Ct ≥ 40), thereby demonstrating a markedly superior analytical sensitivity and an extended lower dynamic range compared with conventional qPCR.

Sample	Dilution	Conc. rxn [cp/µL]	Conc. undiluted [cp/µL]	CI 95%	Valid partitions	Positive	Neg.	Det.
T29	D0	452.7	2586.9	2.3%	25079	7273	17806	Yes
T29	D1	62.67	358.1	5.7%	25471	1170	24301	Yes
T29	D2	5.441	31.09	19.3%	25339	103	25236	Yes
T29	D3	0.479	2.737	65.3%	25097	9	25088	Yes
T29	D4	0.108	0.615	119%	25485	2	25483	Yes
T29	D5	0	0	—	25474	0	25474	**No**
T75	D0	469.6	2683.2	2.3%	25264	7414	17850	Yes
T75	D1	57.59	329.1	6.1%	25506	1027	24479	Yes
T75	D2	4.991	28.52	20.3%	25488	93	25395	Yes
T75	D3	0.374	2.136	74.1%	25417	7	25410	Yes
T75	D4	0.167	0.954	106.4%	25478	3	25475	Yes
T75	D5	0.054	0.311	147.5%	25470	1	25469	Yes
T9	D0	1.075	6.140	43.8%	25460	20	25440	Yes
T9	D1	0.110	0.631	119%	25476	2	25474	Yes
T9	D2	0	0	—	25474	0	25474	**No**
T10	D0	8977.8	5.13×10^4	5.2%	25458	25423	35	Yes
T10	D1	1346.0	7691.6	1.6%	25490	15722	9768	Yes
T10	D2	143.2	818.4	3.9%	25455	2484	22971	Yes
T10	D3	15.07	86.14	11.7%	25463	282	25181	Yes
T10	D4	1.417	8.100	38.4%	25481	26	25455	Yes
T10	D5	0.214	1.221	98.0%	25451	4	25447	Yes
T10	D6	0.157	0.896	106.4%	25402	3	25399	Yes
T10	D7	0	0	—	25478	0	25478	**No**
NTC	—	0	—	—	25395	0	25395	**No**

Complete results of serial dilutions by dPCR (QIAcuity) for the detection of Dirofilaria immitis

Discussions

Digital PCR (dPCR) has emerged as a highly sensitive molecular tool for the detection and genotyping of Dirofilaria immitis, particularly for identifying SNPs associated with macrocyclic lactone resistance, offering absolute quantification without a reference curve, increased resistance to PCR inhibitors, and superior analytical sensitivity compared with qPCR, especially at very low DNA concentrations commonly encountered in vector surveillance studies [Kumar S, 2024; Pietrzak D, 2024].

The experimental results obtained with the QIAcuity dPCR platform demonstrated that dPCR maintained reliable detection of D. immitis DNA at concentrations as low as 0.054 cp/µL, corresponding to levels below the conventional qPCR detection threshold, thereby confirming a markedly extended dynamic range and at least a 10-fold sensitivity advantage over qPCR, which is particularly important in mosquito pool diagnostics where parasite DNA may be highly diluted

Conclusions

Serial dilution experiments performed on the QIAcuity (QIAGEN) dPCR platform demonstrated that Dirofilaria immitis DNA could be reliably detected at concentrations as low as 0.054 cp/µL, establishing a practical detection limit at least one order of magnitude lower than conventional qPCR, while internal controls confirmed reaction quality and contamination-free analysis, supporting the use of dPCR as a reference method for vector surveillance, epidemiological monitoring, and confirmation of borderline qPCR results.