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Comparative evaluation of 5 RT-PCR kits for Dengue virus detection

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ArboNET National Arbovirus Surveillance System

Introduction

- Dengue (DENV) is the most common mosquito-borne viral infection affecting humans worldwide. Dengue disease is caused by an RNA flavivirus, of which 4 serotypes are known: DENV-1, DENV-2, DENV3, DENV-4.
- DENV is a typical tropical and subtropical virus but current climate change increase cases around the world.
- The virus is transmitted to humans through the bites of infected female Aedes mosquitoes, primarily the Aedes aegypti and Aedes albopictus.
- Almost half of the world's population, about 4 billion people, live in areas with a risk of dengue. Dengue is often a leading cause of illness in areas with risk.
- For epidemiological surveillance, ArboNET is a national arboviral surveillance system managed by CDC and state health departments. ArboNET collects data on arboviral infections among people, veterinary animals, mosquitoes, dead birds, and sentinel animals. From ArboNET data, dengue cases in 2024 for travel status were 1217.

Aim of study

- The progressive increase in the number of cases worldwide has highlighted the need for rapid and accurate diagnosis of the infection, achievable by both serological and molecular tests.
- This study compared the diagnostic performance of five commercial RT-PCR kits for the detection of the virus in blood samples.

Material and methods

- During 2023, 25 plasma samples from individuals with symptoms consistent with dengue virus infection were analysed in the regional reference Hygiene laboratory of the Policlinico San Martino Hospital, Genoa.
- Extraction was performed by the automated ELITe InGenius® (ELITechGroup Empowering IVD) platform using the cartridge ready to use ELITe InGenius® SP 200. A volume of 200 µl of sample has been eluted in 100 µl elution buffer. Extraction lasts 30 minutes.
- RNA viral amplification was performed on CFX96™ (Bio-Rad Laboratories, USA). Four commercial kits were used: RealStar dengue PCR 3.0 IVD (Altona Diagnostics, Germany), Clonit'ngo Dengue/Zika/Chikungunya system IVD (Clonit S.r.l., Italy), Dengue Virus Real Time PCR IVD (BioPerfectus, China), Novaplex™ Tropical fever virus Assay RUO (Seegene Inc., South Korea).
- Moreover, all samples were tested on point of care MDx system STANDARD M10, with Standard M10 Arbovirus Panel IVD assay (SD Biosensor, South Korea).
- The turnaround time (TAT) is 1 hour for M10, almost 3 hours for the others. While the Altona kit only detects DENV, the other kits are multiplex RT-PCR's that identify different arboviruses.

Results

- A total of 25 clinical samples were tested in the 4 conventional RT-PCR assays. Of these latter, 15 samples were positive, while the remaining 10 specimens were negative to the Dengue virus.
- The Seegene, Clonit and Altona RT-PCR kits showed a perfect 100% PPA. Conversely, the Bio kit performed worse with a PPA of 84.0%.
- Dengue serotypes' percentage of positive samples is shown in Figure 1.

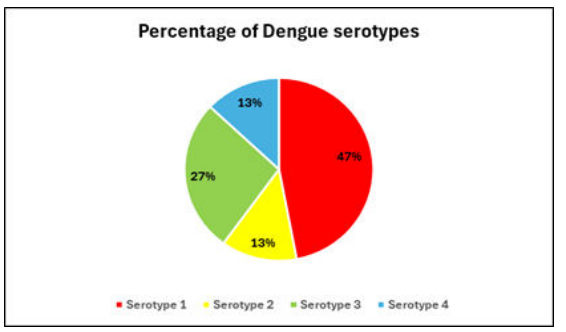

Figure 1: Percentage of Dengue serotypes

- Distribution of Ct values provided by the 5 assays differed significantly. M10 showed comparatively low Ct values, which were significantly lower than those showed by BioPerfectus, Clonit'ngo, and Altona. Conversely, the BioPerfectus kit showed the highest Ct values and all pairwise comparisons were highly significant (Figure 2).

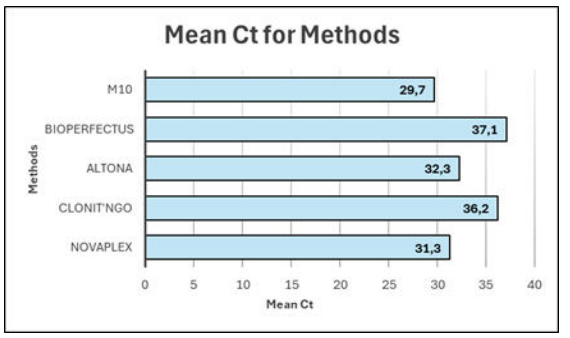

Figure 2: Mean Ct for PCR Methods

- Finally, when 8 serial half-log dilutions (5 replicates for each dilution) of a serotype 1 isolate were tested in the 5 assays, the Altona kit was deemed the most sensitive, while the Bio Perfectus assay was the least sensitive.

Dilution	Parameter	Novaplex	Clonit'ngo	Altona	BioPerfectus	M10
1:1	Detected/Total	5/5	5/5	5/5	5/5	5/5
	Mean Ct	31.3	36.2	32.3	37.1	29.7
1:3.16	Detected/Total	5/5	4/5	5/5	2/5	5/5
	Mean Ct	32.9	38.3	33.9	39.8	31.5
1:10	Detected/Total	5/5	4/5	5/5	2/5	5/5
	Mean Ct	35.0	38.3	35.5	39.6	33.0
1:31.6	Detected/Total	5/5	2/5	5/5	1/5	5/5
	Mean Ct	37.0	39.2	37.3	40.9	34.6
1:100	Detected/Total	4/5	0/5	5/5	0/5	3/5
	Mean Ct	38.1	NA	37.6	NA	35.5
1:316	Detected/Total	0/5	0/5	0/5	0/5	0/5
	Mean Ct	NA	NA	NA	NA	NA
1:1000	Detected/Total	1/5	0/5	2/5	0/5	0/5
	Mean Ct	38.9	NA	37.8	NA	NA
1:3160	Detected/Total	0/5	0/5	1/5	0/5	0/5
	Mean Ct	NA	NA	38.7	NA	NA

Table 1: Detection rates of serotype 1 DENV at different half-long dilutions, by assay

Conclusion

- The results show that these tests can be useful tools for the laboratory diagnosis of dengue and generally showed good diagnostic performance combined with short TAT.
- In addition, the sensitivity of the multiplex assays was found to be almost equivalent to that of the single plex test. In addition, the sensitivity of the multiplex assays was found to be almost equivalent to that of the singleplex test.

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