



INTRODUCTION

Dengue virus (DENV) is an arbovirus spread by the mosquito *Aedes aegypti* and is endemic across tropical and subtropical regions. Dengue is one of the most significant mosquito-borne viral pathogens worldwide [1] with 14.1 million cases reported in 2024 [2]. The virus causes severe disease [3], including dengue haemorrhagic fever and dengue shock syndrome [4]. Comprising four serotypes (DENV-1 to DENV-4), dengue presents challenges for research and public health due to its antigenic diversity and complex immunological interactions [5]. The increasing geographical spread of dengue is driven by the range expansion of the vector caused by climate change, urbanisation, and increased global travel [6].

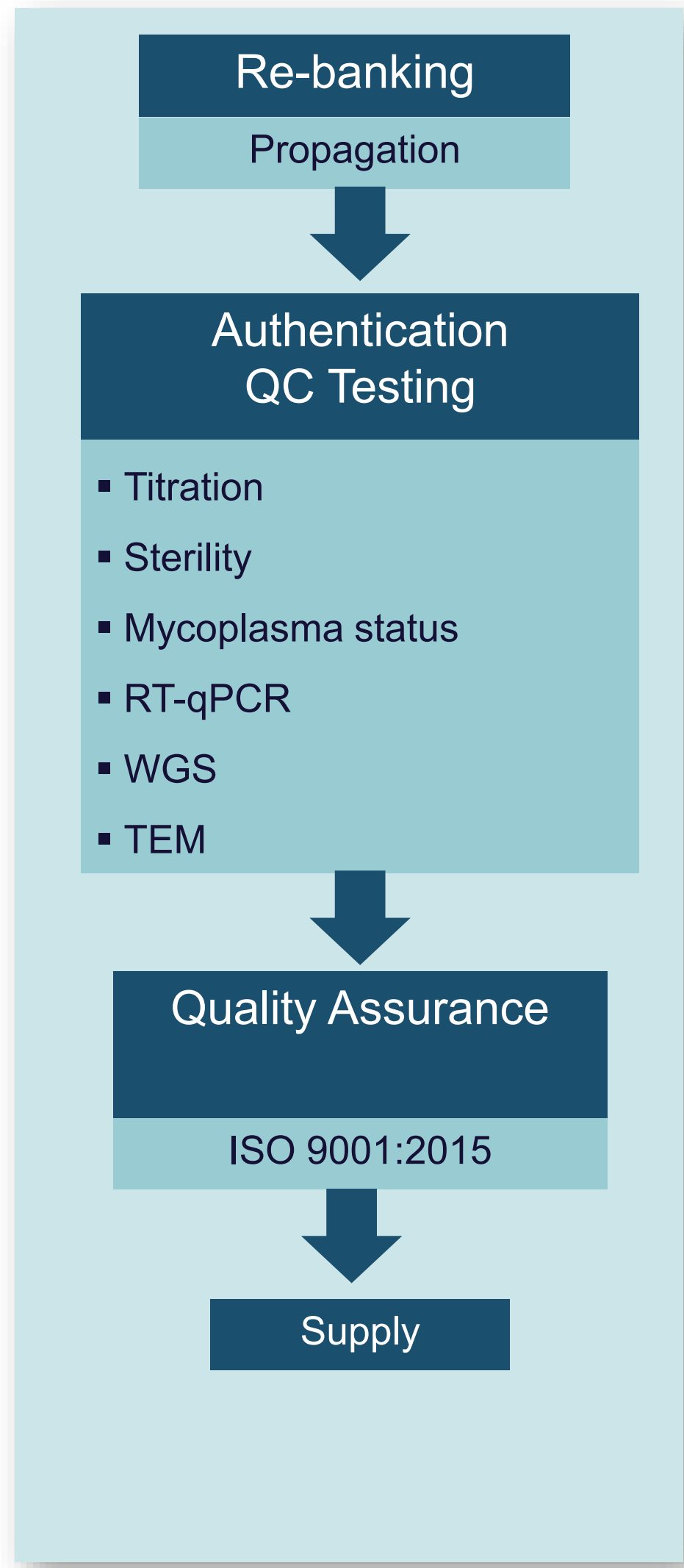
The National Collection of Pathogenic Viruses (NCPV), Culture Collections was established in 2000 and preserves, authenticates, and distributes high-quality viral materials to the scientific community. NCPV holds twelve dengue virus strains representing all four serotypes. The generation of high-titre dengue virus stocks in NCPV has historically been challenging due to variable replication efficiency in cell culture, strain-dependent growth characteristics, sensitivity to culture conditions and low-titre parent stocks.

Improved methodologies for the culture of DENV-3 have been developed, focusing on optimising propagation conditions to enhance viral yield while maintaining genetic integrity and phenotypic authenticity.

METHODS

Dengue 3 virus (NCPV 0411283v) was cultured on Vero E6 (kidney) cell line (ECACC 85020206). Two T175 flasks were seeded at 6×10^4 cell/cm². After 24 hours, the cell monolayer was infected with a 1:6 dilution of virus in MEM (Gibco, Thermo Fisher Scientific), with a total absorption volume of 5 mL. A negative flask was prepared by adding 5 mL of media. Both flasks were incubated for 180 minutes at 33° C before adding 30 mL MEM supplemented with 2% Foetal Bovine Serum (Sigma-Aldrich, Merck, Cat. No. F2442). The flasks were incubated at 33° C with 5% CO₂ and observed regularly under a microscope for cytopathic effect (CPE). CPE was allowed to develop, and no passage or infectious splits were carried out. When ~ 70 % of cells were refractile round and floating, 18 days post-infection, the flask was freeze-thawed (-80° C) and harvested by low-speed centrifugation at 1409 x g for 10 minutes.

The bank was tested for the presence of bacteria or fungi by incubation with Tryptone Soya Broth at 37° C and Fluid Thioglycollate Medium at ~21° C for 7 days. The virus was titred using an optimised TCID₅₀. Nucleic acid was extracted using the QIAmp Viral RNA Mini Kit (Qiagen). The extract was tested for the presence of mycoplasma by qPCR [7] and subject to RT-qPCR using an in-house adaptation of the method of Drosten *et al.* [8] targeting the 3'UTR. Whole genome sequence analysis was achieved by random amplification of cDNA using an in-house SISPA method followed by Illumina sequencing. A sample was inactivated in EM grade formaldehyde, final volume >4% and imaged by Transmission Electron Microscopy (TEM).



RESULTS

- A historical DENV-3 isolate (NCPV 0411283v) was propagated following optimisation of culture conditions, including increased inoculum volume, extended adsorption time and incubation at 33° C without passage resulting in observation of CPE 18 days post-infection.
- The resulting virus stock reached a titre of 4.68×10^6 TCID₅₀/mL.
- Quality control testing confirmed the stock was free from detectable bacterial or fungal contamination and contained no detectable mycoplasma DNA.
- Dengue specific RT-qPCR confirmed a high viral load, with detection at Ct 17 for 1:100 and Ct 21 for 1:1000 dilutions
- Genomic analysis generated a high-quality consensus sequence (99.64% at 20x). Nexclade placed it within DENV-3 clade 3V. The nucleotide BLAST results indicated it was most closely related to AF317645.1, a reference sequence from China for DENV-3, strain 80-2.

Table 1: Summary statistics mapping to M93130.1 (strain 80-2)	value
Total reads	5,246,266
Total reads mapped	3,489,644
Proportion of reads mapped	66.52%
Cov 1x	100%
Cov 20x	99.64%

Figure 1 – Table summarising the statistics of mapping the dengue 3 NCPV 0411283v against reference sequence M93130.1 (strain 80-2).

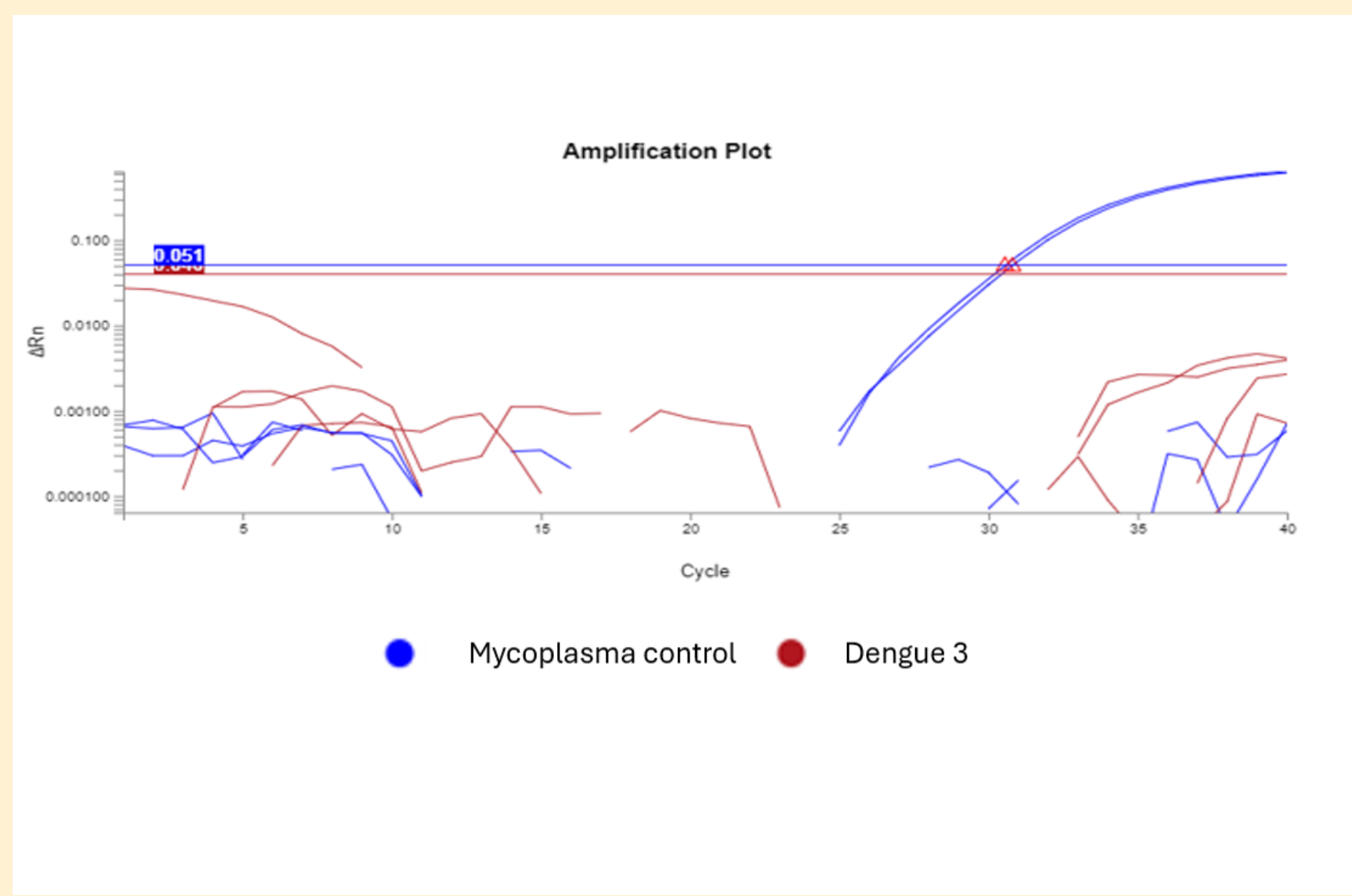


Figure 2 – qPCR graph of dengue 3 NCPV 0411283v for mycoplasma DNA detection. No amplification occurred.

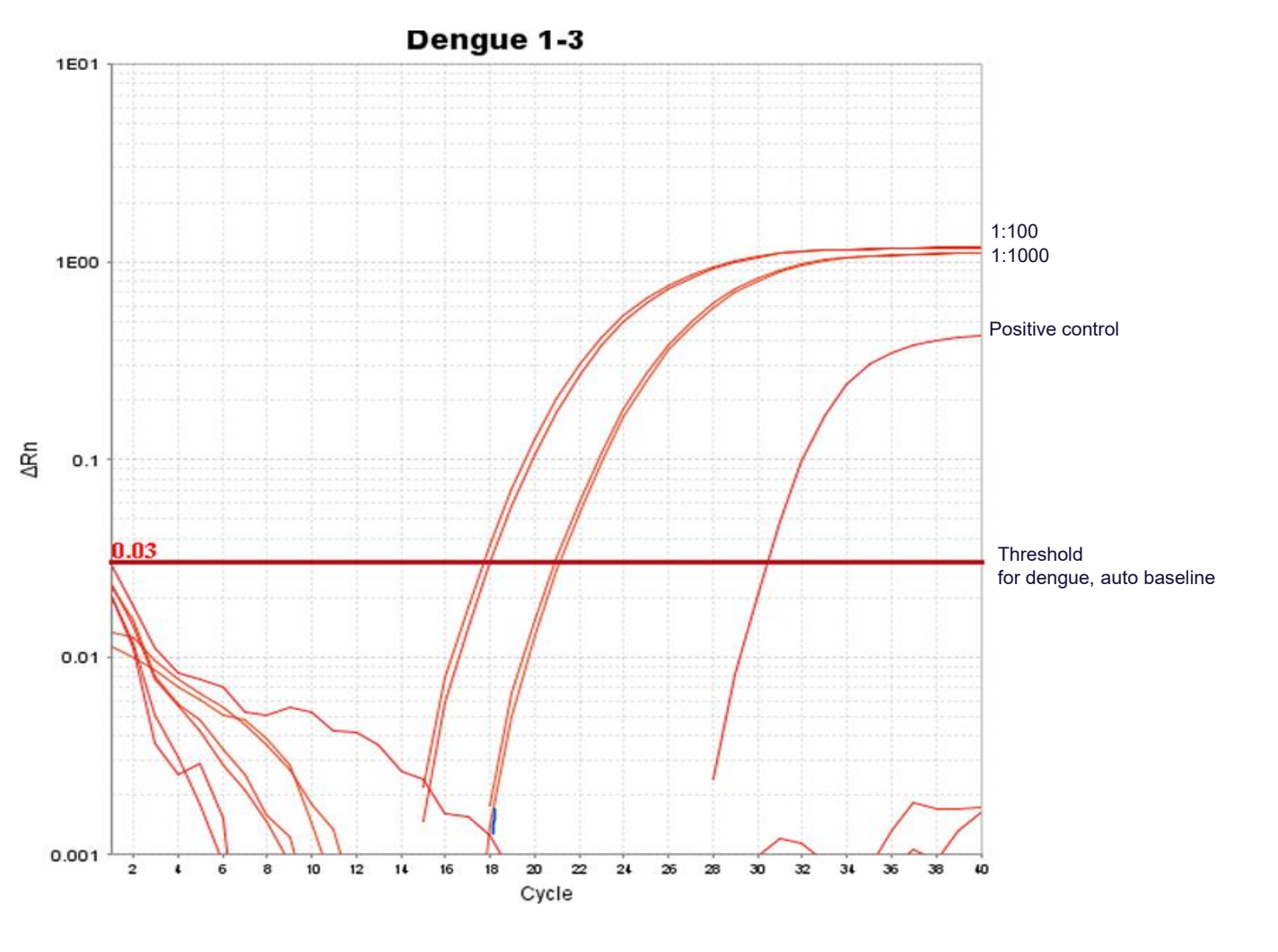


Figure 3 – RT-qPCR of duplicate dilutions (1:100 and 1:1000) of dengue 3 nucleic acid NCPV 0411283v using PCR platform ABI QuantStudio 7 Flex.

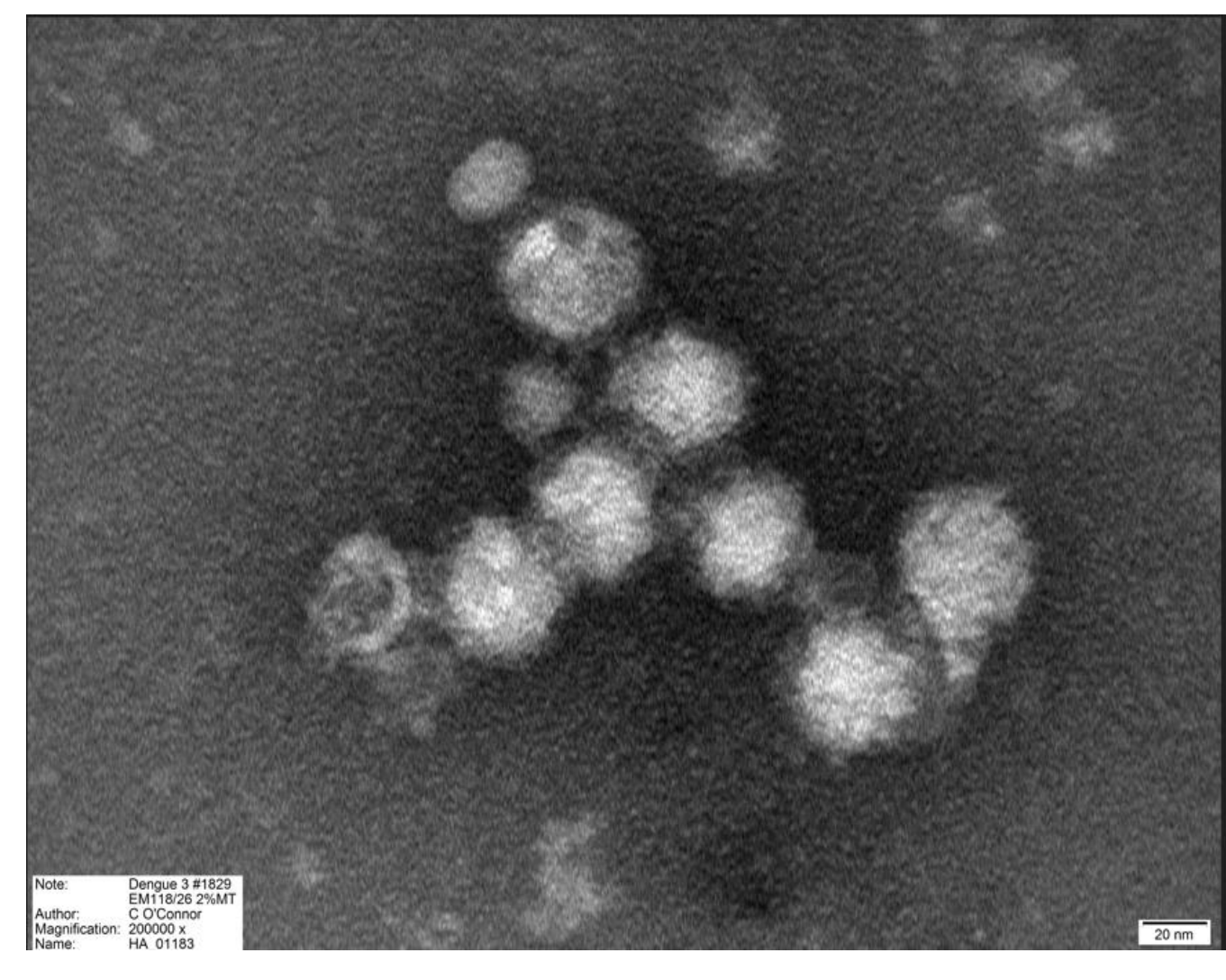


Figure 4 – TEM image of Dengue 3 virus NCPV 0411283v.

DISCUSSION

Growing historical and low passage isolate virus stocks can present challenges for Collections such as NCPV. Reduced recovery may arise from low multiplicity of infection (MOI) frequently linked to the intrinsically low titre of the sample. Often, insufficient deposit is available to allow virus titration before growing a larger bank. In addition, poor adaptation to *in vitro* cell culture systems can limit successful propagation. A common contributing factor is the gradual loss of viability over time, compounded by sometimes unavoidable freeze–thawing of the virus.

CONCLUSIONS

- Authenticated virus reference materials are essential for research and Public Health applications.
- Recovery of historical low-passage virus isolates can be challenging due to reduced viability, low titre, and poor cell culture adaptation, but targeted optimisation of propagation conditions can successfully overcome these limitations.

ACKNOWLEDGEMENTS

European Collection of Authenticated Cell Cultures and Rare Imported Pathogens Laboratory, UKHSA Porton Down, Salisbury.

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