

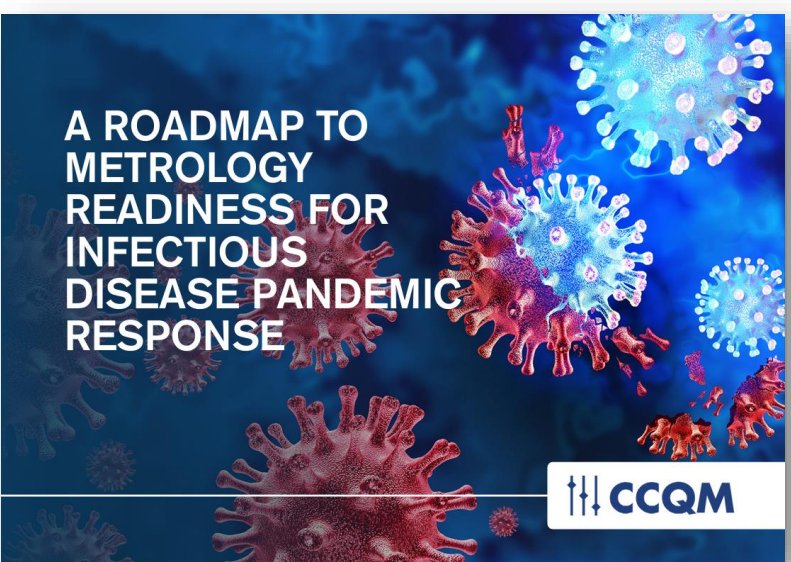
Supporting laboratory services in response to emerging biosecurity threats

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Introduction

- As observed in the COVID-19 pandemic, PCR based diagnostics were the cornerstone in early disease detection.
- The 100 day mission (<https://ippsecretariat.org/>) identified that the first 100 days in responding to a pandemic/epidemic threat are crucial in its control and to change the outcome. Alongside appropriate therapeutics and vaccines, accurate and reliable diagnostics are equally important to be available.
- However it was identified in the COVID-19 pandemic that the approaches to assure quality of these diagnostic tests were lacking in the early stages and may have hindered the response to the disease.
- The metrology community are uniquely placed to support this area in underpinning the measurements to enable confidence. As identified through the CCQM pandemic roadmap the rapid provision of test materials is recommended to ensure quality of the diagnostic tests.



(<https://www.bipm.org/en/-/2022-06-16-ccqm-pandemic-roadmap>)

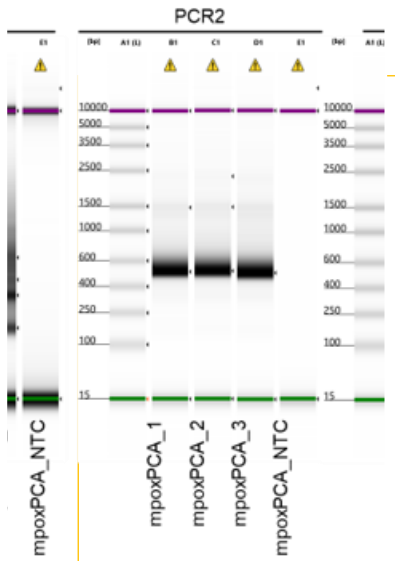
- As a pathogen emerges, either through genetic variation (changes in its genetic sequence e.g. emergence of variants of concern (VOCs) during the COVID-19 pandemic) or other mechanisms, a considerable threat is posed to the diagnostic tests, a rapid approach is essential to assess, fast assessment of the analytical performance of existing assays in clinical use and/or testing of new assays is necessary to ensure readiness of public health response at the earliest opportunity and ultimately control transmission.
- A mechanism to test out these new or existing assays could consist of rapid production of a synthetic material containing the sequence of this completely new or new variant of an existing organism to be prepared and distributed at speed to national health agencies and the end user clinical laboratories.
- This approach would allow the health agencies and laboratories to evaluate the analytical performance of the assays in use/development and further assessment of the limit of detection of said assays which underlies the test sensitivity.
- This process could take place in advance of the clinical presentation of the disease itself, does not require considerations around biosafety containment and can prepare the laboratories should the national deployment of the assays be necessary in a potential local outbreak.

Method

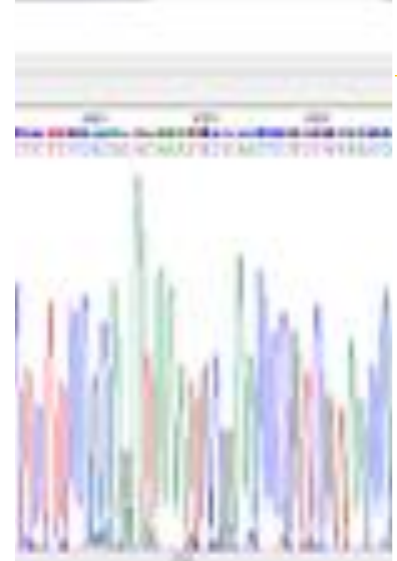
Overlapping oligonucleotides were designed and synthesised using the SYNTAX system (DNAScript). A ~ 550 base pair (bp) DNA molecule was assembled using Polymerase Cycling Assembly (PCA). This test material was evaluated using a range of methods (Figure 1).

Figure 1

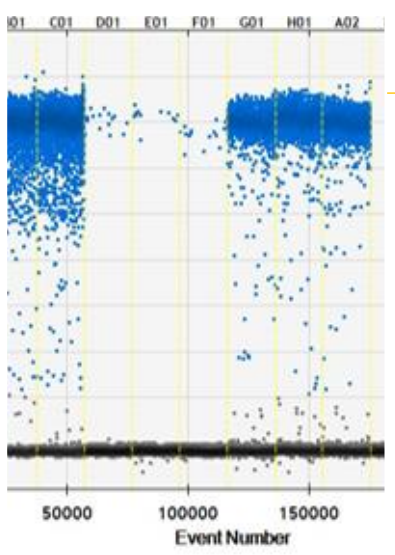
Quality control assessment of the test material, included sizing using the TapeStation (Agilent), Sanger sequencing, MinION (Oxford Nanopore technology) next generation sequencing (NGS) and digital PCR (dPCR) (QX200 system (Bio-rad)).



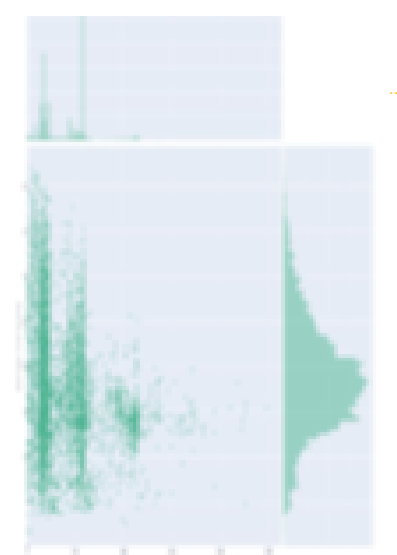
Capillary electrophoresis to check the size of the assembled construct



Sanger sequencing to check that the assembled material matches the intended designed reference material



dPCR to check assay performance and quantify the reference material



NGS to assess the purity of the assembled material (accuracy per base)

Results from mpox test material QC

The results relating to the material specification as performed by the methods in Figure 1 are described in table 1.

Table 1: The QC specification of the mpox test material

Category:	Result:
Qubit Quantification	Mean = 93.0 ± 1.9 ng/μL
TapeStation	Mean length = 552 ± 4 bp. Within sizing accuracy of ± 10%
Sanger Sequencing	390 bp target insert consensus sequence agrees 100% with the construct reference
dPCR quantitation	Concentration (Copies/μL) = 2.52E+06 (± 6.30E+05)
dPCR homogeneity	Between unit RSD = <25 %, within unit RSD = <10 %
Next-Generation Sequencing^a	Full-length Reads ^b = 84.7% ± 0.8%
Sequencing^a	Consensus Sequence showed 100% sequence identity to construct insert reference sequence (depth ≥ 50,000X) Accuracy/base ^c ≥ 99.0% ± 0.9%

^aHigh-throughput long-read nanopore sequencing was carried out on a MinION Mk1D device (Q20+ Kit V14 chemistry kit; high accuracy basecalling).
^bdefined as % of full-length construct insert reads in relation to the total number of reads in a sample. Mean ± Standard deviation (SD) values determined after replicate analysis.
^cdefined as % of bases identical to the construct insert reference sequence base in each given position. Mean ± SD values are shown. SD as measurement of variation across 390 bp region.

Results from clinical laboratory

A serial dilution of the material, ranging from 6.30E+04 to 6.30E+01 copies/μL, was tested by real time quantitative PCR (qPCR) on an ABI 7500 Fast System (Thermo Fisher Scientific) (Figure 2) by the clinical laboratory demonstrating that their assay could detect the new outbreak strain of mpox.

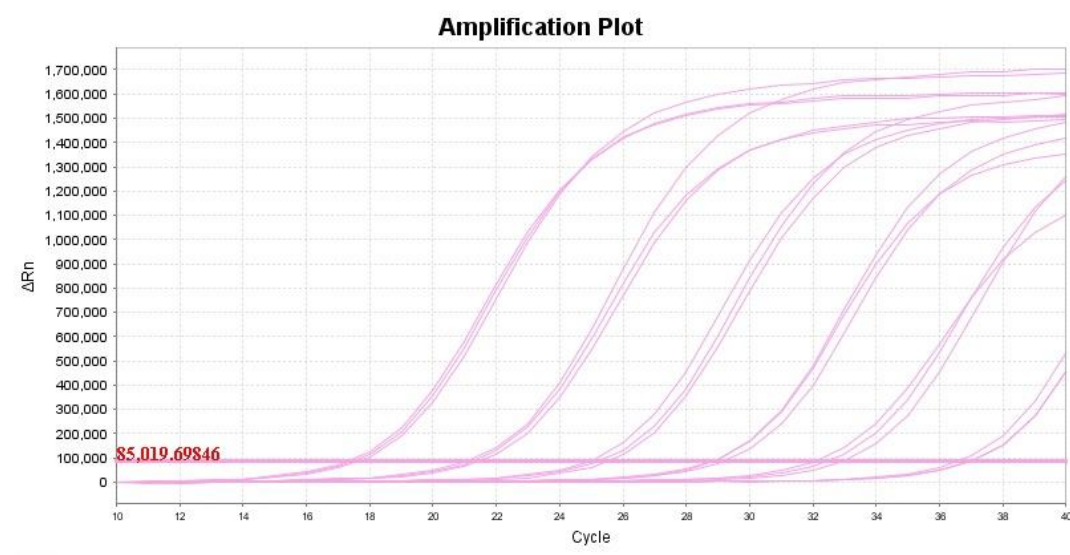


Figure 2

Raw Amplification plot from qPCR demonstrating dilutions of the test material

The Study

To develop the protocols for producing a test material, we conducted a study to produce a DNA test material relevant for the August 2024 outbreak strain of mpox, when there was currently no material which could be obtained, which was available for testing by clinical laboratories to prepare for possible local presentation of the outbreak strain if required.

<https://www.who.int/news/item/14-08-2024-who-director-general-declares-mpox-outbreak-a-public-health-emergency-of-international-concern>



Conclusions

We produced a DNA test material, with a copy concentration and associated measurement uncertainty of < 25 % representing the August 2024 outbreak strain of mpox, including quality control steps in the workflow to confirm the DNA sequence. This material was produced ready for sharing to clinical laboratories to evaluate their diagnostic tests to ensure preparedness should this particular mpox clade present locally. This workflow has the potential to be applied to any targets of interest which could present threat to national biosecurity and will be further developed with stakeholders to meet requirements to ensure a rapid response.