Monkeypox virus (MPXV) outbreak in Berlin – Implementation of molecular diagnostics

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BACKGROUND

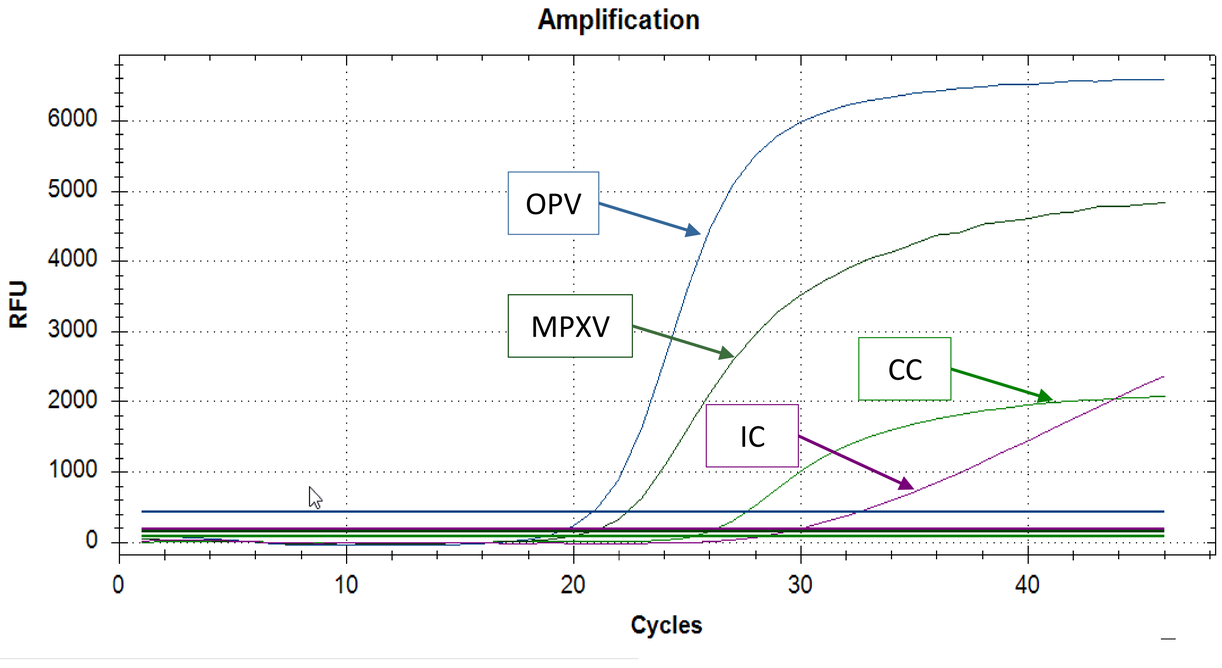
In May 2022 an increasing number of MPXV infections has been reported throughout Europe. With increasing requests for MPXV diagnostics we implemented a multiplex PCR assay testing for Orthopox-Virus (OPV) and Monkeypox-Virus. For quality assessment an internal control and a cell control were included. The assay was validated against the national reference laboratory with more than 100 patient samples.

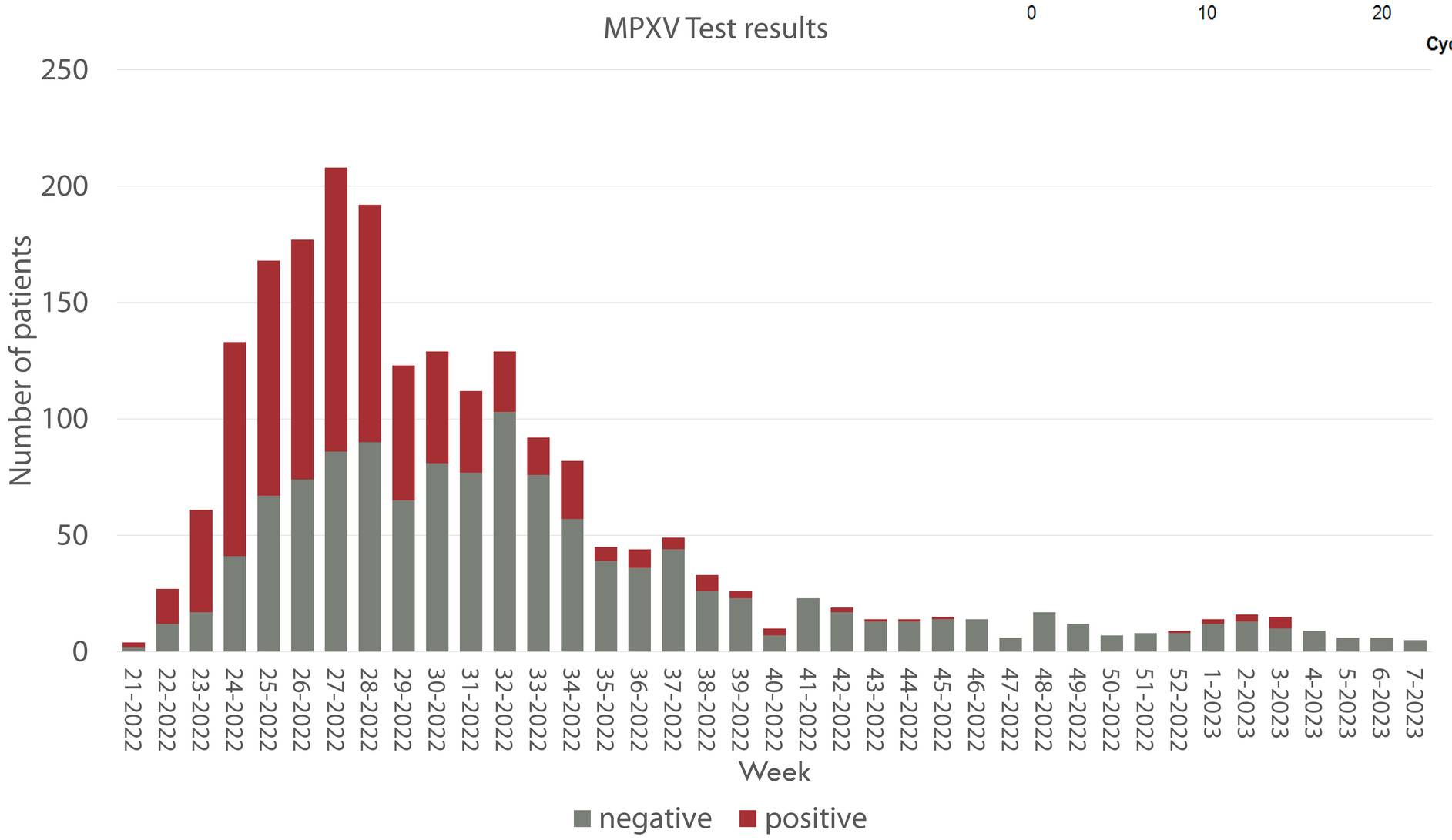
METHODS

Outpatients with contact to confirmed cases or showing symptoms of MPXV infection were tested since end of May 2022. Samples consisted of swabs from lesions, genital, rectal and oro-pharyngeal swabs. To reduce the potential of false negative results we decided for a dual target approach including not only a PCR specific for MPXV but also including OPV. PCR primers and probe for OPV, MPXV and internal control (PhHV-1, IC) from TIB Molbiol (Berlin) were combined with primers and probes for ß-globin (cellular control, CC) in one multiplex PCR. On a Biorad Cfx96 cycler using the TaqPath™ ProAmp™ Multiplex Master Mix run duration was 1:14 h.

RESULTS

2069 samples were tested for MPXV with 837 positive results. Turn around time was below 24 hours for more than 95% of the samples. The lower limit of detection was confirmed on an EQA panel to be below 60 cop./mL. Most of the positive results were detected in June and July with decreasing rates of tested samples in August and September (see figure). In some cases, with low concentration of MPXV DNA only one of the target genes could be detected (either MPXV or OPV). These cases were all confirmed by additional testing from a new swab. In 28 of the samples neither MPXV-DNA nor cellular DNA could be detected.





CONCLUSIONS

We developed a rapid multiplex PCR system to improve patient care and allow better management of infection control. Despite information and vaccination campaigns since July 2022 we still detect new cases of MPXV infection including four cases of vaccination breakthroughs (single dose of vaccine) with high viral loads of up to 4 Mio. copies/mL. High sensitivity of the assay is of great importance as quality of the swabs is divergent.
Rapid molecular diagnostic is an important tool in efficient diagnosis of new emerging infectious diseases. Efficient control of infections is driven by early diagnosis and identification of groups at risk to tailor specific and practically feasible vaccination programs.