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Optimisation of frontline diagnostic testing algorithm in response to the current clade 2.3.4.4b high pathogenicity avian influenza epizootic in the United Kingdom

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Introduction
The avian influenza (AI) epizootic caused by the clade 2.3.4.4.b high pathogenicity AI virus (HPAIV) H5N1 is the largest ever AI outbreak recorded to date in the United Kingdom (UK). Running from October 2021, more than 320 premises have been infected across commercial and backyard sectors, with more than 850 detections of HPAIV H5N1 in wild birds. During the peak of the epizootic, official samples from 5-7 statutory notifiable avian disease ('report case') investigations were regularly submitted to the laboratory for virological investigation. This report highlights the refinements made to the standard diagnostic testing algorithm to facilitate the testing and characterisation of the sheer volumes of samples received for investigation whilst maintaining test sensitivity and virus characterisation opportunities.

Methods
The introduction of the HPAIV H5 ('HP H5') real-time reverse transcription polymerase chain reaction (rRT-PCR) assay (James et al., 2022) for specific pathotyping of viral RNA (vRNA) in swabs from "found-dead" wild birds was followed by the successful validation of the assay for front-line diagnostic application in poultry during the 2021/2022 AI epizootic. Application of the 'HP H5' detection rRT-PCR significantly reduced the time-to-pathotype (from ~36 hours down to ~6 hours from sample receipt) and the cost of the testing by removing the need to follow our previous algorithm of performing conventional Sanger sequencing on H5 PCR-positive samples. While the current AI epizootic with clade 2.3.4.4.b HPAIV H5N1 in the UK continues, the 'HP H5' rRT-PCR is run in tandem with the generic influenza A virus screening rRT-PCR (Nagy et al., 2021), the N1-specific AIV rRT-PCR (Payungporn et al., 2006) and the generic avian paramyxovirus type 1 (APMV-1) screening rRT-PCR (Sutton et al., 2019) as an enhanced diagnostic workflow. Submission of heads instead of full carcasses from galliforme species along with oropharyngeal (OP) and cloacal (C) swabs from both living and dead birds has facilitated full confirmation of HPAIV H5N1 to be made without the need for detailed post-mortem examination and testing of other standard tissues. This optimised algorithm has also removed the need for serological analysis of whole blood from galliformes. Analyses of viral shedding from several infected premises showed that the detection of viral RNA in OP swabs was more prevalent than seen in C swabs, thereby facilitating a targeted sampling strategy of surveillance testing.

Results
For the current AI epizootic, the refinements made to the standard AI outbreak testing algorithm have facilitated timely and high throughput testing and virological characterisation of report case and surveillance samples

Conclusions
Constant refinement of the frontline diagnostic algorithm has significantly added value to the customer, with improved timelines and test sensitivity. Further improvements to the AI outbreak testing algorithm are planned, including the deployment of frozen PCR master mixes and efforts to multiplex the generic influenza A virus screening rRT-PCR with the HP H5 or APMV-1 detection rRT-PCR to reduce (by up to 50%) the numbers of rRT-PCR tests required for statutory confirmation of HPAIV H5N1.