The **Reference** in **Prevention** for **Animal Health**

SUCCESSFUL IMPROVEMENT OF THE MOLECULAR DIAGNOSIS OF INFECTIOUS BURSAL DISEASE IN A HIGH THROUGHPUT LABORATORY

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INTRODUCTION

The Infectious Bursal Disease (IBD) represents one of the main health threats for the poultry industry, mainly due to the severe immunosuppression induced by the virus (IBDV) in the affected birds. Diagnosis by means of molecular tools such as qPCR and sequencing is essential for controlling the disease, as well as for flock monitoring. Avoiding sample cross-contamination, increasing sequencing success rate, and reducing turnaround time for results are some of the challenges for high throughput diagnostic laboratories dealing with IBD diagnosis. The aim of this study is to assess the benefits of some methodological improvements in a busy diagnostic laboratory, in terms of efficiency, and costs reduction.

MATERIALS & METHODS

FTA-imprints (n=935) of bursa of Fabricious (Figure 1) from 17 different poultry production countries worldwide were analyzed.

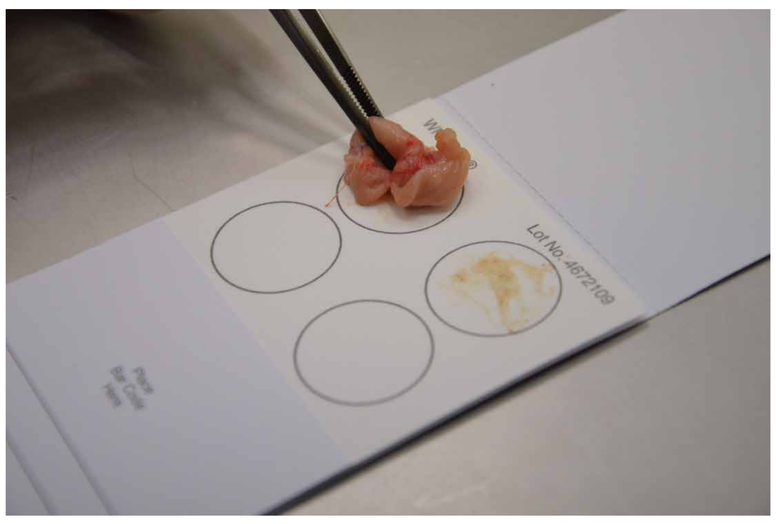

Figure 1. Imprinting the mucosal surface of a bursa of Fabricious onto an FTA card.

Samples were collected for outbreak confirmation or IBD vaccination follow up during two different time periods; before (A) and after (B) the implementation of new laboratory procedures (Table 1); period A with 766 specimens (January 2015 - April 2018) and period B with 169 specimens (August 2018 - March 2019).

Table 1. Laboratory procedures used to detect and characterize IBDV in two different time periods.

Procedure	Time period	
	A	B
Genetic material purification	Column-based	Column-based
Genetic material amplification	End-point PCR	Real Time PCR (qPCR)
Cleanup of the qPCR amplification product	Column-based	Enzymatic
Sequencing	Outsourced	Insourced
Data analysis	Geneious Pro software	Geneious Pro software

Total RNA was extracted and purified by using the RNeasy Mini kit, following the RNA cleanup protocol as per the manufacturer's instructions (QIAGEN, Germany) in an automatic robot (QIAcube, QIAGEN). Subsequently, conventional PCR and qPCR[1, 2] were used to amplify the hypervariable region of the VP2 gene in IBDV. The PCR products were purified using the QIAquick (QIAGEN) and the ExoSAP-IT Express kit (ThermoFisher Scientific) during periods A and B, respectively.

Sequence determinations were carried out by Sanger methodology[3]. Purified PCR products obtained within time period A were submitted to an external sequencing facility, and were sequenced on an ABIPRISM3730 using the Big Dye Terminator v3.1 cycle sequencing Ready Reaction kit. In-house sequencing within time period B was performed using the SeqStudio Genetic Analyzer (ThermoFisher Scientific) instrument, with the the Big Dye Terminator v3.1 cycle sequencing kit, and the BigDye X Terminator Purification Kit, as per manufacturer's instructions.

Nucleotide sequences were analysed using the Geneious Pro software (Biomatters, Ltd. New Zealand) (Figure 2). For typing purposes, the obtained consensus sequences were compared with IBDV strains retrieved from public databases (GenBank).

Results and the time to completion were registered in a Laboratory Information Management System (LIMS) software.

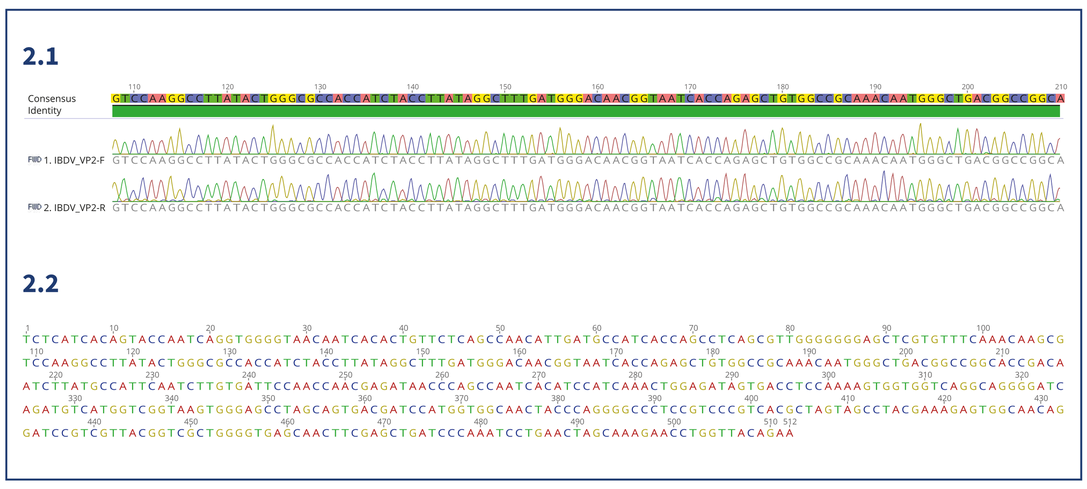

Figure 2. Electropherogram of forward and reverse nucleotide sequences (2.1) and consensus nucleotide sequence of partial VP2 gene (from nt 753 to nt 1332) (2.2). Images were obtained from Geneious Pro Software R11.1.3 version.

RESULTS

Sequence determination was possible in 610/766 (79'6%) and 168/169 (99'4%) samples within time periods A and B, respectively (Figure 3). Turnaround time for results (from sample reception to reporting) was 35 and 17 calendar days for samples received within period A and B, respectively.

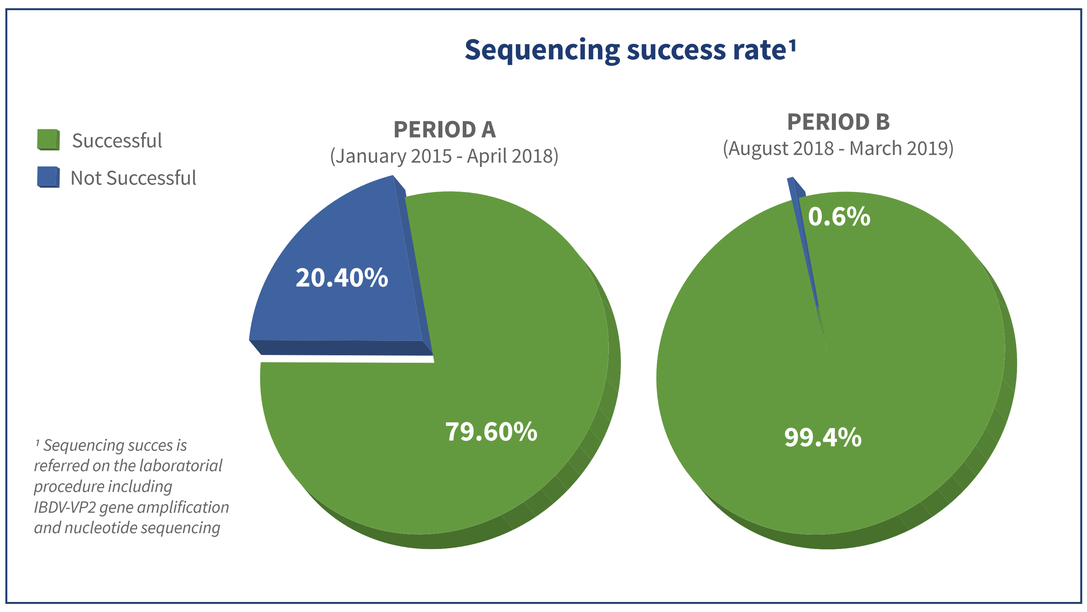

Figure 3. IBDV VP2-gene sequencing success rates obtained before and after the implementation of new laboratory procedures.

CONCLUSIONS

Replacing conventional for real time PCR, column-based for enzymatic PCR purification, and outsourcing for in-house Sanger sequencing, allowed remarkable improvements in sequencing success and turnaround time for results. The cost reduction is due to the fact of not having to transport samples to external laboratories, and to eliminate payment for outsourced sequencing. Finally, the possibility to trace all the phases of the analysis increases the quality of the results, allowing decisions to be made based on valid results.

ACKNOWLEDGEMENTS

The authors would like to thank UCAM-Diagnos staff for sample processing and genetic analysis performance.

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2019-0226