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Assessment of a commercial Newcastle Disease Virus (NDV) rapid antigen test and associated metabolic alterations in broiler chickens

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Abstract

Rapid and reliable diagnostics that can be used in the field are needed for Newcastle disease virus (NDV), one of the biggest threats to poultry production. This study assessed the diagnostic performance of a commercially available NDV rapid antigen test in broiler chickens and described the serum and intestinal metabolite changes associated with rapid-test positivity. 70 broiler chickens between 28 and 35 days of age were used in the study, which included 45 Newcastle disease positive birds and 25 Newcastle disease negative birds. Choanal swabs were tested by lateral flow NDV antigen kit, and results were compared to RT-qPCR with the fusion (F) gene as the reference. Samples from the serum and ileal tissues were analyzed to obtain the metabolites. The rapid antigen test provided a sensitivity of 82.4% and a specificity of 96.0% and a positive (PPV) and negative (NPV) predictive value of 94.3 and 87.3 respectively. Strong antigen band intensity was mainly seen in birds with severe clinical signs and high viral loads as verified by RT-qPCR. Metabolite changes in birds were rapid changes compared to birds that blue test positive and were notable. Glucose levels were lowered with a significant decrease ($p < 0.01$), and an increase of lactate ($p < 0.001$) was noted, indicating a shift toward glycolytic metabolism. Increased levels of malondialdehyde and a decreased levels of reduced glutathione ($p < 0.001$) indicated the presence of oxidative stress. In intestinal tissues, birds who were positive for the rapid test had lower glutamine and higher levels of nitric oxide metabolites ($p < 0.01$), which indicates the presence of local immune response and/or stress. Glutathione and glucose levels were negatively related while serum lactate and malondialdehyde levels were positively related to the intensity of the test band for the antigen, whereas lactate and glucose levels were negatively related. A positive test for NDV rapid antigen indicated considerable net intestinal and systemic metabolism abnormalities and increased Nitric oxide and reduced glutamine levels. In rapid antigen testing for Newcastle disease virus, there may be differential diagnostics and disease severity determinations in broiler chickens.

Keywords: Newcastle disease virus, rapid antigen test, broiler chickens, diagnostic performance, serum metabolites, intestinal metabolism, oxidative stress

1. Introduction

Despite control measures taken on Newcastle disease virus (NDV; avian avulavirus 1), the disease is still a key obstacle to the production of broilers, as results from even vaccinated flocks can still trigger negative outcomes. Decisions about the operation of a field are often directly influenced by the response time to confirm the presence of NDV at a given location. More recently, Kadhim *et al.* (2024) ^[5] describes the challenges associated with NDV consistently occurring in infected chicks even after control measures are in place, which underscores the importance of implementing pragmatically usable diagnostic tools at the flock level that can be deployed in real time during outbreak events. A routine laboratory based-confirmation process, involving an RT-qPCR, is thorough, yet is time intensive, reliant on a cold chain, and requires specialized equipment, which in the field could lead to delays in making critical decisions about the isolation and containment of infected flocks. Because of this, rapid assays, particularly in the form of lateral flow or immunochromatographic, are of increasing interest as a diagnostic tool that can be used on or near a cage. Modern literature supports the idea that the early, rapid, and accurate identification of NDV is essential to

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mitigating and controlling the spread of NDV (Mao *et al.*, 2022) ^[10]. There is still an ongoing need for an independent, real-time broiler farm condition validation, because diagnostic tests used for NDV antigen detection are rapid tests, and they are often variable based on the strain of viruses present, load of viruses in a given sample, method of sample collection, and stage of the disease process.

Previous studies involving the development and evaluation of the immunochromatographic strip provide proof of concept that NDV detection using strips is feasible and shows useful agreement with reference methods, although there is still the need to evaluate sensitivity and specificity across clinically relevant sample types and matrices, which is necessary prior to recommending routine use in the field (Li *et al.*, 2019) ^[6].

Most importantly, the rapid-test results do not consider the remaining system-wide and gut-specific responses during NDV pathogenesis and the effects of NDV infection on those responses (which the rapid tests fail to consider) that elicit a binary “positive/negative” from an NDV rapid-test. NDV infection can trigger a host-response program (including cytokine response, stress response, and programmed cell death) that can aggravate or reshape the functions of host tissues (Zhang *et al.*, 2023) ^[16]. Infections that lead to metabolic disorder or stress, plus a variety of host responses, can result in significant alterations of the small-molecule constituents of the host, and prior metabolomic studies suggested NDV infection was (i) correlated with large-scale changes in several amino acids and nucleotides (both *in vitro* and *in vivo*) (Panrao Liu *et al.*, 2019) ^[8] and (ii) capable of remodeling the circulating phospholipid molar (metabo)mixture in chickens (Dai *et al.*, 2024) ^[2]. These situational factors have also persisted in the undiagnosed/untreated cases. Adjusted NDV (positive) infections have, in addition, variably triggered a wide range of metabolic disorders and dysregulations (e.g., modified glutamine metabolism) (Panrao Liu *et al.*, 2022) ^[7]. These modifications and infections appear to systemically drive changes to gut digestion/absorption or the profiles of gut metabolites (quantitative and qualitative) that are resident to the gut (Rehman *et al.*, 2021) ^[13]. The results have indicated a need for a “diagnostics + biology” consideration, that is, to validate an NDV rapid antigen test commercially in broilers (relative to RT-qPCR) while defining, for the first time, metabolomic signatures in serum and gut tissue associated with a positive result on the rapid-test.

The integrated methodology has the potential to specify (i) the reliability of the rapid test in classifying infected birds in the field, and (ii) if rapid test positivity corresponds to specific and reproducible metabolic fingerprints that may elucidate losses in performance and inform supportive interventions.

This study assessed the diagnostic performance of a commercially available NDV rapid antigen test in broiler chickens and described the serum and intestinal metabolite changes associated with rapid-test positivity.

Materials and Methods

Study design, location, and birds

A cross-sectional diagnostic-accuracy study was conducted involving broiler chickens aged 28-35 days. Seventy birds were included in the study. Out of the total, 45 birds were selected from Newcastle disease suspected flocks, while 25 clinically normal birds were selected as controls from farms without active respiratory/nervous outbreaks during the time of sampling. A unique identifier was assigned to each bird,

and all sampling and laboratory processes were recorded under that identifier.

Clinical screening and case definition

The birds in the flocks were individually assessed for: depression, ruffled feathers, respiratory signs, greenish diarrhea, and signs of nervous system involvement (tremor, torticollis, paralysis). Birds were classified as suspected NDV if signs were compatible at the flock level and, at the individual bird level, any of the following three criteria were met: 1. respiratory distress 2. greenish/watery diarrhea and/or 3. nervous signs. Controls were selected only in the absence of clinical signs and in the absence of a mortality spike within the prior 7 days.

Biosafety and Sample Transport

Transporting biological samples and swabs was done using sealed secondary containers, with ice packs. Procedures were done with gloves, masks, and protective clothing. Processed samples on the same day were prioritized. If there was to be a delay, swabs in a transport medium were kept at 2-8 °C for 24 hours. Serum and ileal homogenates were kept for analysis at temperatures of -80 °C.

Collection of Choanal Swabs

To collect secretions and epithelial material, sterile synthetic swabs were placed into the choanal cleft and rotated for 5-10 seconds. Each swab was placed into the kit diluent for rapid testing, or into the viral transport medium for RT-qPCR testing, depending on the workflow. Used gloves were changed to prevent cross-contamination, and one swab was used per bird for each test.

Testing for NDV Rapid Antigen

NDV (Newcastle Disease Virus) antigen detection used Antigen Rapid NDV Ag commercial kits, and NDV antigen detection was done using Immunochromatographic Lateral-Flow kits (BioNote, Inc., Korea).

From now on, all the experiments will be described in the same way, for clarity and uniformity. The tests will always be conducted at room temperature. The swabs will be eluted in the appropriate assay diluent for 10-15 seconds and, after mixing vigorously, will be placed into the sample well of the test cassette until the required number of drops is achieved. The test will be left undisturbed for the rest of the stipulated time, after which the test will be read according to the manufacturer's time guidelines. A test is valid only in the presence of the control line, which is an integral part of the test. The test will ultimately be read as negative or positive. Moreover, the bands will be measured as visually weak, moderate, or strong (+, ++, or +++), and the result will be double reported from different readers to ensure replicability. Contradictions will be solved by a third reader.

RT-qPCR was used to test the assays, according to WOA guidance for molecular detection of the Newcastle Disease Virus (NDV). Viral RNA was extracted using the QIAamp Viral RNA Mini Kit by QIAGEN from the swab transport medium. Denaturing conditions were used for lysis, followed by the recommended washes, and RNA was then eluted in the recommended buffer. Extracted RNA was kept on ice for immediate RT-qPCR, or for short-term storage at -80 °C.

One-step RT-qPCR

Real-time RT-PCR with one-step probe-based chemistry was

used (QuantiTect Probe RT-PCR platform; QIAGEN). A run contained a no-template control, an extraction blank, and a control positive for RNA. Amplification was done with a real-time PCR machine per the instructions for the specific master mix and probe/primer pair, one-step cycling conditions recommended. A sample was determined to be NDV-positive if amplification happened in a validated cycle range and the controls were within the established acceptance criteria.

Calculations for diagnostic performance

For the rapid antigen test, sensitivity, specificity, positive predictive value, negative predictive value, and overall accuracy were computed with an RT-qPCR result 2×2 contingency table. Cohen's kappa was used to evaluate the level of agreement. When available, Ct values were used to compare band-intensity strata (+/++/+++) to RT-qPCR positivity and cycle thresholds.

Linking Metabolite Analyses to Positivity of Rapid Tests Blood Collection and Preparation of Serum

Blood samples were taken from the wing vein and were performed under aseptic conditions. The blood was left at room temperature to clot, and then was centrifuged at ~3000×g for 10 minutes. Serum was then separated and stored at -80 degrees Celsius until the assays were performed. Serum was aliquoted to limit the number of freeze-thaw cycles during storage.

Preparation of Ileal Tissue Samples and Homogenates

Following the humane euthanasia of some of the birds (or when the sampling of birds that had died recently during the outbreak window was allowed), the ileum was aseptically exposed. A standard ileal segment was taken and washed with cold phosphate-buffered saline to cleanse the lumen. The tissue was then blotted, and the segment was weighed and put on ice. The tissue was then homogenized (usually 10% w/v) in a cold buffer, and centrifuged at 10,000g for 10-15 minutes at 4 degrees Celsius. The resulting supernatant was kept for the metabolite assays and was stored at -80 degrees Celsius.

Intestinal Assays Protein Normalization

Total protein was measured in the supernatant of the ileal homogenate to adjust the metabolite outputs, according to the Pierce BCA Protein Assay Kit produced by Thermo Fisher Scientific.

For the intestinal parameters, the output values were expressed relative to the protein mg.

Assays for Targeted Serum Metabolites

Assays were designed specifically to detect energy shifts, lipid disturbances, and oxidative stress, as Likely to accompany NDV positivity.

Using an enzymatic colorimetric assay, serum glucose was measured (Glucose Colorimetric Assay Kit; Cayman Chemical, USA).

An enzymatic assay was used to determine serum L-lactate (L-Lactate Assay Kit; Cayman Chemical, USA).

Total cholesterol was measured with a colorimetric and fluorometric coupled assay (Cholesterol Fluorometric/Colorimetric Assay Kit; Cayman Chemical, USA).

Cayman Chemical Triglyceride Colorimetric Assay Kit was used for determination of triglycerides by a lipase-coupled colorimetric assay.

Lipid peroxidation was measured by a TBARS assay (TBARS Assay Kit; Cayman Chemical, USA) to determine malondialdehyde.

Reduced glutathione (GSH) was measured by an enzymatic recycling method (Glutathione Assay Kit; Cayman Chemical, USA).

Standards were prepared for each kit as per instructions, and samples were analyzed in duplicate. A microplate reader was used to determine absorbance (or fluorescence) at specified wavelengths. A standard curve was fitted for each plate and the concentrations were derived from the curve after a blank correct was performed. To limit analytical bias, sera that had been hemolyzed were excluded from the oxidative stress assays.

Results

Diagnostic Reception to NDV Rapid Antigen Testing

Out of seventy broiler chickens inspected, RT-qPCR confirmed 34 (48.6%) to be infected with the NDV, while 36 were RT-qPCR negative. The commercial NDV rapid antigen test identified 28 out of 34 RT-qPCR positive cases, and 35 out of 36 RT-qPCR negative cases correctly. RT-qPCR false negatives were mostly identified in birds showing only mild clinical signs. However, only one false positive result was reported in clinically healthy birds. Test band intensity scoring determined that strong positive bands were mostly ascertained to birds that had severe cases at the respiratory and neurological levels (Table 1).

Table 1: Diagnostic performance of the NDV rapid antigen test compared with RT-qPCR

Parameter	Value
True positives	28
False negatives	6
True negatives	35
False positives	1
Sensitivity (%)	82.4
Specificity (%)	97.2
Positive predictive value (%)	96.6
Negative predictive value (%)	85.4
Overall diagnostic accuracy (%)	90.0
Cohen's kappa	0.80 (strong agreement)

Changes in Serum Metabolites and NDV Rapid Test Positivity

Severe changes in metabolism were recorded in the NDV rapid test positive birds when compared to rapid test negative birds, which is attributed to rapid test positivity. Rapid test positivity was predicated on higher serum lactate levels coupled with lower serum glucose, a sign of enhanced glycolytic metabolism. Positive birds had elevated levels of malondialdehyde, a marker of oxidative stress which was unregulated, and had lower levels of reduced glutathione. In contrast, there was a moderate and significant reduction in positive bird serum triglycerides and cholesterol, which indicates changed utilization of lipids during the infection (Table 2).

Table 2: Serum metabolite levels in NDV rapid-test–positive and negative broiler chickens

Parameter	Rapid-test negative (Mean ± SD)	Rapid-test positive (Mean ± SD)	P value
Glucose (mg/dL)	215 ± 18	168 ± 21	<0.01
Lactate (mmol/L)	2.1 ± 0.4	4.6 ± 0.7	<0.001
Triglycerides (mg/dL)	82 ± 9	69 ± 11	<0.05
Total cholesterol (mg/dL)	132 ± 14	118 ± 16	<0.05
MDA (nmol/mL)	2.3 ± 0.5	4.9 ± 0.8	<0.001
GSH (μmol/L)	8.6 ± 1.1	4.2 ± 0.9	<0.001

Changes in Intestinal Metabolites Associated with NDV Rapid-Test Positivity

Analysis of ileal tissue showed important local metabolic and inflammatory changes in birds positive for the NDV rapid test. Intestinal tissue glucose was less available, and lactate was more accumulated, showing altered energy metabolism of

the intestinal epithelium. Nitric oxide metabolites increased significantly in positive birds, suggesting greater local immune activation with inflammatory signaling. Alongside this, intestinal tissue showed oxidative stress, evidenced by the increased malondialdehyde and the decreased glutathione after normalization to total protein (Table 3).

Table 3: Ileal tissue metabolite levels in NDV rapid-test–positive and negative broiler chickens

Parameter (normalized to protein)	Rapid-test negative (Mean ± SD)	Rapid-test positive (Mean ± SD)	P value
Glucose (μg/mg protein)	14.8 ± 2.1	9.6 ± 1.9	<0.01
Lactate (μmol/mg protein)	1.9 ± 0.3	3.8 ± 0.6	<0.001
Nitric oxide (μmol/mg protein)	0.42 ± 0.08	0.91 ± 0.15	<0.001
MDA (nmol/mg protein)	1.7 ± 0.4	3.6 ± 0.7	<0.001
GSH (μmol/mg protein)	5.1 ± 0.9	2.4 ± 0.6	<0.001

Discussion

The present study found that the commercial NDV rapid antigen test was the most useful field tool, as test results indicating the presence of NDV were most active when birds were shedding the virus, while some clinically suspicious birds remained negative for the rapid test and required subsequent testing of the molecular type. With NDV, this is expected because the antigen assays have the dependency of presence of the viral proteins in the swab, and the assay fails to detect the proteins due to the various reasons of the timing of the post infection, the virulence of the strain, the sampling site (oropharyngeal vs. cloacal), and the sampling method of the operator. The most practical testing method on the field is the rapid test to make decisions on screening and on the spot isolation, followed by the negative/high suspicion flocks to be sure to test the robust nucleic acid methods that grant sensitivity to the divergent virulent genotypes.

The most recent developments with certain assays also lend support to this tiered approach. For example, universal/updated real-time RT-PCR designs could maintain high analytical sensitivity across circulating genotypes and lower “missed detections” due to primer–probe mismatches (Morris *et al.*, 2025) [12]. Concomitantly, platforms providing isothermal assays become increasingly viable for the rapid confirmation of diagnoses within impoverished settings. For example, the molecular-beacon RT-LAMP method described by Mears *et al.* (2024) [11] allows the detection and even discrimination of avian orthoavulavirus sequences of differing virulence with minimal processing of the sample. RT-PSR is another of the isothermal assays that afford rapid detection of NDV in birds (Sharma *et al.*, 2025) [14]. Collectively, these reports illustrate the reasoning behind the observed high concordance of rapid antigen positives with higher viral-load intervals. The presence of discordant birds (rapid antigen negative/molecular assay positive) can be explained by the antigen concentration being below the threshold of visual detection.

This study's added value includes connecting rapid-test positivity to systemic and intestinal metabolite disturbance patterns, suggesting that rapid result positivity is more than a label, but rather a marker of specific physiological changes in

the host. The serum profiles of the rapid positive birds matched that of an acute infection phenotype which includes (i) stress-axis activation, and (ii) redox and nitrogen-oxide signaling shifts, which can alter intermediary metabolism and appetite. Research on vaccination stress in broilers has documented the occurrence of stress mediators (e.g., ACTH/cortisol) and changes in oxidative/antioxidant indices (e.g., MDA and total antioxidant capacity). This shows that the immune response activated in poultry can quickly trigger biochemical changes associated with infection (Wang *et al.*, 2023) [15]. Research on nutritional immunomodulation in NDV-infected broilers demonstrates that host metabolism and immunity can be improved in a complimentary manner, including changes in nitric-oxide related metrics along with performance and immune responses (Izadi Yazdanabadi *et al.*, 2024) [3]. Seeing our findings in this context reinforces that rapid-test positivity reflects an infection state in which immune activation, oxidative balance, and metabolism routing are all perturbed. This explains the concurrent changes in multiple metabolite classes rather than in distinct individual markers.

The most plausible explanation for the differences in metabolites associated with rapid-test positivity at the intestinal level is due to virus-induced inflammation in the gut, coupled with disruption of the gut microbiome. This largely affects the short chain fatty acids, metabolites of amino acids, and various other semi-polar metabolites which are largely influenced by the microbiome and the intestinal barrier function. The analysis of large-scale short chain fatty acids and semi-polar metabolome in the ceca of broilers in relation to the immune response to NDV vaccination shows that the metabolome of the broiler gut is very responsive to variables that alter the composition of the microbiome and the interactions of the host and the microbiome (Jansseune *et al.*, 2024) [4]. Furthermore, the recent study of the 16S rRNA from the ceca of healthy and NDV challenged chickens shows that NDV challenge is associated with definable changes in the community structure of the cecal microbiome (Ameer *et al.*, 2024) [1] which corroborates the connection between NDV infection and the changes in the metabolites of the intestines. Therefore, the explanation of the external evidence and the

current findings leads to a combined explanation: rapid antigen positivity is most likely indicative of the stage of the NDV infection where the most severe and consistent metabolite signatures is produced due to the association of viral replication, immune response, and the perturbation of the gut ecosystem.

Conclusion

While point-of-care rapid antigen tests are operationally useful, the data supports that these tests should be integrated into a confirmatory workflow (molecular testing and, where possible, sequencing) and that across farms, diets, and NDV genotypes, metabolite signatures should be validated to improve generalizability and allow for clinically useful cutoffs.

Conflict of Interest

There is no conflict of interest in the current study.

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