Iraqi Journal of Veterinary Sciences

www.vetmedmosul.com

Novel CRISPR/Cas13- based assay for diagnosis of avian infectious bronchitis

M.A. Khudeir[1], A.T. Alsultan[2] and Y.I. Khudhair[2]

[1]Department of Pathology and Poultry Diseases, [2]Department of Internal and Preventive Medicine, College of Veterinary Medicine, University of Al-Qadisiyah, Al- Dewaniyah, Iraq

Article information

Article history:
Received 14 May, 2023
Accepted 30 July, 2023
Available online 15 December, 2023

Keywords:
Poultry
Infectious bronchitis
CRISPR

Correspondence:
A.T. Alsultan
amjed.talib@qu.edu.iq

Abstract

Infectious bronchitis is an acute respiratory disease of poultry associated with reduced egg production and heavy economic losses in chicken flocks. Rabid and accurate detection of IB virus (IBV) is essential for controlling and preventing the infection. In this study, we developed a rapid, accurate, and instrument less assay to detect IBV. For the first time, reverse transcription- Recombinase polymerase amplification (RT-RPA) coupled with CRISPR/Cas13 (SHERLOCK) was used to rapidly visualize IBV. The novel assay was tested in timing, sensitivity, and specificity. The spike gene (S gene) was used as a target gene for detecting the virus. Three samples were used to optimize the assay; sample form confirmed infected chickens with IB, positive sample (full synthesis of S gene), and negative sample from free IB infected chickens. The results show that the Sherlock-based Cas13 platform is a highly specificity and sensitivity assay for detecting infectious bronchitis virus. The assay detected ten copies per µL of the input RNA. No false positives or cross-reactions were seen when bovine coronavirus (BCV) was used instead of IBV in the tested sample. Readout of the results needs just fifty minutes, including RNA extraction. Furthermore, No instrument was used, and amplification of the virus's nucleic acid was performed at room temperature. Sherlock-based Cas13 should clinically use for rapid diagnosis of infectious bronchitis in chickens. However, further studies and experiments are needed to perform the assay at the sample base without extraction of RNA.

DOI: 10.33899/ijvs.2023.140335.3040, ©Authors, 2024, College of Veterinary Medicine, University of Mosul.
This is an open access article under the CC BY 4.0 license (http://creativecommons.org/licenses/by/4.0/).

Introduction

Infectious bronchitis (IB) is a highly contagious and acute disease of the upper respiratory tract and urogenital tracts of chickens (1). IB is caused by a single standard RNA virus belonging to the Nidovirales order, Coronaviridae family, Coronavirinae subfamily, Gammacoronavirus genus, and species Avian coronavirus (2). The virus genome consists of a 27.6-kb kb RNA molecule that encodes for dozens of non-structures and four structural proteins. Spike (S) glycoprotein, membrane (M), small envelope (E), and nucleocapsid (N) proteins. The S protein is formed by post-translational cleavage of S into two separate polypeptide components, S1 and S2, the spike glycoprotein S1 subunit is responsible for the induction of neutralization antibody, hemagglutination, cell tropism, and attachment (3-5). The disease causes severe economic losses in the poultry industry and consider as a second most damaging disease after avian influenza (6-8). IBV outbreaks occur dramatically in both vaccinated and unvaccinated chicken flocks due to vaccination program failure, emerging novel IB variants, and lack of comprehensive genotyping studies (9-11). Therefore, rapid and accurate assay for the detection of IBV is essential for early detection and prevention of the disease. Nucleic acid detection-based assays such as PCR and qPCR are the gold standard for disease diagnosis; however, this method

needs expensive infrastructure and well-trained persons (12-14). Clustered, regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated protein (Cas) is a novel molecular platform that has expanded rapidly. Several rapid nucleic acid diagnostic kits have been developed and validated using Cas9, Cas12, and Cas13 proteins (15-17). The first diagnostic platform based on CRISPR-Cas13 a system is formed from integrates isothermal amplification nucleic acid-based Recombinase polymerase amplification (RPA), and CRISPR-Cas13 is termed SHERLOCK (specific high sensitivity enzymatic reporter unlocking). Recently, SHERLOCK-based Cas13 were used for rapid and accurate diagnosis of Covid-19 infection in human (18-22).

We optimized the SHERLOCK-based Cas13 assay for the first time to detect IBV infection in the chicken.

Materials and methods

Ethical approve

The primary studies under which the samples were collected received ethical clearance from Veterinary Medical Ethics Committee of University of Al-Qadisiyah with approval number 360/2022.

Sampling

Fifteen RNA samples were extracted from confirmed infected chickens with IB, S gene of the virus was synthesized by Macrogen® and used as a positive control for the test. While 10 RNA samples were extracted from non-infected chicken with IB and used as a negative control. RNA extraction was performed according to the manufacturer's instructions (QIAwave RNA Mini Kit®).

RPA and crRNA primer design and synthesis

Primers were designed to amplify the gene encoding to the S protein (spike protein: NP_040831.1). The conservative area of the gene was selected for specific detection of the virus (Table 1). T7 sequence was added to the S gene forward primer to *in vitro* transcription of the target gene using the T7 transcriptase enzyme (HiScribe™ T7). Primers were designed according to the instruction described in TwistAmp Assay Design. Two parts of cRNA were designed. The designed crRNA consists of two parts (Table 1), one for recognition of RNA by Cas13 protein and the other for detecting the S gene (complement to target gene). Labeled RNA reporter was also designed to read out in vitro cleavage of the target gene. All primers were synthesized by Macrogen®.

Table1: primers used in the Sherlock-based cas13 assay

Primer name	Sequence
RPAIB F (avian S gene)	GAAATTAATACGACTCACTATAGGGCAATGTAATTTTGCTATAGAGAGTGTGC
RPAIB R (avian S gene)	TATATTTCTGCACCATACGGTAGATATT
RPABCV F (Bovine S gene)	GAAATTAATACGACTCACTATAGGGGTTTATTAGAACTGGAAGTTGGTGGA
RPABCV R (Bovine S gene)	GCCAGTACCTAGTTTTATACCTTGCATG
Cas13a Guide RNA	GAUUUAGACUACCCCAAAAACGAAGGGGACUAAAACGGUCUGGUUCACACUU
RNA reporter	5'-/56-FAM/mArArUrGrGrCmAmArArUrGrGrCmA/3Bio/-3

Positive control of the S gene

The Conservative sequence of the M gene was synthesized to use it as a positive control. 175bp of the gene was synthesized by Macrogen® (Seoul, Korea). The Gene fragment was cloned to pMG (cloning vector). In vitro transcription of the fragment using T7 RNA transcriptase enzyme (HiScribe™ T7) RPA was used to amplify the fragment using primer pair. As mentioned before T7 prompter sequence was inserted to the 5 ends of the forward primer.

Establishment of RT-RPA assay

Reverse transcription and amplification of the target gene (S gene) were performed using RT-RPA assay without any instrument. TwistAmp® Basic kit (TwistDx®, UK) was used to amplify the S gene. The reaction was set out according to the following formula: 4.5 ul of template, 5 ul of S gene RPA primer mix, and 0.5 of reverse transcriptase (ProtoScript®), 8 ul distilled water, and 29.5 ul of re-suspension buffer. The reaction mixes gently, then the whole mixture is added to a TwistAmp® basic reaction. Finally, 2.5ul of magnesium acetate (MgOAC) was added to the mixture and incubated the reaction at 39 C for 20 minutes.

Establishment of RT-RPA with Cas13a assay

Cas13a protein was provided by Genscript® (Cat. No. Z03486). According to Zhang *et al.* (24), RNA-guided protein (Cas13a) was used for nucleic acid detection of IBV. The detection reaction was set out in one tube as fallow: 2 ul of RT-RPA reaction, 1 ul Labeled RNA reporter (20 uM), 4 ul of diluted Cas13a protein with cleavage buffer (400mM Tris pH 7.4), 1 ul of Ribonucleotide Solution, 0.6 ul T7 RNA Polymerase (NEB®, M0251S), 1 ul of M gene- crRNA, 9.6 ul of ddH_2O and 1 ul of $MgCl_2$. The reaction was mixed gently and then incubated for 30 minutes at 37°C.

Readout of cleavage activity using a later flow device (LFD)

LFD was used as an endpoint essay to read the signal of fluorescence-labeled RNA reporter (5'-/56-FAM/ mArAr

UrGrGrCm AmArArUrGrGrCmA/3Bio/-3). HybriDetect Dip-stick ((Milenia HybriDetect, TwistDx®) and the labeled reporter have been used to visual detection of cleavage activity of Cas13a.

Results

In this study, we developed a rapid, accurate, and novel assay for the detection of IBV using cas13-based SHERLOCK assay; then, we demonstrated the sensitivity and specificity of the assay using samples collected from chickens infected with infectious bronchitis. As figure 1 shows, the SHERLOCK assay was performed by combining RT-RPA (amplification of the target gene) with CRISPR-cas13 (detection of target) and LFD for visual virus detection.

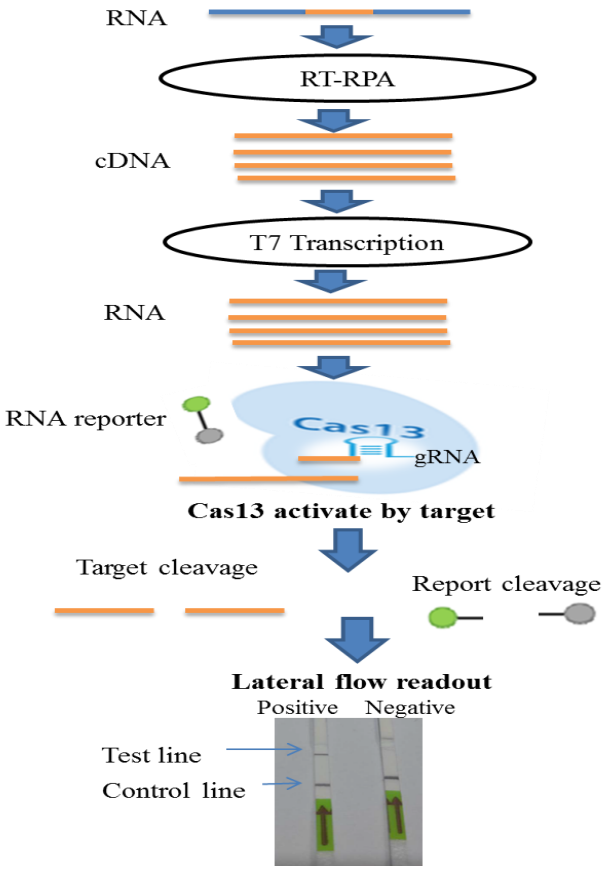

Figure 1: SHERLOCK assay workflow. RNA extracted from chicken with infectious bronchitis. RNA was converted to cDNA using two pair of spike protein RPA primers (RT-RPA) then cDNA was converted to RNA using T7 RNA polymerase. Target RNA connects to its complement segment in the guide RNA, which activates the cas13 enzyme. The enzyme cut the target RNA and fluorescently labelled reporter RNA (collateral cleavage). Cleavage activity was reported using a lateral flow dipstick.

Experimental design and assay optimization

As mentioned in the methods section, the gene encoding spike protein (S gene) of the IB virus was selected. The selected gene was routinely used as a target gene in the nucleic acid-based detection assays (PCR and qPCR) to diagnose IBV. The Conservative area of the gene was selected using Basic Local Alignment Search Tool (BLAST). RPA primers were designed for the IBV S gene. S gene of the bovine coronavirus (BCV) was also used in terms of detected specificity of the assay in the detection of IBV. Four groups were used to perform the assay, including the clinical sample group (chicken clinically infected with IBV), negative group (chicken free of IBV infection), Specificity detecting group- BCV (BCV Kindly provided by Bei resources), and positive control group (S gene were synthesized using gene-arts). Total RNAs were extracted from the first three groups, while the last group synthetic DNA was in vitro Transcripted to RNA using T7 RNA polymerase. Three stages of the assay were performed to detect IBV, including amplification, detection, and visualization. Total RNAs from the four groups were reverse Transcripted and amplified using one tube RT-RPA reaction. At the same time, total RNAs were reverse Transcripted and then amplified using PCR. Figure 2 shows that the shiny band on agarose gel belongs to the S gene of IBV in the case of RT-RPA, while RT-PCR shows a less brilliant band. Amplifying the S gene using RT-RPA takes 20 minutes, while RT-PCR takes 3H; moreover, no machines were used in the case of RT-RPA, and the reaction was performed in one tube and at room temperature. In the next step, the virus was detected using CRISPR/ cas13a. RPA reaction of the S gene and controls were used in the detection reaction. In one tube reaction apart from the RPA reaction, T7 polymerase, crRNA (S gene), CAS13A, and RNA reporter were mixed as mentioned in the method section; firstly, S gene cDNA was converted to RNA with the aid of T7 RNA polymerase. crRNA contains the complement part of the S gene and part for cas13a recognition; when crRNA binds to its complement, this lead to activate of cas13a, which ac to the cutting of the specific part of the S gene (cleavage) and nonspecific cleavage of neighbor RNA fluorescence-labeled RNA reporter (collateral activity cleavage). The Cleavage activity was reported by fluorescent emission from the reporter, which was visually detected by HybriDetect Dip-stick (Figure 1). The detection reaction was incubated for 30 minutes at 37, then HybriDetect Dip-stick was dipped in the reaction, and results were read within 5-10 minutes. As shown in Figure 2, The result shows two lines (control and test) appeared on the LFD dipstick in the case of the IBV positive and positive control samples (synthetic S gene). In contrast, one line (control line) appeared on the dipstick in the IBV negative sample (control negative) case. The mean time from RNA extraction until the results were read out was fifty minutes.

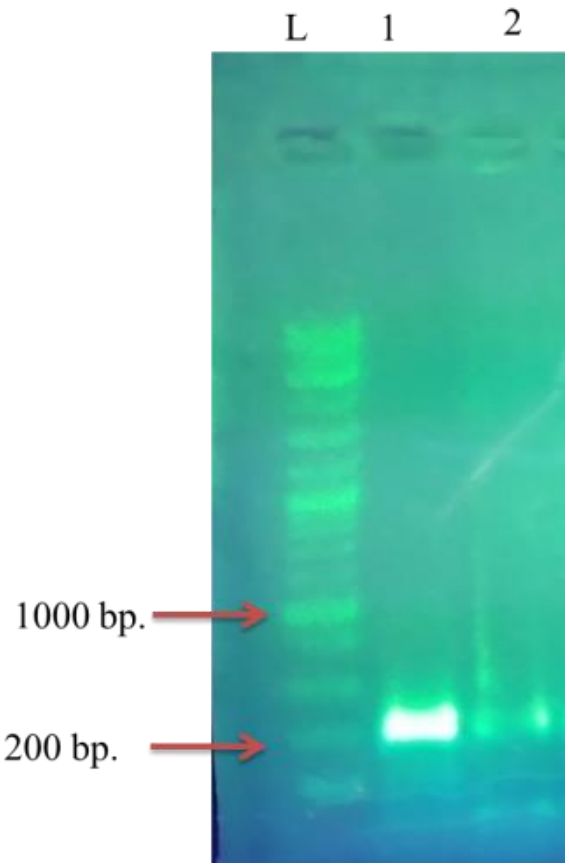

Figure 2: Agarose gel electrophoresis of RPA and PCR product of S gene. L line represents DNA standard marker, Shiny band in line 1 represents the RPA product of the spike gene. Line 2 shows the PCR product of the spike gene.

Specificity and Sensitivity of the established cas13-based SHERLOCK assay

The specificity of the SHERLOCK assay was determined using an RNA sample extracted from bovine coronavirus (BCV). RNA extracted from chicken infected with infectious bronchitis and synthetic S gene of avian coronavirus (ACV) was used as a positive control, while H_2o was used as a negative control**.** Only the sample extracted from clinically infected chicken with infectious bronchitis and positive control produced two visible bands (test and control band) (Figure 3). A 6-fold serial dilution of the input RNA (from positive control) ranging from 0 to 1000 copies per microliter has been used to determine the sensitivity of the SHERLOCK assay. As shown in figure4, 10 copies of RNA per microliter is the minimum concentration that can be detected by the assay. These results demonstrated that cas13-based SHERLOCK assay is a highly specificity and sensitive rapid assay for detecting nucleic acid of avian coronavirus (Figure 4).

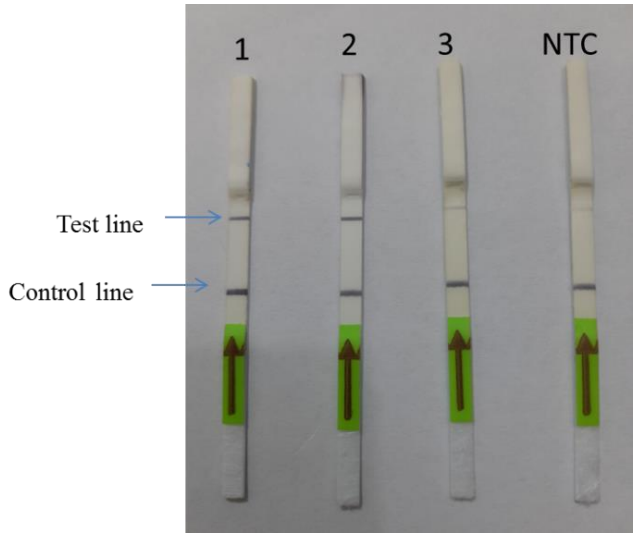

Figure 3: Lateral flow dipstick readout. The cleavage activity of cas13 was read out using LFD from left dip stick no. 1, showing positive results (two bands) of RNA sample extracted from chicken with infectious bronchitis. Synthetic S gene RNA (positive control) results positively (dipstick 2). Dipstick 3 showed negative results for were Guide S gene of IBV used with an RNA template of bovine coronavirus to test the assay specificity dipstick 3 show negative results. NTC is a negative control and shows negative results.

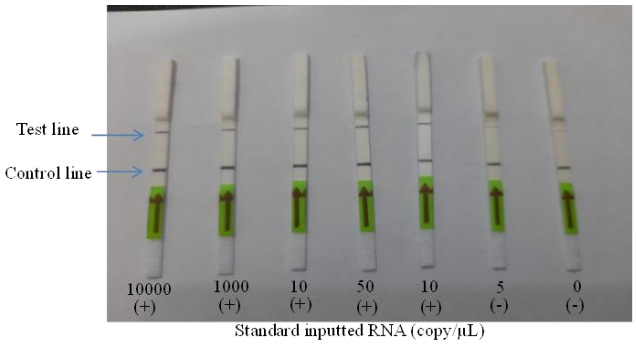

Figure 4: Sensitivity of the SHRLOCK assay to detecting infectious bronchitis virus. Different RNA concentrations were used to measure the assay's sensitivity to detecting the IBV. The figure shows that the assay can detect 5 copies per µL of RNA.

Discussion

Detection of the nucleic acid of pathogens, including viruses, by PCR or qPCR, is a slandered and accurate method for diagnosing animal and human diseases. However, this method needs expensive equipment and well-trained personnel. Furthermore, the PCR method needs time and is not considered a rapid test (23-26). Serological tests based

onantigen-antibody reactions are low-cost and rapid tests widely used to detect viruses and bacteria. However, these tests are inaccurate and have many errors related to false positives and low specificity (27-30). Therefore, a new rapid assay based on nucleic acid detection is urgently needed to accurately and rapidly detect pathogens, including viruses (31,32). Infectious bronchitis is one of the important respiratory diseases of chickens that cause several economic losses in the Poultry industry. Newcastle disease and chicken flu are respiratory diseases that also infect chickens and have similar clinical signs with avian infectious bronchitis (33-37). Therefore, a rapid and specific diagnosis of infectious bronchitis is needed for control and immunization against the disease. Recently CRISPR-based Cas 13 were used as a rabid and accurate assay for diagnosing infections such as Covid-19 (38-41). In this study, for the first time, we developed a nucleic acid-based detection assay to diagnose infectious infection.

SHERLOCK-based cas13 was used to detect the S gene, which was used as a target gene for detecting the virus. The assay was performed in three steps; in the first step, the extracted RNAs were converted to DNA and amplified using RT-RPA and primer of the S gene then cDNA was converted again to RNA. The cas13 enzyme (Cleavage) and guide RNA detected the target sequence. Labeled RNA reporter was used to indicating of cleavage (collateral cleavage). Finally, LFD was used to visualize the results. The assay's ability to diagnose infectious bronchitis was estimated by measuring the sensitivity and specificity of the assay. The results show the assay's high specificity and sensitivity to infectious bronchitis virus detection.

Furthermore, no devices were used to perform the assay. Moreover, the assay can be considered a rapid test and should be clinically used to diagnose infectious bronchitis in chickens. Performing the assay directly on the clinical sample without extracting RNA is needed to develop the assay and is widely used in clinical applications. Also, further studies are needed to use CRISPR assay for genotyping infectious bronchitis virus and screening variants and mutations in the virus genome.

Conclusion

SHERLOCK is a nucleic acid-based detection assay recently used as a novel, accurate, rapid test during the cov-19 pandemic. Newcastle disease, infectious bronchitis, and mycoplasma infection are respiratory diseases of chicken that have similar clinical signs. Differentiating infectious bronchitis from other respiratory diseases is required to treat and control the disease. Therefore, a rapid and accurate test is needed to diagnose the disease. In this study, we first used nucleic acid-based detection assay (SHERLOCK) to diagnose avian infectious bronchitis. The result shows high specificity and sensitivity of SHERLOCK based for diagnosis of avian infectious bronchitis. It's a rapid and accurate assay and should be used in the clinic to diagnose the disease. One important limitation that needs to be considered is that assay needs extracted RNA. Therefore, further studies are needed to make the assay work on clinical samples without RNA extraction.

Acknowledgments

We thank the veterinary hospital team in Al-Dewanyiah province, Iraq ,for their help in achieving this study.

Conflict of interest

The authors declare that there is no conflict of interest.

References

1. Egaña-Labrin S, Hauck R, Figueroa A, Stoute S, Shivaprasad HL, Crispo M, Corsiglia C, Zhou H, Kern C, Crossley B, Gallardo RA. Genotypic characterization of emerging avian reovirus genetic variants in California. Sci Rep. 2019;9(1):9351. DOI: 10.1038/s41598-019-45494-4
2. Liu IL, Lin YC, Lin YC, Jian CZ, Cheng IC, Chen HW. A novel immunochromatographic strip for antigen detection of avian infectious bronchitis virus. Int J Mol Sci. 2019;20(9):2216. DOI: 10.3390/ijms20092216
3. Icochea E, González R, Castro-Sanguinetti G, Maturrano L, Alzamora L, Sesti L, Chacón J, More-Bayona J. Genetic analysis of infectious bronchitis virus S1 gene reveals novel amino acid changes in the GI-16 lineage in Peru. Microorganisms. 2023;11(3):691. DOI: 10.3390/microorganisms11030691
4. Thai TN, Yoo DS, Jang I, Kwon YK, Kim HR. Dynamics of the emerging genogroup of infectious bursal disease virus infection in broiler farms in South Korea: A nationwide study. Viruses. 2022;14(8):1604. DOI: 10.3390/v14081604
5. Shirvani E, Paldurai A, Manoharan VK, Varghese BP, Samal SK. A Recombinant Newcastle disease virus (NDV) expressing S protein of infectious bronchitis virus (IBV) protects chickens against IBV and NDV. Sci Rep. 2020;10(1):762. DOI: 10.1038/s41598-018-30356-2
6. Ulkarni AB, Resurreccion RS. Genotyping of newly isolated infectious bronchitis virus isolates from northeastern Georgia. Avian Dis Digest. 2010;5(4):e3-4. DOI: 10.1637/9543-935810-digest.1
7. Gu K, Song Z, Ma P, Liao Z, Yang M, Zhou C, Li C, Zhao Y, Li H, Yang X, Lei C, Wang H. A novel nanobody-horseradish peroxidase fusion based-competitive ELISA to rapidly detect avian corona-virus-infectious bronchitis virus antibody in chicken serum. Int J Mol Sci. 2022;23(14):7589. DOI: 10.3390/ijms23147589
8. Bóna M, Kiss I, Dénes L, Szilasi A, Mándoki M. Tissue tropism of H9N2 low-pathogenic avian influenza virus in broiler chickens by immunohistochemistry. Animals. 2023;13(6):1052. DOI: 10.3390/ani13061052
9. Ma H, Shao Y, Sun C, Han Z, Liu X, Guo H, Liu X, Kong X, Liu S. Genetic diversity of avian infectious bronchitis coronavirus in recent years in China. Avian Dis Digest. 2012;7(1):e6-7. DOI: 10.1637/10000-980411-digest.1
10. Lin SY, Chen HW. Infectious bronchitis virus variants: Molecular analysis and pathogenicity investigation. Int J Mol Sci. 2017;18(10):2030. DOI: 10.3390/ijms18102030
11. Muhsen H, Alaraji F, Alhatami AO, khudhair YI. Real time PCR detection, sequencing, and phylogenetic tree analysis of Newcastle diseases virus isolated from an outbreak in layer flocks in Baghdad capital, Iraq. Indian J Public Health Res Dev. 2019;10(8):2077. DOI: 10.5958/0976-5506.2019.02162.4

12. Srivastava S, Upadhyay DJ, Srivastava A. Next-generation molecular diagnostics development by CRISPR/Cas tool: Rapid detection and surveillance of viral disease outbreaks. Front Mol BioSci. 2020;23(7):582499. DOI: 10.3389/fmolb.2020.582499
13. Al-Jameel W, Al-Mahmood SS. Similarities and differences of COVID-19 and avian infectious bronchitis from molecular pathologist and poultry specialist view point. Iraqi J Vet Sci. 2020;34(2):223-31. DOI: 10.33899/ijvs.2020.126984.1426
14. Raquib A, Uddin A, Nurozzaman SM, Uddin MM, Ahsan G, Rahman MM, Rahman MM. Seroprevalence of *Mycoplasma gallisepticum* infection in layer chickens of Bangladesh. Iraqi J Vet Sci. 2022;36(1):9-13. DOI: 10.33899/ijvs.2020.127511.1506
15. Corman VM, Landt O, Kaiser M, Molenkamp R, Meijer A, Chu DK, Bleicker T, Brünink S, Schneider J, Schmidt ML, Mulders DG. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Euro Surveill. 2020;25(3):2000045. DOI: 10.2807/1560-7917.es.2020.25.3.2000045
16. Palaz F, Kalkan AK, Can O, Demir AN, Tozluyurt A, Ozcan A, Ozsoz M. CRISPR-Cas13 system as a promising and versatile tool for cancer diagnosis, therapy, and research. ACS Synth Biol. 2021;10(6):1245-67. DOI: 10.1021/acssynbio.1c00107
17. Xiang X, Qian K, Zhang Z, Lin F, Xie Y, Liu Y, Yang Z. CRISPR-Cas systems based molecular diagnostic tool for infectious diseases and emerging 2019 novel coronavirus (COVID-19) pneumonia. J Drug Target. 2020;28(7-8):727-31. DOI: 10.1080/1061186x.2020.1769637
18. Esbin MN, Whitney ON, Chong S, Maurer A, Darzacq X, Tjian R. Overcoming the bottleneck to widespread testing: A rapid review of nucleic acid testing approaches for COVID-19 detection. RNA. 2020;26(7):771-83. DOI: 10.1261/rna.076232.120
19. Myhrvold C, Freije CA, Gootenberg JS, Abudayyeh OO, Metsky HC, Durbin AF, Kellner MJ, Tan AL, Paul LM, Parham LA, Garcia KF. Field-deployable viral diagnostics using CRISPR-Cas13. Sci. 2018;360(6387):444-8. DOI: 10.1126/science.aas8836
20. Shariq M, Khan MF, Raj R, Ahsan N, Singh R, Kumar P. CRISPR-based diagnostic approaches: Implications for rapid management of future pandemics (Review). Mol Med Rep. 2023;27(6):118. DOI: 10.3892/mmr.2023.13005
21. Huang YY, Zhang XY, Zhu P, Ji L. Development of clustered regularly interspaced short palindromic repeats/CRISPR-associated technology for potential clinical applications. World J Clin Cases. 2022;10(18):5934-5945. DOI: 10.12998/wjcc.v10.i18.5934
22. Hillary VE, Ceasar SA. A Review on the mechanism and applications of CRISPR/Cas9/Cas12/Cas13/Cas14 proteins utilized for genome engineering. Mol Biotechnol. 2023;65(3):311-325. DOI: 10.1007/s12033-022-00567-0
23. Compton SR. PCR and RT-PCR in the diagnosis of laboratory animal infections and in health monitoring. J Am Assoc Lab Anim Sci. 2020;59(5):458-68. DOI: 10.30802/aalas-jaalas-20-000008
24. Zhang X. Development of CRISPR-mediated nucleic acid detection technologies and their applications in the livestock industry. Genes. 2022;13(11):2007. DOI: 10.3390/genes13112007
25. Chakraborty J, Chaudhary AA, Khan SU, Rudayni HA, Rahaman SM, Sarkar H. CRISPR/Cas-based biosensor as a new age detection method for pathogenic bacteria. ACS Omega. 2022;7(44):39562-39573. DOI: 10.1021/acsomega.2c04513
26. Chavez M, Chen X, Finn PB, Qi LS. Advances in CRISPR therapeutics. Nat Rev Nephrol. 2023;19(1):9-22. DOI: 10.1038/s41581-022-00636-2
27. Vainionpää R, Leinikki P. Diagnostic techniques: Serological and molecular approaches. In: Mahy BJ, Van Regenmortel MV, editors. Encyclopaedia of virology. USA: Academic Press; 2008. 29-37 p. DOI: 10.1016/b978-012374410-4.00585-9
28. Park HM, Park Y, Berani U, Bang E, Vankerschaver J, Van Messem A, De Neve W, Shim H. In silico optimization of RNA-protein interactions for CRISPR-Cas13-based antimicrobials. Biol Direct. 2022;17(1):27. DOI: 10.1186/s13062-022-00339-5
29. Zhou Q, Chen Y, Wang R, Jia F, He F, Yuan F. Advances of CRISPR-Cas13 system in COVID-19 diagnosis and treatment. Genes Dis. 2022. DOI: 10.1016/j.gendis.2022.11.016
30. Gao H, Shang Z, Chan SY, Ma D. Recent advances in the use of the CRISPR-Cas system for the detection of infectious pathogens. J Zhejiang Univ Sci B. 2022;23(11):881-898. DOI: 10.1631/jzus.B2200068
31. Quansah E, Chen Y, Yang S, Wang J, Sun D, Zhao Y, Chen M, Yu L, Zhang C. CRISPR-Cas13 in malaria parasite: Diagnosis and prospective gene function identification. Front Microbiol. 2023;14:1076947. DOI: 10.3389/fmicb.2023.1076947
32. Zhao L, Qiu M, Li X, Yang J, Li J. CRISPR-Cas13a system: A novel tool for molecular diagnostics. Front Microbiol. 2022;13:1060947. DOI: 10.3389/fmicb.2022.1060947
33. Isa RH, Abdo JM, Al-Barzinji YM. Genotyping of avian infectious bronchitis virus in broiler farms in Duhok province, north of Iraq. Iraqi J Vet Sci. 2022;36(1):171-5. DOI: 10.33899/ijvs.2021.129635.1670
34. Al-Jameel W, Al-Mahmood SS. Similarities and differences of COVID-19 and avian infectious bronchitis from molecular pathologist and poultry specialist view point. Iraqi J Vet Sci. 2020;34(2):223-31. DOI: 10.33899/ijvs.2020.126984.1426
35. Yehia N, Salem HM, Mahmmod Y, Said D, Samir M, Mawgod SA, Sorour HK, AbdelRahman MA, Selim S, Saad AM, El-Saadony MT, El-Meihy RM, Abd El-Hack ME, El-Tarabily KA, Zanaty AM. Common viral and bacterial avian respiratory infections: An updated review. Poult Sci. 2023;102(5):102553. DOI: 10.1016/j.psj.2023.102553
36. Zhao J, Zhao Y, Zhang G. Key aspects of coronavirus avian infectious bronchitis virus. Pathogens. 2023;12(5):698. DOI: 10.3390/pathogens12050698
37. Yehia N, Salem HM, Mahmmod Y, Said D, Samir M, Mawgod SA, Sorour HK, AbdelRahman MA, Selim S, Saad AM, El-Saadony MT. Common viral and bacterial avian respiratory infections: An updated review. Poult Sci. 2023;102(5):102553. DOI: 10.1016/j.psj.2023.102553
38. Ali Z, Mahas A, Mahfouz M. CRISPR/Cas13 as a tool for RNA interference. Trends Plant Sci. 2018;23(5):374-8. DOI: 10.1016/j.tplants.2018.03.003
39. Bot JF, van der Oost J, Geijsen N. The double life of CRISPR-Cas13. Curr Opin Biotechnol. 2022;78:102789. DOI: 10.1016/j.copbio.2022.102789
40. Lou J, Wang B, Li J, Ni P, Jin Y, Chen S, Xi Y, Zhang R, Duan G. The CRISPR-Cas system as a tool for diagnosing and treating infectious diseases. Mol Biol Rep. 2022;49(12):11301-11311. DOI: 10.1007/s11033-022-07752-z
41. Abbas G, Yu J, Li G. Novel and alternative therapeutic strategies for controlling avian viral infectious diseases: Focus on infectious bronchitis and avian influenza. Front Vet Sci. 2022;9:933274. DOI: 10.3389/fvets.2022.933274

اختبار مبتكر معتمد عل تقنية كرسبر/كاس١٣ لتشخيص الالتهاب القصبات الفيروسي في الدواجن

محاسن عبد الرزاق خضير[١]، أمجد طالب السلطان[٢] و يحيى إسماعيل خضير[٢]

[١]فرع الأمراض وامرض الدواجن، [٢]فرع الطب الباطني والوقائي، كلية الطب البيطري جامعة القادسية، الديوانية، العراق

الخلاصة

يصيب مرض التهاب القصبات المعدي مجوعة من الطيور ومن ضمنها الدواجن وتتنج الاصابة عن فايروسات التاج (كورونا) ويسبب المرض خسائر اقتصادية كبيرة في قطعان الدواجن مرتبطة بانخفاض إنتاج البيض. يعد الكشف الدقيق عن الفايروس المسبب للمرض أمرا ضروريا للسيطرة على العدوى والوقاية منها. لأجل ذلك صممت هذه الدراسة حيث قمنا بتطوير اختبار سريع ودقيق وبدون استخدام أجهزة للكشف عن المرض. حيث تم استخدام النسخ العكسي - تضخيم البوليمر الإنزيمي المقترن بتقينه كرسبر - كاس ١٣ للكشف المرئي عن وجود الحامض النووي للفايروس وباستخدام أشرطة التدفق السطحي. تم اختبار فعالية الاختبار في كشف المرض من خلال قياس حساسية وخصوصية الاختبار للكشف عن الفايروس هذا بالإضافة الى تحديد الوقت اللازم لقراءة النتائج. اختير جين السبايك للفايروس للدلالة على المسبب. حيث تم اختبار فعالية الفحص من خلاص جمع عينات من دواجن مصابة بفايروس وأخرى غير مصابة وأيضا استخدام عينات سيطرة موجبة من خلال تصنيع جين السبايك الخاص بالفايروس. أظهرت النتائج أن فحص كرسبر - شيرولوك كاس ١٣ قدرة عل تشخيص الفايروس بخصوصية عالية إضافة الى انه اختبار حساس جدا حيث يمكن تشخيص التركيز القليل من الحامض النووي لحد عشر نسخ لكل مايكروليتر. هذا بالإضافة الى أن الفحص يعتبر من الفحوصات السريعة مقارنة بالفحوصات الأخرى مثل تفاعل البلمرة المتسلسل حيث يحتاج فقط الى خمسون دقيقة لقراءة النتيجة. لا يتطلب تشخيص الفايروس المرض أجهزة وجميع تفاعلات الاختبار تجري بدرجة حرارة الغرفة. إن النتائج الخاصة باختبار فعالية الفحص تشير الى انه يمكن استخدام هذا الفحص لتشخيص الحقلي للمرض غير ان هنالك حاجة للمزيد من الدراسات والتجارب للتطوير هذا الاختبار.