Heliyon 7 (2021) e08471

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Sensitivity and specificity of anti-double-stranded RNA immunofluorescence for universal detection of viral infection in respiratory specimens

Kornthara Kawang [a,b,1], Udsanee Naoudom [a,1], Ekasit Kowitdamrong [c,d], Stephen J. Kerr [e], Kiat Ruxrungtham [f,g], Voraphoj Nilaratanakul [a,h,*]

[a] Division of Infectious Diseases, Department of Medicine, Faculty of Medicine, Chulalongkorn University and King Chulalongkorn Memorial Hospital, Thai Red Cross Society, Bangkok, 10330, Thailand
[b] Interdisciplinary Program of Biomedical Sciences, Graduate School, Chulalongkorn University, Bangkok, 10330, Thailand
[c] Department of Microbiology, Faculty of Medicine, Chulalongkorn University, Bangkok, 10330, Thailand
[d] Applied Medical Virology Research Unit, Chulalongkorn University, Bangkok, Thailand
[e] Biostatistics Excellence Centre, Faculty of Medicine, Chulalongkorn University, Bangkok, 10330, Thailand
[f] Chulalongkorn Vaccine Research Center (Chula VRC), Department of Medicine, Faculty of Medicine, Chulalongkorn University, Bangkok, 10330, Thailand
[g] Thai Red Cross AIDS Research Centre (TRC-ARC), Thailand
[h] Healthcare-associated Infection Research Group STAR (Special Task Force for Activating Research), Chulalongkorn University, Bangkok, 10330, Thailand

HIGHLIGHTS

- Double-stranded RNA (dsRNA) is the only common antigen shared among most viruses.
- Anti-dsRNA immunofluorescence (IF) is simple and cost-effective.
- Anti-dsRNA IF can detect unknown viruses in clinical respiratory specimens.
- Compared to microarray, anti-dsRNA IF has acceptable accuracy (85.1%).

ARTICLE INFO

Keywords:
Respiratory virus
Double-stranded RNA
Immunofluorescence

ABSTRACT

Background: Emerging viruses could be detected before reaching pandemic level if universal viral detection screening was routinely used. Double-stranded RNA (dsRNA) is the only common antigen across most viral families. Anti-dsRNA immunofluorescence has shown promising results *in vitro*; however, its diagnostic value in respiratory specimens has not been evaluated.

Methods: Consecutive inpatient cases of suspected respiratory viral infections were prospectively enrolled. Respiratory samples were collected and divided for anti-dsRNA immunofluorescence (index test) and 19-subtypes respiratory virus microarray (reference standard). Using fluorescence microscopy, positive or negative anti-dsRNA IF results were determined independently by two raters.

Results: By microarray, 108 and 87 samples were positive and negative for viruses, respectively. The anti-dsRNA IF sensitivity was 83.3% (95%CI 76.1%–90.2%), while specificity was 87.4% (95%CI 80.8%–93.7%).

Conclusions: Anti-dsRNA IF is simple to perform, with acceptable accuracy, and suitable for point-of-care respiratory virus screening. Unlike most molecular techniques, known viral genome sequences are not required.

1. Introduction

Many respiratory viruses, especially influenza and coronavirus, have continuously evolved in wildlife and successively attacked humans and livestock on a global scale [1]. To prevent future pandemics, the Global Virome Project was established, focusing on early identification of the pandemic-potential of viruses found in wild animals [2]. Despite the tremendous manpower and resources required by this project, this effort alone was unable to prevent the current pandemic of SARS-CoV-2.

* Corresponding author.
E-mail address: voraphoj.n@chula.ac.th (V. Nilaratanakul).
[1] These authors contributed equally.

https://doi.org/10.1016/j.heliyon.2021.e08471
Received 16 October 2021; Received in revised form 21 November 2021; Accepted 22 November 2021

Viral adaptation to a human host is a rate-determining step before these zoonotic viruses efficiently spread from human to human and reach pandemic stage [3, 4]. It may be possible to effectively detect emerging viruses at early stages of adaptation, if most patients suspected of viral infections are routinely screened.

Many molecular techniques have already been developed for this purpose. The majority are polymerase chain reaction (PCR)-based and, more specifically, multiplex PCR assays, that make the diagnosis of viral infections in the respiratory system faster and broader [5]. One such method is the microarray technique, where amplified viral genetic material (amplicons) from multiplex PCR are hybridized to the microarray to identify the type of virus [6]. Unfortunately, due to their complexity, relatively high price and long turn-around time, microarray technology and other multiplexing methods for virus panels are only available at some tertiary and university hospitals. In addition, this technique is not applicable if the viral genome is unknown, as is the case with a newly emerging virus.

There is therefore an urgent need for a rapid, affordable, and broad diagnostic test for unknown respiratory viruses, that can be deployed in most settings. This would facilitate increased understanding of virus epidemiology and provide an opportunity for early detection of newly emerging and unknown viruses. From a public health perspective, earlier diagnosis of respiratory viral infections would afford the opportunity for early initiation of specific anti-viral treatments, and prompt isolation of the patients.

Double-stranded RNA (dsRNA) is generated as a by-product of viral replication in cells infected by most viruses, with a few exceptions including retroviruses and hepatitis B virus [7]. Since long dsRNA are pathogen-associated molecular patterns (PAMPs) not generally found in normal human cells [8], anti-dsRNA immunofluorescence (IF) may be able to detect virus-infected cells. Originally known as a 'pan--enterovirus antibody', anti-dsRNA antibodies detect cells infected by most viruses due to their characteristic binding to any long dsRNA, independent of their sequence [9]. Unlike single-stranded RNA, dsRNA is not easily degraded and can survive better in poor storage conditions [10].

In previous studies, direct and indirect IF assays against viral proteins have been used for detection of respiratory viral infections. Reported sensitivity and specificity range from 65-86% and 99–100%, respectively [11, 12]. Though anti-dsRNA antibody has been widely used *in vitro* for many viruses [7, 13, 14], it has rarely been applied to clinical specimens. One report observed positive anti-dsRNA immunoassay results in formalin-fixed paraffin-embedded (FFPE) cardiac tissues autopsied from children with enteroviral myocarditis [15]. Tissue diagnosis, however, is not a practical choice for most viral infections. This study aimed to explore the feasibility and accuracy of anti-dsRNA IF, as a triage test for viral infections in direct respiratory samples from inpatient settings, compared to the commercially available microarray technique.

2. Materials & methods

2.1. Specimen collection

All patients whose respiratory specimens were tested for respiratory virus microarray by request from their primary physician were prospectively and consecutively enrolled. Specimens were taken from both upper and lower respiratory tracts including nasal swab, nasopharyngeal swab, throat swab, sputum, tracheal suction, and bronchoalveolar lavage. After delivery of specimens to the laboratory for routine detection of 19 subtypes of respiratory viruses with microarray, an amount of 0.5 ml from the 2 ml total from each specimen in viral transport medium (2% fetal bovine serum, 10 units/ml penicillin, and 10 mcg/ml streptomycin in phosphate buffered saline) was secured and stored at 4 °C for further anti-dsRNA immunofluorescence. The specimens were labeled with random numbers and the microarray results were concealed from the investigators.

2.2. Cytocentrifugation and fixation

Each specimen was prepared on a microscope glass slide in a biosafety cabinet class II type A2. One hundred microliters of each specimen was cytocentrifuged (Hettich Rotina 380R with cytospin adaptor and 1 ml cyto chambers) at 4,000 rpm for 5 min onto a 30 mm^2 surface area of each slide, air-dried, then fixed with cold acetone (−20 °C) for 10 min. The fixed slides were air-dried again at room temperature and stored at -20 °C. We had previously compared between heat, formaldehyde, and cold acetone fixation with or without proteinase K digestion to determine the best method. Cold acetone fixation without proteinase K was chosen due to its superior performance and simplicity. (Supplementary Appendix).

2.3. Anti-dsRNA immunofluorescence (IF) staining

The prepared clinical respiratory specimen slides, a negative control, and a positive control were stained simultaneously. The negative and positive controls were prepared from a BHK (baby hamster kidney) cell line and Chikungunya virus infected BHK, respectively. Blocking with 10% normal goat serum (NGS) made no difference in the signal to noise ratio, or reduction of non-specific signals. Thus, the blocking step was omitted. The primary anti-dsRNA antibody [J2] monoclonal antibody from Scicon® (product number 10010500 at 1:200 dilution) in phosphate buffered saline (PBS) with 0.04% TritonX and 5% normal goat serum (NGS) was applied to the prepared slides. The slides were incubated at room temperature for 60 min. After 3 washes with PBS, the secondary antibody (Goat anti-mouse IgG-AF488 from Invitrogen® (ab 150117) at 1:200 dilution plus 100 ng/ml of DAPI) in PBS with 0.04% TritonX and 5% NGS was then applied and the slides were incubated at room temperature for 30 min. After 3 additional washes with PBS, each slide was mounted with Prolong Diamond Antifade Mountant from Invitrogen® (Catalog number P36970) and a coverslip.

2.4. Interpretation

The fluorescent signal was visualized with a fluorescent microscope (Zeiss Axio Imager M1m) using the FITC channel for dsRNA and the DAPI channel for the nucleus. The images were captured with a Zeiss AxioCam MRm 1.4 megapixels monochrome microscope camera and Zeiss Axio Vision SE64 Rel. 4.9.1 software. The signal from each slide was compared to positive and negative controls to determine the positivity and negativity. All slides were independently visualized and interpreted by two trained investigators, blinded from the microarray results. A dsRNA positive result was determined by the presence of a group of intracellular bright green puncta in the FITC channel. We excluded any signals that were outside the cell boundaries seen in the DAPI channel (nucleus) and brightfield (cytoplasm). Fluorescent signals from artifacts like crystals were also ruled out. If the signal in the FITC channel was also seen in the TRITC channel, it would be considered as autofluorescence, which generally emits its fluorescent signal across all channels. When the results were inconsistent between the two investigators, those particular slides were adjudicated by a third investigator. The results were then compared to the microarray as a reference standard, for calculation of sensitivity and specificity.

2.5. Reference standard

Automated microarray (The NxTAG® Respiratory Pathogen Panel) is a molecular technique routinely used in our hospital to detect viruses in respiratory specimens. The microarray can detect 19 viruses simultaneously, including adenovirus, human bocavirus, coronaviruses (HKU1, 229E, NL63, and OC43), human metapneumovirus, enteroviruses/rhinoviruses, influenza A viruses (H1 and H3), influenza B virus, parainfluenza viruses (types 1, 2, 3, and 4), and respiratory syncytial virus (A and B).

2.6. Data collection

Written informed consent was obtained from patients. The anti-dsRNA immunofluorescence results were recorded as positive or negative, and the cellularity content for each slide was graded from 1 + to 4+. The grading system was as follows: 1 + represented average cells on a slide of less than 11 cells per low power field (LPF), 2 + for 11–20 cells per LPF, 3 + for 21–50 cells per LPF, and 4 + for more than 50 cells per LPF. Types or sources of respiratory specimens were also recorded. After the results of the anti-dsRNA immunofluorescence from all slides were finalized by the investigative team, the random number labels were unmasked. The investigators reviewed the enrolled patients' charts and recorded each patient's information, such as gender, age, underlying diseases, antiviral drugs, antibiotics, and immunosuppressants, duration of onset, microarray results, and the final diagnosis at the time of patient discharge.

2.7. Statistical analysis

Sensitivity, specificity, accuracy, positive predictive value (PPV), negative predictive value (NPV), accuracy and likelihood ratios of dsRNA detection with immunofluorescence were calculated against standard test (microarray) results as a gold standard. Clustered bootstrapping with 1000 replications was used to derive 95%CI around each parameter and account for correlation in patients with >1 test result during the study [16]. Inter-rater agreement was determined with Cohen's kappa (κ). Software used for analysis was STATA 16 (Statacorp, College Station, TX).

2.8. Sample size calculation

We hypothesized that the anti-dsRNA IF accuracy would be similar to that described in previous viral protein antigen immunostaining studies. Assuming a sensitivity and specificity of 85, we aimed to estimate these parameters with a precision of ±5% using the following formula: [$n = \frac{Z_{\alpha/2}{}^2 pq}{d^2}$]. Since the prevalence of positive microarray for respiratory virus in our hospital was approximately 60% of all specimens collected, the calculated targeted sample size was 195 (n/0.6).

In accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki), this study was approved by the Institutional Review Board (IRB number 168/61), Institutional Biosafety Committee (CU-IBC number 008/2018), Faculty of Medicine, Chulalongkorn University, and registered in Thai Clinical Trial Registry (TCTR20180129003).

2.9. Role of the funding source

All funding sources had no involvement in the study design, specimen collection, analysis, interpretation, and preparation/submission of the manuscript.

3. Results

3.1. Demographic data and clinical characteristics

A total of 195 specimens from 148 unique patients from October 2018 to March 2019 were consecutively enrolled in this study; 24, 5, 3 and 1 patients had 2, 3, 4 and 5 tests, respectively. Demographic and clinical characteristics are summarized in Table 1. Median patient age was 3.4 years (interquartile range (IQR) 0.83–55; range 0.10–91 years). One hundred and seventy-two (88.2%) patients reported a symptom onset of less than 7 days at specimen collection, with the majority less than 3 days prior to investigation.

One hundred and thirty-two patients (89.2%) had comorbidities. Forty-seven (24.1%) patients received immunosuppressive agents. Thirty (15.4%) patients were taking antiviral drugs before specimen collection, and 150 (76.9%) patients received multiple antibiotics prior to specimen collection. The majority of specimens were collected from the upper respiratory tract, including 167 (85.6%) nasopharyngeal swabs and 2 (1.0%) throat swabs.

Table 1. Demographic and clinical characteristics of patients.

Characteristic	N (%)*
Age	
< 15 years	90 (60.8%)
≥ 15 years	58 (39.2%)
Sex	
Male	90 (60.8%)
Female	58 (39.2%)
Onset of symptoms	
1-3 days	152 (78.0%)
4-6 days	20 (10.2%)
≥7 days	23 (11.8%)
Comorbidity**	
Chronic lung disease	17 (11.5%)
Congenital abnormalities	53 (35.8%)
Connective tissue disease	5 (3.4%)
Hematologic disorder	2 (1.4%)
Hematologic malignancy	17 (11.5%)
Solid malignancy	11 (7.4%)
Metabolic disorder	14 (9.5%)
Post transplantation	6 (4.1%)
Preterm	11 (7.4%)
Other	12 (8.1%)
No comorbidity	16 (10.8%)
Received immunosuppressive agents	47 (24.1%)
Received antiviral drugs before specimen collection	30 (15.4%)
Received antibiotics before specimen collection	150 (76.9%)
Source of specimens	
Nasopharyngeal swab	167 (85.6%)
Nasal swab	1 (0.5%)
Throat swab	2 (1.0%)
Bronchoalveolar lavage (BAL)	20 (10.3%)
Sputum	1 (0.5%)
Tracheal suction	4 (2.1%)
Grade of specimens	
1 + (less than 11 cells per LPF)	29 (14.9%)
2 + (11-20 cells per LPF)	43 (22.1%)
3 + (21-50 cells per LPF)	60 (30.8%)
4 + (more than 50 cells per LPF)	63 (32.3%)
Median (IQR) days from first to subsequent test in patients with > 1 sample	
2nd sample (n= 33 patients)	18 (3– 33) days
3rd sample (n= 9 patients)	67 (38 – 81) days
4th sample (n = 4 patients)	68 (50 – 92) days
5th sample (n = 1 patient)	98 days

* For age, sex, and comorbidity, the denominator is patient at first admission or test, for other characteristics, the denominator is over all admissions or tests.

** The comorbidities are not mutually exclusive. Some patients had more than 1 comorbidity.

3.2. Respiratory viruses detected by microarray

A total of 108 of 195 specimens (55.3%, 95%CI 47.2%–63.5%) tested positive for a virus with the respiratory virus 19 subtypes detection (microarray). Viruses included enterovirus/rhinovirus (60, 55.56%), parainfluenza virus (11, 10.19%), influenza virus (9, 8.33%),

coronavirus (5, 4.63%), metapneumovirus (3, 2.78%), bocavirus (1, 0.93%), and mixed viral infection (19, 17.59%) (Table S1, Supplementary Appendix).

3.3. Sensitivity and specificity of the anti-dsRNA immunofluorescence compared to the respiratory virus 19 subtypes detection (microarray)

One hundred and one (51.8%) of 195 specimens were positive for dsRNA as detected by anti-dsRNA immunofluorescence (Figure 1).

There were 29 discordant results from the microarray and immunofluorescence assays. The positive and negative concordant and discordant results are shown in Table 2.

Compared to the molecular technique (microarray), the immunofluorescence method had a sensitivity of 83.3% (95% CI 76.1%–90.2%), specificity of 87.4% (95% CI 80.8%–93.7%), positive predictive value of 89.1% (95% CI 82.5%–94.9%), negative predictive value of 80.9% (95% CI 73.1%–88.9%) and an accuracy of 85.1% (95% CI 80.1%–89.9%). The positive likelihood ratio (+LR) was 6.6 (95%CI 4.3–13.5) and the negative likelihood ratio was 0.2 (95%CI 0.1–0.3). Changes in positive and negative predictive values with changing prevalence, and the post-test probability of disease changes are shown in Figures S1 and S2 (Supplementary Appendix), Subgroup performance of anti-dsRNA IF is shown in Figure 2 and Table S2 (Supplementary Appendix).

3.4. Interrater agreement

All slides were visualized and interpreted by two trained investigators independently. Expected agreement between the investigators was 50.0% and actual agreement was 69.7%, $\kappa = 0.394$ indicating fair agreement unlikely due to chance ($P < 0.001$).

Table 2. Contingency table showing the concordance of anti-dsRNA immunofluorescence results and respiratory virus 19 subtypes detection (microarray).

dsRNA	Microarray	
	Positive	Negative
Positive	90	11
Negative	18	76

4. Discussion

In our study, the accuracy of anti-dsRNA IF was comparable to viral protein detection rates reported in previous studies [11, 12]. The major advantages of anti-dsRNA IF are its simplicity, short turn-around time of approximately 2.5 h, and low cost (less than $3 US reagent cost per test), making it possible to be used for a point-of-care screening and epidemiologic studies. The test results could be available in less than an hour if we omitted the secondary antibody step with a dye-conjugated anti--dsRNA as the primary antibody and incubation time was further optimized. Importantly, this technique also has the potential to detect emerging or unknown viruses, since the knowledge of the specific sequence of virus is not required. The positive and negative likelihood ratios were consistent with a 35% increase or decrease in the post-test probability of disease, respectively [17].

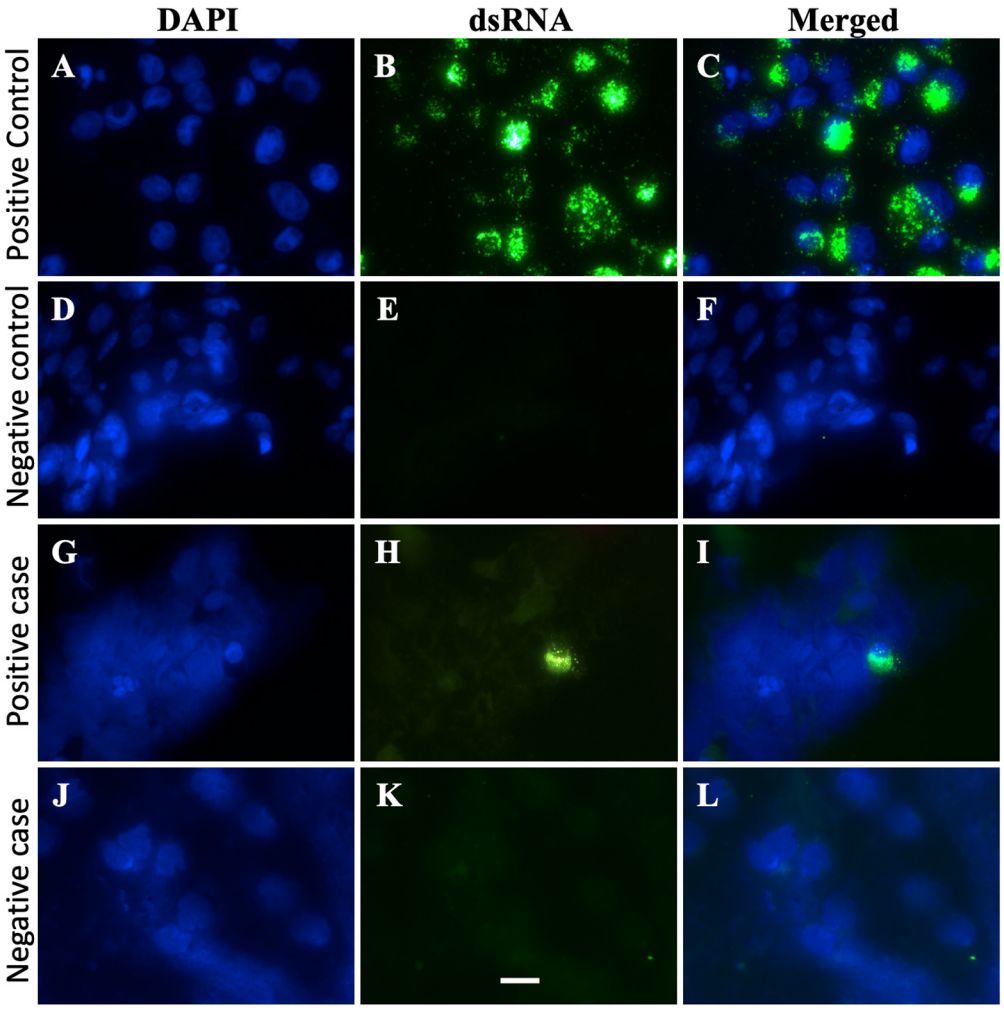

Figure 1. The examples of anti-dsRNA IF microscopic images are compared between positive control (A–C), negative control (D–F), positive clinical specimen (G–I) and negative clinical specimen (J–L). The images in the left column are from DAPI channel (A, D, G, J) for DAPI (nucleus) staining. The images in the middle column are from FITC channel (B, E, H, K) for dsRNA staining (AF-488 dye). The right column shows the merged images of the left and middle columns. Chikungunya-infected BHK cells are used for the positive control. The positive clinical specimen, which had Entero/Rhinovirus (microarray), shows one cell with a bright fluorescent signal from a group of intracytoplasmic puncta (H, I). The signal from an extracellular large dot, seen in image K and L, is considered to be an artifact and not counted as a positive signal. The scale bar of 20 microns is shown in image K.

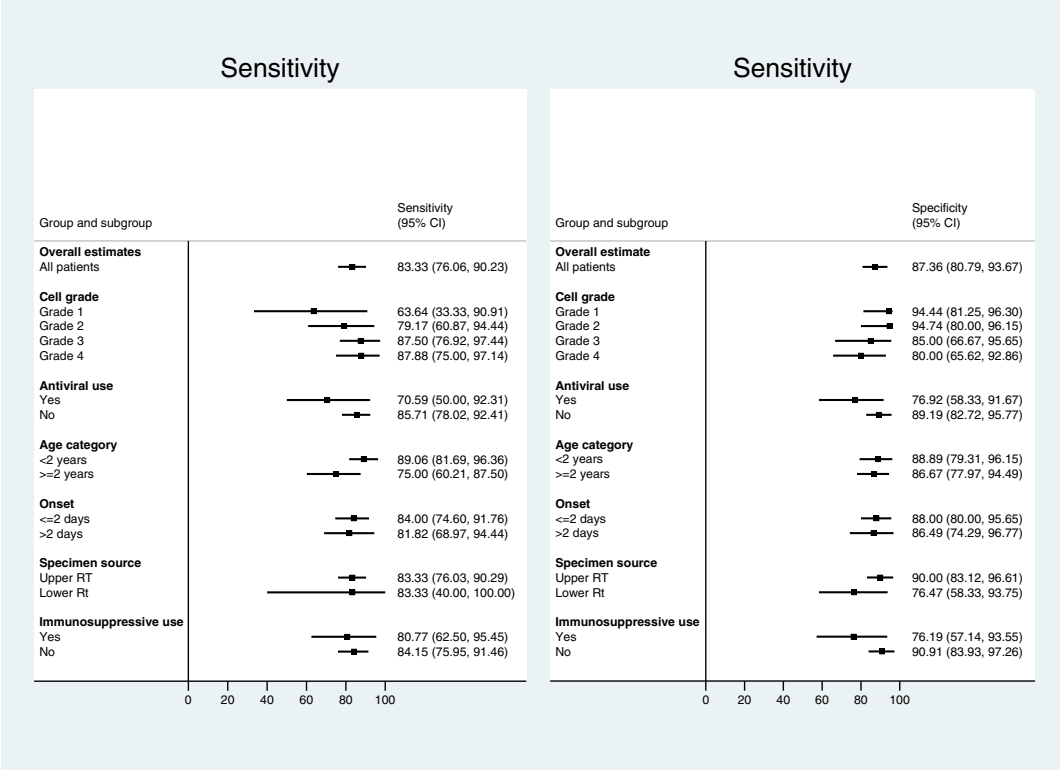

Figure 2. Forest plot showing sensitivities and specificities of anti-dsRNA IF versus respiratory virus 19 subtypes detection microarray.

Seventy-three samples were positive for enterovirus/rhinovirus (+ssRNA virus), the most common virus detected by microarray in this study. Out of these 73 samples, 64 (87.67%) were positive for anti-dsRNA IF. The sensitivity dropped to 74.23% with other viruses, mainly -ssRNA and DNA viruses. The increased sensitivity in the infant group (<2 years, Figure 2) was most likely due to a higher percentage of enterovirus/rhinovirus (78.13%) in this groups.

False negatives were inevitable due to the paucity of infected cells that were positive for dsRNA signal found on each slide. Subgroup analysis showed higher sensitivities as the cellularity grade increased. However, the specificity consequently decreased because of more false positive results from autofluorescence or non-specific staining that were observed in the higher cellularity groups. This could be improved by counting the cell numbers in each sample and adjusting the volume accordingly by cytocentrifugation to arrive at the proper cellularity level (grade 3+, 21–50 cells/LPF, Figure 2). Decreased sensitivity was also observed in the group given anti-viral treatment (Figure 2).

False positive signals might originate from bacterial flora or bacteriophages harboring dsRNA. In rare cases with mutations in the *PNPT1* gene, which controls dsRNA degradation in mitochondria, dsRNA accumulation in mitochondria could be detected with IF [18]. Positive anti-dsRNA IF in novel viral infection, undetected by microarray, is also possible.

Recently, anti-dsRNA immunostaining has shown positive results in cells and formalin-fixed paraffin-embedded (FFPE) animal tissues experimentally infected with SARS-CoV-2 [19]. Unfortunately, our study detected coronaviruses in only six samples by microarray, an insufficient number to draw robust conclusions regarding anti-dsRNA IF and coronaviruses.

The major disadvantage of our approach is low reliability. The interpretation of the fluorescent signals is heavily based on the rater's experience. Unlike Gram's stain or other simple staining techniques, there is no reference textbook to guide IF staining of dsRNA. We chose the virus-infected/non-infected cell cultures for rater training. Control respiratory virus slides were also purchased for training, but the anti-dsRNA IF on these slides showed very poor signals unlike our freshly prepared cells. We also prepared some positive and negative clinical specimens for training, but this was not very helpful due to a high variation of clinical specimens and a very low number of infected cells. The overall results showed fair agreement between raters (Kappa = 0.394, $p < 0.0001$). The interrater agreement did improve over time despite the lack of awareness by the raters about the microarray results until the end of the study. Subgroup analysis showed that the agreement was of moderate degree in the second half (96 samples, Kappa = 0.439, $p < 0.0001$), an improvement from the first half (99 samples, Kappa = 0.323, $p = 0.0006$). In the future, machine learning on digital files of whole slide images might overcome the low reliability problem with human raters.

One limitation of anti-dsRNA IF is that it cannot differentiate types of viruses. Rather than being a replacement of a molecular test, it should be an add-on test to raise a clinician's suspicion on unknown viral infection, of which further investigation is required. More cases may be correctly diagnosed as viral rather than bacterial infections, which would in turn reduce the inappropriate use of antibiotics and the problem of multi-drug-resistant pathogens.

Similar to 16S rRNA gene PCR and sequencing for universal identification of both known and unknown bacteria, dsRNA enrichment by immunoprecipitation for PCR and sequencing (dsRNA-seq) has successfully detected unknown viruses in *in vitro*, plants, and animals [20, 21]. This might be worthy of further investigation in human specimens.

In summary, our current study demonstrates that the anti-dsRNA IF test may be used in respiratory samples with more than 80% accuracy, if better reliability is achieved by more training. Further improvements in sensitivity and specificity can likely be achieved by suitable pre-treatment of samples, optimizing and standardizing cell numbers on the slides, and developing as well as validating methods for computer-based slide reading and interpretation. In resource limited settings where molecular testing might not be readily affordable, the anti-dsRNA IF technique might provide a sentinel signal if a new virus was to emerge somewhere.

Declarations

Author contribution statement

Kornthara Kawang and Udsanee Naoudom: Performed the experiments; Wrote the paper.

Ekasit Kowitdamrong: Contributed reagents, materials, analysis tools or data.

Stephen J. Kerr and Kiat Ruxrungtham: Analyzed and interpreted the data; Wrote the paper.

Voraphoj Nilaratanakul: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Funding statement

This work was supported by the Ratchadapiseksompotch Fund, Faculty of Medicine, Chulalongkorn University (Grant Number RA61/061, RA-MF-10/63, and RA-MF-55/64), the Thailand Research Fund, and the Higher Education Commission (MRG6180177). The cytocentrifuge equipment was supported by a grant for the Healthcare-associated Infection Research Group STAR (Special Task Force for Activating Research), Chulalongkorn University (STF 6100430002-1). Voraphoj Nilaratanakul was supported by Grant for Development of New Faculty Staff, Ratchadaphiseksomphot Endowment Fund (DNS 64_002_30_001_2). Kornthara Kawang was supported by Research Assistant Scholarship, Graduate School, Chulalongkorn University (GCUGE17).

Data availability statement

Data will be made available on request.

Data sharing

All of individual participant data are available from the corresponding author, [VN], upon reasonable request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

Supplementary content related to this article has been published online at https://doi.org/10.1016/j.heliyon.2021.e08471.

Acknowledgements

The control cells and infected cells for protocol optimization were prepared from RNA and DNA viruses in cell cultures; positive-sense single-stranded RNA virus – Chikungunya and dengue viruses in BHK cells courtesy of Professor Padet Siriyasatien (Department of Parasitology, Faculty of Medicine, Chulalongkorn university), Assistant Professor Siwaporn Boonyasuppayakorn (Department of Microbiology, Faculty of Medicine, Chulalongkorn University), and Associate Professor Wanla Kulwichit (Division of Infectious Diseases, Department of Medicine, Faculty of Medicine, Chulalongkorn University); negative-sense single-stranded RNA virus – H3N2 influenza A virus in MDCK cells courtesy of Associate Professor Ekasit Kowitdamrong (Department of Microbiology, Faculty of Medicine, Chulalongkorn University); DNA virus – herpes simplex virus I in vero cells courtesy of Professor Parvapan Bhattarakosol (Department of Microbiology, Faculty of Medicine, Chulalongkorn university).

The authors thank the research team of the Department of Medicine, Faculty of Medicine, Chulalongkorn University for editing the final manuscript.

References

[1] A.F. Franchini, F. Auxilia, P.M. Galimberti, M.A. Piga, S. Castaldi, A. Porro, COVID 19 and Spanish flu pandemics: all it changes, nothing changes, Acta Biomed. 91 (2) (2020) 245–250.

[2] D. Carroll, P. Daszak, N.D. Wolfe, G.F. Gao, C.M. Morel, S. Morzaria, et al., The global Virome project, Science 359 (6378) (2018) 872–874.

[3] L.H. Moncla, G. Zhong, C.W. Nelson, J.M. Dinis, J. Mutschler, A.L. Hughes, et al., Selective bottlenecks shape evolutionary pathways taken during mammalian adaptation of a 1918-like avian influenza virus, Cell Host Microbe 19 (2) (2016) 169–180.

[4] K. Voskarides, E. Christaki, G.K. Nikolopoulos, Influenza virus-host Co-evolution. A predator-prey relationship? Front. Immunol. 9 (2018) 2017.

[5] S. Murali, A.A. Langston, F.S. Nolte, G. Banks, R. Martin, A.M. Caliendo, Detection of respiratory viruses with a multiplex polymerase chain reaction assay (MultiCode-PLx Respiratory Virus Panel) in patients with hematologic malignancies, Leuk. Lymphoma 50 (4) (2009) 619–624.

[6] F. Raymond, J. Carbonneau, N. Boucher, L. Robitaille, S. Boisvert, W.K. Wu, et al., Comparison of automated microarray detection with real-time PCR assays for detection of respiratory viruses in specimens obtained from children, J. Clin. Microbiol. 47 (3) (2009) 743–750.

[7] K.N. Son, Z. Liang, H.L. Lipton, Double-stranded RNA is detected by immunofluorescence analysis in RNA and DNA virus infections, including those by negative-stranded RNA viruses, J. Virol. 89 (18) (2015) 9383–9392.

[8] S. Jensen, A.R. Thomsen, Sensing of RNA viruses: a review of innate immune receptors involved in recognizing RNA virus invasion, J. Virol. 86 (6) (2012) 2900–2910.

[9] J. Schonborn, J. Oberstrass, E. Breyel, J. Tittgen, J. Schumacher, N. Lukacs, Monoclonal antibodies to double-stranded RNA as probes of RNA structure in crude nucleic acid extracts, Nucleic Acids Res. 19 (11) (1991) 2993–3000.

[10] J.H. Strauss Jr., R.B. Kelly, R.L. Sinsheimer, Denaturation of RNA with dimethyl sulfoxide, Biopolymers 6 (6) (1968) 793–807.

[11] P. Bakerman, L. Balasuriya, O. Fried, D. Tellez, P. Garcia-Filion, H. Dalton, Direct fluorescent-antibody testing followed by culture for diagnosis of 2009 H1N1 influenza A, J. Clin. Microbiol. 49 (10) (2011) 3673–3674.

[12] C.F. Shafik, E.W. Mohareb, F.G. Youssef, Comparison of direct fluorescence assay and real-time rt-PCR as diagnostics for respiratory syncytial virus in young children, J. Trop. Med. 2011 (2011) 781919.

[13] F. Weber, V. Wagner, S.B. Rasmussen, R. Hartmann, S.R. Paludan, Double-stranded RNA is produced by positive-strand RNA viruses and DNA viruses but not in detectable amounts by negative-strand RNA viruses, J. Virol. 80 (10) (2006) 5059–5064.

[14] C.A. O'Brien, J. Hobson-Peters, A.W. Yam, A.M. Colmant, B.J. McLean, N.A. Prow, et al., Viral RNA intermediates as targets for detection and discovery of novel and emerging mosquito-borne viruses, PLoS Neglected Trop. Dis. 9 (3) (2015), e0003629.

[15] S.J. Richardson, A. Willcox, D.A. Hilton, S. Tauriainen, H. Hyoty, A.J. Bone, et al., Use of antisera directed against dsRNA to detect viral infections in formalin-fixed paraffin-embedded tissue, J. Clin. Virol. 49 (3) (2010) 180–185.

[16] X-h Zhou, D.K. McClish, N.A. Obuchowski, Statistical Methods in Diagnostic Medicine, second ed., Wiley, Hoboken, N.J., 2011 xxx, 545 pp.

[17] D.A. Grimes, K.F. Schulz, Refining clinical diagnosis with likelihood ratios, Lancet 365 (9469) (2005) 1500–1505.

[18] A. Dhir, S. Dhir, L.S. Borowski, L. Jimenez, M. Teitell, A. Rotig, et al., Mitochondrial double-stranded RNA triggers antiviral signalling in humans, Nature 560 (7717) (2018) 238–242.

[19] F.Z.X. Lean, M.M. Lamers, S.P. Smith, R. Shipley, D. Schipper, N. Temperton, et al., Development of immunohistochemistry and in situ hybridisation for the detection of SARS-CoV and SARS-CoV-2 in formalin-fixed paraffin-embedded specimens, Sci. Rep. 10 (1) (2020) 21894.

[20] A.G. Blouin, H.A. Ross, J. Hobson-Peters, C.A. O'Brien, B. Warren, R. MacDiarmid, A new virus discovered by immunocapture of double-stranded RNA, a rapid method for virus enrichment in metagenomic studies, Mol. Ecol. Resour. 16 (5) (2016) 1255–1263.

[21] C.J. Decker, H.R. Steiner, L.L. Hoon-Hanks, J.H. Morrison, K.C. Haist, A.C. Stabell, et al., dsRNA-seq: identification of viral infection by purifying and sequencing dsRNA, Viruses 11 (10) (2019).