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SHORT REPORT

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An international review of the characteristics of viral nucleic acid-amplification testing (NAT) reveals a trend towards the use of smaller pool sizes and individual donation NAT

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Abstract

Background and Objectives: Nucleic acid-amplification testing (NAT) is used for screening blood donations/donors for blood-borne viruses. We reviewed global viral NAT characteristics and NAT-yield confirmatory testing used by blood operators.

Materials and Methods: NAT characteristics and NAT-yield confirmatory testing used during 2019 was surveyed internationally by the International Society of Blood

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Transfusion Working Party Transfusion-Transmitted Infectious Diseases. Reported characteristics are presented herein.

Results: NAT was mainly performed under government mandate. Human immunodeficiency virus (HIV), hepatitis C virus (HCV) and hepatitis B virus (HBV) NAT was performed on all donors and donation types, while selective testing was reported for West Nile virus, hepatitis E virus (HEV), and Zika virus. Individual donation NAT was used for HIV, HCV and HBV by ~50% of responders, while HEV was screened in mini-pools by 83% of responders performing HEV NAT. Confirmatory testing for NAT-yield samples was generally performed by NAT on a sample from the same donation or by NAT and serology on samples from the same donation and a follow-up sample.

Conclusion: In the last decade, there has been a trend towards use of smaller pool sizes or individual donation NAT. We captured characteristics of NAT internationally in 2019 and provide insights into confirmatory testing approaches used for NAT-yields, potentially benefitting blood operators seeking to implement NAT.

Keywords
NAT, transfusion safety, virus

Highlights

- Human immunodeficiency virus, hepatitis C virus and hepatitis B virus nucleic acid-amplification testing (NAT) was performed on all donors and donation types, whereas selective testing was reported for West Nile virus, hepatitis E virus and Zika virus.
- In the last decade, there has been a trend towards the use of smaller pool sizes or individual donation NAT.
- Confirmatory testing for NAT-yield samples was generally performed by NAT on a sample from the same donation or by NAT and serology on samples from the same donation and a follow-up sample.

INTRODUCTION

Blood-borne viruses can be transmitted through blood transfusion. Blood operators employ a myriad of tools to reduce this risk including nucleic acid-amplification testing (NAT). Since the introduction of hepatitis C virus (HCV) and human immunodeficiency virus (HIV) NAT in the 1990s, NAT has been implemented for other viruses, including hepatitis B virus (HBV), hepatitis E virus (HEV), West Nile virus (WNV) and Zika virus (ZIKV) [1, 2]. NAT was initially performed mainly on mini-pools (MPs), but technological advances and reduced costs of NAT have led to the introduction of individual donation (ID)-NAT [1]. Procedures for confirming NAT-yield samples (donations testing reactive by NAT, but negative by serology) can be complex. Variability remains in the different approaches to NAT and indeed in the ways that NAT-yield samples are confirmed, amongst blood operators internationally.

A survey of NAT usage and yield amongst blood operators internationally was recently undertaken by the International Society of Blood Transfusion Working Party Transfusion-Transmitted Infectious Diseases (ISBT WP-TTID) [2]. The characteristics of blood donation viral NAT and NAT-yield confirmatory testing was surveyed amongst blood operators in 2019, the analysis of which is presented herein.

MATERIALS AND METHODS

This is a sub-analysis of data collected for an international review of NAT performed by ISBT WP-TTID [2]. The present study focused on reviewing international practices for viral blood donation NAT and NAT-yield confirmatory testing in 2019.

Given some regions within a country (representing different blood operators within a country) reported different responses to some survey questions, percentages were based on the proportion of survey responses, not the proportion of countries involved. For analyses of MP size, where a range of MPs were provided for a virus by the same responder or same country (for comparison with 2008 data [1]), the largest number was used. The 2008 data were not available for all countries that responded to the 2019 survey.

Descriptive analyses were performed, with reported variables expressed as frequencies and percentages. Comparisons of MP size per country in 2019 with that from 2008 [1] were performed with a Mann–Whitney test, using GraphPad Prism. Ordinal logistic regression analysis was performed to determine whether possible predictors including world bank income category (data from [3]), region, HIV incidence (data from [4]), HCV viraemic prevalence (data from [5]), HBV incidence (data from [4]) or number of donations tested for HIV were associated with the use of ID- or MP-NAT or MP size (where a MP of 1 was used for ID-NAT). For quantitative predictors, correlations were first performed to determine multi-collinearity. These analyses were performed using R Statistical Software version 2023.06.0 [6].

Confirmatory testing procedures for NAT yields were initially examined via frequency analysis; however, given the large number of separate responses, data were re-categorized by sample and assay type. Categorization by sample type was separated into three groups: same donation (re-testing the same or alternate sample only, where an alternate sample was defined as another tube from the same donation or sample from the retrieved plasma unit), donor follow-up (testing a donor follow-up sample only), donation and follow-up (any combination of testing the same/alternate sample and a donor follow-up sample). Categorization by assay type was separated into three groups: NAT (NAT assay only, where the NAT assay was defined as the same assay or an alternate NAT assay with comparable or increased sensitivity), serology (serology only) and NAT and serology (combination of NAT assays and serology testing). Responses that could not be grouped by confirmatory assay type were omitted.

RESULTS

In 2019, 38 survey participants, representing 27 countries, performed blood donation NAT for at least one virus (a total of 43 survey responses were received from 32 countries; 5 countries indicated they were not performing NAT [2]). Of these survey responders, 25/38 were from high-income countries, 10/38 were from upper-middle-income countries and 3/38 were from lower-middle-income countries. Most responders performing NAT did so under government mandate (Figure 1a).

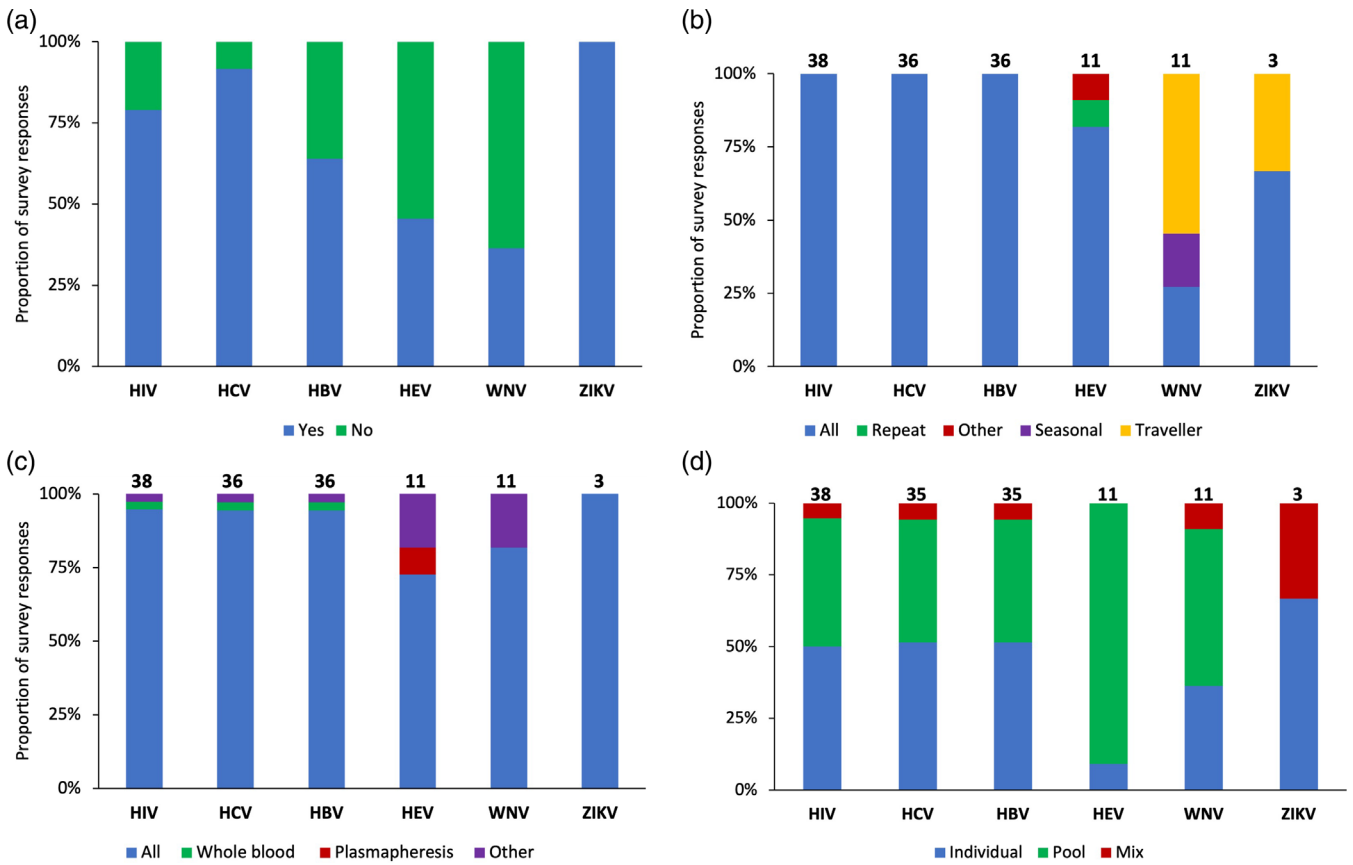

FIGURE 1 Characteristics of blood screening nucleic acid-amplification testing (NAT), 2019, amongst survey responders, organized by (a) whether NAT is government mandated, (b) by donor type, (c) by donation type and (d) by sample type (individual, pooled or a mix of individual and pooled). The number of survey responders performing NAT for each virus with suitable data available for analysis is shown above each bar. Complete data were not available from all respondents. HBV, hepatitis B virus; HCV, hepatitis C virus; HEV, hepatitis E virus; HIV, human immunodeficiency virus; WNV, West Nile virus; ZIKV, Zika virus.

All survey participants performed HIV, HBV and HCV on all donor types (Figure 1b), whereas 82% ($n = 9$) of responders tested all donor types for HEV (in one country where HEV RNA screening is undertaken, it was only performed on donors of plasma intended for fractionation). WNV and ZIKV testing was performed on donors who travelled to endemic regions (55% ($n = 6$) and 33% ($n = 1$), respectively), whereas 18% ($n = 2$) of responders performed WNV NAT only on a seasonal basis.

For all viruses, most responders indicated that all donation types were tested (Figure 1c). One responder indicated that HIV, HCV and HBV NAT were not performed on plateletpheresis or granulocyte donations, which are short shelf-life products. HEV NAT was performed only on whole blood and plasma donations by one responder. For WNV, one responder indicated that all donation types except plasmapheresis donations for fractionation were tested.

For HIV, HCV, HBV and WNV, approximately 50% of responders performed ID-NAT (Figure 1d). For responders in which donations were pooled prior to testing, MP sizes ranged from 4 to 96, with 6 being the most frequently reported pool size for HIV, HBV, HCV and WNV (Table S1). For WNV NAT, responders from one country reported a combination of ID and MP testing, switching between the two testing approaches depending on circumstances (e.g., triggering conversion to individual donation NAT based on detection of WNV RNA by MP-NAT in defined geographical regions in the United States). For HEV NAT, most responders performed MP-NAT with a similar range in pool sizes, but with MPs of 16 samples most frequently reported (Table S1). ZIKV was primarily tested by ID-NAT by responders.

Comparing ID- and MP-NAT usage for HIV within countries between 2008 and 2019, approaches were either maintained (ID-NAT [$n = 5$]; same MP-NAT size [$n = 2$]), changed from MP- to ID-NAT ($n = 7$) or reduced MP size ($n = 7$) (Figure 2). No country moved from ID- to MP-NAT or increased MP size. These observations were consistent for all regions. The median MP size for HIV NAT in 2019 was smaller than in 2008 (6; range: 6–96 versus 16; range: 6–96, respectively; $p = 0.0331$).

We wanted to explore whether using ID- or MP-NAT, as well as MP size, was associated with income category, region, HIV incidence in the general population, HCV viraemic prevalence in the general population, HBV incidence in the general population or the number of donations tested for HIV. No significant correlations were observed between the quantitative variables, hence no multi-collinearity

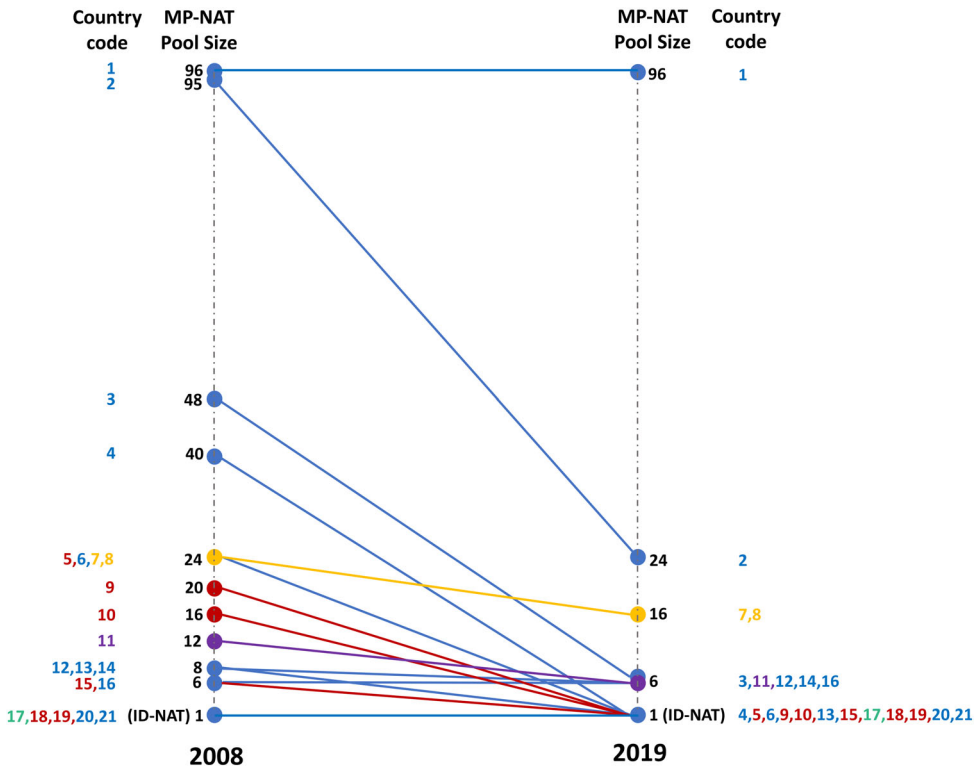

FIGURE 2 Schematic showing changes in the use of individual donation (ID) and mini-pool (MP) blood donation human immunodeficiency virus (HIV) nucleic acid-amplification testing (NAT) in 2008 and 2019. Country data from 2008 [1] were compared with country data from 2019 survey responses ($n = 21$). Where a combination of ID- and MP-NAT or a range of MPs were provided by the same responder, or by the same country, the highest number was used. Data from 2008 were not available for all countries that responded to the 2019 survey. Country codes: 1, Germany; 2, United Kingdom; 3, Netherlands; 4, Switzerland; 5, Republic of Korea; 6, France; 7, Canada; 8, United States; 9, Japan; 10, Australia; 11, Brazil; 12, Belgium; 13, Ireland; 14, Spain; 15, Thailand; 16, Poland; 17, South Africa; 18, New Zealand; 19, Singapore; 20, Denmark; 21, Greece. Colour-coding of countries by region: Africa, green; Asia/Pacific, red; Europe, blue; North America, yellow; South America, purple. Schematic adapted from one made with Flourish (available from https://flourish.studio).

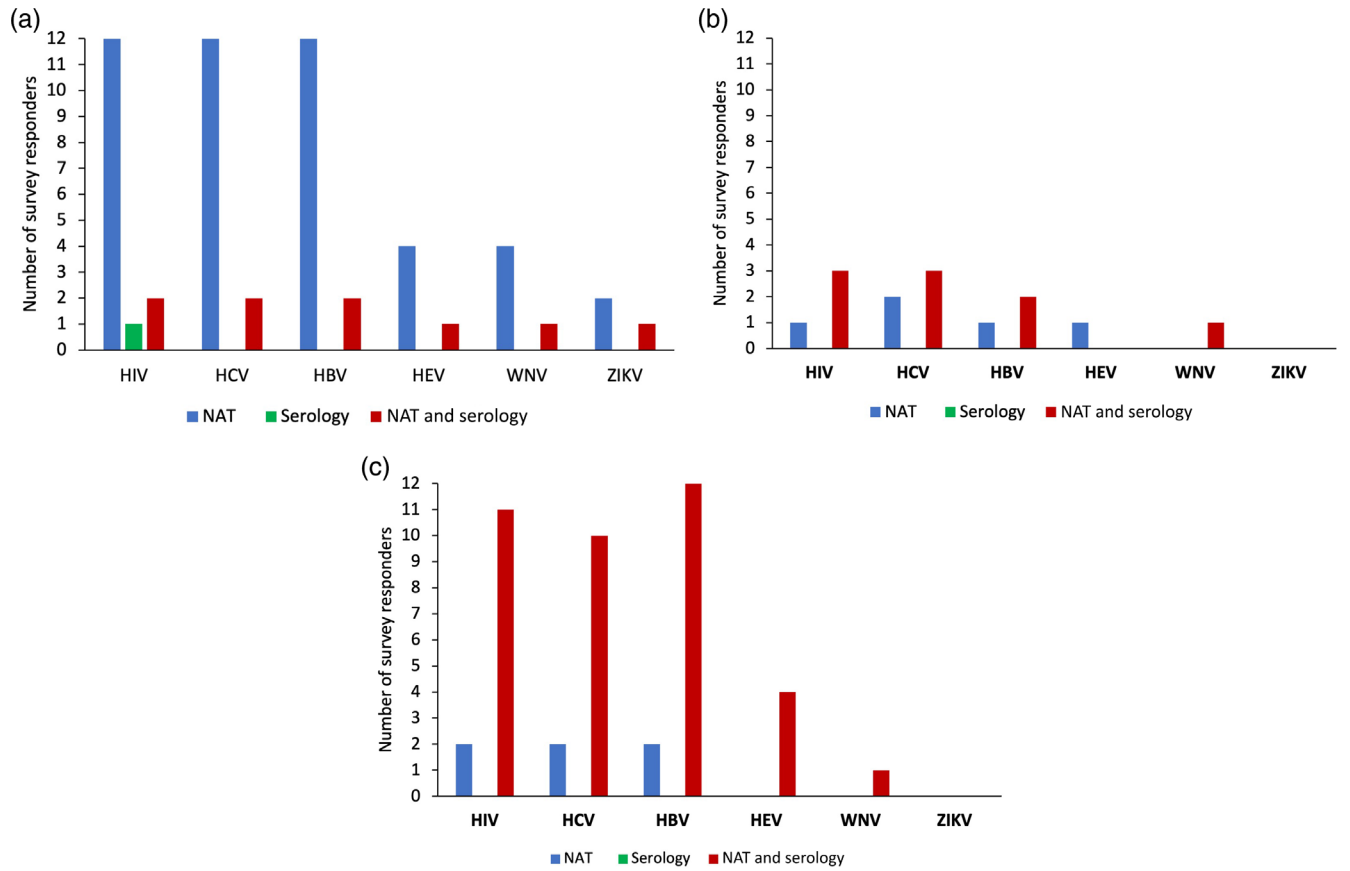

FIGURE 3 Confirmatory testing approaches for nucleic acid-amplification testing (NAT) yield samples, 2019, amongst survey responders, organized by whether testing is performed on a sample from (a) the same donation ($n = 15$), (b) a follow-up donation ($n = 4$) or (c) both the same donation and a follow-up donation ($n = 13$). Complete data were not available from all respondents. HBV, hepatitis B virus; HCV, hepatitis C virus; HEV, hepatitis E virus; HIV, human immunodeficiency virus; WNV, West Nile virus; ZIKV, Zika virus.

observed, and no association was observed between each outcome and the predictors ($p > 0.05$ for all; data not shown).

Confirmatory testing for NAT-yield samples were classified based on the sample type tested and types of assays used as described above. Confirmatory testing was generally performed on a sample from the same donation using NAT (Figure 3a) or on both a sample from the same donation and a follow-up sample using NAT and serology-based assays (Figure 3c). Using NAT and serology-based assays on the same donation or only on a follow-up sample with any assay type were less frequent (Figure 3a,b). Timing of follow-up samples ranged from 72 h following a reactive result up to 6 months (data not shown).

DISCUSSION

We outlined characteristics of blood donation viral NAT and NAT-yield confirmatory testing used by 38 blood operators from 27 countries. HIV, HCV and HBV NAT were primarily undertaken under government mandate and were performed on all donor and donation types. More selective testing was performed for other viruses, such as HEV, in the absence of a government mandate, or WNV and ZIKV, for donors who travelled to countries not endemic for these viruses. The tailored approaches used for HEV, WNV and ZIKV reflect localized factors such as regional epidemiology, viral incidence, lack of screening mandates, resource availability and use of other risk management strategies.

There appears to be a move towards use of smaller MP sizes or ID-NAT since 2008. This may be driven by the introduction of individual triplex HIV/HCV/HBV NAT assays on higher throughput instruments and/or the higher sensitivity of ID-NAT assays compared to their MP counterparts. Our observation is consistent with reports of blood operators transitioning from MP- to ID-NAT, whereby such a transition can result reduction in reported transfusion-transmitted infections as recently reported by Japan for HBV [7]. Moreover, there is interest in moving to ID-NAT for HEV in a combined HIV-1/HIV-2/HCV/HBV/HEV assay that has comparable sensitivities to existing assays [8]. Future studies could investigate the impact of this transition to ID-NAT on the occurrence of transfusion-transmitted infections internationally. Income, region, HIV incidence in the general population, HCV viraemic prevalence in the general population and HBV incidence in the general population or the number of donations tested were not associated with whether a blood operator performs ID- or MP-NAT or with MP size; however, only a small number of

lower-middle-income countries ($n = 3$) reported performing NAT in this survey. Other factors, such as individualized level of risk acceptance, history or additional cost may be responsible for the decision to perform ID- or MP-NAT, and if the latter, pool size.

Confirmatory testing, when done, for NAT-yield samples is complex, with a large amount of highly diverse testing algorithms. Our study suggests that such testing was generally performed by NAT on a sample from the same donation or by NAT and serology on samples from the same donation and also a follow-up sample. When follow-up samples were used for confirmatory testing, the timing of sampling was also varied. Although we have attempted to summarize current confirmatory testing approaches for NAT-yield samples, given the high degree of variability used amongst blood operators, additional studies focusing on the specifics of confirmatory testing algorithms, and how this relates to donor management, are needed.

Our study is not without limitations, including those outlined previously [2]. In addition, where different ID- and MP-NAT sizes were reported by a survey responder or within a country, the larger MP size was used, which may have led to estimates of larger MP sizes thus underestimating the reduction in MP size. Comparisons of ID- and MP-NAT between 2008 and 2019 were made for a whole country rather than for a specific blood operator; in instances where a different blood operator responded to the 2019 survey compared with the 2008 survey, changes may have been incorrectly assigned; however, this may have impacted only a small number of responders. The operating characteristics of the assays used may have differed between survey responders as well as over time; however, investigation of this was not a focus of the present study.

To our knowledge, this is the only comprehensive assessment of NAT characteristics and confirmatory testing approaches used by blood operators in the past 10 years. It is anticipated that the data presented herein will assist blood operators planning to implement viral NAT to augment blood transfusion safety in their local setting.

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H.M.F., C.O., B.C., M.B. and S.L.S. conceived the study and prepared the survey; E.V. performed statistical analyses; H.M.F. prepared the first draft of the manuscript; all authors contributed to study

design, data analysis, data interpretation, manuscript editing and approval of the final manuscript. Open access publishing facilitated by University of the Sunshine Coast, as part of the Wiley - University of the Sunshine Coast agreement via the Council of Australian University Librarians.

CONFLICT OF INTEREST STATEMENT

Helen M. Faddy has received research funding and/or honoraria from Grifols Diagnostic Solutions Inc. and Roche Diagnostic Solutions in the past. Brian Custer and/or the organization he is employed by have received research funding from Grifols Diagnostic Solutions Inc. and Roche Diagnostic Solutions. Susan L. Stramer has received research funding and/or honoraria from Grifols Diagnostic Solutions Inc. and Roche Diagnostic Solutions in the past. Christian Erikstrup has received unrestricted research grants from Abbott Diagnostics and Novo Nordisk, which are both administered by Aarhus University Hospital and Aarhus University, respectively. Christian Erikstrup has not received any personal fees from these or other entities. Silvia Sauleda has received research funding from Grifols Diagnostic Solutions in the past. Pierre Gallian has received honoraria for lectures from Grifols Diagnostic Solutions Inc. and Roche Diagnostic Solutions in the past. Aneta Kopacz has received honoraria for lectures from Grifols Diagnostic Solutions Inc. and Roche Diagnostic Solutions in the past. Magdalena Łętowska has received honoraria for lecture from Grifols Diagnostic Solutions Inc. in the past. The remaining authors have no relevant conflict of interest to declare.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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