

Survey results on nucleic acid tests of infectious diseases: present status and need for rapid and patient near diagnostics

Umfrage zu Nukleinsäureamplifikationstests bei Infektionserkrankungen: Stand und Bedarf für eine schnelle und patientennahe Diagnostik

Abstract

This survey will discuss current and emerging isothermal and rapid polymerase chain reaction (PCR) based nucleic acid amplification methods for patient near diagnostics.

To assess the clinical need of rapid diagnostics for infectious diseases based on nucleic acid tests (NATs) we performed and analysed a questionnaire among laboratories participating in corresponding INSTAND ring trials for external quality assurance. The questions concerning new amplification technologies like isothermal nucleic acid amplification, potentially suited to significantly decrease turnaround times, were complemented by questions to evaluate the present status of NATs. Besides end-users, companies were also addressed by sending out a manufacturer specific questionnaire.

Analysis of the answers from 48 laboratories in 14 European countries revealed that a much shorter turnaround time is requested for selected pathogens compared to about 2 h or longer when applying temperature cycling amplification, i.e. PCR. In this context, most frequently mentioned were MRSA, norovirus, influenza A and B viruses, cytomegalovirus (CMV) as well as hepatitis B virus (HBV) and hepatitis C virus (HCV). At present, 8% of the laboratories having participated in this survey apply isothermal amplification of nucleic acid to identify infectious pathogens.

Keywords: nucleic acid tests, infectious diseases, virus detection, bacteria detection, isothermal nucleic acid amplification, status report, questionnaire, NAT, PCR

Zusammenfassung

Die vorliegende Studie gibt eine Übersicht über derzeit verwendete und in der Entwicklung befindliche Methoden der isothermalen und der schnellen, auf einer Polymerase-Kettenreaktionen (PCR) beruhende Nukleinsäure-Verstärkung für die patientennahe Diagnostik.

Um den klinischen Bedarf einer schnellen Diagnostik auf der Grundlage von Verfahren zur Nukleinsäure-Verstärkung (NATs) abzuschätzen, wurde eine Umfrage bei Laboratorien, die an INSTAND-Ringversuchen zur externen Qualitätssicherung teilnehmen, durchgeführt. Die Umfrage zu neuen DNA-Amplifikationstechniken wie die isothermale Nukleinsäure-Verstärkung wurde ergänzt durch Fragen zum aktuellen Stand von NATs. Neben den Endanwendern wurden Herstellern spezifische Fragebögen zugesandt.

Die Analyse der Antworten von 48 Laboratorien aus 14 europäischen Ländern zeigt, dass für bestimmte Pathogene eine deutlich geringere Durchlaufzeit erforderlich ist als die für eine auf Temperaturzyklen beruhende Nukleinsäure-Verstärkung (d.h. PCR) benötigten 2 h oder noch längeren Zeiten. In diesem Zusammenhang wurden am häufigsten MRSA, Norovirus, Influenza A und B Viren, Cytomegalievirus (CMV) sowie

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Hepatitis B Virus (HBV) und Hepatitis C Virus (HCV) genannt. Derzeit setzen etwa 8% der Laboratorien, die sich an der Abfrage beteiligten, isothermale DNA-Amplifikationstechniken zum Nachweis von Infektionserregern ein.

Schlüsselwörter: Nukleinsäure-Tests, Infektionserkrankungen, Virusnachweis, Bakteriennachweis, isothermale Nukleinsäure-Amplifikation, Statusbericht, Umfrageergebnisse, NAT, PCR

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Introduction

The aim of the study presented in this contribution is to give an overview of the presently applied methodologies for nucleic acid tests (NATs) for infectious diseases in clinical and analytical laboratories and to discuss emerging technologies for NATs potentially suited to support rapid and point-of-care (POC) diagnostics. Near patient instruments will directly influence clinical outcomes for patients and allow to manage patients in one consultation rather than require further follow-ups. This can allow treatment of the patient which should be appropriate and timely. The most relevant POC tests for monitoring infectious diseases involve rapid polymerase chain reaction (PCR) based approaches and novel isothermal amplification methods. The development of these nucleic acid based methods has improved the analytical performance, i.e. sensitivity and specificity, of methods in the area of infectious diseases. These methods have the potential to complement microbiological methods for molecular pathogen detection, which are time consuming and may lack sensitivity.

Important for POC applications are low costs and an acceptable turnaround time [1], [2], [3]. The turnaround time is correlated to the term “rapid diagnostics”, which has been subject to dramatic changes with the availability of PCR and isothermal nucleic amplification technologies. Whereas MRSA detection based on chromogenic agar technology typically requires 2 days to 3 days, application of PCR allows the reduction of the time to obtain the result in a few hours [1], [2]. The transport time from sample collection to the laboratory contributes significantly to turn around times in PCR based examination [2], which could be avoided using POC tests. Using isothermal nucleic amplification techniques, thermocycling necessary for PCR is avoided and the time to result can be reduced even further to less than 60 min or even to 20 min [3]. Isothermal amplification technology has the potential for NATs integrated in disposable microchips [4], [5], [6], [7] and instrumentation for isothermal amplification and detection of the reaction is simpler compared to PCR. Hence, besides rapid diagnostics in industrial countries application of POC nucleic acid detection based on isothermal nucleic amplification is of high interest in devel-

oping countries, where easy handling shall be taken into account.

Isothermal nucleic amplification methods

The literature study revealed that a variety of methods for the detection of nucleic acids are developed, which are not based on thermo-cycling PCR (Table 1). Commercially available are instruments and related kits based on branched chain DNA signal amplification (bDNA) [8], loop-mediated isothermal amplification (LAMP) [9], [10] and recombinase polymerase amplification (RPA) [7].

The bDNA method was first demonstrated to provide reliable results in 1995 [11] by quantitative measurements of HIV-1. Later, the performance of bDNA was evaluated by comparing different NAT methods using the VERSANT HCV RNA Assay and the Siemens System 340 bDNA analyser (Siemens Healthcare Diagnostics) [12], [13]. The LAMP method is used in the real time turbidimeter developed by Eiken Chemical Co., Ltd (Japan) and applied e.g. for the diagnosis of H5N1 avian influenza viruses [14]. Lumora Ltd. (United Kingdom) also implemented the LAMP method in a portable small instrument (PQD) and a high throughput development system. In contrast to the turbidimetric approach, the amplification of the nucleic acid is monitored by fluorescence measurements, called bioluminescent assay in real-time (BART) [15]. The instruments provided by OptiGene also rely on the LAMP methodology and isothermal master mix kits (licensed for LAMP by Eiken Chemical Company) are provided. The recombinase polymerase amplification (RPA) method [7] is used by TwistDx, Ltd (United Kingdom) in their Twista® portable real-time fluorometer and reagent kits are provided for food safety.

The development of improved variants for LAMP and RPA is still in progress. Instead of using displacement primers “Stem primers” are used for LAMP [16] and a chemical heating approach was applied to detect HIV-1 [17]. An advantage of RPA is that no initial heating (e.g. to 95 °C to obtain single stranded DNA) is required and only 37 °C are needed compared to 65 °C for LAMP; this was exploited in a microfluidic lab-on-a-foil device [18]. Further-

Table 1: Non-cycling methodologies for the amplification of nucleic acid

Abbreviation	Method	Reference
bDNA	Branched chain DNA	[8, 11, 24, 25]
HDA	Helicase-dependent amplification	[26, 27]
LAMP	Loop mediated isothermal amplification	[9, 10, 14, 15, 16, 17, 28]
NASBA	Nucleic acid sequence based amplification	[29]
NEAR	Nicking enzyme amplification reaction	[30, 31]
RCA	Rolling circle amplification	[27, 28, 32, 33]
RAM	Ramification amplification	[28, 34, 35]
RPA	Recombinase polymerase amplification	[7, 23, 27]
SDA	Strand displacement amplification	[27, 28, 36]

more, an active field of research of particular interest for rapid and POC diagnostics is the miniaturisation of nucleic acid amplification systems [19] including the adaption of NATs in microfluidic chips [20].

Analysis of questionnaires

Concept of the survey

Two questionnaires (see Attachment 1 and Attachment 2) were created comprising manufacturer specific or end-user specific inquiries. In addition, questions for both, companies and analytical laboratories were included in each of the questionnaires. The end-user specific survey was distributed by INSTAND to 1,000 analytical laboratories in Germany regularly participating in ring trials for external quality assessment addressing detection of infectious pathogens by NATs. In addition, INSTAND contacted 336 laboratories from other European and Non-European countries. These laboratories perform infectious disease diagnostics in the fields of virology, bacteriology, mycobacteriology, parasitology and mycology. Many of these laboratories offer diagnostic services in several of the above fields.

We received replies from 48 laboratories in 14 European countries. All except 2 laboratories offer analytical test in infectious disease medicine in several fields. Three quarters of the laboratories having replied perform more than 1,000 NAT based analyses per month. The numbers of laboratories from the respective countries that participated in the survey are shown in Figure 1. Most participants came from Germany and Scandinavian countries, but we received also answers from Baltic and East-European countries. In total, 36 manufacturers were contacted, of which 12 responded. Only 8 forms returned could be included in the analysis, since 4 manufacturers focus on the development of new instrumentation or kits and are not directly in contact with analytical laboratories. The results of the survey, summarised in Table 2, Table 3, Table 4 and Table 5 are presented in the following

paragraphs. In these tables, the most frequent answers are marked in grey.

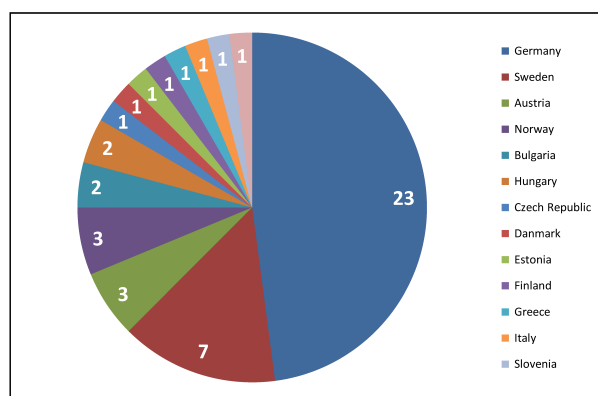


Figure 1: Number of participating analytical laboratories from different countries. In total, 48 questionnaires were included in the analysis.

Reply from manufacturers

The answers for manufacturer specific questions are summarised in Table 2. The majority of manufacturers offer both, instrumentation and specific kits (Table 2, row 1). A quarter of the answers are from companies producing instrumentation only and few firms (12%) focus on the development of kits for nucleic acid amplification. With respect to the number of kits supplied (Table 2, row 2) for the detection of human cytomegalovirus (CMV), human immunodeficiency virus (HIV), hepatitis B and C viruses (HBV/HCV) most of the manufacturers (89%) provide more than 1,000 tests per month and the remaining companies (11%) supply 100 to 1000 tests per month.

Half of the fluidic handling systems are based on conventional technique, whereas 38% of the companies claim that they apply microsystem technology (Table 2, row 3). Interpretation of these answers is difficult since the term "microsystem technology", i.e. application of ultra precision milling or lithography for production of embossing

Table 2: Results of questionnaire for manufacturer specific questions indicating the product spectrum of manufacturers, characteristics of instrumentation and number of kits provided

1	Supply of			
	instrumentation 25%	test kits 12%	instrumentation and test kits 63%	
2	Number of nucleic acid tests provided (for CMV, HIV, HBV, HCV)			
	< 100 / month 0%	100 / month – 1000 / month 11%	> 1000 / month 89%	
3	Use of microsystem technology for liquid handling			
	yes 38%	no 50%		
4	Total volume used in amplification chamber / cuvette			
	< 10 µL 0%	10 µL – 20 µL 38%	20 µL – 50 µL 38%	> 50 µL 13%
5	Time for nucleic acid amplification			
	< 5 min 0%	5 min – 25 min 0%	> 25 min 88%	

tools of injection molds yielding surface roughness in the 10 nm region, was not defined in the questionnaire. More significant is the volume of the cuvette or chamber used for the amplification since less material is required and shorter amplification times are expected for smaller volumes, in particular if PCR is used. For cuvette volumes between 10 µL to 20 µL and 20 µL to 50 µL the same numbers (38%) are given and in few cases (13%) volumes above 50 µL are required (Table 2, row 4). However, in the context of nucleic acid amplification, it follows from the answers concerning the amplification time that no substantial reduction is reached, all responses state times above 25 min (Table 2, row 5). Hence, the contribution of amplification times to the turnaround time cannot be neglected and its reduction is highly desirable.

Current status of NATs derived from manufacturers’ and end-user responses

The market shares of kits and instruments according to the answers of end-users are depicted in Figure 2. According to Figure 2a the amplification is based on in house tests (blue colour, 14 laboratories) and to an equal amount kits provided by Roche (red, 14 nominations). Other suppliers were quoted less frequently. For each vendor, the same colour was chosen in Figure 2a and 2b to visualise the correlation between kits applied for nucleic amplification tests and instrumentation. In Figure 2b, equipment for nucleic acid amplification as well as apparatuses for DNA/RNA extraction and purification is accounted for. It follows from Figure 2b that – besides supply of kits – Roche (red, 24 laboratories) is also the market leader for equipment. The use of “no standard equipment” is accordingly interpreted as “in house tests” and indicated in blue. Other companies providing kits and instrumentation are Cepheid (8/11), Abbott (5/8), Qiagen (4/10), Applied Biosystems (1/4) and Becton Dickinson

(1/4). The other companies referred to as either manufacturer of kits (Altona Diagnostics, Amplex Biosystems, Fisher Scientific) or manufacturer of instruments (BioRad, bioMérieux, Hologic, SensoQuest) are marked in different grey values.

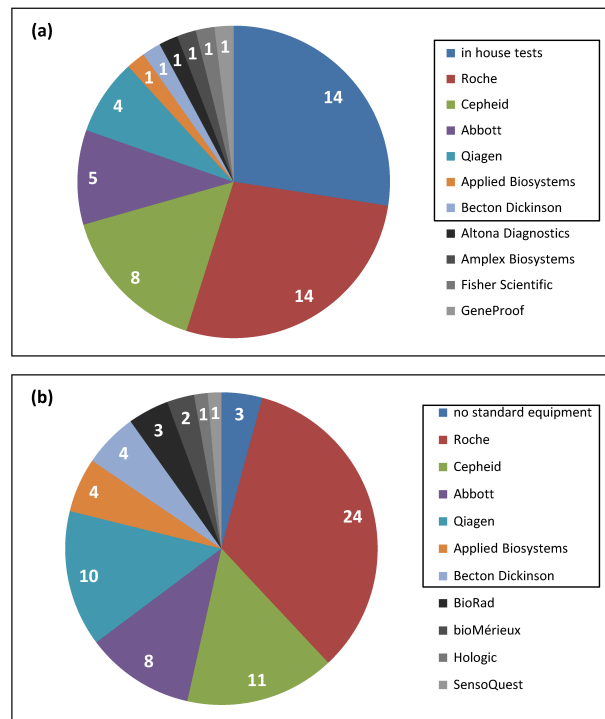


Figure 2: Kits (a) and instrumentation (b) used for nucleic acid amplification tests based on the response of analytical laboratories

In Table 3 and Table 4 the answers of the end-users (Table 3) and the manufacturers’ responses (Table 4) with respect to technologies applied for nucleic acid amplification, pathogens, sample type, workflow, turnaround time, sensitivity and price are listed.

Table 3: Response of end-users giving an overview on the current status of preparation and measurement procedures and wishes regarding sensitivity and costs of NATs

1	Amplification method			
	temperature cycling (PCR) 98%	isothermal amplification 8%	branched DNA amplification 0%	
2	Target bacteria / viruses			
	<i>M. tuberculosis</i> 27%	MRSA 71%	SARS 6%	
	CMV 40%	Adenovirus 35%	Influenza A / B 65%	
	HIV 48%	EBV 40%	HBV / HCV 60%	
3	Other target bacteria / viruses not explicitly specified in the questionnaire			
	<i>Chlamydia trachomatis</i> (CT) 44%	Norovirus 27%	HSV-1 / HSV-2 27%	
	Varicella zoster virus (VZV, HHV-3) 23%	<i>Neisseria gonorrhoeae</i> (NG) 23%	<i>Mycoplasma genitalium</i> 17%	
4	Type of sample			
	Blood / plasma 60%	swab 83%	bioptic tissue 38%	urine 44%
	BAL 25%	faeces 33%	CSF 21%	sputum 13%
5	Mainly used workflow for sample preparation and nucleic acid amplification			
	manual preparation 33%	complete tests, automated preparation and amplification in one / different instruments 63%		complete tests (Cartouche) 21%
6	Turn around time for pathogen detection			
	< 60 min 2%	1 h – 2 h 23%	> 2 h 77%	
7	Requested limit of detection or analytical sensitivity in pure control samples			
	< 10 copies mL ⁻¹ 29%	10 copies mL ⁻¹ – 50 copies mL ⁻¹ 26%	50 copies mL ⁻¹ – 100 copies mL ⁻¹ 31%	100 copies mL ⁻¹ – 500 copies mL ⁻¹ 14%
8	Reasonable costs per test for selected targets			
	< 1€ 17%	1€ – 4€ 49%	4€ – 6€ 34%	> 6€ 0%

PCR is the leading technology applied by almost all end-users (98%) and offered by most companies (75%). Isothermal amplification of nucleic acid is used in 8% of the analytical laboratories and 25% of the companies produce corresponding instruments and/or kits. None of the participants, neither end-users nor manufacturers indicated the application of branched DNA methodology. Microarray hybridisation was also listed (Table 4, row 1), but it is less relevant in the context of analysis of pathogens due to low potential for quantification.

In Figure 3, we summarise responses of analytical laboratories (blue bars) with respect to the target bacteria and viruses, which were explicitly listed in the questionnaire as multiple choice. The red bars in Figure 3 indicate the answers given by the manufacturers concerning the supply of corresponding kits. The end-users (Table 3, row 2) reported that in more than 50% of the laboratories methicillin-resistant *Staphylococcus aureus* (MRSA), in-

fluenza A and B viruses and hepatitis B and C viruses (HBV/HCV) are diagnosed. *M. tuberculosis* (27%), human cytomegalovirus (CMV) (40%), adenovirus (35%), human immunodeficiency virus (HIV) (48%) and Epstein-Barr virus (EBV) (40%) are also frequently analysed. On the other hand, only a few (6%) laboratories provide molecular diagnostics for SARS-associated coronavirus (SARS-CoV) causative for severe acute respiratory syndrome. For all these NATs commercial kits are available and provided by some of the manufacturers. In particular, according to Table 4, row 2, kits for *M. tuberculosis* are offered by 25% of the responding companies, 38% supply kits for CMV, 50% for influenza A and B viruses and 38% for EBV. A smaller fraction of the companies provide kits for MRSA (38%), SARS-CoV (13%), adenovirus (13%), HIV (25%) and HBV/HCV (25%). *M. tuberculosis*, tested in 27% of the analytical laboratories, is reported to reveal a much higher notification rate in WHO Member States in the east

Table 4: Response of manufacturers concerning the current status of preparation, measurement procedures and costs of NATs

Amplification method																			
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than in the west [21]. In addition, the WHO tuberculosis surveillance report reveals a slight increase in the multidrug resistant tuberculosis between 2009 and 2010 as well as a small continued decrease of treatment success rate from 2005 to 2010. The relative use of diagnostic tests by the replying laboratories as well as the supply of commercial diagnostic tests by the responding manufacturers seem to reflect the diagnostics needs of the corresponding methods for public health in the countries from where replies were received (part of northern, eastern and mid Europe). This correlation may be true for MRSA, as well as HBV/HCV and influenza A and B viruses. Interestingly, replying laboratories and manufactures hang on to diagnostic tests for the detection of SARS-CoV, although there have not been any known cases of SARS-CoV infection reported anywhere in the world since 2004 (<http://www.cdc.gov/coronavirus/about/index.html>). Apart from the pathogens explicitly listed in the questionnaire, the participants were also asked to note other important target bacteria and viruses. The most frequent answers are summarised in row 3 of Table 3. Besides *Chlamydia trachomatis*, detected in 44% of the laboratories, norovirus (27%), HSV-1/HSV-2 (27%), varicella

zoster virus (23%), *Neisseria gonorrhoeae* (23%) and *Mycoplasma genitalium* (17%) are analysed.

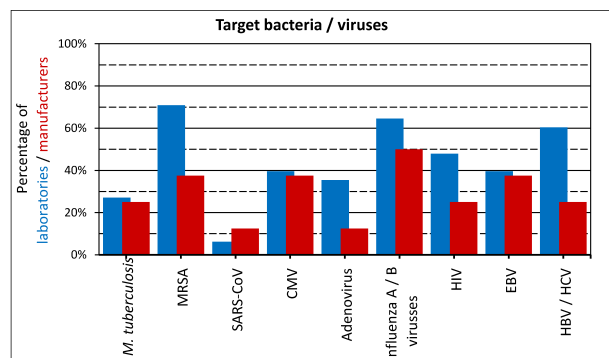


Figure 3: Relative number of laboratories which perform NATs for the listed pathogens (blue bars). Percentage of manufacturers providing corresponding kits (red bars). The data are taken from Tab. 3 and 4. SARS-CoV: severe acute respiratory syndrome associated coronavirus; CMV: human cytomegalovirus; HIV: human immunodeficiency virus; EBV: Epstein-Barr virus; HBV / HCV: hepatitis B and C viruses.

The distribution of samples for NATs is depicted in Figure 4. It follows from the response of the end-users (Figure 4, blue bars; Table 3, row 4) that most often swab (83%) and blood/blood plasma (60%) is used. Besides these samples, urine (44%), tissue from biopsies (38%), faeces (33%), bronchoalveolar lavage (BAL) (25%), cerebrospinal fluid (CSF) (21%) and sputum (13%) serve for nucleic acid tests. The percentage of manufactures providing kits for these materials (Table 4, row 3) is shown as red bars in Figure 4. Most nominations concern faeces (50%), swab (46%) and blood/blood plasma (36%), followed by biopptic tissue (22%). These samples are among the five materials most frequently reported by the analytical laboratories.

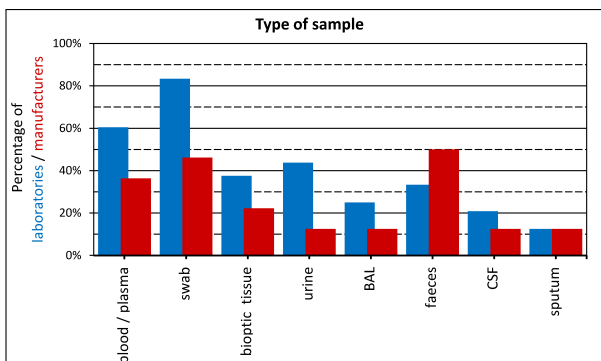


Figure 4: Percentage of laboratories (blue bars) using the listed specimen and relative number of manufactures (red bars) providing corresponding test kits. BAL: bronchoalveolar lavage; CSF: cerebrospinal fluid.

In the questionnaire, we offered three options for the workflow of nucleic acid tests. The manual preparation requires DNA/RNA extraction, purification and pipetting of reagents, complete tests include automated preparation and amplification in one or different instruments and the third selection is for complete, cartouche or cassette based test which contain all reagents needed and only the sample has to be fed in. As presented in Figure 5 and Table 3, row 5, in analytical laboratories, manual preparation is done in 33% of cases. At present, tests which include automated preparation (63%) of samples are being preferred. Fully automated tests, based on cartouche or cassette systems are applied in 21% of the laboratories. The sum exceeds 100% since in some laboratories various modalities are being used. The most common indication of manufactures (Table 4, row 4) concerns the manual preparation (63%), automated preparation is reported with 25% and cartouche based workflows in 13% of cases. Compared to the end-users, the order is reverse with respect to manual and automated preparation, possibly because most of the laboratories have a high throughput which requires the highest possible degree of automation. On the other hand, both groups are ranking complete, cartouche based tests on the third rank, the relative market share is estimated to be in the range of 10% to 20%.

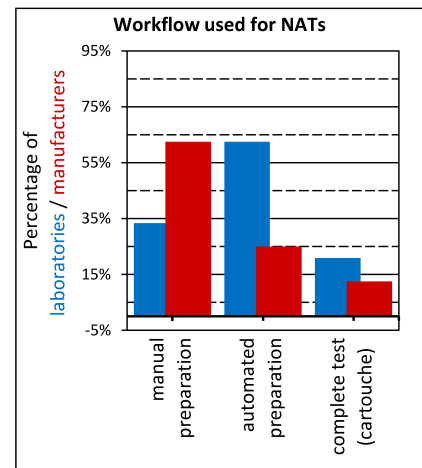


Figure 5: Comparison of different workflows applied for NATs based on responses of analytical laboratories (blue bars) and manufactures (red bars)

The turnaround time for pathogen detection using NATs is generally above 2 h (77%) according to the analytical laboratories (Table 3, row 6), 23% and 2% claimed turnaround times between 1 h to 2 h or even below 60 min, respectively. The analytical sensitivity or limit of detection in pure samples or calibrators requested by most of the end-users for reliable pathogen detection is about the same for <10 copies mL^{-1} (29%), 10 copies mL^{-1} to 50 copies mL^{-1} (26%) and 50 copies mL^{-1} to 100 copies mL^{-1} (31%) (Table 3, row 7). The broad range given by the end-users are probably due to the fact that pathogen and sample specific sensitivity and limit of detections are required for reliable diagnostics. The manufactures' responses to these questions are in close concordance with the end-users requirements and show a trend to lower turnaround times (38% quote the range 1 h to 2 h) (Table 4, row 5) and to higher sensitivity (50% state a sensitivity of 10 copies mL^{-1} to 50 copies mL^{-1}) (Table 4, row 6). However, the costs per tests requested by the analytical laboratories are lower compared to the manufactures' announcements. The range up to 4 € is mentioned by 66% of the end-users (Table 3, row 8) and 42% of the companies (Table 4, row 7). The upper limit stated by the laboratories is 6 €, but the manufactures indicate that the costs for several kits (57%) exceed 8 €. Again, the broad spread in acceptable costs and cost-covering prices indicate that for different pathogens and samples the complexity of test kits and the corresponding developments are crucial.

Need for rapid NATs in clinical diagnostics

An overview on the response to the questions specific for analytical laboratories is given in Table 5. As already mentioned when discussing the high percentage of laboratories using automated preparation and cartouche based tests (see Table 3, row 5: sum 84%), most of the participating analytical laboratories (75%) investigate more than 1000 samples with respect to infectious

Table 5: Result of questionnaire for end-user specific questions giving the number of NATs per month and indicating the need for rapid NATs for infectious diseases

1	Number of all analyses (including non-NATs) for diagnostics of infectious diseases			
	< 100 / month 4%	100 / month – 1000 / month 21%	> 1000 / month 75%	
2	Relative share of NATs with respect to all analyses			
	< 25% 54%	25% – 50% 8%	50% – 75% 10%	> 75% 23%
3	Use of rapid PCR / NAT			
	yes 38%	no 60%		
4	For which target Target bacteria / viruses is an urgent need for rapid PCR / NATs ?			
	<i>M. tuberculosis</i> 2%	MRSA 54%	SARS 0%	
	CMV 10%	Adenovirus 2%	Influenza A / B 21%	
	HIV 2%	EBV 2%	HBV / HCV 13%	Norovirus 27%
5	Requested turn around time for selected pathogens			
	< 10 min 16%	< 15 min 72%	< 60 min 12%	
6	Estimated number of tests applying rapid NATs			
	<i>M. tuberculosis</i> > 50 tests / month	MRSA > 50 tests / month	SARS n.a.	
	CMV 10 – 50 tests / month	Adenovirus > 50 tests / month	Influenza A / B > 50 tests / month	
	HIV 10 – 50 tests / month	EBV < 10 tests / month	HBV / HCV 10 – 50 tests / month	Norovirus > 50 tests / month
7	Are quantitative rapid NATs required ?			
	qualitative tests sufficient 31%	quantitative tests required 2%	dependent on pathogen 44%	
8	Do you intend to purchase equipment for rapid NATs?			
	no 50%	next year 15%	next three years 8%	later 19%

pathogens (Table 5, row 1). For high throughput applications automation is necessary to provide analyses in short times at reasonable costs. About 21% of the users perform 100 tests per month to 1000 tests per month. The number of analyses is less than 100 tests per month in 4% of the analytical laboratories. The relative share of NATs at all analyses covers the complete range from <25% to >75% with the most common indication for <25%, given by 54% of the laboratories (Table 4, row 2). The distribution reveals a second (relative) maximum, 23% of the laboratories perform in more than 75% of the analyses nucleic acid tests. This survey result demonstrates the increasing importance of nucleic acid tests, used in more than half of the laboratories (54%) in addition to other diagnostic methods and applied as leading procedure in specialised facilities (23%).

About 40% (Table 5, row 3) of the end-users report the application of “rapid” PCR/NATs when answering the corresponding question of the survey sheet. However,

this statement is not supported by the answers concerning the turnaround times, since only 2% reach times below 60 min (Table 3, row 6). These 2% of the laboratories seem to better represent the current situation than the 38% given in Table 5, row 3 with respect to “rapid” rapid diagnostic. We interpret these discrepancies as non-conform definitions and suggest the use of the term “rapid” PCR/NAT as indicative for turnaround times less than 30 min to better account for the present state of the art of NATs.

The bacteria and viruses for which the end-users declare an urgent need of rapid nucleic acid tests are shown in Figure 6 (Table 5, row 4). The percentage of laboratories requesting a rapid test is plotted on a logarithmic scale to account for the large variations. Apart from SARS, for all pathogens explicitly listed in the questionnaire there seems to be an interest for significantly shorter turnaround times, specified to be <15 min by the majority of users (Table 5, row 5). However, the number of laborator-

ies requesting such rapid NATs ranges from only 2% for *M. tuberculosis*, adenovirus, HIV and EBV to 54% for MRSA. Besides MRSA, a large proportion of the answers indicate the demand for rapid tests with respect to norovirus (27%) and influenza A and B viruses (21%). About 10% identify the need for rapid CMV and HBV/HCV nucleic acid tests. In Figure 6, we include the expected number of rapid tests (Table 5, row 6) per analytical laboratory – based on the most frequent statements – as colour code, violet represents <10 tests per month, light-brown indicates 10 tests per month to 50 tests per month and green stands for >50 tests per month. Alternatively, instead of using the most frequent answers for each bacterium or virus, we summarized the expected numbers of tests stated by each laboratories for all pathogens which are analysed. It follows from Figure 7 that most of the end-users, who intend to apply rapid NATs expect more than 50 tests per month. Please note that the total number of nominations (60) exceeds the number of participating laboratories (48), since generally more pathogens are analysed in each analytical laboratory. Compared to the typical throughput of >1,000 analyses per month the relative contribution of rapid test would be in the order of 5%.

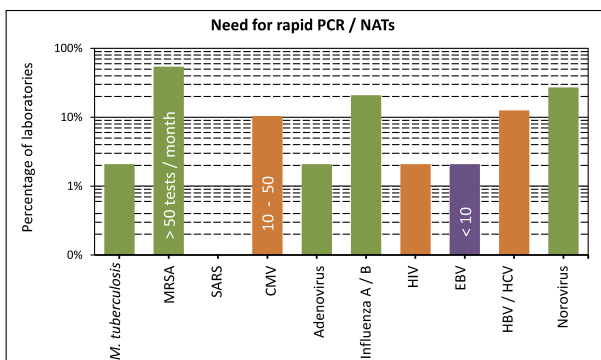


Figure 6: Percentage of laboratories requesting rapid nucleic acid tests for the listed pathogens. The colour indicates the expected number of tests per month in one analytical laboratory for the respective bacterium or virus.

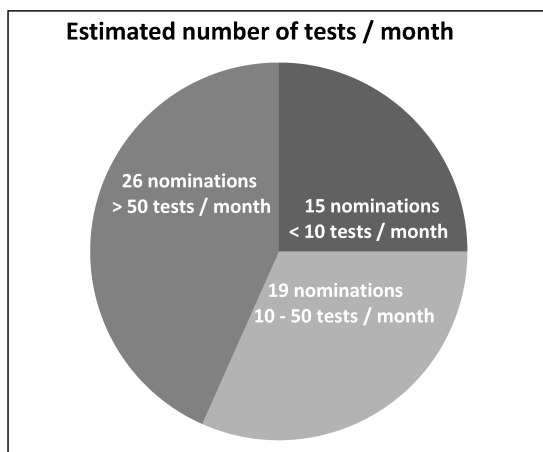


Figure 7: Number of predicted rapid nucleic acid tests per month in each laboratory for specific target pathogens

The question whether qualitative tests or quantitative tests are needed yielded different opinions. According to Table 5, row 7, most of the users (44%) prefer to choose between a qualitative or quantitative tests dependent on the pathogen to be detected, 31% would be satisfied with a qualitative result.

The answers concerning the purchase of equipment (Table 5, row 8) and the establishment of rapid nucleic acid analyses in analytical laboratories (50% do not intend to introduce rapid tests and 19% responded with “later than the next three years”) reflect that the end-users are currently waiting, possibly because the technologies for rapid NATs are still being developed and improved by corresponding research activities.

Summary and conclusion

The results of this survey refer to the answers of 48 analytical laboratories, most located in Germany. About a quarter of the responses were from Scandinavian countries. With respect to the current state of the art of NATs reasonable consensus was observed except for the costs between end-users and manufacturers, albeit only 8 companies responded.

The pathogens most frequently analysed in more than half of the laboratories are MRSA, influenza A and B viruses and HBV/HCV, followed by HIV, EBV, CMV and adenovirus detected in about 40% of the laboratories. *M. tuberculosis* was mentioned as target by 27% of the end-users. Apart from these pathogens, which were explicitly listed in the questionnaire as multiple choice option, *Chlamydia trachomatis*, norovirus and *Neisseria gonorrhoeae* are also frequently (in 20% to 40% of the laboratories) analysed.

The majority of the participating analytical laboratories perform more than 1000 analyses per month to detect infectious diseases. Hence, a high degree of automation is required to achieve high sample throughput applying complete conventional or cartridge based tests. The relative share of in-house tests for nucleic acid extraction/purification and amplification is about 27% while 73% utilize kits provided by various manufacturers. In this context, internal and external quality assurance is highly relevant to ensure that results from different laboratories are in agreement within defined limits of equivalence, regulated for selected pathogens in the corresponding guidelines [22] of the German medical association.

The questionnaire revealed a non-consistent use of the term “rapid” analysis, due to the dramatic reduction of turnaround times from several days required by cultures to typically 2 h - 4h after the introduction of PCR. It follows from the questionnaire that the current state of the art for the turnaround time of PCR based nucleic acid tests is still >2 h. The next step towards rapid point of care tests is expected by the implementation of complete tests involving isothermal methodology for NATs. Such a setting might allow turnaround times below 30 min and possibly

between 10 min – 15 min. Hence we suggest the definition of a rapid NAT as analysis with turnaround times <30 min. The most promising technology to produce integrated microfluidic chips as complete test for a rapid NAT for a specific pathogen is possibly RPA [7], [23], because the amplification can be carried out at about 37 °C.

The majority of the laboratories (75%) points out the need for rapid nucleic tests for certain pathogens. Of particular interest is MRSA for more than 50% of the end-users, 27% and 21% demand for rapid NATs to detect Norovirus and influenza A/ B, respectively (Table 5, row 4). According to about 10% of the laboratories, further pathogens for which rapid tests are needed are HBV HCV and CMV. The desired turnaround time is <15 min (Table 5, row 5) for rapid nucleic acid tests of these disease-causing agents. Slightly more laboratories (44% compared to 31%, Table 4, row 7) indicated that – dependent on the pathogen – quantitative test would be preferable to qualitative analyses. The results of the questionnaire allow the estimation that the relative share of rapid NATs is expected to be around 5% compared to the total number of analysis per month. It should be noted that the bacteria and viruses for which rapid tests are requested are being frequently examined at present in the participating laboratories utilising PCR.

Besides conventional nucleic amplification technique by PCR, only few laboratories (8%) apply isothermal amplification. This demonstrates that research and development is still necessary to overcome the drawbacks of isothermal methodologies for nucleic acid amplification. In particular, the possibility to obtain quantitative results would certainly accelerate the application of isothermal tests in routine laboratories and for point of care applications. In addition, integration of isothermal tests in disposable cartridges is essential to improve handling, reproducibility and to reduce the turnaround time to the requested range of 15 min. Because of the ongoing development most of the end-users (85%) are still waiting before purchasing corresponding equipment to utilise rapid (isothermal) nucleic analysis. However, we conclude from the questionnaire that for certain applications rapid NATs are needed to improve the measurement support for diagnostic and therapeutic decisions.

Notes

Competing interests

The authors declare that they have no competing interests.

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