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Comparative analytical evaluation of the respiratory TaqMan Array Card with real-time PCR and commercial multi-pathogen assays☆

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A B S T R A C T

In this study, a multicenter evaluation of the Life Technologies TaqMan® Array Card (TAC) with 21 custom viral and bacterial respiratory assays was performed on the Applied Biosystems ViiA™ 7 Real-Time PCR System. The goal of the study was to demonstrate the analytical performance of this platform when compared to identical individual pathogen specific laboratory developed tests (LDTs) designed at the Centers for Disease Control and Prevention (CDC), equivalent LDTs provided by state public health laboratories, or to three different commercial multi-respiratory panels. CDC and Association of Public Health Laboratories (APHL) LDTs had similar analytical sensitivities for viral pathogens, while several of the bacterial pathogen APHL LDTs demonstrated sensitivities one log higher than the corresponding CDC LDT. When compared to CDC LDTs, TAC assays were generally one to two logs less sensitive depending on the site performing the analysis. Finally, TAC assays were generally more sensitive than their counterparts in three different commercial multi-respiratory panels. TAC technology allows users to spot customized assays and design TAC layout, simplify assay setup, conserve specimen, dramatically reduce contamination potential, and as demonstrated in this study, analyze multiple samples in parallel with good reproducibility between instruments and operators.

1. Introduction

Molecular methods, including real-time PCR, have become the preferred approaches for the prompt testing of respiratory tract specimens for the presence of viral and bacterial pathogens. Conventional techniques are frequently labor intensive, time consuming, less sensitive, and often require specialized expertise and/or facilities but remain valuable in cases where culture-independent tests have reduced sensitivity. Examples include the

☆ The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

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emergence of a new type or subtype that evades molecular detection or the cumulative effect of genetic drift that reduces detection sensitivity. Culture methods are also useful in clinical microbiology, such as for antimicrobial susceptibility testing. Rapid reporting of real-time test results gives health professionals greater flexibility in patient management and provides epidemiologists time to recognize and respond to an outbreak. In addition, test results may allow for appropriate antimicrobial treatment that helps prevent the spread of secondary infection and development of multiple drug resistant species. Real-time PCR has demonstrated enhanced sensitivity, specificity, and utility when compared to traditional methods such as culture/DFA (Mahony, 2008), including the ability to simultaneously detect multiple pathogens (Mengelle et al., 2014; Babady, 2013; Pillet et al., 2013; Choudhary et al., 2013; Weinberg et al., 2013; Kodani et al., 2011), quantify pathogen load, and potentially identify new pathogens. While multiplex PCR is attractive in terms of throughput and conservation of reagents and specimen, it does require extensive optimization and is not easily amenable to changes in the components without an entire re-validation of the performance of the assays (Henegariu et al., 1997; Markoulatos et al., 2002).

Several commercial platforms and assays exist for the simultaneous detection of multiple respiratory pathogens in a specimen, including the Luminex xTAG Respiratory Viral Panels (Luminex Corporation, Austin, Texas), Qiagen ResPlex v2.0 (Qiagen, Hilden, Germany), and TessArae RPM 3.1 Flu (TessArae LLC, Potomac Falls, Virginia), along with other systems (Biofire Diagnostics, 2015; Hologic, 2015; GenMark diagnostics, 2015; PathoFinder, 2015; Fast-Track Diagnostics, 2015; Nanosphere, 2015). These platforms differ in a variety of features including the type and number of analyses that can be performed (viral, bacterial, mixed, influenza specific), detection technology and need for dedicated instrumentation, complexity, sensitivity and throughput, and cost per specimen. Comparative evaluations of several of these panels show that they all have good specificity and varying levels of sensitivity across pathogens (Sakthivel et al., 2012; Renaud et al., 2012; Popowitch et al., 2013; Babady et al., 2012; Gadsby et al., 2010; Rand et al., 2011; Driscoll et al., 2014). One common theme among all the commercial platforms is that the end user does not have control over the menu of assays included in the panel or the ability to customize individual assays without incurring significant developmental costs. At the time of preparation of this manuscript the Qiagen ResPlex v2.0 panel and TessArae were no longer available.

As an alternative, the TaqMan® Array Card (TAC; Life Technologies, Carlsbad, California) is a low density microfluidic card arranged in an 8 specimen × 48 assay array for a total of 384 individual real-time PCR reactions. The studies presented in this manuscript were carried out using a TAC partitioned into an 8 × 24 array where each sample was unique and assays were performed in duplicate as shown (Fig. 1). TACs and associated positive control materials (Kodani and Winchell, 2012) have been designed for the detection of respiratory pathogens (Kodani et al., 2011; Waller et al., 2014; Steensels et al., 2015), hepatitis genomes (Kodani et al., 2014), enteropathogens (Liu et al., 2014, 2013; Platts-Mills et al., 2014), and bioterrorism agents (Weller et al., 2012; Rachwal et al., 2012). Assays are independent of each other, so that modification of one primer/probe set will not require revalidation of the entire TAC. Thus, in the event that an assay is updated, new TACs would only have to be manufactured that incorporate the design change. The design of the TAC also helps to minimize specimen handling and requires less input template nucleic acid than an equivalent number of individual PCR reactions.

In this study we compared the analytical sensitivity and reproducibility of TACs spotted with oligonucleotides for the detection of 21 individual respiratory pathogens against individual laboratory developed tests (LDTs) and three different multi-pathogen commercial assays. Six different state public health laboratories (PHLs) participated in the study, including the Wadsworth Center at the New York State Department of Health, (NY), Washington State Public Health Laboratories (WA), Massachusetts Department of Public Health (MA), Wisconsin State Laboratory of Hygiene (WI), Iowa State Hygienic Laboratory (IA), and the Hawaii State Department of Health (HI).

Table 1
Viral and bacterial reference standards.

Assay Name	Abbreviation	Strain/Type/Subtype/ Lineage
Adenovirus	ADEV	1
Influenza A virus	FLUA	A/California/07/2009 (H1N1)pdm09
Influenza B virus	FLUB	B/Nevada/03/2011 Victoria Lineage
Human coronavirus 229E	HCV229E	229E
Human coronavirus NL63	HCVNL63	NL63
Human coronavirus OC43	HCVOC43	OC43
Human metapneumovirus-A1[a]	HMPV-A1	A1
Human metapneumovirus-B2[a]	HMPV-B2	B2
Parainfluenza virus	PIV1	1
Parainfluenza virus	PIV2	2
Parainfluenza virus	PIV3	3
Parainfluenza virus	PIV4	4
Respiratory syncytial virus	RESV	A
Respiratory enterovirus	REV	Coxsackie A9
Rhinovirus	RHIV	5
B. pertussis	BOP	1
C. pneumoniae	CHPN	NA
S. pyogenes	GAST	NA
H. influenzae	HIAT	NA
L. pneumophila	LSPP	Sg1
M. pneumoniae	MYPN	NA
S. pneumoniae	STPN	NA

NA – not applicable.
[a] The human metapneumovirus TAC assay detects subgroups A1 and B2.

2. Materials and methods

2.1. Reference standards

All viral and bacterial reference standards used in this study, with the exception of influenza A, influenza B, *Streptococcus pneumoniae*, and *Streptococcus pyogenes*, were obtained from Quality Control for Molecular Diagnostics (QCMD; Glasgow, Scotland). Influenza A and B were obtained from the Influenza Reagent Resource (IRR; Manassas, VA). *S. pneumoniae*, and *S. pyogenes* were obtained from Bacteriology Laboratory, Wadsworth Center. Assay names and abbreviations for the reference standards are shown in Table 1.

2.2. Assay design and performance assessment

Laboratory developed tests (LDTs) for the detection of viral and bacterial respiratory pathogens were designed in Clinical Laboratory Improvement Amendments certified laboratories at the Centers for Disease Control and Prevention (CDC, Atlanta, Georgia) or at two different state public health laboratories (PHLs). These individual real-time PCR assays served as reference standards against which TAC performance could be compared. Pathogen specific assays on the TACs were the same CDC or PHL LDTs adapted for use with TAC. Commercial assays included the xTAG® Respiratory Viral Panel Fast, ResPlex v2.0, and TessArray® RPM 3.1-Flu. Extraction protocols used in this study were validated in multiple PHLs and determined to have comparable extraction efficiencies for different pathogens in various matrices. Sensitivity was assessed using quantified reference viral and bacterial standards to determine the

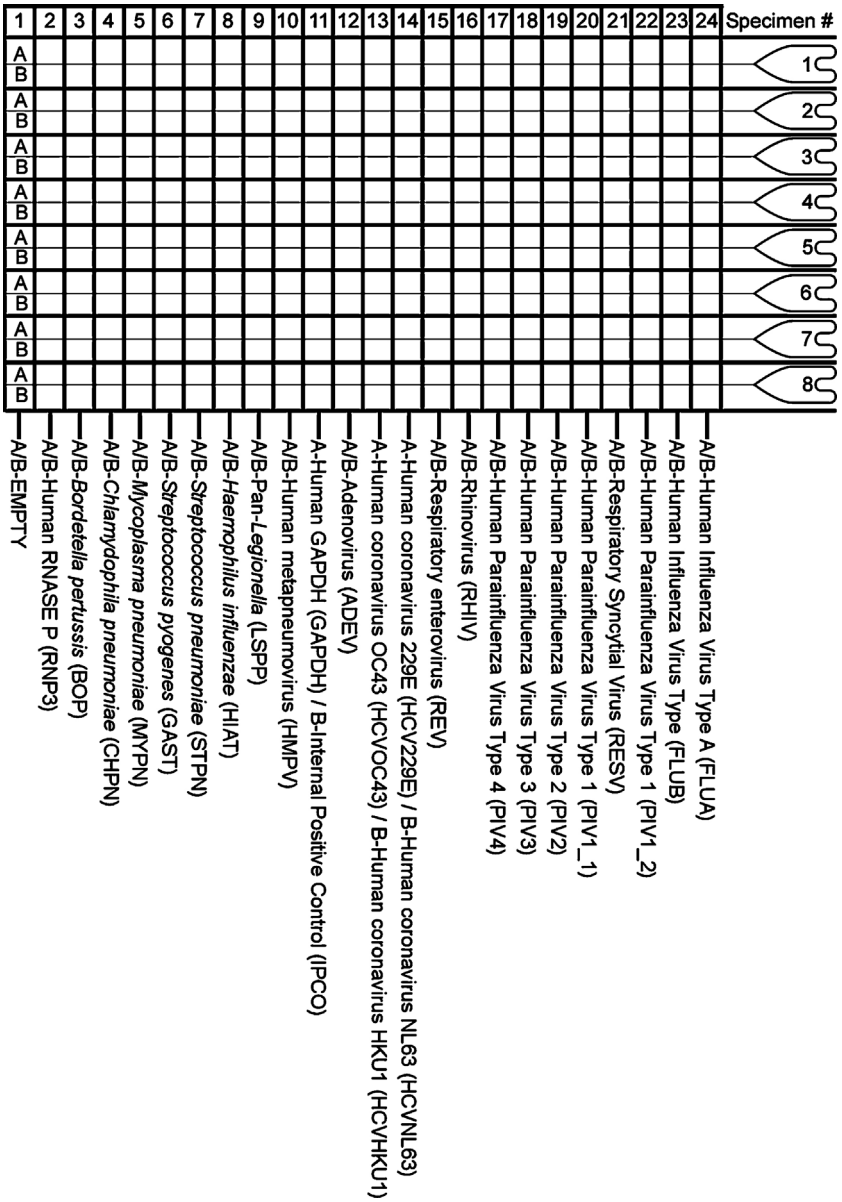

Fig. 1. TAC layout for CDC respiratory assays including 16 viral and 7 bacterial targets. At maximum density the cards are arranged in an 8 specimen × 48 assay array for a maximum total of 384 individual real-time PCR reactions. TACs can also be manufactured in other formats. In this study the cards were partitioned into 8 specimen × 24 assay sub-arrays where each assay was performed in duplicate in adjacent wells (squares A and B). Each column represents a single assay (abbreviation shown in parenthesis), with the exception of the internal positive control (IPCO), manufacturing control (GAPDH), and human coronavirus assays which were spotted in single wells within a column (columns 11, 13, and 14). Each card also includes an RNA control (RNP3). Sample port one was reserved for use as a negative control, permitting seven samples to be analyzed on each TAC.

Limit of Detection (LoD) for each assay according to the published user instructions or package insert.

2.3. Commercial panels

The quantitative performance of individual assays that are part of the Qiagen ResPlex II Panel v2.0, Luminex xTAG Respiratory Viral Panel Fast, and TessArray RPM 3.1 Flu panel was established in two steps. First, a range finding experiment was performed to identify the endpoint at which no detection was evident. Next, the LoD was determined by testing replicate samples at several different dilutions. Reference standards were serially diluted in viral transport media and stored at −20 °C prior to extraction. Instrumentation and reaction conditions are summarized in Table S1. Duplicate samples were extracted and purified nucleic acid was pooled and stored at −20 °C. Analysis of Qiagen ResPlex and Luminex xTAG data was performed using mean fluorescence intensity unit (MFI) cutoff values listed in Table S2. The LoD was then determined by analysis of 15 replicate samples at three different dilutions; one log above the endpoint, the endpoint, and one log below the endpoint whereby ≥90% (14 of 15) of the replicates tested positive for detection. Analysis of TessArray data was performed using C3 scores and/or *E*-values listed in Table S2. Dilutions of each reference standard were initially tested on five individual arrays and the LoD was subsequently determined by analysis of 10 replicate samples (20 arrays) at two different dilutions; the endpoint and one log below the endpoint whereby ≥80% (8 of 10) of the replicates tested positive for detection.

2.4. Pathogen specific CDC laboratory developed tests (CDC LDTs)

The quantitative performance of CDC LDTs was established in a manner similar to the commercial panels described above. A range finding experiment was first performed to bracket the detection endpoint and the LoD was subsequently determined by analysis of 20 replicate samples at three different dilutions. The LoD was calculated to indicate the dilution whereby ≥95% (19 of 20) of the replicates tested positive for detection. Instrumentation and reaction conditions are summarized in Table S1. Primer and probe sequences, along with final oligonucleotide concentrations are given in Table S3. All assays were performed using 5 μL of diluted extracted nucleic acid in a 25 μL reaction.

2.5. Public health laboratory developed tests (PHL LDTs)

Laboratory developed tests for the detection of individual respiratory pathogens were independently designed at NY and IA. The quantitative performance of these assays was established in the same manner as for the CDC LDTs. Instrumentation and reaction conditions are summarized in Tables S4 and S5. Primer and probe sequences, along with final oligonucleotide concentrations are given in Table S6. All assays were performed using 5 μL of diluted extracted nucleic acid.

2.6. TaqMan array card (TAC) assays

A custom configured TAC (Kodani et al., 2011; Diaz et al., 2013) was designed to detect 16 viral and 7 bacterial targets in two adjacent wells for the same sample (squares A and B in Fig. 1). During TAC manufacturing, target specific primer:probe solution is lyophilized in each well. Extracted nucleic acid (46 μL) was combined with AgPath-ID One-Step RT-PCR Reagent (ThermoFisher Scientific, Waltham, MA, 54 μL) and the reaction mixture was loaded into one of the sample ports. After all ports were loaded the reaction mixtures were distributed throughout the TAC by low speed centrifugation. This also rehydrates the lyophilized oligonucleotides. The assay for parainfluenza virus 1, PIV1_1, on the TAC was updated to PIV1_2 for better performance at the 60 °C annealing/extension temperature used in this study (see Table S3), therefore performance data are presented for the PIV1_2 assay only. Each TAC also included an assay for human coronavirus HKU1; however, the assay was not characterized due to the lack of an appropriate reference standard. In most cases each column represents a single assay spotted in duplicate wells as shown in Fig. 1; however the IPCO, GAPDH, and human coronavirus assays were spotted in single wells within a column (columns 11, 13, and 14). Duplicate testing reduces the probability of false negatives due to sampling variation near the LoD and can compensate for a single defect in the microfluidics channel on the TAC. Sample port one was reserved for use as a negative control, permitting seven samples to be analyzed on each TAC. Since the identity of each reference standard used in the studies was known a combined positive control was not included to verify individual assay performance.

The quantitative performance for individual pathogen specific viral and bacterial assays on the TAC was established in the same manner as for the CDC LDTs except that the LoD was calculated to indicate the dilution whereby ≥94.4% (17 of 18) of the replicates tested positive for detection. Samples were prepared by serial dilution into universal transport media (Copan Diagnostics, Murrieta, CA) and stored at −20 °C prior to extraction. Each participating state PHL used their preferred extraction method, either the Roche MagNA Pure LC System with the Total Nucleic Acid Isolation Kit I. (HI, MA, WA, and WI) or the bioMerieux NucliSENS EasyMag (IA and NY). Iowa used an input/elution volume of 140 μL/100 μL, while all other sites used 100 μL/100 μL. Primer and probe sequences, along with final oligonucleotide concentrations are given in Table S3. TACs were run on the ABI ViiA 7 real-time PCR instrument configured with a TAC heating block using the following cycling parameters: 45 °C for 10 min, 94 °C for 10 min, followed by 45 cycles of 94 °C for 30 s and 60 °C for 1 min.

Real-time fluorescence intensity data were examined manually for each TAC to confirm positive amplification of the internal positive controls and negative amplification for all no template controls (NTC). Provenance for the reference viral and bacterial standards was not supplied by the manufacturer, therefore the absence or presence of amplification for RNase P was examined but the results were not used to qualify the analysis. Assay integrity was confirmed by examining each TAC for cross-amplification. TACs were also monitored for the presence of anomalous baseline fluorescence and to note the reproducibility of amplification in adjacent wells (Fig. 1, squares A and B). Atypical results may be caused by defects in spotting of the oligonucleotides for different assays or in the microfluidics channels that distribute specimen throughout the TAC during centrifugation.

3. Results

3.1. Performance comparison of CDC and PHL LDTs with three different commercial multi-pathogen respiratory panels

The LoD for CDC LDTs was evaluated at two different state laboratories and on two different real-time PCR instruments, the ABI 7500 Fast Dx and ViiA7, with a 96 well heating block. Results are presented in Table 2 as the highest dilution (lowest concentration) of extracted reference standard that met acceptance criteria for the experiment. Calculated LoD between the two instruments was identical (columns A–D) for each site, with the exception of the rhinovirus and adenovirus LDTs on the ViiA7 (column B) that did not meet the 95% acceptance criteria. The 12 CDC LDTs also demonstrated good reproducibility (±1 log) on the ViiA7 across six state PHLs as shown in columns B, D, E, F, G, and H, apart from the human parainfluenza virus 2 assay which also did not meet the necessary criteria at the WI site. As compared to LDTs developed by the New York State Department of Health (column I), the CDC LDTs again demonstrated similar sensitivities except for the CDC human parainfluenza virus 1 LDT whose LoD was 2–3 logs higher. Sensitivity of the TessArray, Qiagen, and Luminex panels was generally 1–3 logs lower than for the CDC and PHL LDTs, except for the Qiagen RVPIIv2 respiratory enterovirus assay which was one log more sensitive.

3.2. Performance comparison of CDC and PHL bacterial LDTs

LoD and reproducibility were also evaluated for seven different CDC and PHL LDTs designed to detect bacterial pathogens associated with common respiratory infections. Selected assays were performed at six state PHLs and the results are presented in Table 3. Sensitivity was similar between the CDC and IA LDTs (columns A and B), with the exception of the CDC *Legionella* spp. LDT that did not meet the statistical threshold for inclusion (≥95% positive detection). Several of the NY LDTs demonstrated 10-fold better sensitivity, including *Haemophilus influenzae*, *Legionella pneumophila*, *Mycoplasma pneumoniae*, and *S. pneumoniae*. Precision across the six state PHLs was within 1 log (columns A, D, E, and F).

3.3. Analytical sensitivity and precision of CDC LDTs in the TAC format

The performance of 21 different CDC designed LDTs in the TAC format was evaluated at six participating state PHLs. LoD was determined for each assay at three different laboratories and each site

Table 2
Limit of detection for CDC and PHL viral respiratory LDTs in 96 well format and commercial multi-respiratory panels. Results that did not meet the acceptance criteria (at least 19 out of 20 replicates tested positive for detection) were considered indeterminate. LoD is expressed as the highest dilution (lowest concentration) of extracted reference standard that met acceptance criteria for the experiment.

Column	A	B	C	D	E	F	G	H	I	J	K	L
State PHL	IA		WA		MA	WI	HI	NY		MA	WI	HI
Assay type	CDC	CDC	CDC	CDC	CDC	CDC	CDC	CDC	LDT	TessArrayRPM Flu3.1	QiagenResplex II v2	Luminex xTAG
Instrumentation	7500	ViiA7	7500	ViiA7	ViiA7	ViiA7	ViiA7	ViiA7	IQ5	Affymetrix Genechip	Luminex 200	Luminex 200
7500 = ABI 7500 Fast												
ViiA7 = ABI ViiA7												
IQ5 = Bio-Rad IQ5												
Adenovirus	−5	a	−4	−4	−5	NA	−5	−5	−5	−3	NA	−4
Human coronavirus 229E	NA	NA	−5	−5	−5	−5	NA	−6	−6	−2	−5	NA
Human coronavirus NL63	NA	NA	−6	−6	−6	−6	NA	−7	−6	−3	−4	NA
Human coronavirus OC43	NA	NA	−5	−5	−5	−4	NA	−6	−6	−2	−4	NA
Human metapneumovirus A1	−5	−5	−5	−5	−4	−5	−5	−5	−5	d	−3	−4
Human parainfluenza virus 1	−4	−4	NA	NA	−3	−3	NA	−3	−1	−1	−3	NA
Human parainfluenza virus 2	−6	−6	NA	NA	−5	c	NA	−7	−7	−3	−5	NA
Human parainfluenza virus 3	−6	−6	NA	NA	−6	−6	NA	−6	−7	−2	−4	NA
Human parainfluenza virus 4	NA	NA	−3	−3	−2	−3	NA	−3	−3	−1	−1	NA
Respiratory enterovirus (CoxA9)	NA	NA	−6	−6	−6	−6	NA	NA	NA	−5	−7	NA
Respiratory syncytial virus	−6	−6	NA	NA	−5	−6	−6	−6	−7	−3	−3	−3
Rhinovirus	−5	b	−5	−5	−6	−6	−5	−6	−6	ND	−4	−5

a – 12/20 positive (60%) detection at −6.
b – 17/20 positive (85%) detection at −5.
c – 12/20 positive (60%) detection at −6.
d – 1/10 positive (10%) detection at −1.
NA – assay not assigned to site.
ND – panel not designed to detect Rhinovirus.

Table 3
Limit of detection for CDC and PHL bacterial respiratory LDTs in 96 well format. LoD is expressed as the highest dilution (lowest concentration) of extracted reference standard that met acceptance criteria for the experiment.

Column	A	B	C	D	E	F
State PHL	IA		NY	WA	WI	HI
Assay type	CDC	LDT	LDT	CDC	CDC	CDC
Instrument7500 = ABI 7500 Fast	7500	7500	7500	7500	7500	7500
B. pertussis	−7	−7	−7	NA	−7	NA
C. pneumoniae	−3	−3	−5	−3	NA	NA
H. influenzae	NA	NA	−6	−5	NA	-5
Legionella spp.	A	−5	−6	−5	NA	NA
M. pneumoniae	−5	−5	−6	−5	NA	NA
S. pneumoniae	NA	NA	−6	−5	−4	NA
S. pyogenes	NA	NA	−5	−5	−5	NA

A – 15/20 positive (75%) detection at −6.
NA – assay not assigned to site.

Table 4
Limit of detection for CDC viral and bacterial respiratory assays in TAC format. LoD is expressed as the highest dilution (lowest concentration) of extracted reference standard that met acceptance criteria for the experiment.

Pathogen	State PHL					
	NY	WA	MA	WI	IA	HI
Adenovirus				−3	−4	−5
Human coronavirus 229E				−5	−5	−5
Human coronavirus NL63	−5	−5	−5			
Human coronavirus OC43				−4	−5	−5
Human metapneumovirus A1				−4	−4	−4
Human metapneumovirus B2	−4	−4	−3			
Influenza A	−5	−5	−5			
Influenza B	−6	−6	−6			
Human parainfluenza virus 1				−3	−3	−3
Human parainfluenza virus 2	−4	−4	−5			
Human parainfluenza virus 3				−5	−5	−5
Human parainfluenza virus 4	−3	−2	−2			
Respiratory enterovirus (CoxA9)	−6	−6	−5			
Respiratory syncytial Virus	−6	−5	−5			
Rhinovirus				−5	−5	−5
B. pertussis				−5	−7	−6
C. pneumoniae	−3	−2	−3			
H. influenzae	−4	−4	−4			
Legionella spp.				−6	−6	−5
M. pneumoniae				−4	−5	−4
S. pneumoniae	−3	−4	−4			
S. pyogenes				−4	−5	−5

tested a combination of viral and bacterial assays as shown in Table 4. As compared to the LoD data for each LDT in a 96 well format (Tables 2 and 3) sensitivity in the TAC format was generally one or two logs lower depending on the assay and testing site. Precision also varied depending on the assay, with the human coronavirus 229E, human coronavirus NL63, human metapneumovirus A1, influenza A, influenza B, human parainfluenza virus 1, human parainfluenza virus 3, rhinovirus, and *H. influenzae* assays having the best reproducibility, while the adenovirus and *Bordetella pertussis* assays had a standard deviation of 1 log.

4. Discussion

In this study Association of Public Health Laboratories (APHL) and CDC, in cooperation with six PHLs, conducted a multicenter evaluation of TAC technology with viral and bacterial respiratory assays. The overall goals were to evaluate the analytical performance of CDC developed reference assays on TACs in comparison with individual CDC laboratory developed tests (LDTs), LDTs from two different state PHLs, and three commercial multi-target respiratory panels. Sensitivity was generally one log lower in the TAC format than for individual LDTs, consistent with previous studies (Kodani et al., 2011). This may be due to the oligonucleotide concentrations not being fully optimized for TAC in addition to the smaller reaction volume (1–2 μL per assay) as compared to typical real-time assays (20–30 μL). Smaller reaction volumes conserve specimen in comparison with individual or multiplexed real-time PCR assays, leaving greater residual specimen volume to be used for further molecular or microbial characterization. Additionally, the CDC viral LDTs were optimized for an annealing temperature of 55 °C, while the bacterial LDTs were designed to use 60 °C. The bacterial LDTs did not perform as well at 55 °C (data not shown), while the viral LDTs were acceptable at the higher annealing

temperature. Since both types of assays were included on the TACs, a compromise was made by running all of the assays at an annealing temperature of 60 °C.

Previous studies have compared the sensitivity, specificity, and reproducibility of TACs for the detection of viral and bacterial pathogens capable of causing respiratory infections against individual real-time PCR assays in a 96 well format (wet assays) using clinical specimens. In one study examining 192 virus positive specimens the sensitivity and specificity of TAC was 95% and 98%, respectively (Kodani et al., 2011); whereas in a different study testing 942 specimens these values were 83% and 99% (Weinberg et al., 2013). Detection sensitivity as described in these previous studies varied depending on the target, with the adenovirus, human parainfluenza virus 2, *M. pneumoniae*, and *Chlamydophila pneumoniae* assays demonstrating lower performance. Reproducibility of cycle threshold (Ct) values within and across TACs in these two studies was also good, with an average coefficient of variation below 2.91%. Finally, positive and negative predictive performance in both studies was also similar between TAC and traditional individual wet assays. A separate follow up TAC study found that sensitivity near the LoD could be further improved by optimizing the entire testing procedure, including evaluating different enzyme systems, adjusting oligonucleotide concentrations, and by increasing the number of replicates from two to four (Diaz et al., 2013).

In comparison to the two studies cited above, the purpose of the current study was to evaluate the analytical sensitivity of TAC assays against their individual LDTs using reference standards and to measure reproducibility between multiple sites. Regardless of the specimen type assayed, TAC demonstrated sensitivity, specificity, and reproducibility equivalent to other multi-pathogen detection platforms. One limitation of the current study is that the LoD was not calculated in terms of infectious dose 50% (ID_{50}) or copies/mL. Future studies will evaluate TAC performance against validated laboratory developed tests using clinical specimens of known titer. Several different extraction instruments, combinations of oligonucleotide primers and probes, PCR enzyme master mixes, and real-time instruments were used by multiple laboratories in this study and the consistency of the results demonstrated the robustness of the technique when assays are carefully designed and thoroughly validated. Taken collectively, the data underscore the utility of TAC for multi-pathogen testing performed on a variety of specimen types using various extraction and amplification chemistries. Although inherent logistical limitations exist when attempting to compare technologies, the results of the current study demonstrate that the TAC is an ideal platform for testing specimens using a customized panel of targets and supports the suitability of TAC for future studies in a variety of formats and settings.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jviromet.2015.11.020.

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