Comparison of FTD® respiratory pathogens 33 and a singleplex CDC assay for the detection of respiratory viruses: A study from Cameroon

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ABSTRACT

Introduction: This study compares the detection of 14 common respiratory viruses by two different real-time reverse transcription polymerase chain reaction (rRT-PCR) methods: in house singleplex tests developed by the Centers for Disease Control and Prevention and the commercially available Fast Track Diagnostic (FTD®) Respiratory Pathogens 33 multiplex test. Methods: A total of 217 nasopharyngeal swabs were analyzed using CDC singleplex rRT-PCR and FTD® Respiratory Pathogens 33 multiplex assays, for the detection of 14 respiratory viruses. Results: The results showed that 179/217 (82.5%) samples were positive with the singleplex method and 183/217 (84.3%) with the FTD® Respiratory Pathogens 33 multiplex test. Excellent or satisfactory agreement was obtained for all viruses (k > 0.6) except Parainfluenzavirus 4 (k = 0.24) and influenza B (k = 0.51). Conclusion: Although the multiplex FTD kits were more expensive than the singleplex assay, the FTD kits yielded rapid results in a shorter timeframe, increasing efficiency of diagnosis.

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1. Introduction

Acute respiratory infections (ARI) are one of the leading causes of hospitalization, morbidity and mortality worldwide. Data from a meta-analysis study shows that approximately 1 million of children under 5 years worldwide died due to these infections in 2013 (Liu et al., 2015). The burden of ARIs is particularly high in sub-Saharan Africa, which has recorded nearly half of all these deaths (0.4 million) (Liu et al., 2015). Available data indicate that viral agents play an important role globally in the causes of ARIs (Cilla et al., 2008).

Antigen detection by immunofluorescence, enzyme-linked immunosorbent assay (ELISA), and culture are still occasionally used to confirm infection with respiratory viruses (Ginocchio, 2007). These traditional methods have several limitations. Viral culture, for example, can be time consuming, labor intensive and requires substantial technical expertise to maintain and evaluate cell culture monolayers (Leland and Ginocchio, 2007). Currently, nucleic acid amplification is the method of choice for the diagnosis of respiratory infections (Bierbaum et al., 2014; Driscoll et al., 2014; Kenmoe et al., 2016; Lagare et al., 2015; Malhotra et al., 2016). Molecular tests are widely recognized to contribute to a better efficiency, sensitivity and speed compared to traditional methods for the diagnosis of respiratory infections. Several in house methods have been described, including a panel of singleplex assays for detecting respiratory viruses from Centers for Disease Control and Prevention (CDC) (Njouom et al., 2012; Sakthivel et al., 2012). Analyses of respiratory pathogens using individual real-time reverse transcription polymerase chain reaction (rRT-PCR), however, can be laborious and time consuming.

Recently, several multiplex rRT-PCR commercial methods have become available for the detection of respiratory pathogens (Anderson et al., 2013; Barratt et al., 2017; Driscoll et al., 2014; Malhotra et al., 2016). At this time, multiplex panels capable of simultaneously detecting more than three respiratory pathogens are widely available: Luminex NxTAG® Respiratory Pathogen Panel (Luminex Molecular Diagnostics, Austin, TX), FilmArray® Respiratory Panel (Biofire Diagnostics, Salt Lake City, UT), Allplex and Anyplex Respiratory Virus Panel Assays (Seegene), RespFinder® SMART and 2SMART (Patho Finder), Respiratory Multi Well System MWS r-gene® Range (BioMerieux) and Fast-Track Diagnostics FTD-21, -21+ & -33 (FTD®).

The introduction of these new multiplex methods allows the analysis of a wider range of respiratory pathogens coupled with simplification of procedures, reducing time to detection, sample volume requirements and risk of contamination. A number of studies have compared the performance of FTD® Respiratory Pathogens kits with that of in house respiratory panels or commercial panels, such as Luminex NxTAG® Respiratory Pathogen Panel (Luminex Molecular Diagnostics, Austin, TX), TaqMan Array Card® (Thermo Fischer Scientific) and Anyplex® Respiratory Virus Panel Assays (Seegene) (Anderson et al., 2013; Barratt et al., 2017; Bierbaum et al., 2014; Driscoll et al., 2014; Malhotra et al., 2016; Sakthivel et al., 2012). Overall, these reports showed that FTD® Respiratory Pathogens kits yield similar results as other multiplex pathogen detection kits. To date, there are no available data on the performance of these new multiplex tests when used in Africa. Africa is a continent with a diversity of socio-economic and cultural contexts, yet it bears the greatest burden of ARI both in terms of morbidity and mortality (Liu et al., 2015). An evaluation of these molecular assays in the context of Cameroon can help guide priorities in assay selection for routine detection of these respiratory viruses. Multiplex tools enable the detection of multiple viruses within a single specimen. While co-detection data offers insight into the viruses present in the sample, it is less clear how these patterns or virus combinations correlate with clinical manifestations (US FOOD and DRUG ADMINISTRATION, 2007; Ortiz et al., 2009). Co-detection represents the multiple organisms present in a sample, whereas co-infection suggests that these organisms contribute in part to pathogenicity (Koon et al., 2010). Comparing multiplex data from patients with varying clinical severity, offers an opportunity to examine potential differences in co-detection patterns.

In this study we (1) compare the performance of FTD® Respiratory Pathogens 33 with the singleplex CDC assay for the detection of respiratory viruses using respiratory samples collected as part of sentinel surveillance of influenza virus in Cameroon; (2) compare the time and cost necessary to run the FTD® Respiratory Pathogens 33 and the singleplex CDC assays in Cameroon; and (3) examine co-detection patterns associated among hospitalized and non-hospitalized patients.

2. Materials and methods

2.1. Description of the study

All testing for this study was performed in Cameroon's National Influenza Center, Centre Pasteur Cameroon (CPC). This retroprospective and cross-sectional study was conducted with 217 banked nasopharyngeal swabs collected from sentinel sites conducting influenza surveillance in Cameroon representing 6 regions of South Cameroon (Fig. 1). Patients were recruited from October 2016 to August 2017. The samples selected in the study originated from ambulatory and hospitalized patients with acute respiratory infections of all ages. Influenza like illness (ILI) was defined as any ambulatory patient with fever, cough and/or sore throat with onset of symptoms during the 5 previous days (Ortiz et al., 2009). Severe acute respiratory infections (SARI), in addition to the ILI clinical presentation, required hospitalization. The consent of all participants in the study was obtained as part of the routine influenza virus surveillance. The study was dispensed from institutional ethical clearance as this is part of a public health surveillance activity.

2.2. Amplification of influenza viruses by singleplex CDC assay

Archived nasopharyngeal swabs processed as previously described (Sakthivel et al., 2012) were included in this study. Nucleic acids extracted from these samples were prospectively pre-screened for influenza A, influenza B, and RNase P internal control using singleplex CDC methods (Kenmoe et al., 2016). Samples were subsequently stored at −80 °C until a second analysis with FTD® Respiratory Pathogens 33 and singleplex CDC assays. Two new extractions of total nucleic acid

were performed from 140 μL of each sample and 2 μL of the internal control present in the FTD® Respiratory Pathogens 33 kit. All extracts were stored in the refrigerator until the manipulations were complete (2–4 days) to avoid degradation of nucleic acids by freezing/thawing. The extractions were conducted with the QIAamp Viral RNA Mini kit (Qiagen, Hilden, Germany) and according to the manufacturer's recommendations.

2.3. Amplification by FTD® respiratory pathogens 33 multiplex and CDC singleplex assay

All samples were tested with singleplex CDC techniques according to the previously described protocol (Sakthivel et al., 2012). Briefly, the samples were tested for the following additional 12 respiratory viral targets: Rhinovirus (RV), Human Coronavirus (HCoV) [HCoV-229E, HCoV-OC43, HCoV-NL63, HCoV-HKU1], Human Parainfluenzavirus (HPIV) [HPIV-1, HPIV-2, HPIV-3, HPIV-4], Human Metapneumovirus (HMPV), Human Respiratory Syncytial Virus (HRSV) and Human Adenovirus (HAdV). The singleplex CDC assays are designed to detect all virus subtypes including HRSV-A/B, and HMPV-A/B. Twenty samples were analyzed per 96-well plate for 4 viral targets. Singleplex CDC assays were performed with 5 μL of extract.

Each extract was also tested by the FTD® Respiratory Pathogens 33 method using 10 μL of extract with 10 samples per 96-well PCR plate according to the manufacturer's instructions. The FTD® Respiratory Pathogens 33 method was performed in 5 tubes covering 18 viruses, including influenza A, influenza B, influenza A H1N1 2009, RV, HCoV-229E, HCoV-OC43, HCoV-NL63, HCoV-HKU1, HPIV -1, HPIV-2, HPIV-3, HPIV-4, HMPV-A/B, HBoV, HRSV-A/B, HPeV, EV, and HAdV, and one bacteria, Mpneu. The 3 remaining assay pools in the FTD kit focus on bacterial detection, and data from these assay pools were not tested in this study. The results of both methods were considered positive for all sigmoidal curve and cycle thresholds (Ct) <40 curves.

The per specimen cost in US dollars of the singleplex CDC method and the FTD® Respiratory Pathogens 33 kit were calculated based on commercially available prices for primers and enzyme, as well as the FTD kit respectively. The costs of plastic consumables were not included in these calculations, the labor and validation costs were estimated at $16 per hour. The time needed to prepare and analyze 12 specimens using each method was determined according to the experiences at CPC.

2.4. Statistical analysis

The targets that were included only in the FTD® Respiratory Pathogens 33 kit (influenza A H1N1 2009, HBoV, EV, HPeV and Mpneu) were not compared in the performance evaluation. The positive and negative percentage agreements (PPA and NPA, respectively) were obtained by the following two formulas (U.S. FOOD and DRUG ADMINISTRATION, 2007): PPA = $100*a/(a + c)$; NPA = $100*d/(d + b)$. In the formulas a represents the number of positive samples by both tests, b the number of samples determined negative by FTD® Respiratory Pathogens 33 and positive by singleplex CDC, c the number of samples positive by FTD® Respiratory Pathogens 33 and negative by singleplex CDC, and d the number of negative samples by the two assays. The agreement between the two methods was estimated by Cohen's kappa coefficient. Kappa values [−1–0], [0–0.2], [>0.2–0.4], [>0.4–0.6], [>0.6–0.8] and [>0.8–1] represented a high disagreement, a very weak agreement, a weak agreement, a medium agreement, a satisfactory agreement and an excellent agreement respectively. The statistical difference between the two compared methods was estimated using the McNemar test.

First, data from the FTD® Respiratory Pathogens 33 method alone were utilized to compare co-detection in SARI versus ILI patients because this method tested for more respiratory pathogens. Separately, data from the CDC singleplex method were utilized to compare co-detection. A χ^2 test was used to estimate the statistical difference of
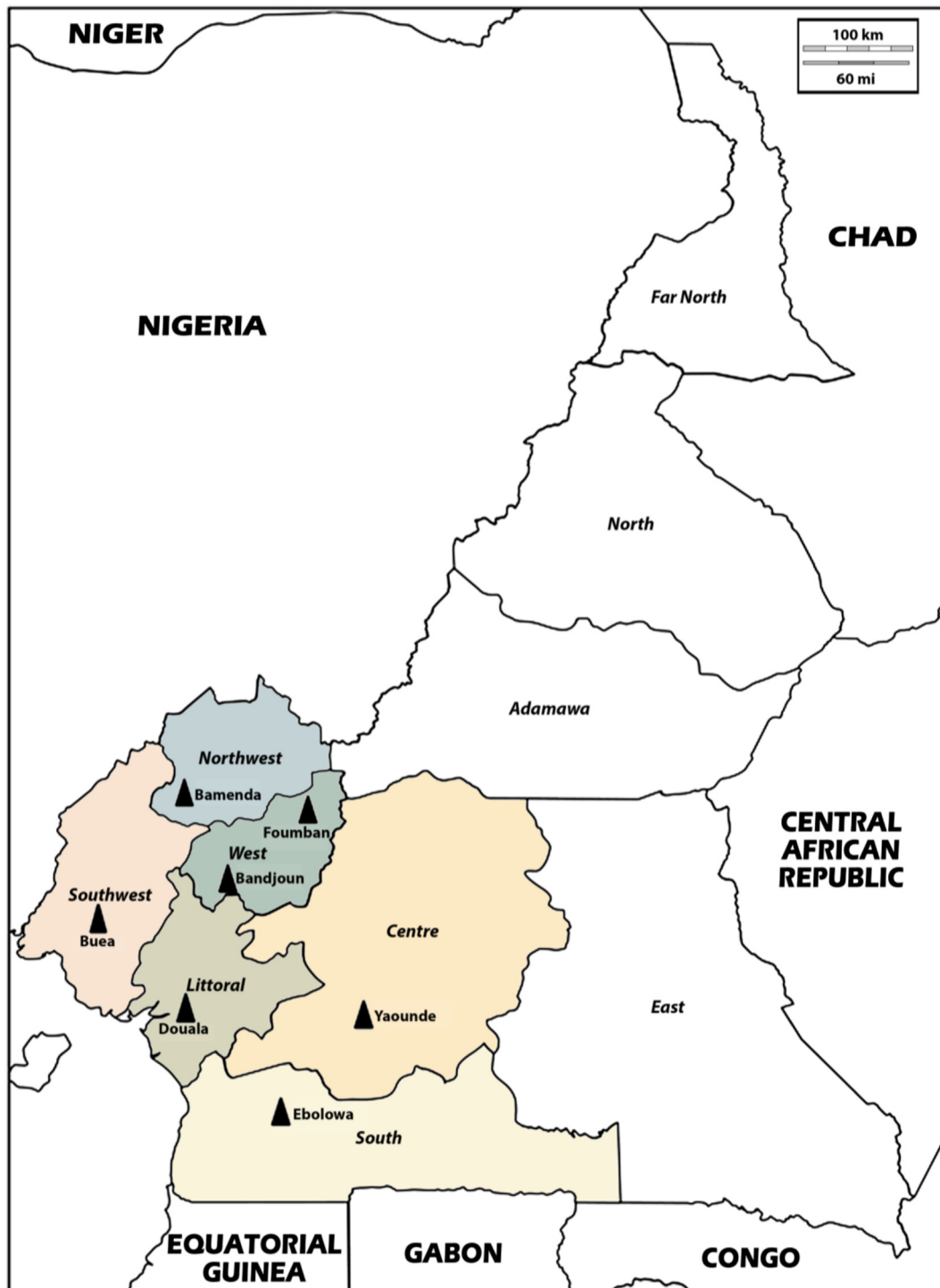
Fig. 1. Location of the influenza sentinel surveillance sites (▲) included in this study.

the proportion of co-detection in SARI versus ILI patients. Using an independent t-test, the mean number of viruses was compared between all SARI specimens, and an equal number of randomly sampled ILI specimens. For all analyses, *P*-values <0.05 were considered statistically significant. All statistical analyzes were conducted using R version 3.4.1 (R Core Team, 2017).

3. Results

Of the 1026 patients recruited from influenza surveillance sentinel sites between October 2016 and August 2017, a total of 217 (21.1%)

nasopharyngeal specimens (about 20 per month) were randomly selected for this study according to age, location, and sex. The majority of samples originated from Yaoundé (86; 39.6%) followed by Bandjoun (44; 20.3%), Douala (20; 9.2%), Buea (19; 8.8%), Foumban (19; 8.8%), Bamenda (17; 7.8%) and Ebolowa (12; 5.5%). The mean age of study participants was 5.3 +/−12.5 years, with a range of 0.1–85 years. The study included 104 female (49.3%) and 107 male (50.7%) patients. The study population consisted of 138 (63.6%) ILI (ambulatory patients) and 79 (36.4%) SARI (hospitalized patients).

We included 14 respiratory virus targets tested in common by the FTD® Respiratory Pathogens 33 and singleplex CDC assays in the

comparative analysis (Table 1). The targets of HCoV-229E, HCoV-NL63, HCoV-HKU1 and influenza A showed 100% agreement between the FTD® Respiratory Pathogens 33 and singleplex CDC results. For the remaining pathogens, the positive percent agreement ranged from 25% for HPIV-4 to 97.3% for HRSV. The negative percent agreement ranged from 90.6% for HAdV to 99.5% for HPIV-2. An excellent agreement (k > 0.8–1) was obtained for the HRSV, HMPV, HPIV-2, HAdV, RV, 229E, NL63, HKU1 and influenza A targets. The agreement between the FTD® Respiratory Pathogens 33 and singleplex CDC was satisfactory (k > 0.6–0.8) for the targets HPIV-1 (k = 0.77), HPIV-3 (k = 0.75) and OC43 (k = 0.69). The agreement in the detection of influenza B and HPIV-4 was average (k > 0.4–0.6) and low (k > 0.2–0.4), (k = 0.51 and k = 0.24, respectively). According to the McNemar test, the FTD® Respiratory Pathogens 33 and singleplex CDC tests show significant differences in detection for the OC43 and influenza B targets. A total of 24% (52/217) of the tested samples showed discrepant results between the FTD® Respiratory Pathogens 33 and singleplex CDC techniques. The majority of discrepancies (44/52; $P < 0.001$) were recorded for samples with multiple pathogen detections. Discrepancies were predominant in the detection of HAdV (8.8%; 19/217) followed by RV (6%; 13/217) and influenza B (4.1%; 9/217). The Ct of the FTD® Respiratory Pathogens 33 and singleplex CDC discordant tests are reported in Fig. 2A and B. Most discordant results were observed for samples with low viral load (median Ct > 35) except HPIV-4, RV and influenza B targets.

The costing calculation estimated that the FTD® Respiratory Pathogens 33 was 1.6 times more expensive than testing 14 pathogens using the singleplex CDC assay, yet 2 times as fast. During this study, the singleplex CDC tests required approximately 7 hours for extraction and amplification of 12 samples including controls for 14 viral targets in two manipulations. The FTD® Respiratory Pathogens 33 technique, on the other hand, required about 3.5 hours for the extraction and amplification of 12 samples, including the controls of the 21 targets for a single manipulation. Table 2 describes the theoretical reagent and labor costs associated with conducting the two described methods for 12 samples at the CPC. Total cost per specimen was $42.66 and $26.83 for the FTD® Respiratory Pathogens 33 technique and the singleplex CDC method respectively. The cost of the singleplex CDC assay is even lower when testing for fewer than 14 viruses. Both techniques had a similar duration for analysis of the amplification results.

Overall, one or more respiratory viruses were found in 179 (82.5%) and 183 (84.3%) samples by the singleplex CDC and FTD® Respiratory Pathogens 33 assays, respectively. A single virus was detected in 94 and 93 samples by the singleplex CDC and FTD® Respiratory Pathogens 33 assays, respectively. Multiple detections were present in 85 samples by the singleplex CDC assay (67 double detections, 14 triple detections and 4 quadruple detections) and 90 samples by the FTD® Respiratory Pathogens 33 assay (60 double detections, 23 triple detections, 5 quadruple detections and 2 quintuple detections). Except Inf A H1N1 2009 and Mpneu, all other respiratory pathogens tested for were detected. The respiratory virus most commonly detected by singleplex CDC assays (85; 39.2%) and FTD® Respiratory Pathogens 33 (78; 35.9%) was HAdV followed by HRSV and RV. Table 1 presents the detection frequencies of the respiratory pathogens found by the singleplex CDC and FTD® Respiratory Pathogens 33 assays during this study.

Using the FTD multiplex, co-detection was more commonly observed in ambulatory outpatients when compared to hospitalized patients ($\chi^2 = 7.16$; $P = 0.007$). There were 21/79 (26%) SARI specimens and 62/138 (45%) ILI specimens with multiple viruses detected in each sample. When comparing co-detection patterns from the 79 SARI specimens with a random sampling of 79 ILI specimens, the detections in hospitalized patients had 16 different viral combinations (10 combinations of 2 viruses, and 6 combinations of 3 viruses), compared to the out-patient specimens with 26 different viral combinations (13 combinations of 2, 10 combinations of 3, and 3 combinations of 4). When testing for 27% fewer organisms using the CDC singleplex assays, the same strong co-detection pattern was not observed (24/59, 40.7% SARI and 60/118, 50.8% ILI specimens had multiple viruses, $\chi^2 = 1.63$; $P = 0.202$). A random sample of 59 SARI specimens had 13 viral combinations and ILI specimens had 16 viral combinations.

4. Discussion

In this study, singleplex CDC assays were compared to commercial FTD® Respiratory Pathogens 33 multiplex tests for the detection of respiratory viruses collected from SARI and ILI cases from six regions in Cameroon. Results of the study showed that the two methods had excellent or satisfactory correlation for the detection of many viral pathogens tested, except for two viruses HPIV-4 (k = 0.24) and influenza B (k = 0.51). The singleplex assays took twice as long as the multiplex, but were approximately half of the cost.

About 80% of the 217 samples tested by both techniques showed the presence of at least one respiratory virus. This detection rate is relatively

Table 1
Viral detection by in house singleplex CDC and commercial FTD® Respiratory Pathogens 33 in 217 respiratory specimens collected in Cameroon.

Targets	FTD-33		Singleplex CDC		FTD-33/singleplex CDC				PPA	NPA	kappa [CI 95%]	P-value*
	+	-	+	-	+/+	−/−	+/−	−/+				
RSV	74 (34.1)	143 (65.9)	73 (33.5)	144 (66.5)	72	142	2	1	97.3	99.3	0.97 [0.92–1]	1
HMPV	5 (2.3)	212 (97.7)	7 (3.2)	210 (96.8)	5	210	0	2	100	99.1	0.83 [0.49–1]	0.5
PIV 1	10 (4.6)	207 (95.4)	13 (6)	204 (94)	9	203	1	4	90	98.1	0.77 [0.53–0.94]	0.3
PIV 2	6 (2.8)	211 (97.2)	7 (3.2)	210 (96.8)	6	210	0	1	100	99.5	0.92 [0.66–1]	1
PIV 3	17 (7.8)	200 (92.2)	17 (7.8)	200 (92.2)	13	196	4	4	76.5	98	0.75 [0.53–0.9]	1
PIV 4	4 (1.8)	213 (98.2)	4 (1.8)	213 (98.2)	1	210	3	3	25	98.6	0.24 [−0.02–0.66]	1
AdV	78 (35.9)	139 (64.1)	85 (39.2)	132 (60.8)	72	126	6	13	92.3	90.6	0.81 [0.73–0.88]	0.1
RV	58 (26.7)	159 (73.3)	55 (25.3)	162 (74.7)	50	154	8	5	86.2	96.9	0.84 [0.75–0.92]	0.5
HCoV-229E	2 (0.9)	215 (99.1)	2 (0.9)	215 (99.1)	2	215	0	0	100	100	1 [1–1]	1
HCoV-OC43	7 (3.2)	210 (96.8)	13 (6)	204 (94)	7	204	0	6	100	97.1	0.69 [0.38–0.89]	0.03
HCoV-NL63	1 (0.5)	216 (99.5)	1 (0.5)	216 (99.5)	1	216	0	0	100	100	1 [1–1]	1
HCoV-HKU1	1 (0.5)	216 (99.5)	1 (0.5)	216 (99.5)	1	216	0	0	100	100	1 [1–1]	1
Influenza A	2 (0.9)	215 (99.1)	2 (0.9)	215 (99.1)	2	215	0	0	100	100	1 [1–1]	1
Influenza B	13 (7.4)	204 (92.6)	6 (2.8)	211 (97.2)	5	199	8	1	38.5	99.5	0.51 [0.17–0.76]	0.03
Influenza A H1N1 2009	0 (0)	217 (100)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
HBoV	15 (6.9)	202 (93.1)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
EV	17 (7.8)	200 (92.2)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
PeV	2 (0.9)	215 (99.1)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Mpneu	0 (0)	217 (100)	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

  * *P*-value for McNemar test. PPA = Positive Percentage Agreement; NPA = Negative Percentage Agreement; RV = Rhinovirus; HCoV = Human Coronavirus (HCoV-229E; HCoV-OC43; HCoV-NL63; HCoV-HKU1); HPIV = Human Parainfluenzavirus (HPIV-1; HPIV-2; HPIV-3; HPIV-4); HMPV = Human Metapneumovirus; HRSV = Human Respiratory Syncytial Virus; HAdV = Human Adenovirus; HBoV = Human Bocavirus; EV = Enterovirus; PeV = Parechovirus; Mpneu = *Mycoplasma pneumoniae*.
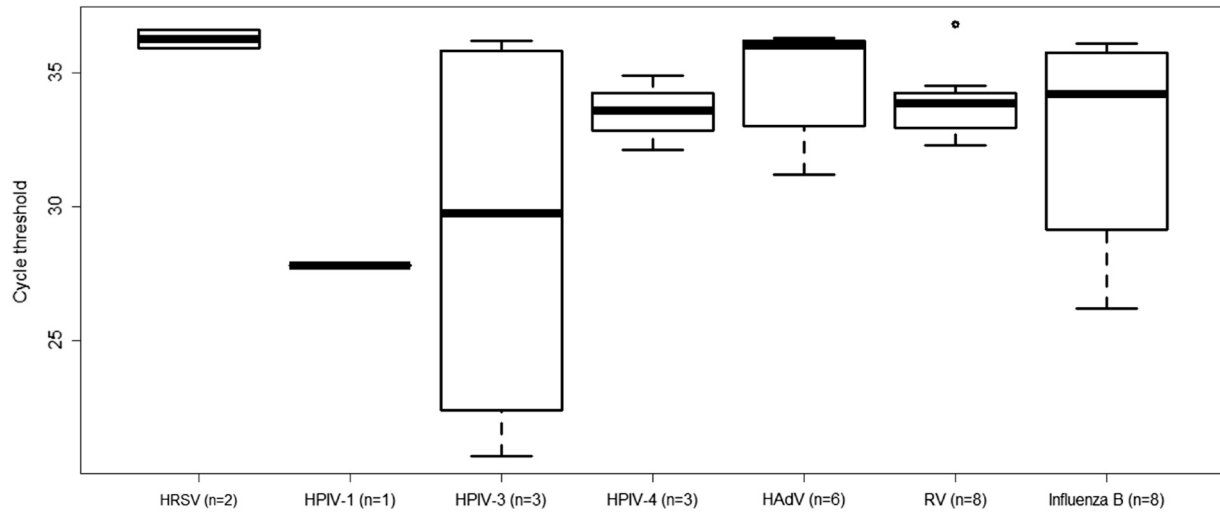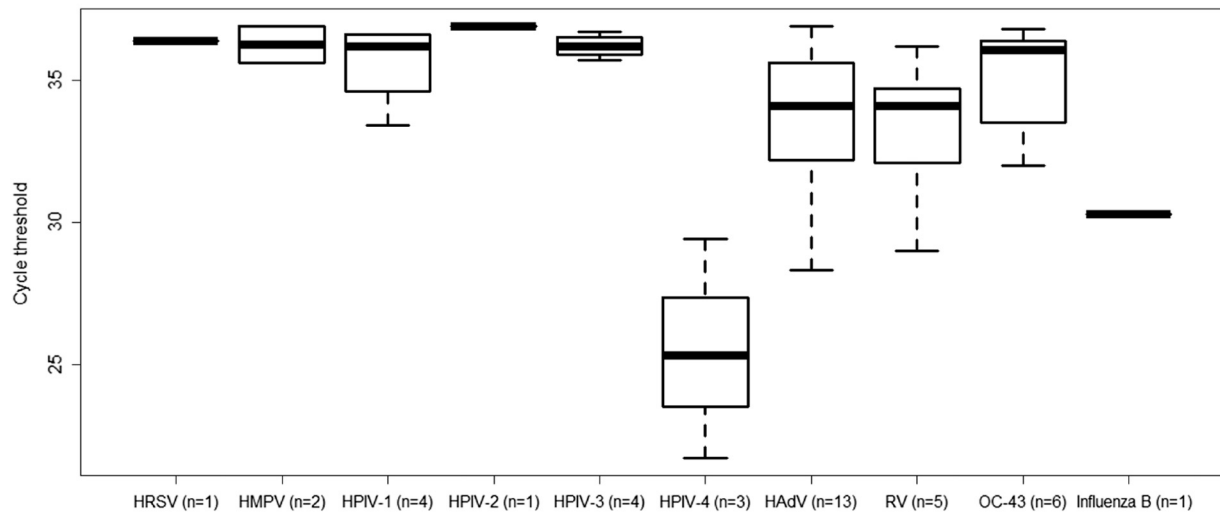
Fig. 2. A and B: Ct values for discrepant detections between CDC and FTD® Respiratory Pathogens 33 assays.

higher than the rates obtained in previous studies in Cameroon and other Central African countries. This could be explained by the higher number of respiratory viruses tested for in this study; 21 compared to only 8 for the Nakouné et al. study, which reported a prevalence of

Table 2
The commercial reagent and labor costs associated with conducting the singleplex CDC assay for 14 viral targets and FTD® Respiratory Pathogens 33 molecular tests at the Centre Pasteur Cameroon, in Yaoundé, Cameroon. Costs are reported in US dollars.

	Singleplex CDC	FTD® respiratory pathogens 33
Primer/reagent costs	($50.00) * 14 = $700.00	$1900.00
Number of specimens that can be tested	1000	50
Enzyme costs	$1200.00/1000 reactions	(included in kit)
Reagent cost per specimen	**$17.50**	**$38.00**
Labor in hours to test 12 specimens	7	3.5
Labor and validation costs at a rate of $16/h	$112.00	$56.00
Labor cost per specimen	**$9.33**	**$4.66**
Total cost per specimen	**$26.83**	**$42.66**

only 14.9% in ambulatory and hospitalized children recruited in Central African Republic in 2010 (Nakouné et al., 2013). Still in this study, detection of some respiratory viruses may have been missed. Detection of pathogens from the lower respiratory tract was not possible, since only nasopharyngeal specimens were analyzed, and depending on the sampling conducted each month certain pathogens may have been under or over represented. Overall, however, all viral targets showed comparable detection rates by both techniques, with the exception of influenza B and HCoV-OC43 which were significantly different. Even though the kappa value was low for HPIV-4 ($k = 0.24$), its low prevalence may have limited the ability to detect a significant difference between the two diagnostic tests. The detection frequencies ranged from 35.9% for HAdV to 0.5% for both HCoV-NL63 and HCoV-HKU1 species. These detection rates of the different viral targets are consistent with those of previous studies in Cameroon and in other African countries (Hoffmann et al., 2012; Kenmoe et al., 2016; Lagare et al., 2015; Lekana-Douki et al., 2014; Nakouné et al., 2013; Njouom et al., 2012). However, it is noteworthy according to previous study in Africa (Dia et al., 2014; Kenmoe et al., 2016), to mention that this study confirms the predominant frequency of HAdV in acute respiratory infections this region.

Twelve (HCoV-229E, HCoV-NL63, HCoV-HLU1, influenza A, HRSV, HPIV-2, RV, HMPV, HAdV, HPIV-1, HPIV-3, and HCoV-OC43) of the 14 viral targets tested revealed an excellent or satisfactory agreement (kappa value >0.6–1) between the FTD® Respiratory Pathogens 33 and singleplex CDC assays. Similarly, other previous studies comparing FTD kits and other commercial or personalized tests, singleplex or multiplex, have shown similar results (Anderson et al., 2013; Barratt et al., 2017; Bierbaum et al., 2014; Driscoll et al., 2014; Malhotra et al., 2016; Sakthivel et al., 2012). A comparison of the sensitivities between CDC singleplex assays and FTD assays for respiratory viruses by testing 308 prospective specimens was previously conducted showing similar sensitivities for most assays with exceptions of the FTD assays for HAdV, RSV, and RV having lower sensitivity (Ortiz et al., 2009). It must be noted that in the study conducted by Sakthivel et al a different FTD assay kit was used; however the FTD® 21 kit referenced has overlapping assays with the FTD® 33 kit discussed in this study. The good concordance observed for HAdV, HCoV-OC43, EV/HPeV and HPIV-2 in this current study was different from that found by Sakthivel et al., and Bierbaum et al. in their studies comparing the same techniques (Bierbaum et al., 2014; Sakthivel et al., 2012). This result could be explained by an update of the primer and probe sequences in the version FTD® Respiratory Pathogens 33 which was different from the version FTD® Respiratory Pathogens 21 which had been used in prior studies. An important feature of molecular detection assays is the modification of the primer and probe sequences following mutations of the viral genome sequences in the region of primer and probe binding. Unlike the current study, HAdV and HBoV revealed a weak agreement when comparing for FTD® Respiratory Pathogens 21 to the Easyplex® (EP) respiratory pathogen 12 in a study by Anderson et al., in New Zealand (Anderson et al., 2013). In contrast to this study, where the HPIV-4 (k = 0.24) and influenza B (k = 0.51) targets had a low agreement, most of the previous studies showed good agreement (Barratt et al., 2017; Malhotra et al., 2016; Sakthivel et al., 2012). Similar to previous studies, the majority of discrepancies were observed in samples with high Ct values (Bierbaum et al., 2014; Sakthivel et al., 2012). We hypothesized that, the higher sample input volume used for the FTD® Respiratory Pathogens 33 (10 μL) versus 5 μL for the singleplex CDC assay could better explain these observed discrepancies in samples with high Ct values rather than differences in sensitivity of the two assays. Although the volume difference is minimal, when specimen titer is near the limit of detection by PCR even a small difference could affect detection. In contrast, RV, influenza B and HPIV-4 targets showed discordance in samples with low Ct. Rhinovirus strains are known to exhibit primer specificity problems and it is difficult to design a single pair of primers that can detect all RV types while excluding all EV types (Faux et al., 2011; Sakthivel et al., 2012). Although it is important to resolve all these discrepancies with more sensitive and specific molecular techniques, we were unfortunately unable to address this during our study due to insufficient sample volume. On the other hand, during this study the low number of positives obtained for the HPIV-4, influenza A, 229E, NL63 and HKU1 targets could reduce accuracy of the analysis performed for these targets. Therefore additional studies based on a larger number of samples are important to support the results obtained in this study for these targets.

The costing analyses comparing the two methods were consistent with the literature showing that personalized singleplex assay are less expensive than commercial multiplex kits (Bierbaum et al., 2014; Malhotra et al., 2016). The findings, however, must be considered in the context of the intended use of these reagents. For routine surveillance, the benefits gained from a multiplex kit, i.e. multiple pathogen detection and rapid results, may not be worth the additional financial cost. In contrast, during an emergency or outbreak situation, the benefits of a multiplex commercial kit may be well worth the additional price. Future studies should consider examining laboratory testing algorithms used during different outbreak or routine surveillance scenarios to better understand this cost–benefit tradeoff.

In addition to comparing the performance and cost of these two diagnostic platforms, we were able to compare viral co-detection patterns using both the multiplex and singleplex data from specimens from hospitalized and out-patient individuals. When using the multiplex platform to test for more pathogens, viral co-detection patterns in non-hospitalized ILI patients were more heterogeneous than the hospitalized patients who often had single viruses, or had fewer viral combinations when co-detection was observed. These findings contrast some of the existing literature such as a meta-analysis by Asner et al., which did not find differences in clinical severity between single and co-detections (Asner et al., 2014). This pattern of heterogeneity in out-patients was not observed, however, when testing for fewer viruses using the singleplex assays. The clinical consequences of co-detection with respiratory viruses are not well understood, and as our study shows, co-detections can be relatively common for ILI and SARI. Some limitations, however, should be considered, namely case selection included both adults and children and lacked control for co-morbidities.

5. Conclusion

Overall the two methods compared in this study showed excellent or satisfactory agreement for the detection of respiratory viruses with the exception of HPIV-4 and influenza B. Each approach offered contrasting advantages and drawbacks in terms of number of pathogens detected, speed of analysis, and per specimen cost. In order to select the most suitable diagnostic platforms, laboratories should consider the value of each of these assays in the context of routine surveillance and outbreak situations to better understand the resource-benefits.

Conflicts of interest

There is no conflicts of interest.

Disclaimer

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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