Clinical utility of Multiplex PCR Syndromic testing: Respiratory Infections
Winter is coming! Is your lab prepared?

Multiply the possibilities for Respiratory Infection Detection

As with every year, winter and cold season brings an excess of demand for respiratory testing.

To meet budgets and higher demand and throughput, labs need kits to be used on existing molecular instruments at lower cost per pathogen tested.

Fast Track Diagnostics, a Siemens Healthineers Company, with 12 years of experience in delivering a platform agnostic approach to multiplexing of clinically relevant pathogens, can help in making ends meet.

“Real-Time Syndromic Multiplex PCR is a robust diagnostic approach, enabling the rapid and accurate identification of life threatening pathogens. It provides the absolute solution to the everyday laboratory challenge “do more with less”, detecting all different types of pathogens highly efficiently, shortening the turnaround times to result, reducing complexity and cost while at the same time improves patients’ outcome with faster clinical decisions. Real-Time Syndromic Multiplex PCR is the diagnostic method of choice, particularly during the winter season, when laboratories are overwhelmed with respiratory samples”.

Professor George Sourvinos, PhD
Head of the Laboratory of Clinical Virology
Medical School, University of Crete, Heraklion, Greece

A Patient-Oriented Approach
For Your Laboratory
Lab Operations with Confidence and Limited Investment
A Patient-Oriented Approach

- 19 different kits cover 41 different relevant respiratory pathogens, including viruses, bacteria, and fungi.
- Clinically relevant combinations of pathogens: carefully designed kits provide tailored configurations to detect and distinguish between viral, bacterial, and other infections.
- Singleplex- to large multiplex-configured kits help to effectively manage outbreaks and seasonal pathogen screening as well as detect community- and healthcare-associated respiratory infections.
- Short turnaround time: Multiplex PCR assays deliver fast results to ensure timely management of patients.

For Your Laboratory

- FTD kits can be used in almost any laboratory without disrupting routine operations. FTD kits are compatible with most extraction and PCR platforms on the market today, including the automated, high-throughput VERSANT® kPCR Molecular System.
- Don’t lose precious time waiting for a sufficient number of samples to run. With FTD, 1 to 96 samples can be processed in one run, depending on the activity of your laboratory.
- Choose from large selection of assay and pathogen combinations. We offer a broad and comprehensive range of PCR multiplexing kits.
- FTD kits are available in liquid and lyophilized formats with identical performance to accommodate all workflows.

*Check our compatibility list to find out more about compatible devices.
Lab Operations with Confidence...

- With over 12 years of experience, FTD is a pioneer in multiplex real-time PCR and the syndromic approach to molecular diagnostics of infectious diseases.
- Clinically proven products: more than 40 peer-reviewed publications that show the clinical value of FTD Respiratory assays are available on the FTD website.
- Accurate, specific, and reliable: since 2012, performance of QCMD panels has been documented at 98.08% accuracy.
- Billions of samples around the world have been tested with FTD kits, making FTD a leading global supplier of real-time PCR multiplexing kits.
- Full access to raw data prevents the black-box effect and allows the laboratory to review patient results.

...and Limited Investment

- No specific equipment required: FTD assays can be run on several platforms already in your laboratory, expediting implementation while saving space and cost.
- Low reagent cost: test several pathogens at the same time from a single sample and on the same plate while minimizing reagent waste and increasing laboratory efficiency compared to cartridge-based technologies.
- Train laboratory personnel once: all FTD assays have the same pipetting and PCR protocol, streamlining operational procedures.
- Lyophilized kits save consumables and technician time and can be stored in the refrigerator.

“The Respiratory pathogens 21 assay allows my laboratory to produce results in a timely fashion for prompt diagnosis whilst allowing us to test for atypical pathogens, which otherwise would not be tested for.”

Customer, Freiburg, Germany

Enabling better outcomes at lower costs
Eight good reasons...

FTD offers **19** different kits for respiratory infections, detecting **41** different pathogens.

**Available in lyophilized or liquid formats.**

**All respiratory kits are CE-labeled.**

Each kit has been carefully designed to include a clinically relevant combination of pathogens.

From singleplex to large multiplex, FTD kits enable the management of seasonal pathogen screening and outbreaks, as well as detection of community- and healthcare-associated respiratory infections.

All kits are available in two sizes—**32 or 64** reactions—to adapt to individual needs and testing volume.

The tailored assay configurations allow for the detection and identification of viral, bacterial, and fungal infections.

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**...to check FTD Respiratory Infections panel**

|-----------|--------------------|----------------------|------------------|-----|-------------|------------|-----------|------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|----------------|----------------|----------------|------------------|----------------|------------------|----------------|----------------|----------------|----------------|

All assays can be processed in the same run, streamlining busy laboratory operations.

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[Table of respiratory pathogens]
How to request your kit for evaluation purposes

For evaluation purposes only, a limited number of kits with Respiratory Infections Multiplexing Assay are available upon request.

A Siemens Healthineers specialist will contact you and work with you in your laboratory on your existing Instrumentation.

Available only until December 15th

Evaluation kit options

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<thead>
<tr>
<th>Kit name</th>
<th>Targets</th>
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<tr>
<td>FTlyo FLU/HRSV</td>
<td>influenza A virus</td>
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<td>human metapneumoviruses A/B</td>
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<td>human rhinovirus</td>
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Request your FTD Respiratory Infections Multiplexing Assay evaluation kit

To evaluate the role of multiplex PCR analysis in children with febrile seizures, FTD Respiratory pathogens 21 was used to analyze respiratory samples of children admitted to University Children’s Hospital.

FTD Respiratory pathogens 21 simultaneously detects 20 viruses and 1 bacterium involved in respiratory tract infections.

Background
The aim of this study was to assess multiplex PCR analysis in detecting causative viruses in children with febrile seizures.

Methods
The study was a retrospective analysis comparing data from a pre-multiplex era (2009) with a period after the introduction of routine respiratory multiplex analysis (2010-2013) in children with febrile seizures.

Results
We included 200 children with febrile seizures (mean age: 29.5 ± 1.4 months; 104 male) in the study. In 2009, in 10 out of 49 (20 %) children, microbiology testing (bacterial/fungal) was positive compared with a rate of 74 out of 151 (49 %) children during 2010-2013 (p < 0.01). The rate of positive virological studies increased from 10 (20 %) in 2009 to 73 (48.3 %) in the period 2010-2013 (p < 0.01). Multiplex PCR analysis confirmed viral infections in 52 of 73 cases (71.2 %).

Conclusion
Routine multiplex PCR analysis fosters the detection of respiratory viruses in children with febrile seizure. The precise role of multiplex analysis in the management of these children awaits further clarification.

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Summary
Human adenovirus (HAdV) infection can result in a severe respiratory disease. The aim of this study was to identify HAdV types detected in patients hospitalized for severe respiratory illness. The study population consisted of 743 patients with severe respiratory disease admitted to four major hospitals in Kuwait between January 2013 and December 2016. Respiratory specimens were retrospectively screened for 20 respiratory viruses by real-time PCR. The HAdV hexon gene was amplified and directly sequenced, and HAdV types were identified by performing Bayesian phylogenetic analysis. HAdV DNA was detected in 27 (3.6%) patients, with peaks in November and March. Most patients were infants and young children suffering from pneumonia or acute bronchiolitis. The detected HAdV types were C1, C2, C5, B3, and B7. Clusters of HAdV C1, C2, and C5 were observed with high posterior probability. All patients infected with HAdV C5 and 50% of patients infected with HAdV C2 or B7 were admitted to the intensive care unit (ICU). Co-infection with other viruses was detected in 44.4% of patients. The most common co-infecting virus was rhinovirus (HRV). HAdV/HRV co-infection was detected in two children who presumably developed disseminated HAdV infection and died. This is the first report describing the circulation of HAdV types associated with severe respiratory outcomes in Kuwait. These findings highlight the need for a national surveillance system to monitor changes in predominant HAdV types and increased numbers of severe respiratory infections.

Background
Influenza A(H1N1)pdm09 virus infections often manifest severe respiratory symptoms, particularly in patients with a past history of allergic disease. Most of these findings were reported during the 2009 pandemic. The purpose of this study was to detail the clinical characteristics of influenza virus-induced lower respiratory infection (LRI) during the A(H1N1)pdm09–predominant 2015–2016 season.

Methods
We retrospectively reviewed the clinical characteristics of influenza-induced LRI cases in children admitted to a tertiary children’s hospital. Molecular diagnostic evaluation was performed on samples obtained from the most severe cases.

Results
We identified 66 patients with influenza-associated hospitalization and included 21 patients with influenza virus-induced LRI for analyses. Twelve patients (57%) were admitted to the pediatric intensive care unit, seven (33%) required mechanical ventilation, and three (14%) required extracorporeal membrane oxygenation. Plastic bronchitis (PB) was identified in six patients (29%), among whom a past medical history of asthma or food allergy were noted in all six patients. A past history of allergic disease was more common among patients with, than among those without, PB (p = 0.009). A(H1N1)pdm09 was detected from all the PB cases, and phylogenetic analyses of the hemagglutinin and neuraminidase genes demonstrated that this virus belonged to subclades 6B.1 and 6B.2. In the six PB cases, we found one patient with H275Y mutation in neuraminidase.

Conclusion
Allergic disease was a risk factor for developing PB due to influenza A(H1N1)pdm09 infection during the 2015–16 season.
Detection of enterovirus D68 in patients hospitalised in three tertiary university hospitals in Germany, 2013 to 2014


Summary
Enterovirus D68 (EV-D68) has been recognized as a worldwide emerging pathogen associated with severe respiratory symptoms since 2009. We here report EV-D68 detection in hospitalized patients with acute respiratory infection admitted to three tertiary hospitals in Germany between January 2013 and December 2014. From a total of 14,838 respiratory samples obtained during the study period, 246 (1.7%) tested enterovirus-positive and, among these, 39 (15.9%) were identified as EV-D68. Infection was observed in children and teenagers (0–19 years; n=31), the majority (n=22) being under five years-old, as well as in adults > 50 years of age (n=8). No significant difference in prevalence was observed between the 2013 and 2014 seasons. Phylogenetic analyses based on viral protein 1 (VP1) sequences showed co-circulation of different EV-D68 lineages in Germany. Sequence data encompassing the entire capsid region of the genome were analyzed to gain information on amino acid changes possibly relevant for immunogenicity and revealed mutations in two recently described pleconaril binding sites.

Detection of enterovirus D68 in patients hospitalised in three tertiary university hospitals in Germany, 2013 to 2014

Genotyping of human rhinovirus in adult patients with acute respiratory infections identified predominant infections of genotype A21


Scientific Reports. 2017;7(41601).

Summary
Human rhinovirus (HRV) is an important causative agent of acute respiratory tract infections (ARTIs). The roles of specific HRV genotypes in patients suffering from ARTIs have not been well established. We recruited 147 adult inpatients with community-acquired pneumonia (CAP) and 291 adult outpatients with upper ARTIs (URTIs). Respiratory pathogens were screened via PCR assays. HRV was detected in 42 patients, with 35 species A, five B and two C. Seventeen genotypes were identified, and HRV-A21 ranked the highest (9/42, 21.4%). The HRV-A21-positive infections were detected in four patients with CAP and in five with URTIs, all without co-infections. The HRV-A21 genome sequenced in this study contained 12 novel coding polymorphisms in viral protein (VP) 1, VP2 EF loop, VP3 knob and 3D regions. The infections of HRV-A21 virus obtained in this study could not be neutralized by antiserum of HRV-A21 prototype strain (VR-1131), indicating remarkable antigenic variation. Metagenomic analysis showed the HRV-A21 reads were dominant in bronchoalveolar lavage fluid of the three HRV-A21-positive patients with severe CAP, in which two dead. Our results highlight an unexpected infection of genotype HRV-A21 in the clinic, indicating the necessity of precise genotyping and surveillance of HRVs to improve the clinical management of ARTIs.
Summary
The Pneumonia Etiology Research for Child Health study was conducted across 7 diverse research sites and relied on standardized clinical and laboratory methods for the accurate and meaningful interpretation of pneumonia etiology data. Blood, respiratory specimens, and urine were collected from children aged 1–59 months hospitalized with severe or very severe pneumonia and community controls of the same age without severe pneumonia and were tested with an extensive array of laboratory diagnostic tests. A standardized testing algorithm and standard operating procedures were applied across all study sites. Site laboratories received uniform training, equipment, and reagents for core testing methods. Standardization was further assured by routine teleconferences, in-person meetings, site monitoring visits, and internal and external quality assurance testing. Targeted confirmatory testing and testing by specialized assays were done at a central reference laboratory.

The multiplex PCR FTD Respiratory pathogens 33 kit was used in the PERCH study, one of the largest pneumonia etiology studies ever undertaken. FTD Respiratory pathogens 33 simultaneously detects 11 bacteria, 1 fungus, and 21 viruses potentially involved in respiratory tract infections.

Objectives
The aim of the study was assessment of the usefulness of multiplex real-time PCR tests in the diagnostic and therapeutic process in children hospitalized due to pneumonia and burdened with comorbidities.

Methods
The study group included 97 children hospitalized due to pneumonia at the Karol Jonscher Teaching Hospital in Poznan, in whom multiplex real-time PCR tests (FTD Respiratory pathogens 33; Fast Track Diagnostics) were used.

Results
Positive test results of the test were achieved in 74 patients (76.3%). The average age in the group was 56 months. Viruses were detected in 61 samples (82% of all positive results); bacterial factors were found in 29 samples (39% of all positive results). The presence of comorbidities was established in 90 children (92.78%). On the basis of the obtained results, 5 groups of patients were established: viral etiology of infection, 34 patients; bacterial etiology, 7 patients; mixed etiology, 23 patients; pneumocystis, 9 patients; and no etiology diagnosed, 24 patients.

Conclusions
Our analysis demonstrated that the participation of viruses in causing severe lung infections is significant in children with comorbidities. Multiplex real-time PCR tests proved to be more useful in establishing the etiology of pneumonia in hospitalized children than the traditional microbiological examinations.

Evaluation of the performance of FTD Respiratory pathogens 33 in comparison to microbiological culture tests on throat and nasal swabs of children hospitalized due to pneumonia. FTD Respiratory pathogens 33 proved to be more useful in establishing the etiology of pneumonia in hospitalized children than the traditional microbiological examinations. FTD Respiratory pathogens 33 simultaneously detects 11 bacteria, 1 fungus, and 21 viruses potentially involved in respiratory tract infections.
Objectives
Viruses and atypical pathogens can cause significant respiratory illness in immunocompromised patients. Multiplex polymerase chain reaction (MPCR) has improved the diagnostic yield of pathogens, and it is easier to identify the co-infections also. The present study was done to evaluate the performance of MPCR on bronchoalveolar lavage (BAL) samples in immunocompromised patients.

Methods
A total of 177 BAL specimens collected over a 19 months period from immunocompromised patients with respiratory illness were analyzed with the MPCR and aerobic culture. Patients were divided into four according to the pathogens. Category V (only viral), Category NV (nonviral, i.e., bacteria and atypical), Category M (mixed, i.e., both viral and nonviral pathogen), and Category UK (unknown etiology).

Results
MPCR identified the causative pathogen in 59.3% of patients while culture could identify only in 37.8% of patients. Most frequent etiological agent was Klebsiella pneumoniae (32%), followed by cytomegalovirus (21%), and Pneumocystis jirovecii (10%). Numbers of patients in each category were Category V (9.6%), Category NV (43.5%), Category M (19.8%), and Category UK (27.1%). Mortality was significantly higher in patients of Category M having mixed infections.

Conclusion
MPCR is highly sensitive and rapid tool which can be considered in the routine diagnostic algorithm of respiratory illness in immunocompromised patients.
With Fast Track Diagnostics
You’re Ready for Winter!

Find out more at
www.fast-trackdiagnostics.com