
Clinical Evaluation of a Multi-parameter Customized Respiratory Taqman® Array Card Compared to Conventional Methods in Immunocompromised Patients
Syndromic approach
Why syndromic approach?


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• The same clinical symptoms can be the result of different infecting etiologic agents
• Infections in infants, the elderly and the immunocompromised host can present differently than in an otherwise healthy individual
• Under-diagnosis of co-infections
• Unnecessary medical procedures
• Positive impact on growing problem of antibiotic resistance
• Clinicians are able to reassure anxious parents
• Assist the public health authorities in investigating outbreaks
• Cost effective

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Commercial

MP PRODUCTS

Seegene

GeneFinder

Sacace BIOTECHNOLOGIES

BD

fast-track DIAGNOSTICS
TRUE POSITIVES. TRUE NEGATIVES.

Luminex

RANDOX

curetis

FilmArray®
The fastest way to better results.

Zettia GENOMICA

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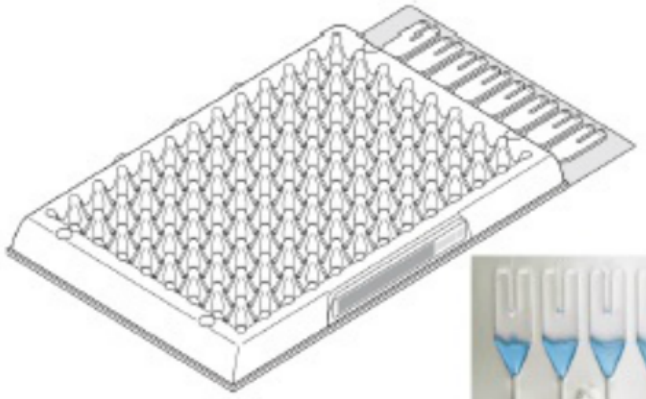
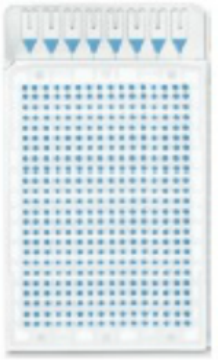
In house





Taqman Array Card Technology

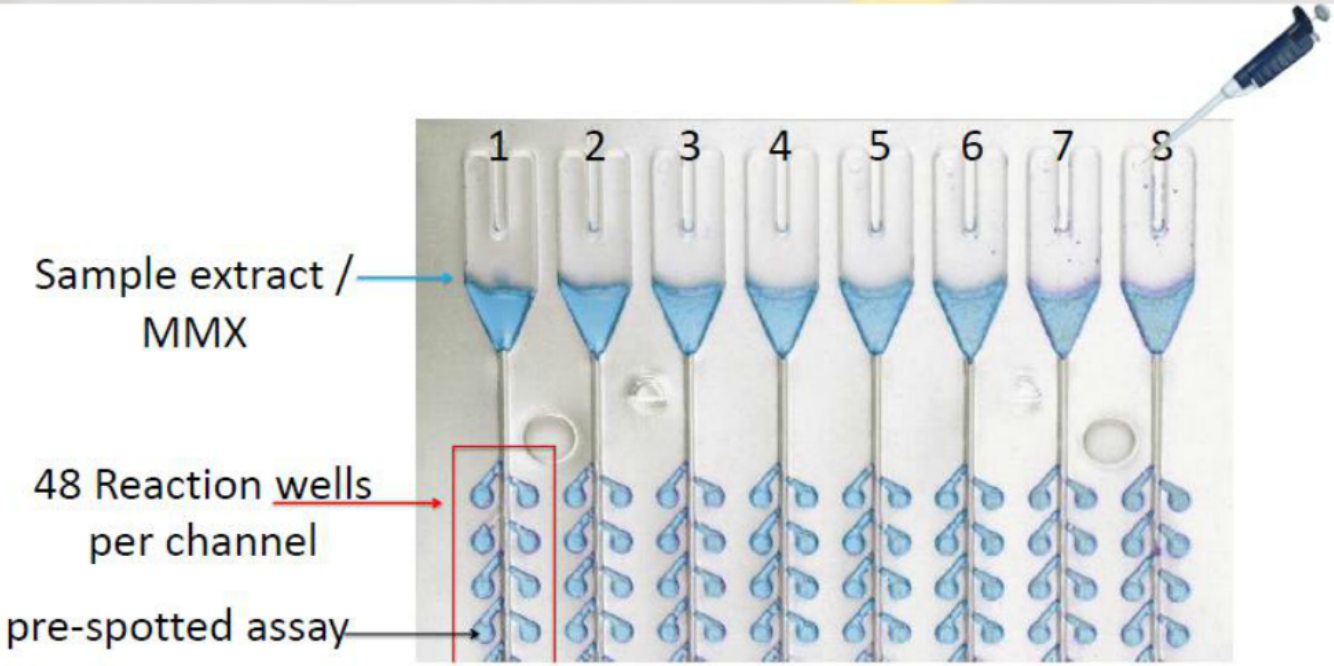
Advantages

- completely custom-made => great flexibility (choice of pathogens, choice and number of gene targets per pathogen to be included on the card)
- spatial separation of the 48 reaction wells => easily changed without the need for extensive re-optimisation and validation of a highly parallel multiplex assay
- real-time PCR => semi-quantitative Ct-value for each separated target
- workflow: simple and easy
- TAT: 1-2h
- cost per test: relatively low

Microfluidic technology

Principle

TaqMan® Array Card

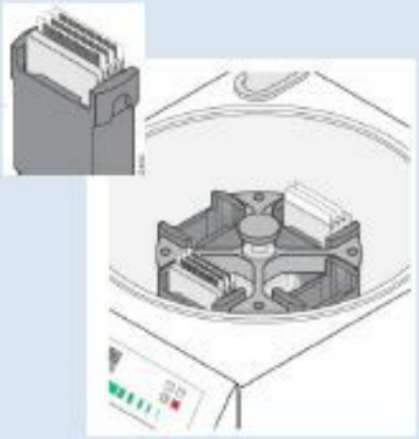


1well = 1µl reaction volume = 1 Real Time PCR reaction

Fill the TAC

- 78 µl extract
- 26 µl TaqMan® Fast Virus 1-Step Master Mix

Centrifugation



- 2 x 2min 1200xg

Sealing

Trim the fill strip

Amplification

- 52 min run time

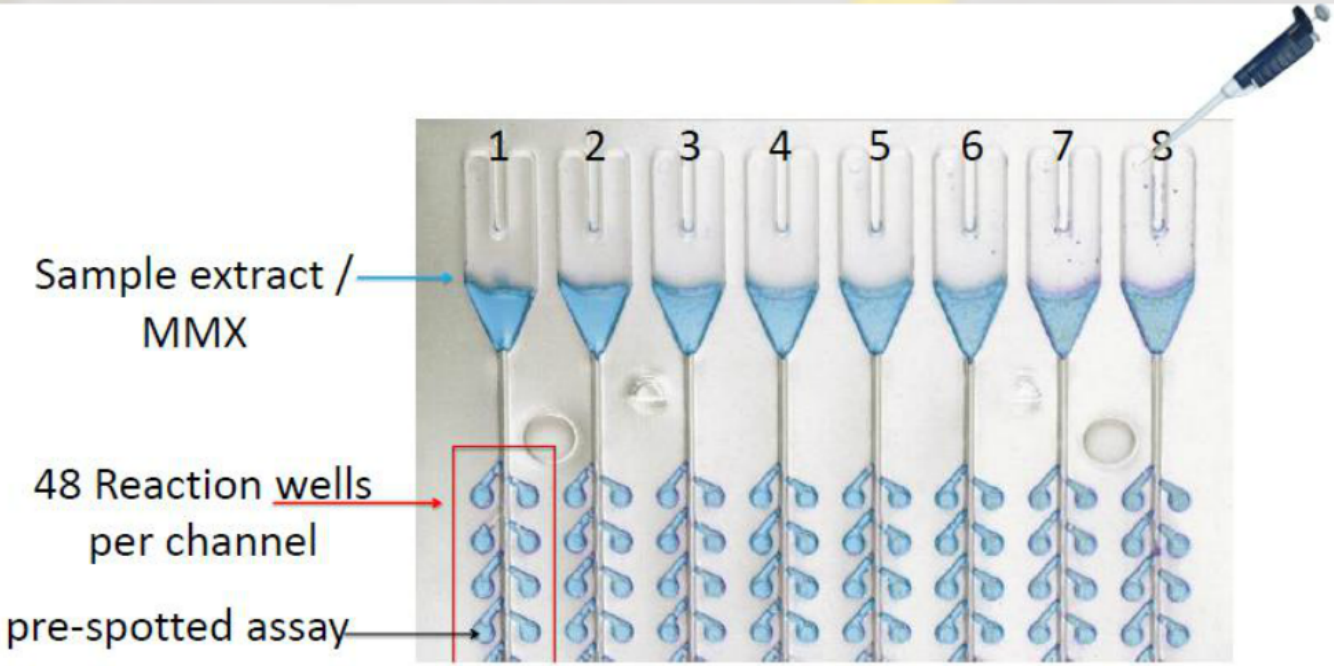
Data analysis

- 10 min

Microfluidic technology

Principle

TaqMan® Array Card



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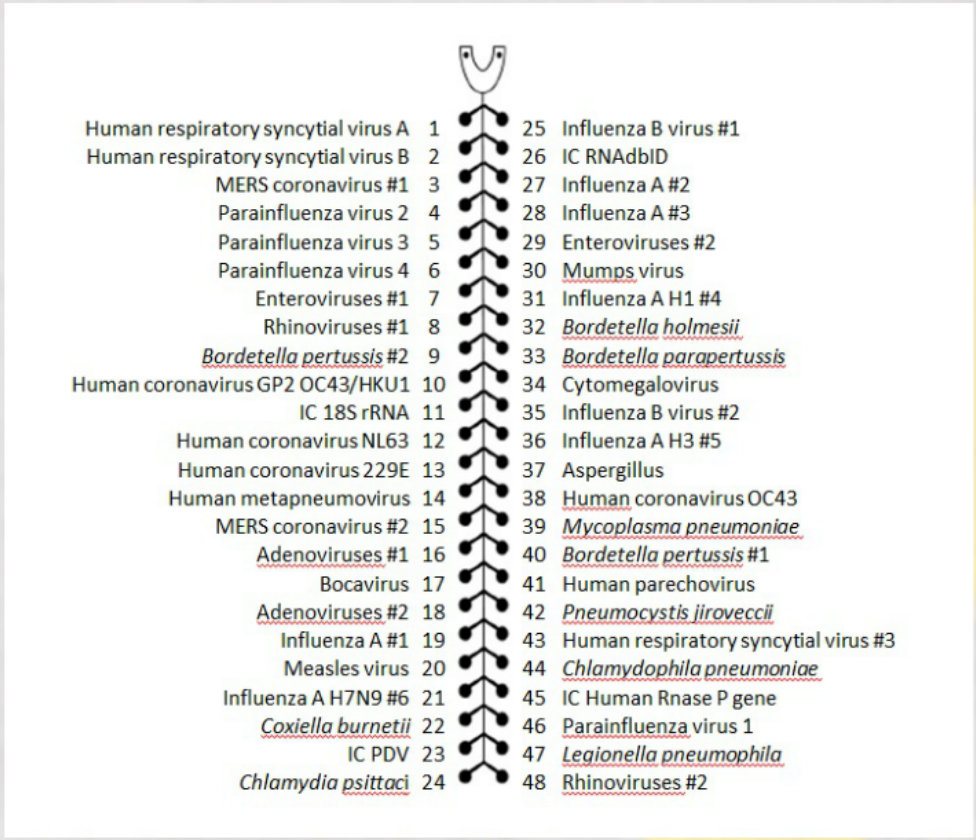
Study n°1

Respi TAC v7: developed and validated by Cambridge and Brugge – not published



	Viruses
1	Rhinoviruses #1
2	Rhinoviruses #2
3	Enteroviruses #1
4	Enteroviruses #2
5	Influenza A CDC DC
6	Influenza A H1 2009 ABI #1
7	Influenza A H3 seasonal CFI
8	Influenza A H7N9
9	Influenza A Quad AM2
10	Influenza A Quad Y
11	Influenza B Bruges
12	Influenza B Quad
13	Human respiratory syncytial virus A
14	Human respiratory syncytial virus B
15	Human respiratory syncytial virus
16	Parainfluenza 1
17	Parainfluenza 2
18	Parainfluenza 3
19	Parainfluenza 4

	Viruses
20	Human adenovirus (all types)
21	Human adenovirus (all types)
22	Human metapneumoviruses
23	Human parechovirus
24	Bocavirus
25	Cytomegalovirus
26	Human coronavirus 229E
27	Human coronavirus GP2 OC43/HKU1
28	Human coronavirus NL63
29	Human coronavirus OC43
30	Measles virus
31	Mumps virus
32	MERS CoV ABI (2)
33	MERS CoV ORF 1b
34	MERS CoV cam

34 pathogens

	Bacteria
35	*Bordetella holmesii IS 1001*
36	*Bordetella parapertussis IS 1001*
37	*Bordetella pertussis IS481*
38	*Bordetella pertussis ptxS1*
39	*Mycoplasma pneumoniae*
40	*Chlamydophila pneumoniae*
41	*Legionella pneumophila*
42	*Coxiella burnetii*
43	*Chlamydophila psittaci*
	Fungi
44	*Aspergillus fumigatus 28S*
45	*Pneumocystis jiroveci*
	Controls
46	18S
47	PDV control
48	Human Rnase P gene

Human respiratory syncytial virus A	1	25	Influenza B virus #1
Human respiratory syncytial virus B	2	26	IC RNAdbID
MERS coronavirus #1	3	27	Influenza A #2
Parainfluenza virus 2	4	28	Influenza A #3
Parainfluenza virus 3	5	29	Enteroviruses #2
Parainfluenza virus 4	6	30	Mumps virus
Enteroviruses #1	7	31	Influenza A H1 #4
Rhinoviruses #1	8	32	*Bordetella holmesii*
Bordetella pertussis #2	9	33	*Bordetella parapertussis*
Human coronavirus GP2 OC43/HKU1	10	34	Cytomegalovirus
IC 18S rRNA	11	35	Influenza B virus #2
Human coronavirus NL63	12	36	Influenza A H3 #5
Human coronavirus 229E	13	37	Aspergillus
Human metapneumovirus	14	38	Human coronavirus OC43
MERS coronavirus #2	15	39	*Mycoplasma pneumoniae*
Adenoviruses #1	16	40	*Bordetella pertussis* #1
Bocavirus	17	41	Human parechovirus
Adenoviruses #2	18	42	*Pneumocystis jiroveccii*
Influenza A #1	19	43	Human respiratory syncytial virus #3
Measles virus	20	44	*Chlamydophila pneumoniae*
Influenza A H7N9 #6	21	45	IC Human Rnase P gene
Coxiella burnetii	22	46	Parainfluenza virus 1
IC PDV	23	47	*Legionella pneumophila*
Chlamydia psittaci	24	48	Rhinoviruses #2

	Viru
1	Rhin
2	Rhin
3	Ente
4	Ente
5	Influ
6	Influ
7	Influ
8	Influ
9	Influ
10	Influ
11	Influ
12	Influ
13	Hum
14	Hum
15	Hum
16	Para
17	Para
18	Para
19	Para

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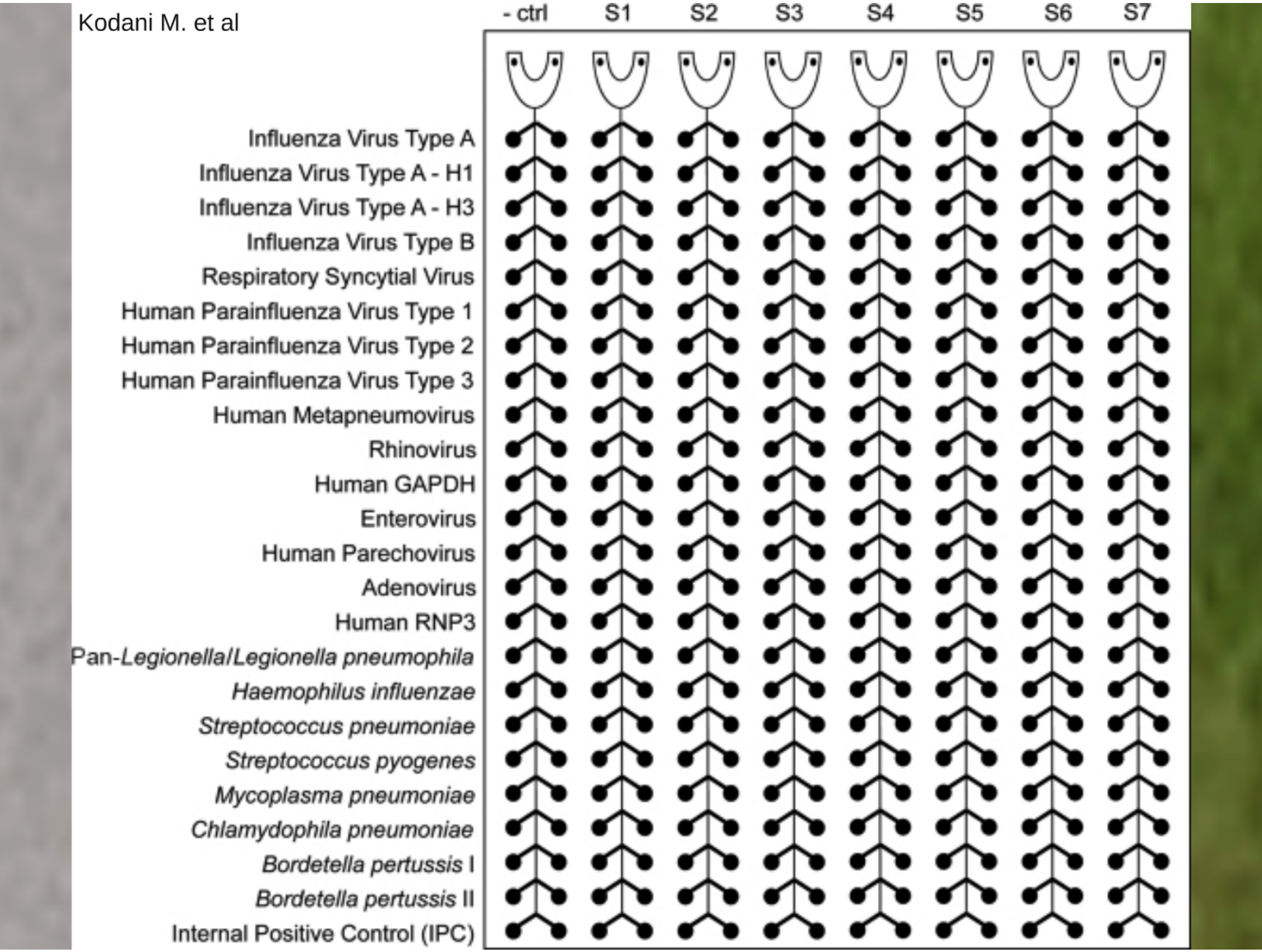
Kodani M. et al



Objectives

To characterize the performance of the TAC assay (premarket version Cambridge-Brugge – not published) on BAL and NTS samples in the immunocompromised host population in comparison to standard clinical testing for respiratory viruses (Erasme).

Materials & methods

Patients and samples.

- between December 2014 and April 2015
- 120 adult immunocompromised patients
- symptoms of an upper or lower respiratory tract infection
- electronic medical records were reviewed for clinical details
- after conventional testing, the samples were aliquoted and stored at -80°C until study testing
- approved by the ethical committee of the Erasme hospital

Conventional testing.

- DFA respiratory virus tests: Influenza A and B viruses, adenovirus, respiratory syncytial virus (RSV), parainfluenza viruses 1, 2, and 3 (PIV1, -2, -3) and human metapneumovirus (hMPV)
- rapid viral culture (Shell vial LLC-MK2) for adenovirus, parainfluenza, RSV and Influenza A/B
- conventional viral culture (A549 and MRC-5) for BAL samples

Nucleic acid extraction.

DSP viral pathogen midi kit on QiaSymphony

TAC testing.

78µL of nucleic acid extract + 26µL of Taqman Fast Virus 1-step mastermix

Verification PCR testing.

discordance => further verification testing using validated and accredited real-time PCR assays
also performed for non-viral pathogens detected by the TAC assay

Statistical analysis.

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Statistical analysis.

Results (1)

Patients and samples.

Conventional testing (A).

- 27/143 samples with one pathogen each (18.9%)

TAC testing (B).

- 77 samples with one or more viral respiratory pathogens (53.8%)
- + 13 samples with *Pneumocytis jirovecii*, 2 samples with *Aspergillus* species and 1 sample each with *Bordetella parapertussis*, *Mycoplasma pneumoniae* and *Legionella pneumophila*.
- Co-infection rate of 11.9% (viral + non-viral pathogens)

=> The TAC assay was significantly more likely to detect a respiratory virus than routine conventional testing (McNemar P <0.0001)

=> When TAC assay results for viruses that could not be detected by conventional testing (coronavirus, rhinovirus, CMV in NTS samples) (n= 18) and conventional testing results for HSV (n= 1) that could not be detected by TAC testing were excluded from analysis, the difference in diagnostic performance was still significant (P <0.0001).

Table 1: Baseline characteristics of 120 patients from whom respiratory samples were collected

Characteristic	Value
Median age, yrs (range)	58.5 (22, 94)
No. (%) male	64 (53.3)
No. (%) with underlying condition	
– solid organ transplantation	59 (49.2)
– solid malignancy	26 (21.7)
– hematological malignancy	21 (17.5)
– other underlying disease needing long-term corticosteroids therapy or immunosuppressive therapy	12 (10.0)
– HIV CD4 < 200/mm3	2 (1.7)
No. (%) with type of solid organ transplantation	
– lung transplant	23 (19.2)
– kidney transplant	19 (15.8)
– liver transplant	7 (5.8)
– heart transplant	7 (5.8)
– combined transplant*	3 (2.5)
* lung + kidney, lung + heart, kidney + liver	

Table 2: Clinical characteristics of the 143 respiratory samples collected for clinical indications

Sample characteristic	No. (%) of samples (c)
Type of sample	
NTS (a)	108 (75.5)
BAL (b)	35 (24.5)
Clinical indication for test	
upper respiratory tract infection	29
lower respiratory tract infection	93

(a) NTS, nose-throat swab
(b) BAL, bronchoalveolar lavage
(c) Samples obtained from 120 patients, of whom 22 patients with more than one sample:

- 20 patients with NTS and BAL for the same clinical indication
- One patient with one NTS during first respiratory episode, 1 month later NTS and BAL for the same clinical indication
- One patient with NTS and BAL for different clinical indications

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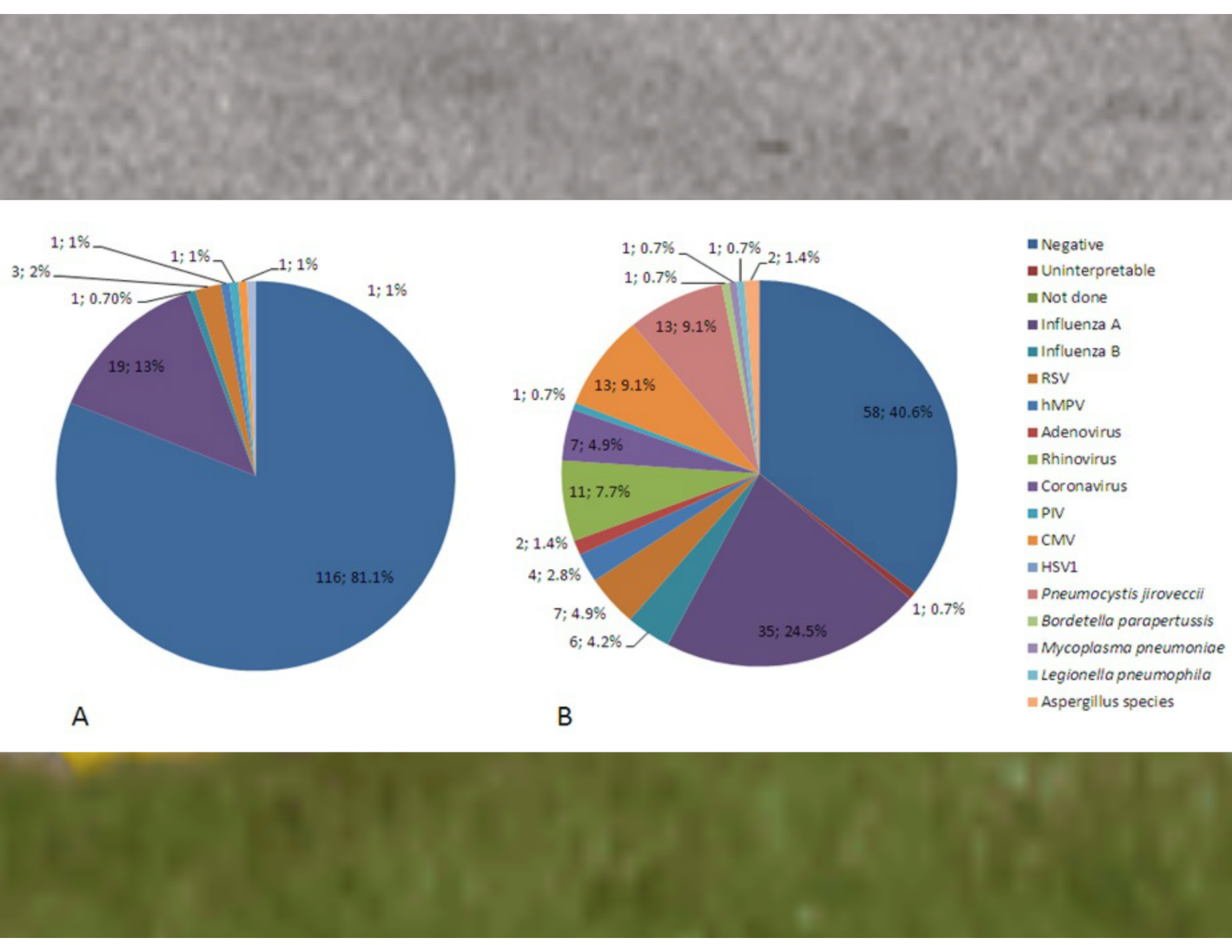
1; 1%
1; 1%
1; 1%
3; 2%
1; 0.70%
1; 1%
19; 13%
116; 81.1%
A
1; 0.7%
1; 0.7%
2; 1.4%
1; 0.7%
13; 9.1%
13; 9.1%
1; 0.7%
58; 40.6%
7; 4.9%
11; 7.7%
2; 1.4%
4; 2.8%
7; 4.9%
6; 4.2%
35; 24.5%
1; 0.7%
B
Negative
Uninterpretable
Not done
Influenza A
Influenza B
RSV
hMPV
Adenovirus
Rhinovirus
Coronavirus
PIV
CMV
HSV1
Pneumocystis jiroveccii
Bordetella parapertussis
Mycoplasma pneumoniae
Legionella pneumophila
Aspergillus species

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Results (2)

Verification PCR testing.
58 samples on which the two techniques disagreed for viral pathogens
viral disease
present in 75 samples (52.4%)
absent in 68 samples (47.6%)

cPPV not significantly different (P =0.25)
cNPV TAC assay (96.7%) >>> routine conventional testing (57.8%) (P <0.0001)

11/13 samples positive for *P. jirovecii* confirmed => median cycle threshold 34 for the 11 P. jiroveccii confirmed by verification testing versus 28
2/2 Aspergillus species, 1/1 Mycoplasma pneumoniae and 1/1 Bordetella parapertussis
0/1 Legionella pneumophila => detection limit of the PCR assay on the TAC card (CT 32)

Coupled samples
21 patients NTS + BAL during the same episode of respiratory tract infection symptoms:
9/21: NTS + BAL negative by conventional and TAC testing
10/21: same viral pathogen both in the NTS as in the BAL sample with TAC testing, compared to only 1 patient by conventional testing
2/21: one positive for influenza B virus only in the BAL sample, and one for coronavirus solely in the NTS

Table 4: Results from conventional, TAC and verification testing for samples with discordant results

No. of samples (a)	routine testing result	TAC result	verification result
2	negative	Adenovirus	Adenovirus
11	negative	CMV	CMV
1	negative	CMV	negative
7	negative	Coronavirus (b)	Coronavirus (b)
3	negative	hMPV	hMPV
15	negative	Influenza A	Influenza A
1	HSV1	negative (c)	HSV1
3	negative	Influenza A (d)	negative
1	Influenza A (e)	negative	Influenza A (e)
1	Influenza A (f)	negative	negative
4	negative	Influenza B	Influenza B
1	negative	Influenza B (g)	negative
9	negative	Rhinovirus	Rhinovirus
1	negative	Rhinovirus (h)	negative
4	negative	RSV	RSV

(a) n= 64 (58 samples in total, 6 samples with more than one discordant result)

(b) Coronavirus OC43 (n= 2), Coronavirus 229E (n= 4), Coronavirus NL63 (n= 1)

(c) HSV1 targets not included in TAC assay

(d) two samples with only 1/6 and one sample with 3/6 targets for influenza A weakly positive (Ct-value >30)

(e) only viral culture positive, verification PCR very weakly positive (Ct-value >36)

(f) false positive DFA

(g) only 1/2 targets weakly positive (Ct-value >30)

(h) 2/2 targets for rhinovirus weakly positive (Ct-value >30)

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(h) 2/2 targets for rhinovirus weakly positive (Ct-value >30)

Table 5: Calculated performance characteristics for TAC and conventional testing

	DFA + viral culture	TAC	*P* value
% cSens (95% CI)	34.67 (24.05, 46.54)	97.33 (90.68, 99.60)	<0.0001
% cSpec (95% CI)	98.53 (92.05, 99.75)	91.18 (81.77, 96.67)	0.9703
% cPPV (95% CI)	96.30 (80.97, 99.38)	92.41 (84.19, 97.14)	0.2485
% cNPV (95% CI)	57.76 (48.24, 66.87)	96.88 (89.14, 99.53)	<0.0001

cSens, calculated sensitivity ; cSpec, calculated specificity ; cPPV, calculated positive predictive value ; cNPV, calculated negative predictive value

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Discussion

- practical real-life assessment of the performance of the custom TAC assay in a population for whom rapid and accurate diagnosis of viral pathogens is crucial for appropriate clinical management
- relatively high overall positivity rate (52.4%)
- co-infection rate: 5.6% of samples with more than one viral pathogen, and 11.9% if all included pathogens were considered
- positive molecular assay on a respiratory sample may indicate viral infection, benign (and asymptomatic) colonization, or contamination
  CHALLENGE => develop algorithms to determine which pathogens are primarily responsible for disease, which pathogens can work synergistically to evoke disease, and which pathogens represent carriage
- useful applications to various syndromes beyond respiratory infections, such as diarrhea, sepsis, and meningitis/encephalitis, where a variety of pathogens could be causing similar symptoms


Optimize patient management!


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AKNOWLEDGEMENTS

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- Sylvia Betermiez
- Philippe Gilot
- Sarah Fortems
- Nadia De Schrijver
- Amélie Decuypere

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- Marie-Hélène Jurion
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