

Validation

FTD Respiratory pathogens 33



32 reactions (catalog no. FTD-2P.3-32)



64 reactions (catalog no. FTD-2P.3-64)

Qualitative assay for *in vitro* diagnostics

Validated with:

Applied Biosystems® 7500 (Thermo Fisher Scientific)
Fast-track mastermix (Fast Track Diagnostics)
NucliSENS® easyMAG® (bioMérieux)

CE₀₁₂₃

IVD

REF

FTD-2P.3-32, FTD-2P.3-64



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Validation

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The following data demonstrate that FTD Respiratory pathogens 33 (FTD Resp33) is suitable for its intended purpose and performs according to pre-determined criteria.

The validation file describes specificity, analytical sensitivity, precision (intra- and inter-assays), clinical evaluation studies, QCMD panels, Instand e. V. panels and stability tests.

Principle of the test:

FTD Resp33 is an *in vitro* test for the detection of viral, fungal and bacterial nucleic acid from respiratory samples (throat/nasal/nasopharyngeal swabs, bronchoalveolar lavage and sputum) of human origin as an aid in the evaluation of infections caused by influenza A, influenza B, influenza C, influenza A (H1N1)swl; parainfluenza viruses 1, 2, 3 and 4; coronaviruses NL63, 229E, OC43 and HKU1; human metapneumoviruses A and B; rhinovirus; respiratory syncytial viruses A and B ; adenovirus; enterovirus; parechovirus; bocavirus; *Pneumocystis jirovecii*; *Mycoplasma pneumoniae*; *Chlamydia pneumoniae*; *Streptococcus pneumoniae*; *Haemophilus influenzae* and *Haemophilus influenzae* type B; *Staphylococcus aureus*; *Moraxella catarrhalis*; *Bordetella* spp.*; *Klebsiella pneumoniae*; *Legionella pneumophila* | *Legionella longbeachae*; *Salmonella* spp. including internal control. *except *Bordetella parapertussis*



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Table 1: Table of contents: PP = primer and probe, IC = internal control, PC = positive control, NC = negative control.

	contents	FTD-2P.3-32	FTD-2P.3-64
FluRhino PP	Primer/probe mix for FLUA, FLUB, H1N1 & RV	1 x 48 µl	2 x 48 µl
COR PP	Primer/probe mix for Cor43, 63, 229 & HKU1	1 x 48 µl	2 x 48 µl
ParaEAV PP	Primer/probe mix for HPIV2-4 & IC (EAV)	1 x 48 µl	2 x 48 µl
BoMpPf1 PP	Primer/probe mix for HBoV, Mpneu, HPIV1 & HMPVA/B	1 x 48 µl	2 x 48 µl
RsEPA PP	Primer/probe mix for HRSVA & B, HAdV, EV & HPeV	1 x 48 µl	2 x 48 µl
RespBac PP	Primer/probe mix for Saur, Spneu, Cpneu & HIB	1 x 48 µl	2 x 48 µl
KLePSa PP	Primer/probe mix for Kpneu, Lpneu/Llong, PCP & Salm	1 x 48 µl	2 x 48 µl
MoBoCH PP	Primer/probe mix for Morax, Bord, FLUC & Haeinf	1 x 48 µl	2 x 48 µl
Resp21 PC	Positive control: plasmid pool for FLUA, FLUB, H1N1, RV, Cor63, Cor229, Cor43, HKU, HPIV1-4, HMPV, HBoV, Mpneu, HRSV, HAdV, EV & HPeV	1 x 750 µl	2 x 750 µl
Resp33 PC2	Positive control: plasmid pool for use with PPMixes 6-8. This pool includes Saur, Spneu, Cpneu, HIB, Kpneu, Lpneu/Llong, PCP, Salm, Morax, Bord, FLUC and Haeinf.	1 x 450 µl	2 x 450 µl
NC	Negative control	1 x 4000 µl	1 x 8000 µl
IC	Internal control	1 x 128 µl	2 x 128 µl
Enzyme	25x RT-PCR Enzyme mix (Fast-track mastermix)	1 x 256 µl	2 x 256 µl
Buffer	2x RT-PCR Buffer (Fast-track mastermix)	2 x 1600 µl	4 x 1600 µl

(PP= primer and probe, PC= positive control, IC= Internal control, NC= negative control, FLUA= influenza A, H1N1= influenza A (H1N1)swl, FLUB= influenza B, FLUC= influenza C, HRV= rhinovirus, Cor63= coronavirus NL63, Cor229= coronavirus 229E, Cor43= coronavirus OC43, HKU= coronavirus HKU1, HPIV2= parainfluenza virus 2, HPIV3= parainfluenza virus 3, HPIV4= parainfluenza virus 4, HRV= rhinovirus, HRSV= respiratory syncytial viruses A and B, HAdV= adenovirus, EV= enterovirus, HPeV= parechovirus, HMPV= human metapneumovirus A and B, HPIV1= parainfluenza virus 1, HBoV= bocavirus, Mpneu= *Mycoplasma pneumoniae*, Cpneu= *Chlamydia pneumoniae*, Spneu= *Streptococcus pneumoniae*, Saur= *Staphylococcus aureus*, HIB= *Haemophilus influenzae* B, PCP= *Pneumocystis jirovecii*, Morax= *Moraxella catarrhalis*, Kpneu= *Klebsiella pneumoniae*, Haeinf= *Haemophilus influenzae*, Lpneu/Llong= *Legionella pneumophila*/ *Legionella longbeachae*, Salm= *Salmonella* spp.)



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1. Specificity

The BLAST (www.ncbi.nlm.nih.gov/blast) search shows that the selected primers and probes detect only the corresponding viruses, fungi and bacteria, hence the primers and probes of the different pathogens are not cross-reacting.

There are 3 exceptional cases:

1. The primers and probe for the detection of HRV also detect EV, but not the other way around. If you get a positive signal for HRV and EV the patient is EV positive. If you just detect HRV the patient is HRV positive.
2. The *Bordetella* spp. assay detects *B. pertussis* (100 %) but also target other *Bordetella* species like *B. holmesii* (99-98 %) and some strains of *B. bronchiseptica* (99-96 %) which are also associated with respiratory infections in humans. The assay does not detect *B. parapertussis*.
3. The *K. pneumoniae* assay also detects *Klebsiella variicola* (98 %) which is not important as a human pathogen.

The specificity was validated with various negative samples tested by a reference method. The negative samples did not generate any positive signals with the FTD Resp33.

77 clinical samples containing bacteria and viruses were evaluated with FTD Resp33. No other pathogens than expected were positive with FTD Resp33 (Tables 2 and 3).



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Table 2: Specificity panel of different viruses tested with FTD Resp33.

Pathogens	Amount	FTD Resp33
Adenovirus	4	positive
Astrovirus	1	negative
Coronavirus 229	1	positive
Coronavirus 43	1	positive
Coronavirus 63	1	positive
Cytomegalovirus	1	negative
Enterovirus	2	positive
Epstein-Barr virus	1	negative
Herpes simplex virus1	3	negative
Herpes simplex virus2	3	negative
Human herpes virus 6	1	negative
Human herpes virus 7	1	negative
Human metapneumovirus	1	positive
Influenza A virus	1	positive
Influenza B virus	1	positive
Measles virus	1	negative
Mumps virus	1	negative
Norovirus G1	1	negative
Norovirus G2	1	negative
Parainfluenzavirus 1	1	positive
Parainfluenzavirus 2	1	positive
Parainfluenzavirus 3	1	positive
Parainfluenzavirus 4	1	positive
Parvovirus B19	1	negative
Polyomavirus 1 (BKV)	1	negative
Respiratory syncytial virus A	1	positive
Respiratory syncytial virus B	1	positive
Rhinovirus	1	positive
Rotavirus	1	negative
Sapovirus	1	negative
Varicella-zoster virus	2	negative



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Table 3: Specificity panel of different bacteria tested with FTD Resp33.

Pathogens	Amount	FTD Resp33
<i>Aeromonas hydrophilia</i>	1	negative
<i>Bacillus ssp.</i>	1	negative
<i>Bifidobacterium</i>	1	negative
<i>Bordetella pertussis</i>	1	positive
<i>Campylobacter jejuni</i>	1	negative
<i>Campylobacter coli</i>	1	negative
<i>Chlamydia trachomatis</i>	1	negative
<i>Citrobacter freundii</i>	1	negative
<i>Clostridium difficile</i>	1	negative
<i>Clostridium perfringens</i>	1	negative
EHEC vtx+	1	negative
EIEC	1	negative
<i>Enterococcus faecalis</i>	1	negative
EPEC	1	negative
ETEC	1	negative
<i>Haemophilus influenzae</i>	1	positive
<i>Hafnia alvei</i>	1	negative
<i>Klebsiella oxytoca</i>	1	negative
<i>Klebsiella pneumoniae</i>	1	positive
<i>Legionella pneumophila</i>	1	positive
<i>Legionella ansia</i>	1	negative
<i>Listeria monocytogenes</i>	1	negative
<i>Moraxella catarrhalis</i>	1	positive
<i>Morganella morganii</i>	1	negative
<i>Mycoplasma genitalium</i>	1	negative
<i>Mycoplasma pneumoniae</i>	1	positive
<i>Neisseria gonorrhoe</i>	1	negative
<i>Proteus mirabilis</i>	1	negative
<i>Proteus vulgaris</i>	1	negative
<i>Rhodococcus equi</i>	1	negative
<i>Salmonella typhimurium</i>	1	positive
<i>Shigella boydii</i>	1	negative
<i>Staphylococcus aureus</i>	1	positive
<i>Streptococcus pneumoniae</i>	1	positive
<i>Treponema pallidum</i>	1	negative
<i>Vibrio cholerae</i>	1	negative
<i>Yersinia enterocolitica</i>	1	negative



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2. Analytical sensitivity

The analytical sensitivity was determined by testing a tenfold dilution series of plasmid DNA of known concentrations from 10^9 to 10^2 copies/ml. FTD Resp33 has been compared to singleplex assays.

FLUA, Cor43, *M. pneumoniae*, HMPV, HIB, and PCP showed a detection limit of 10^2 copies/ml, HKU1, HAdV, EV, HPeV, HPIV1-3, FLUB, H1N1, HBoV, Cor63, HRSV, *C. pneumoniae*, *S. aureus*, *S. pneumoniae*, *Salmonella* spp., *K. pneumoniae*, *M. catarrhalis*, *H. influenzae* of 10^3 copies/ml and Cor229, Lpneu, HPIV4, HRV, FLUC, *Bordetella* spp. of 10^4 copies/ml (tables 4a-d).

The comparison of FTD Resp33 to singleplex assays with plasmid dilution series showed no loss of sensitivity except for FLUA, Cor229, HPIV1, HAdV, EV/ HPeV, CMV, *Legionella* spp., *M. catarrhalis*, *Bordetella* spp. and HBoV where the singleplex detected one dilution step further. For HPIV2, Cor43, *S. aureus*, *S. pneumoniae*, *C. pneumoniae* and PCP FTD Resp33 detected even one dilution step further.

Table 4a: Analytical sensitivity evaluated with FTD Resp33 part I.

Plasmid	10^9 cop/ml	10^8 cop/ml	10^7 cop/ml	10^6 cop/ml	10^5 cop/ml	10^4 cop/ml	10^3 cop/ml	10^2 cop/ml
FLUA SP	14.2	17.7	20.7	25.4	28.7	32.3	35.6	37.2
FLUA RE33	15.3	18.4	21.7	26.0	29.2	33.7	34.2	38.2
FLUB SP	13.3	16.2	20.1	23.2	26.8	32.5	neg	neg
FLUB RE33	14.1	17.2	21.0	24.5	27.9	34.8	35.6	neg
H1N1 SP	16.3	19.3	22.4	25.8	29.4	32.5	37.2	neg
H1N1 RE33	16.5	19.5	22.6	25.9	29.4	33.0	37.7	neg
HRV SP	16.5	19.7	23.3	26.8	30.8	34.0	neg	neg
HRV RE33	17.1	20.2	23.9	27.4	31.5	34.1	neg	neg

(FLUA= influenza A, H1N1= influenza A (H1N1)swl, FLUB= influenza B, HRV= rhinovirus, SP= singleplex assay, cop/ml=copies/ml, neg=negative, RE33= FTD Resp33)



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Table 4b: Analytical sensitivity evaluated with FTD Resp33 part II.

Plasmid	10 ⁹ cop/ml	10 ⁸ cop/ml	10 ⁷ cop/ml	10 ⁶ cop/ml	10 ⁵ cop/ml	10 ⁴ cop/ml	10 ³ cop/ml	10 ² cop/ml
HKU1 SP	14.3	17.5	20.7	24.2	27.3	30.6	34.4	neg
HKU1 RE33	14.9	18.2	21.5	25.3	28.3	32.3	36.2	neg
Cor43 SP	16.2	19.4	22.1	26.2	28.9	31.7	38.2	neg
Cor43 RE33	16.5	19.6	22.3	26.6	30.1	33.1	35.2	37.5
Cor63 SP	17.2	20.0	22.5	24.7	29.0	31.7	36.0	neg
Cor63 RE33	17.6	20.5	23.0	25.2	29.7	32.7	35.8	neg
Cor229 SP	16.1	19.6	22.9	26.5	29.7	33.3	37.4	neg
Cor229 RE33	17.1	20.5	23.9	27.4	30.7	34.4	neg	neg
HPIV2 SP	16.6	19.1	23.5	26.6	29.9	33.6	36.3	neg
HPIV2 RE33	17.3	19.8	24.3	27.1	30.7	33.9	37.4	39.0
HPIV3 SP	17.0	19.5	23.1	27.0	29.9	32.6	35.9	neg
HPIV3 RE33	17.4	19.9	23.3	27.2	30.6	33.8	36.3	neg
HPIV4 SP	18.4	22.1	25.4	28.8	32.0	35.4	neg	neg
HPIV4 RE33	19.8	23.7	27.9	30.9	34.5	38.7	neg	neg
Mpneu SP	16.3	19.4	23.5	26.7	29.8	33.4	35.9	37.5
Mpneu RE33	17.0	19.9	23.5	26.8	30.2	33.7	37.0	38.9
HPIV1 SP	15.4	18.0	21.9	25.3	28.6	32.4	36.2	36.3
HPIV1 RE33	16.4	19.2	23.4	26.7	30.6	33.8	36.8	neg
HBoV SP	15.9	19.6	21.7	26.3	28.6	32.1	34.3	36.1
HBoV RE33	16.8	20.0	22.7	27.5	29.9	33.3	36.7	neg
HMPV SP	16.5	19.5	24.0	27.4	30.9	33.2	34.4	37.5
HMPV RE33	17.2	20.2	24.8	28.3	31.9	34.1	35.4	38.4

(HPIV2= parainfluenza virus 2, HPIV3= parainfluenza virus 3, HPIV4= parainfluenza virus 4, Cor63= coronavirus NL63, Cor229= coronavirus 229E, Cor43= coronavirus OC43, HKU1= coronavirus HKU1, HMPV= human metapneumovirus A and B, HPIV1= parainfluenza virus 1, HBoV= bocavirus, Mpneu= *Mycoplasma pneumoniae*, SP= singleplex assay, cop/ml=copies/ml, neg=negative, RE33= FTD Resp33)



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Table 4c: Analytical sensitivity evaluated with FTD Resp33 part III.

Plasmid	10 ⁹ cop/ml	10 ⁸ cop/ml	10 ⁷ cop/ml	10 ⁶ cop/ml	10 ⁵ cop/ml	10 ⁴ cop/ml	10 ³ cop/ml	10 ² cop/ml
HRSV SP	15.1	15.5	21.7	25.0	29.3	36.0	neg	neg
HRSV RE33	16.0	18.9	22.1	25.2	28.9	32.8	36.9	neg
HAdV SP	16.8	18.4	21.6	25.0	29.2	33.6	neg	neg
HAdV RE33	17.1	18.8	22.4	26.2	30.0	32.9	36.0	neg
EV SP	15.4	18.1	21.5	24.9	28.2	31.6	34.6	neg
EV RE33	15.9	18.9	22.2	25.7	29.0	32.4	37.1	neg
HPeV SP	14.9	18.2	21.6	25.0	28.3	32.1	34.3	37.5
HPeV RE33	15.3	18.7	22.1	25.6	28.8	30.9	35.1	neg
HIB SP	13.6	16.7	20.1	23.0	26.6	29.9	33.0	36.5
HIB RE33	13.8	16.8	20.2	23.2	26.7	30.2	33.4	36.5
Spneu SP	21.6	26.1	29.1	32.7	35.6	38.0	neg	neg
Spneu RE33	16.5	19.9	23.0	26.7	30.2	35.7	38.3	neg
Cpneu SP	17.5	20.4	23.6	27.1	33.7	38.1	neg	neg
Cpneu RE33	17.6	20.5	23.7	27.2	30.0	34.3	37.4	neg
Saur SP	18.6	22.7	25.7	29.2	32.5	35.4	neg	neg
Saur RE33	16.8	20.4	23.7	27.4	31.1	34.8	38.4	neg

(HRSV= respiratory syncytial viruses A and B, HAdV= adenovirus, EV= enterovirus, HPeV= parechovirus, Cpneu= *Chlamydia pneumoniae*, Spneu= *Streptococcus pneumoniae*, Saur= *Staphylococcus aureus*, HIB= *Haemophilus influenzae* B, SP= singleplex assay, cop/ml=copies/ml, neg=negative, RE33= FTD Resp33)



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Table 4d: Analytical sensitivity evaluated with FTD Resp33 part IV.

Plasmid	10 ⁹ cop/ml	10 ⁸ cop/ml	10 ⁷ cop/ml	10 ⁶ cop/ml	10 ⁵ cop/ml	10 ⁴ cop/ml	10 ³ cop/ml	10 ² cop/ml
Salm SP	17.8	21.5	24.7	27.7	31.7	35	neg	neg
Salm RE33	18.2	22.5	25.6	29	32.1	33.9	37.8	neg
PCP SP	15	18.3	21.5	24.6	27.9	31.3	35.8	35.8
PCP RE33	14.7	17.8	20.8	24.4	27	30.1	34.3	35.7
Kpneu SP	14.3	17.3	20.4	24	27.1	30.6	36	neg
Kpneu RE33	14.4	17.2	20.5	23.7	26.9	30.5	33.6	neg
Lpneu SP	18.8	22.6	24.9	28.5	32.1	neg	neg	neg
Lpneu RE33	19.1	22.8	25.7	29	30.8	35	neg	neg
Llong SP	19.1	22.4	25.9	29.2	32.5	35.7	neg	neg
Llong RE33	18.6	22.4	25.5	28.6	31.9	neg	neg	neg
Morax SP	14.8	18.0	21.5	24.8	27.9	31.4	34.2	35.2
Morax RE33	14.7	17.6	20.9	24.5	27.9	30.9	36.4	neg
FLUC SP	17.6	20.9	24.7	28.2	31.6	35.0	neg	neg
FLUC RE33	17.1	20.9	24.1	27.4	30.1	34.2	neg	neg
Bord SP	14.2	17.5	20.7	24.1	27.6	31.1	35.7	neg
Bord RE33	14.0	17.1	20.4	23.6	27.2	31.0	neg	neg
Haeinf SP	13.7	16.7	19.8	23.9	27.4	30.0	32.8	neg
Haeinf RE33	13.7	16.5	19.9	23.8	26.8	29.6	32.9	neg

(Salm= *Salmonella* spp., FLUC= influenza C, PCP= *Pneumocystis jirovecii*, Morax= *Moraxella catarrhalis*, Bord= *Bordetella* spp., Kpneu= *Klebsiella pneumoniae*, Lpneu/Llong= *Legionella pneumophila*/*Legionella longbeachae*, Haeinf= *Haemophilus influenzae*, SP= singleplex assay, cop/ml=copies/ml, neg=negative, RE33= FTD Resp33)



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Furthermore we tested for each pathogen 4 dilution steps of the tenfold plasmid dilution series of known concentration around the detection limit in 10 times measurements (tables 5a-c).

Table 5a: Analytical sensitivity of FTD Resp33 determined with 10 time measurements around the endpoint detection limit part I.

	Plasmid	10 ⁵ cop/ml		10 ⁴ cop/ml		10 ³ cop/ml		10 ² cop/ml	
RE33	FLUA	10/10	100%	10/10	100%	9/10	90%	5/10	50%
	Ct	26.6 ¹		29.6 ¹		32.6 ¹		36.8 ¹	
	FLUB	10/10	100%	10/10	100%	7/10	70%	0/10	0%
	Ct	28.0 ¹		30.5 ¹		34.2 ¹		neg	
	H1N1	10/10	100%	10/10	100%	9/10	90%	0/10	0%
	Ct	27.5 ¹		29.9 ¹		34.7 ¹		neg	
	HRV	10/10	100%	10/10	100%	10/10	100%	0/10	0%
	Ct	28.1 ¹		30.3 ¹		34.8 ¹		neg	
	HKU1	10/10	100%	10/10	100%	0/10	0%	0/10	0%
	Ct	28.7 ¹		32.6 ¹		neg		neg	
	Cor43	10/10	100%	10/10	100%	10/10	100%	7/10	70%
	Ct	28.5 ¹		31.9 ¹		34.9 ¹		35.8 ¹	
	Cor63	10/10	100%	10/10	100%	10/10	100%	0/10	0%
	Ct	29.7 ¹		32.8 ¹		37.1 ¹		neg	
	Cor229	10/10	100%	10/10	100%	9/10	90%	0/10	0%
	Ct	29.4 ¹		32.7 ¹		36.3 ¹		neg	
	HPIV2	10/10	100%	10/10	100%	10/10	100%	5/10	50%
	Ct	29.1 ¹		32.5 ¹		35.1 ¹		36.8 ¹	
HPIV3	10/10	100%	10/10	100%	10/10	100%	0/10	0%	
Ct	27.4 ¹		30.8 ¹		34.4 ¹		neg		
HPIV4	10/10	100%	10/10	100%	0/10	0%	0/10	0%	
Ct	33.0 ¹		36.1 ¹		neg		neg		

(FLUA= influenza A, H1N1= influenza A (H1N1)swl, FLUB= influenza B, HRV= Human Rhinovirus, Cor63= Coronavirus NL63, Cor229= Coronavirus 229E, Cor43= Coronavirus OC43, HKU1= Coronavirus HKU1, HPIV2= Human Parainfluenza virus 2, HPIV3= Human Parainfluenza virus 3, HPIV4= Human Parainfluenza virus 4, ¹ = Ct expressed as a mean of 10 reactions, cop/ml= copies/ml, PP= primer and probe mix, RE33= FTD Respiratory pathogens 33)



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Table 5b: Analytical sensitivity of FTD Resp33 determined with 10 time measurements around the endpoint detection limit part II.

	Plasmid	10 ⁵ cop/ml		10 ⁴ cop/ml		10 ³ cop/ml		10 ² cop/ml	
RE33	HRSV	10/10	100%	10/10	100%	0/10	0%	0/10	0%
	Ct	28.8 ¹		32.7 ¹		neg		neg	
	HAdV	10/10	100%	10/10	100%	0/10	0%	0/10	0%
	Ct	29.5 ¹		32.4 ¹		neg		neg	
	EV	10/10	100%	10/10	100%	7/10	70%	0/10	0%
	Ct	26.8 ¹		29.9 ¹		34.2 ¹		neg	
	HPeV	10/10	100%	10/10	100%	8/10	80%	0/10	0%
	Ct	26.2 ¹		29.4 ¹		34.7 ¹		neg	
	Mpneu	10/10	100%	10/10	100%	10/10	100%	7/10	70%
	Ct	28.3 ¹		31.7 ¹		34.6 ¹		36.2 ¹	
	HPIV1	10/10	100%	10/10	100%	10/10	100%	0/10	0%
	Ct	30.8 ¹		33.6 ¹		36.8 ¹		neg	
	HBoV	10/10	100%	10/10	100%	10/10	100%	0/10	0%
	Ct	30.1 ¹		33.5 ¹		36.3 ¹		neg	
	HMPV	10/10	100%	10/10	100%	10/10	100%	10/10	100%
	Ct	28.3 ¹		31.7 ¹		34.6 ¹		36.2 ¹	
	HIB	10/10	100%	10/10	100%	10/10	100%	10/10	100%
	Ct	26.7 ¹		29.6 ¹		33.1 ¹		34.8 ¹	
	Spneu	10/10	100%	10/10	100%	10/10	100%	2/10	20%
	Ct	28.9 ¹		31.9 ¹		36.4 ¹		38.5 ¹	
Cpneu	10/10	100%	10/10	100%	10/10	100%	8/10	80%	
Ct	30.0 ¹		32.9 ¹		36.3 ¹		37.4 ¹		
Saur	10/10	100%	10/10	100%	10/10	100%	4/10	40%	
Ct	30.4 ¹		33.0 ¹		35.6 ¹		37.4 ¹		

(HMPV= Human Metapneumovirus A and B, HRV= Human Rhinovirus, HRSV= Human Respiratory syncytial viruses A and B, HPIV1= Human Parainfluenza virus 1, HAdV= Human Adenovirus, EV= Enterovirus, HPeV= Human Parechovirus, HBoV= Human Bocavirus, Mpneu= *Mycoplasma pneumoniae*, Cpneu= *Chlamydia pneumoniae*, Spneu= *Streptococcus pneumoniae*, Saur= *Staphylococcus aureus*, HIB= *Haemophilus influenzae B*, ¹ = Ct expressed as a mean of 10 reactions, cop/ml= copies/ml, PP= primer and probe mix, RE33= FTD Respiratory pathogens 33)



FTD Respiratory pathogens 33

Table 5c: Analytical sensitivity of FTD Resp33 determined with 10 time measurements around the endpoint detection limit part III.

	Plasmid	10 ⁵ cop/ml		10 ⁴ cop/ml		10 ³ cop/ml		10 ² cop/ml	
RE33	Lpneu	10/10	100%	8/10	80%	0/10	0%	0/10	0%
	Ct	31.8 ¹		35.8 ¹		/		/	
	Llong	10/10	100%	8/10	80%	2/10	20%	0/10	0%
	Ct	33.3 ¹		36.0 ¹		36.2 ¹		/	
	Kpneu	10/10	100%	10/10	100%	10/10	100%	5/10	50%
	Ct	27.6 ¹		31.0 ¹		34.3 ¹		36.8 ¹	
	PCP	10/10	100%	10/10	100%	9/10	90%	1/10	10%
	Ct	28.3 ¹		31.6 ¹		34.7 ¹		36.5 ¹	
	Salm	10/10	100%	9/10	90%	5/10	50%	0/10	0%
	Ct	32.3 ¹		35.5 ¹		36.6 ¹		/	
	Morax	10/10	100%	10/10	100%	10/10	100%	3/10	30%
	Ct	28.5 ¹		31.6 ¹		34.5 ¹		37.8 ¹	
	Bord	10/10	100%	10/10	100%	10/10	100%	9/10	90%
	Ct	22.1 ¹		25.4 ¹		28.5 ¹		32.8 ¹	
	FLUC	10/10	100%	10/10	100%	10/10	100%	7/10	70%
	Ct	26.6 ¹		29.6 ¹		33.2 ¹		36.3 ¹	
Haeinf	10/10	100%	10/10	100%	8/10	80%	0/10	0%	
Ct	24.1 ¹		26.9 ¹		29.9 ¹		32.9 ¹		

(Salm= *Salmonella* spp., FLUC= influenza C, PCP= *Pneumocystis jirovecii*, Morax= *Moraxella catarrhalis*, Bord= *Bordetella* spp., Kpneu= *Klebsiella pneumoniae*, Lpneu/Llong= *Legionella pneumophila*/*Legionella longbeachae*, Haeinf= *Haemophilus influenzae*, ¹ = Ct expressed as a mean of 10 reactions, cop/ml= copies/ml, PP= primer and probe mix)

Additionally, the endpoint detection limit was determined by using diluted positive samples (1:2, 1:5 and 1:10).

The results of the sample limit of detection for the pathogen detected with FTD Resp33 are listed in tables 6a-c.

The comparison of FTD Resp33 to singleplex assays showed no significant loss of sensitivity.



FTD Respiratory pathogens 33

Table 6a: Analytical sensitivity determined with samples and FTD Resp33 part I.

Pathogen		Dil1 (1:10)	Dil2 (1:100)	Dil3 (1:1,000)	Dil4 (1:5,000)
FLUA SP	Ct cop/ml	27.3	29.8	34.4	36.7
FLUA RE33		29.3	31.2	35.2	neg
FLUB SP		28.4	30.7	36.1	36.3
FLUB RE33		31.3	32.3	35.7	37.2
H1N1 SP		30.6	32.8	34.9	neg
H1N1 RE33		32.9	33.8	36.9	neg
HRV SP		33.2	36.5	36.5	neg
HRV RE33		31.1	34.5	36.2	neg
HPIV2 SP		31.9	34.8	38.3	neg
HPIV2 RE33		30.7	33.9	37.0	neg
HPIV3 SP		29.8	33.4	36.3	38.3
HPIV3 RE33		31.0	33.8	36.2	38.1
HPIV4 SP		29.6	32.7	36.9	neg
HPIV4 RE33		33.4	36.6	38.7	neg

(FLUA= Influenza A, H1N1= Influenza A (H1N1)swl, FLUB= Influenza B, HRV= Human Rhinovirus, HPIV2= Human Parainfluenza virus 2, HPIV3= Human Parainfluenza virus 3, HPIV4= Human Parainfluenza virus 4, SP= singleplex assay, Dil= dilution step, cop/ml=copies/ml, neg=negative, RE33= FTD Resp33 kit)



FTD Respiratory pathogens 33

Table 6b: Analytical sensitivity determined with samples and FTD Resp33 part II.

Pathogen		Dil1 (1:10)	Dil2 (1:100)	Dil3 (1:1,000)	Dil4 (1:5,000)
HKU1 SP	Ct cop/ml	28.2	31.7	36.0	37.8
HKU1 RE33		28.1	32.1	36.5	neg
Cor43 SP		23.0	28.2	31.4	neg
Cor43 RE33		24.2	28.3	31.6	35.3
Cor63 SP		26.8	31.4	35.9	38.0
Cor63 RE33		27.4	31.4	36.3	neg
Cor229 SP		26.5	30.6	35.2	38.4
Cor229 RE33		28.2	31.4	37.0	neg
HRSV SP		26.9	29.5	35.3	neg
HRSV RE33		29.7	31.3	35.4	neg
HAdV SP		28.3	30.7	33.2	35.8
HAdV RE33		28.7	30.9	31.9	36.2
EV SP		23.7	27.4	31.8	neg
EV RE33		25.4	30.0	36.7	neg
HPeV SP		32.4	35.8	neg	neg
HPeV RE33		24.7	30.5	35.9	neg

(Cor63= Coronavirus NL63, Cor229= Coronavirus 229E, Cor43= Coronavirus OC43, HKU1= Coronavirus HKU1, HRV= Human Rhinovirus, HRSV= Human Respiratory syncytial viruses A and B, HAdV= Human Adenovirus, EV= Enterovirus, HPeV= Human Parechovirus, CMV= Cytomegalovirus, SP= singleplex assay, Dil= dilution step, cop/ml=copies/ml, neg=negative, RE33= FTD Resp33)



FTD Respiratory pathogens 33

Table 6c: Analytical sensitivity determined with samples and FTD Resp33 part III.

Pathogen		Dil1 (1:10)	Dil2 (1:100)	Dil3 (1:1,000)	Dil4 (1:5,000)
Mpneu SP	Ct cop/ml	29.4	31.7	34.0	37.0
Mpneu RE33		30.2	32.8	36.2	38.8
HPIV1 SP		26.4	30.5	32.4	36.3
HPIV1 RE33		30.6	32.3	34.8	neg
HBoV SP		34.3	36.4	neg	neg
HBoV RE33		35.7	38.4	neg	neg
HMPV SP		30.8	34.2	36.4	neg
HMPV RE33		30.2	33.3	37.0	38.4
HIB SP	Ct	28.8	31.9	36.1	neg
HIB RE33		28.8	32.2	35.5	38.1
Spneu SP		32.3	37.9	39.4	neg
Spneu RE33		30.5	34.7	36.8	38.5
Cpneu SP		31.7	34.8	36.4	39.2
Cpneu RE33		32.2	34.1	35.8	38.2
Saur SP		30.9	33.1	35.8	37.1
Saur RE33		29.1	32.2	35.6	36.9

(HMPV= Human Metapneumovirus A and B, HPIV1= Human Parainfluenza virus 1, HBoV= Human Bocavirus, Mpneu= *Mycoplasma pneumoniae*, Cpneu= *Chlamydia pneumoniae*, Spneu= *Streptococcus pneumoniae*, Saur= *Staphylococcus aureus*, HIB= *Haemophilus influenzae B*, SP= singleplex assay, Dil= dilution step, cop/ml=copies/ml, neg=negative, RE33= FTD Resp33)



FTD Respiratory pathogens 33

Table 6d: Analytical sensitivity determined with samples and FTD Resp33 part IV.

Pathogen		Dil1 (1:16)	Dil2 (1:32)	Dil3 (1:64)	Dil4 (1:128)	
Salm SP	Ct cop/ml	33.1	34.1	33.8	neg	
Salm RE33		32.2	33.2	34.2	35.6	
PCP SP		33.6	35.8	36.4	36.6	
PCP RE33		32.9	32.9	34.4	35.6	
Kpneu SP		34.6	35.2	34.5	neg	
Kpneu RE33		33.6	34.2	34.6	34.7	
Llong SP		35.9	36.2	36.9	38.9	
Llong RE33		34.1	34.6	36.9	neg	
			Dil1 (1:1)	Dil2 (1:4)	Dil3 (1:16)	Dil4 (1:64)
Lpneu SP		30.3	31.7	36.3	neg	
Lpneu RE33		30.6	32.0	33.5	neg	
			Dil1 (1:10)	Dil2 (1:100)	Dil3 (1:1,000)	Dil4 (1:5,000)
Morax SP		29.3	31.7	33.6	36.0	
Morax RE33		28.6	30.4	36.6	Neg	
FLUC SP		31.7	34.9	38.5	Neg	
FLUC RE33		30.4	35.0	Neg	Neg	
Bord SP		26.9	30.5	35.3	Neg	
Bord RE33		26.8	30.3	Neg	Neg	
Haeinf SP		25.9	30.3	32.5	34.4	
Haeinf RE33		25.9	29.9	32.7	neg	

(Salm= *Salmonella* spp., FLUC= Influenza C, PCP= *Pneumocystis jirovecii*, Morax= *Moraxella catarrhalis*, Bord= *Bordetella* spp., Kpneu= *Klebsiella pneumoniae*, Lpneu/Llong= *Legionella pneumophila*/*Legionella longbeachae*, Haeinf= *Haemophilus influenzae*, SP= singleplex assay, Dil= dilution step, cop/ml=copies/ml, neg=negative, RE33= FTD Resp33)



FTD Respiratory pathogens 33

3. Precision (intra- and inter-assay)

To determine precision intra- and inter-assays were performed with clinical samples for each pathogen included in FTD Resp33.

The arithmetic mean (\bar{x}), the standard deviation (σ) and the coefficient of variation (CV %) were calculated.

For the intra-assay 1 positive, 1 low positive and 1 negative patient sample for each pathogen were tested in triplicate on one day with the FTD Resp33. For HRV and HPIV4 only low positive samples and for *H. influenzae* only positive samples were available. (Tables 7a-c)



FTD Respiratory pathogens 33

Table 7a: Intra-assay reproducibility of FTD Resp33 part I.

sample	category	replicate 1	replicate 2	replicate 3	\bar{x}	σ	CV %
FLUA	low positive	36.0	38.0	36.2	36.7	1.1	3.00
	positive	24.9	25.3	25.3	25.1	0.2	0.80
	negative	neg	neg	neg	neg	n.a.	n.a.
FLUB	low positive	34.1	34.2	34.3	34.2	0.1	0.29
	positive	29.0	29.4	29.3	29.2	0.2	0.68
	negative	neg	neg	neg	neg	n.a.	n.a.
H1N1	low positive	33.9	33.0	32.9	33.2	0.5	1.51
	positive	23.1	22.9	22.9	22.9	0.1	0.44
	negative	neg	neg	neg	neg	n.a.	n.a.
HRV	low positive	35.2	37.0	36.5	36.2	0.9	2.49
	low positive	32.6	32.5	32.8	32.6	0.2	0.61
	negative	neg	neg	neg	neg	n.a.	n.a.
HPIV2	low positive	35.1	35.3	35.0	35.2	0.2	0.57
	positive	26.9	26.7	26.7	26.7	0.1	0.37
	negative	neg	neg	neg	neg	n.a.	n.a.
HPIV3	low positive	34.6	34.1	35.2	34.6	0.6	1.73
	positive	24.9	24.8	24.8	24.8	0.1	0.40
	negative	neg	neg	neg	neg	n.a.	n.a.
HPIV4	low positive	34.3	34.4	34.7	34.5	0.3	0.87
	low positive	32.4	32.9	32.5	32.6	0.3	0.92
	negative	neg	neg	neg	neg	n.a.	n.a.
Cor43	low positive	34.7	34.9	34.9	34.8	0.2	0.57
	positive	27.8	27.9	27.8	27.8	0.1	0.36
	negative	neg	neg	neg	neg	n.a.	n.a.
Cor63	low positive	35.3	34.9	35.7	35.3	0.4	1.13
	positive	21.1	21.1	21.1	21.1	0	0.00
	negative	neg	neg	neg	neg	n.a.	n.a.
Cor229	low positive	37.9	38.0	37.8	37.9	0.1	0.26
	positive	31.4	31.4	31.1	31.3	0.1	0.32
	negative	neg	neg	neg	neg	n.a.	n.a.
HKU1	low positive	34.4	34.7	34.8	34.6	0.2	0.58
	positive	28.1	27.7	27.3	27.7	0.4	1.44
	negative	neg	neg	neg	neg	n.a.	n.a.

(FLUA= Influenza A, H1N1= Influenza A (H1N1)swl, FLUB= Influenza B, HRV= Human Rhinovirus, Cor63= Coronavirus NL63, Cor229= Coronavirus 229E, Cor43= Coronavirus OC43, HKU1= Coronavirus HKU1, HPIV2= Human Parainfluenza virus 2, HPIV3= Human Parainfluenza virus 3, HPIV4= Human Parainfluenza virus 4, \bar{x} = mean Ct value of 3 replicates, σ = standard deviation, CV %= coefficient of variation in %, low positive = samples with a Ct value > 33, positive= samples with a Ct value < 33, negative= samples negative for any pathogen, neg = negative, n.a. = not applicable)



FTD Respiratory pathogens 33

Table 7b: Intra-assay reproducibility of FTD Resp33 part II.

sample	category	replicate 1	replicate 2	replicate 3	\bar{x}	σ	CV %
HAdV	low positive	34.3	33.7	35.1	34.4	0.7	2.03
	positive	15.9	15.9	15.9	15.9	0	0.00
	negative	neg	neg	neg	neg	n.a.	n.a.
HRSV	low positive	35.3	34.5	34.4	34.8	0.5	1.44
	positive	21.0	20.7	20.6	20.8	0.2	0.96
	negative	neg	neg	neg	neg	n.a.	n.a.
EV	low positive	34.0	34.0	35.0	34.3	0.6	1.75
	positive	27.7	27.5	26.8	27.3	0.5	1.83
	negative	neg	neg	neg	neg	n.a.	n.a.
HPeV	low positive	34.1	34.0	32.9	33.6	0.7	2.08
	positive	31.5	31.2	31.7	31.4	0.3	0.96
	negative	neg	neg	neg	neg	n.a.	n.a.
HMPV	low positive	35.9	35.6	35.4	35.7	0.3	0.84
	positive	26.6	26.9	26.9	26.8	0.2	0.75
	negative	neg	neg	neg	neg	n.a.	n.a.
HPIV1	low positive	36.5	35.9	36.7	36.4	0.4	1.10
	positive	28.7	29.1	29.4	29.0	0.3	1.03
	negative	neg	neg	neg	neg	n.a.	n.a.
Mpneu	low positive	35.9	36.3	36.4	36.2	0.2	0.55
	positive	23.9	23.9	23.8	23.8	0.1	n.a.
	negative	neg	neg	neg	neg	n.a.	n.a.
HBoV	low positive	35.7	34.9	37.4	36.0	1.3	3.61
	positive	25.4	25.6	25.7	25.5	1.4	5.49
	negative	neg	neg	neg	neg	n.a.	n.a.
HIB	low positive	37.8	36.3	35.9	36.7	1.0	2.72
	positive	25.2	25.2	25.2	25.2	0.0	0.00
	negative	neg	neg	neg	neg	n.a.	n.a.
Spneu	low positive	34.0	32.8	34.3	33.7	0.8	2.37
	positive	26.6	26.9	26.4	26.6	0.2	0.75
	negative	neg	neg	neg	neg	n.a.	n.a.
Cpneu	low positive	35.2	35.0	34.9	35.0	0.1	0.29
	positive	30.8	30.7	30.6	30.7	0.1	0.33
	negative	neg	neg	neg	neg	n.a.	n.a.
Saur	low positive	37.5	36.6	36.0	36.7	0.8	2.18
	positive	27.4	27.7	27.7	27.6	0.2	0.72
	negative	neg	neg	neg	neg	n.a.	n.a.

(HRSV= Human Respiratory syncytial viruses A and B, HAdV= Human Adenovirus, EV= Enterovirus, HPeV= Human Parechovirus, HMPV= Human Metapneumovirus A and B, HPIV1= Human Parainfluenza virus 1, HBoV= Human Bocavirus, Mpneu= *Mycoplasma pneumoniae*, Cpneu= *Chlamydia pneumoniae*, Spneu= *Streptococcus pneumoniae*, Saur= *Staphylococcus aureus*, HIB= *Haemophilus influenzae B*, \bar{x} = mean Ct value of 3 replicates, σ = standard deviation, CV %= coefficient of variation in %, low positive = samples with a Ct value > 31.7, positive= samples with a Ct value < 31.7, negative= samples negative for any pathogen, neg = negative, n.a. = not applicable)



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Table 7c: Intra-assay reproducibility of FTD Resp33 part III.

sample	category	replicate 1	replicate 2	replicate 3	\bar{x}	σ	CV %
Salm	low positive	32.4	32.5	32.1	32.4	0.2	0.6
	positive	28.2	28.0	28.3	28.1	0.2	0.8
	negative	neg	neg	neg	neg	n.a.	n.a.
PCP	low positive	33.6	33.3	33.0	33.2	0.3	1.0
	positive	26.9	27.0	26.9	27.0	0.1	0.2
	negative	neg	neg	neg	neg	n.a.	n.a.
Kpneu	low positive	33.1	33.0	32.9	33.1	0.2	0.7
	positive	25.2	25.2	25.1	25.1	0.1	0.4
	negative	neg	neg	neg	neg	n.a.	n.a.
Lpneu	low positive	30.5	30.5	30.4	30.6	0.2	0.7
	positive	26.5	26.6	26.6	26.6	0.1	0.2
	negative	neg	neg	neg	neg	n.a.	n.a.
Llong	low positive	32.1	32.3	32.3	32.2	0.1	0.3
	positive	30.7	31.2	31.1	30.9	0.4	1.2
	negative	neg	neg	neg	neg	n.a.	n.a.
Morax	low positive	34.7	33.1	33.7	33.8	0.8	2.37
	positive	18.9	18.9	19.0	18.9	0.0	0.00
	negative	neg	neg	neg	neg	n.a.	n.a.
FLUC	low positive	36.3	37.6	35.4	36.4	1.1	3.02
	positive	29.0	29.3	29.4	29.3	0.2	0.68
	negative	neg	neg	neg	neg	n.a.	n.a.
Bord	low positive	31.5	30.4	30.5	30.8	0.6	1.95
	positive	25.7	25.3	25.3	25.5	0.2	0.78
	negative	neg	neg	neg	neg	n.a.	n.a.
Haeinf	positive	29.9	29.9	30.2	30.0	0.1	0.33
	positive	19.9	19.8	19.8	19.8	0.0	0.00
	negative	neg	neg	neg	neg	n.a.	n.a.

(Salm= *Salmonella* spp., FLUC= Influenza C, PCP= *Pneumocystis jirovecii*, Morax= *Moraxella catarrhalis*, Bord= *Bordetella* spp., Kpneu= *Klebsiella pneumoniae*, Lpneu/Llong= *Legionella pneumophila*/ *Legionella longbeachae*, Haeinf= *Haemophilus influenzae*, \bar{x} = mean Ct value of 3 replicates, σ = standard deviation, CV %= coefficient of variation in %, low positive = samples with a Ct value > 31.9, positive= samples with a Ct value < 31.9, negative= samples negative for any pathogen, neg = negative, n.a. = not applicable)

For the inter-assay 1 positive, 1 low positive and 1 negative patient sample for the pathogens were tested on three different days with the FTD Resp33 kit. For HRV and HPIV4 only low positive samples and for *H. influenzae* only positive samples were available (Tables 8a-c).



FTD Respiratory pathogens 33

Table 8a: Inter-assay reproducibility of FTD Resp33 part I.

sample	category	day 1	day 2	day 3	\bar{x}	σ	CV %
FLUA	low positive	36.7	36.1	36.2	36.4	0.3	0.82
	positive	25.1	24.5	24.2	24.6	0.5	2.03
	negative	neg	neg	neg	neg	n.a.	n.a.
FLUB	low positive	34.2	33.8	34.0	34.0	0.2	0.59
	positive	29.2	29.2	29.5	29.3	0.2	0.68
	negative	neg	neg	neg	neg	n.a.	n.a.
H1N1	low positive	33.2	32.9	32.0	32.7	0.6	1.83
	positive	22.9	22.9	23.4	23.1	0.3	1.30
	negative	neg	neg	neg	neg	n.a.	n.a.
HRV	low positive	36.2	34.7	36.0	35.6	0.8	2.25
	low positive	32.6	33.0	31.8	32.5	0.6	1.85
	negative	neg	neg	neg	neg	n.a.	n.a.
HPIV2	low positive	34.6	34.9	34.8	34.8	0.2	0.57
	positive	25.4	25.3	25.7	25.4	0.2	0.79
	negative	neg	neg	neg	neg	n.a.	n.a.
HPIV3	low positive	34.5	34.9	34.6	34.7	0.2	0.58
	positive	25.8	25.5	25.2	25.5	0.3	1.18
	negative	neg	neg	neg	neg	n.a.	n.a.
HPIV4	low positive	34.5	35.7	35.6	35.3	0.7	1.98
	low positive	33.7	33.1	33.4	33.4	0.3	0.90
	negative	neg	neg	neg	neg	n.a.	n.a.
Cor43	low positive	33.5	33.6	33.3	33.4	0.2	0.60
	positive	24.8	24.2	25.2	24.7	0.5	2.02
	negative	neg	neg	neg	neg	n.a.	n.a.
Cor63	low positive	35.4	35.1	34.6	35.0	0.4	1.14
	positive	21.2	20.4	20.6	20.7	0.4	1.93
	negative	neg	neg	neg	neg	n.a.	n.a.
Cor229	low positive	34.5	34.7	35.2	34.8	0.4	1.15
	positive	24.7	25.2	25.9	25.2	0.6	2.38
	negative	neg	neg	neg	neg	n.a.	n.a.
HKU1	low positive	34.0	34.8	33.7	34.2	0.5	1.46
	positive	25.9	25.6	26.6	26.0	0.5	1.92
	negative	neg	neg	neg	neg	n.a.	n.a.

(FLUA= Influenza A, FLUB= Influenza B, HRV= Human Rhinovirus, Cor63= Coronavirus NL63, Cor229= Coronavirus 229E, Cor43= Coronavirus OC43, HKU1= Coronavirus HKU1, HPIV2= Human Parainfluenza virus 2, HPIV3= Human Parainfluenza virus 3, HPIV4= Human Parainfluenza virus 4, \bar{x} = mean Ct value of 3 different days, σ = standard deviation, CV %= coefficient of variation in %, low positive = samples with a Ct value > 33, positive= samples with a Ct value < 33, negative= samples negative for any pathogen, neg = negative, n.a. = not applicable)



FTD Respiratory pathogens 33

Table 8b: Inter-assay reproducibility of FTD Resp33 part II.

sample	category	day 1	day 2	day 3	\bar{x}	σ	CV %
HAdV	low positive	33.9	34.8	33.6	34.1	0.6	1.76
	positive	16.6	16.8	16.9	16.8	0.1	0.60
	negative	neg	neg	neg	neg	n.a.	n.a.
HRSV	low positive	32.4	32.3	32.2	32.3	0.1	0.31
	positive	19.2	18.7	18.8	18.9	0.3	1.59
	negative	neg	neg	neg	neg	n.a.	n.a.
EV	low positive	32.3	33.3	33.2	32.9	0.6	1.82
	positive	26.4	27.0	27.9	27.1	0.7	2.58
	negative	neg	neg	neg	neg	n.a.	n.a.
HPeV	low positive	33.8	33.4	33.3	33.5	0.3	0.90
	positive	28.8	28.9	28.2	28.7	0.4	1.39
	negative	neg	neg	neg	neg	n.a.	n.a.
HMPV	low positive	37.2	37.3	36.3	36.9	0.6	1.63
	positive	24.3	24.9	25.3	24.8	0.5	2.02
	negative	neg	neg	neg	neg	n.a.	n.a.
HPIV1	low positive	36.2	35.9	36.3	36.2	0.2	0.55
	positive	28.7	29.7	29.2	29.2	0.5	1.71
	negative	neg	neg	neg	neg	n.a.	n.a.
Mpneu	low positive	33.4	33.9	34.1	33.8	0.4	1.18
	positive	24.6	24.2	24.1	24.3	0.2	0.82
	negative	neg	neg	neg	neg	n.a.	n.a.
HBoV	low positive	32.0	31.8	32.4	32.1	0.3	0.93
	positive	25.4	25.9	26.3	25.9	0.5	1.93
	negative	neg	neg	neg	neg	n.a.	n.a.
HIB	low positive	36.7	38.1	37.9	37.6	0.8	2.13
	positive	25.2	25.3	25.1	25.2	0.1	0.40
	negative	neg	neg	neg	neg	n.a.	n.a.
Spneu	low positive	33.7	35.9	34.5	34.7	1.2	3.46
	positive	26.6	26.3	26.9	26.6	0.3	1.13
	negative	neg	neg	neg	neg	n.a.	n.a.
Cpneu	low positive	35.0	35.9	36.9	36.0	1.0	2.78
	positive	30.7	31.7	31.8	31.4	0.6	1.91
	negative	neg	neg	neg	neg	n.a.	n.a.
Saur	low positive	36.7	36.1	35.1	36.0	0.8	2.22
	positive	27.6	28.6	27.4	27.9	0.6	2.15
	negative	neg	neg	neg	neg	n.a.	n.a.

(HRV= Human Rhinovirus, HRSV= Human Respiratory syncytial viruses A and B, HAdV= Human Adenovirus, EV= Enterovirus, HPeV= Human Parechovirus, HMPV= Human Metapneumovirus A and B, HPIV1= Human parainfluenza virus 1, HBoV= Human Bocavirus, Mpneu= *Mycoplasma pneumoniae*, Cpneu= *Chlamydia pneumoniae*, Spneu= *Streptococcus pneumoniae*, Saur= *Staphylococcus aureus*, HIB= *Haemophilus influenzae B*, \bar{x} = mean Ct value of 3 different days, σ = standard deviation, CV %= coefficient of variation in %, low positive = samples with a Ct value > 31, positive= samples with a Ct value < 31, negative= samples negative for any pathogen, neg = negative, n.a. = not applicable)



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Table 8c: Inter-assay reproducibility of FTD Resp33 part III.

sample	category	day 1	day 2	day 3	\bar{x}	σ	CV %
Salm	low positive	32.4	30.9	31.6	31.6	0.6	2.0
	positive	28.1	27.0	27.8	27.6	0.5	1.9
	negative	neg	neg	neg	neg	n.a.	n.a.
PCP	low positive	33.2	31.7	32.2	32.3	0.8	2.5
	positive	27.0	26.0	26.1	26.3	0.5	1.8
	negative	neg	neg	neg	neg	n.a.	n.a.
Kpneu	low positive	33.1	33.2	32.9	33.1	0.4	1.1
	positive	25.1	25.3	25.2	25.2	0.1	0.4
	negative	neg	neg	neg	neg	n.a.	n.a.
Lpneu	low positive	30.6	31.4	30.9	31.0	0.4	1.3
	positive	26.6	27.1	27.0	26.9	0.3	1.0
	negative	neg	neg	neg	neg	n.a.	n.a.
Llong	low positive	32.2	31.3	32.1	31.9	0.4	1.4
	positive	30.9	30.3	31.0	30.7	0.4	1.3
	negative	neg	neg	neg	neg	n.a.	n.a.
Morax	low positive	33.2	33.8	33.8	33.6	0.3	0.89
	positive	18.5	18.9	18.7	18.7	0.2	1.07
	negative	neg	neg	neg	neg	n.a.	n.a.
FLUC	low positive	36.6	36.4	39.1	37.4	1.5	4.01
	positive	29.5	30.4	29.3	29.7	0.6	2.02
	negative	neg	neg	neg	neg	n.a.	n.a.
Bord	low positive	32.4	30.8	32.7	32.0	1.0	3.13
	positive	25.9	25.5	25.8	25.7	0.2	0.78
	negative	neg	neg	neg	neg	n.a.	n.a.
Haeinf	positive	30.1	30.0	30.9	30.3	0.5	1.65
	positive	19.8	19.8	20.1	19.9	0.2	1.01
	negative	neg	neg	neg	neg	n.a.	n.a.

(Salm= *Salmonella* spp., FLUC= Influenza C, PCP= *Pneumocystis jirovecii*, Morax= *Moraxella catarrhalis*, Bord= *Bordetella* spp., Kpneu= *Klebsiella pneumoniae*, Lpneu/Llong= *Legionella pneumophila*/*Legionella longbeachae*, Haeinf= *Haemophilus influenzae*, \bar{x} = mean Ct value of 3 replicates, σ = standard deviation, CV %= coefficient of variation in %, low positive = samples with a Ct value > 30.4, positive= samples with a Ct value < 32, negative= samples negative for any pathogen, neg = negative, n.a. = not applicable, PP= primer and probe mix)



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4. Clinical study

4.1 Study 1: Internal study 1

246 nasopharyngeal and pleural effusion samples were analysed with different reference methods such as FTD Bacterial gastroenteritis or in-house singleplex RT-PCR (Fast Track Diagnostics) and FTD Resp33 (former version FTD 2P.2 Resp33).

The reference methods and FTD Resp33 detected 23 FLUA, 20 FLUB, 24 FLUC, HPIV1-4, Cor63, Cor229, Cor43 and HKU1, HMPV, HRV, HRSV, 31 HAdV, EV, HPeV, HBoV, 14 PCP, *M. pneumoniae*, 6 *C. pneumoniae*, 63 *S. pneumoniae*, 18 HiB, 40 *S. aureus*, 29 *M. catarrhalis*, 6 *Bordetella* spp., 20 *K. pneumoniae*, 18 *Legionella* spp., 29 *Salmonella* spp., 40 *H. influenzae*. (Tables 10a and b) 100% sensitivity and specificity was received for the detection of respiratory pathogens with FTD Resp33 and reference methods.

Table 10a: Results for FTD Resp33 compared reference methods. part I

pathogen	reference method	FTD Resp33		
		pos	neg	
Salm	FTD Bacgas	pos	29	0
		neg	0	217
Mpneu	culture	pos	16	0
		neg	0	230
Cpneu		pos	6	0
		neg	0	240
Spneu		pos	63	0
		neg	0	183
HIB		pos	18	0
		neg	0	228
Saur		pos	40	0
		neg	0	206
Legio		pos	18	0
		neg	0	228
Kpneu		pos	20	0
		neg	0	226
Morax		pos	29	0
		neg	0	217
Bord		pos	6	0
		neg	0	240
Haeinf		pos	40	0
		neg	0	206
PCP	in-house singleplex PCR	pos	14	0
		neg	0	232

(Mpneu= *Mycoplasma pneumoniae*, Cpneu= *Chlamydia pneumoniae*, Spneu= *Streptococcus pneumoniae*, Saur= *Staphylococcus aureus*, HIB= *Haemophilus influenzae B*, Salm= *Salmonella* spp., FLUC= Influenza C, PCP= *Pneumocystis jirovecii*, Morax= *Moraxella catarrhalis*, Bord= *Bordetella* spp., Kpneu= *Klebsiella pneumoniae*, Legio= *Legionella* spp., Haeinf= *Haemophilus influenzae*, FTD Resp21plus= FTD Respiratory pathogens 21 plus, FTD Bacgas= FTD Bacterial gastroenteritis)



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Table 10b: Results for FTD Resp33 compared reference methods. part II

pathogen	reference method	FTD Resp33		
		pos	neg	
FLUC	in-house singleplex PCR	pos	24	0
		neg	0	222
HAdV		pos	31	0
		neg	0	215
FLUA		pos	23	0
		neg	0	223
FLUB		pos	20	0
		neg	0	226
HRV		pos	25	0
		neg	0	221
HPIV2		pos	19	0
		neg	0	227
HPIV3		pos	18	0
		neg	0	228
HPIV4		pos	16	0
		neg	0	230
Cor43		pos	23	0
		neg	0	223
Cor63		pos	12	0
		neg	0	234
Cor229		pos	13	0
		neg	0	233
HKU1		pos	6	0
		neg	0	240
HMPV		pos	19	0
		neg	0	227
HBoV		pos	7	0
		neg	0	239
HPIV1		pos	18	0
		neg	0	228
EV		pos	16	0
		neg	0	230
HPeV		pos	14	0
	neg	0	232	
HRSV	pos	22	0	
	neg	0	224	

(FLUA= Influenza A, FLUB= Influenza B, FLUC= Influenza C, HRV= Human Rhinovirus, Cor63= Coronavirus NL63, Cor229= Coronavirus 229E, Cor43= Coronavirus OC43, HKU1= Coronavirus HKU1, HPIV2= Human Parainfluenza virus 2, HPIV3= Human Parainfluenza virus 3, HPIV4= Human Parainfluenza virus 4, HRV= Human Rhinovirus, HRSV= Human Respiratory syncytial viruses A and B, HAdV= Human Adenovirus, EV= Enterovirus, HPeV= Human Parechovirus, HMPV= Human Metapneumovirus A and B, HPIV1= Human Parainfluenza virus 1, HBoV= Human Bocavirus, FTD Resp21plus= FTD Respiratory pathogens 21 plus)



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4.2 Study 2: Internal study 2

Internal Study 2 part 1

A total of 11 throat swabs was spiked with *Chlamydia pneumoniae* ATCC strain TW-183 ($10^{3,5}$ TCID₅₀/0,2 ml) for analysis with FTD respiratory infections 33 (2P.2). Different spiked volumes (Table 11) as well as a dilution series of the respective samples (Table 12) were tested.

Samples were extracted with Nuclisens Easymag platform (BioMérieux), PCR was performed on the ABI7500 (Thermo Fisher).

All samples were identified correctly with FTD Resp33.

Table 11: Results obtained for swab picked with *Chlamydia pneumoniae* samples using RespBac PP mix of the FTD Resp 33.

sample ID	dilution of C. pneu. culture	Ct-value
swab 1	1:200	33,9
swab 1	1:40	32,0
swab 1	1:10	31,4
swab 2	1:2	30,4
swab 2	1:199	34,4
swab 3	1:1	29,1
swab 3	1:199	35,5
swab 4	1:1	29,5
swab 4	1:199	33,4
swab 5	1:199	35,1
swab 6	1:199	33,4

C. pneu = Chlamydia pneumoniae

Table 12: Results obtained for dilution series of swabs spiked with *Chlamydia pneumoniae* sample using RespBac PP mix of the FTD respiratory 33.

dilution steps	original sample and initial dilution		
	sample 2	sample 3	sample 4
1/1	30,4	29,1	29,5
1/2	31,2	30,2	30,5
1/4	32	30,9	31,4
1/8	32,8	31,9	32,3
1/16	33,4	32,5	32,8
1/32	34,2	33,2	33,3
1/64	34,6	31,6	33,3
1/128	35,6	34	34,6
1/256	37,3	35	36,6
1/512	neg	35,1	37,7
1/1024	neg	35,6	neg

neg= negative



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Internal Study 2 part 2

Chlamydia pneumoniae samples of different origin (Brochoalvoelar lavage and culture) were used to spike 17 respiratory swabs (1:10) and analysed with RespBac PP mix. All samples were further diluted in a 1:2 dilution series. In addition, 18 negative swabs, which were of the same origin as swabs used for spiking, were tested. Results are summarized in Tables 13a and b.

Samples were extracted with Nuclisens Easymag platform (BioMérieux); PCR was performed on the ABI 7500 (Thermo Fisher).

All samples were identified correctly with FTD Resp33.

Table 13a: Ct values obtained for *Chlamydia pneumoniae* spiked samples diluted or not using the RespBac PP mix of FTD respiratory 33.

dilution steps	sample														
	1	2	3	4	5	6	7	8	9	10	11	13	13	14	15
1/1	33.3	34.6	neg	35.4	31.5	32.1	34.3	33.4	38.3	31.5	34.8	31.5	28.5	31.5	25.1
1/2	34.1	36.9	36.2	37.1	32.9	32.9	35.1	34.7	neg	32.3	35.3	32.2	29.6	32.6	26.3
1/4	34.9	36.4	neg	35.8	33.1	33.3	neg	35.5	neg	33.5	neg	33	30.5	33.7	27.6
1/8	36.2	36.4	37	36.8	33.6	34.4	neg	37.3	neg	33.8	36.4	33.9	31.3	34.2	28.7
1/16	37	neg	neg	neg	34.1	34.3	neg	neg	neg	34.6	neg	34.7	31.9	34.3	29.4
1/32	neg	neg	neg	neg	34.7	35.4	neg	38.7	neg	36.2	neg	35.9	32.8	34.8	30
1/64	neg	neg	neg	neg	35.9	35	neg	neg	neg	38	neg	neg	33.2	34.8	30.9
1/128	neg	neg	neg	neg	35.1	35.5	neg	neg	neg	neg	neg	37.6	33.9	neg	31.4
1/256	neg	neg	neg	neg	37	neg	neg	neg	neg	neg	neg	38.4	34.5	38.4	32.2
1/512	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	38.2	38.5	32.4
1/1024	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	37.5	neg	33.2
1/2048	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	38.9	38.4	neg	35.6

neg= negative

Table 13b: Results of *Chlamydia pneumoniae* negative samples detection using the RespBac PP mix of FTD respiratory 33.

sample																			
16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg

neg = ngative

Altogether, 125 samples positive for *C. pneumoniae* of different origin and with different bacterial load were analysed by FTD Resp33. Moreover, 18 *C. pneumoniae* negative samples were analysed in this study.



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4.3 Study 3: External study 1 - Canterbury Health Laboratories

10 clinical samples (FLUA H3N2) were analysed with in-house RT-PCR (Canterbury Health Laboratories-CHL) and FTD Resp33 (former version FTD 2P.2 Resp33).

The in-house RT-PCR and FTD Resp33 detected 10 FLUA samples (Table 14).

The sensitivity for the detection of FLUA with FTD Resp33 and an in-house RT-PCR test is listed in Table 15.

Table 14: Results of positive and negative samples determined with FTD Resp33 and in-house CHL RT-PCR.

test	FTD Resp33 Ct value	in-house RT-PCR
pathogen	FLUA	FLUA
1	33.5	positive
2	36.1	positive
3	34.3	positive
4	24.4	positive
5	36.7	positive
6	33.3	positive
7	35.9	positive
8	36.9	positive
9	36.6	positive
10	34.2	positive

(FLUA= Influenza A)

Table 15: Sensitivity of FTD Resp33.

pathogen	sensitivity (%) [*]
FLUA	100 %

*

$$\frac{\text{number of true positives}}{\text{number of true positives} + \text{number of false negatives}} \times 100$$



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4.4 Study 4: External study 2 - John Hopkins University

Clinical specimens/ nasal washes spiked with different virus loads

A series of specimens has been prepared in nasal washings by a laboratory at Johns Hopkins University (Table 16). These specimens have been prepared from culture isolates and some of the specimens contained known concentrations of specific viruses. Some of the specimens had more than one pathogen in order to simulate co-infections. After they spiked known concentrations of these viruses in the nasal washes they were able to detect down to 10TCID₅₀ (tissue culture infective dose) or PFU (plaque forming units).

One problem occurred: When they prepared the pool of negative nasal washings they obviously did not test them for all the pathogens which are detected by FTD Resp33. Unfortunately they had a contamination with HRV, *S. aureus* and *S. pneumoniae* which later on was detected in nearly all spiked samples by the FTD Resp33 (former version FTD 2P.2 Resp33).

100% sensitivity and specificity was received for the detection of respiratory pathogens with FTD Resp33.



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Table 16: Specimen panel tested by John Hopkins Vaccine Initiative/ Boston with FTD Resp33.

number	pathogen	detected pathogens (Ct value)
1	HRSV A 1000 PFU	HRSV 25.1; HRV 30.5; Saur 30.3; Spneu 32.3
2	HRSV A 100 PFU	HRSV 28.0; HRV 28.2; Saur 30.2; Spneu 31.9
3	HRSV A 10 PFU	HRSV 31.4; HRV 28.1; Saur 29.3; Spneu 31.2
4	HRSV B 1000 PFU	HRSV 23.3; HRV 25.8; Saur 28.3; Spneu 30.9
5	HRSV B 100 PFU	HRSV 27.2; HRV 27.5; Saur 28.1; Spneu 30.9
6	HRSV B 10 PFU	HRSV 28.2; HRV 26.5; Saur 28.5; Spneu 31.1
7	FLUA (H1N1 2008) 1000 TCID50	FLUA 23.6; HRV 26.9; Saur 28.8; Spneu 30.4
8	FLUA (H1N1 2008) 100 TCID50	FLUA 28.1; HRV 26.9; Saur 28.8; Spneu 30.4
9	FLUA (H1N1 2008) 10TCID50	FLUA 31.1; HRV 27.01; Saur 28.4; Spneu 31.0
10	FLUA (H3N2) 1000 TCID50	FLUA 24.6; HRV 27.5; Saur 28.4; Spneu 31.0
11	FLUA (H3N2) 100 TCID50	FLUA 32.3; HRV 33.2; Saur 30.5; Spneu 32.1
12	FLUA (H3N2) 10 TCID50	FLUA 33.2; HRV 27.3; Saur 28.3; Spneu 32.1
13	FLUB 1000 TCID50	FLUA 25.4; HRV 27.5; Saur 29.8; Spneu 31.0
14	FLUB 100 TCID50	FLUB 27.7; HRV 27.8; Saur 29.4; Spneu 31.3
15	FLUB 10 TCID50	FLUB 30.3; HRV 29.2; Saur 28.9; Spneu 30.6
16	HMPV 1000 PFU	HMPV 24.4; HRV 30.1; Saur 30.4; Spneu 33.7
17	HMPV 100 PFU	HMPV 26.9; HRV 29.4; Saur 29.3; Spneu 32.6
18	HMPV 10 PFU	HMPV 29.6; HRV 28.8; Saur 27.9; Spneu 31.4
19	HPIV1 1000 PFU	HPIV1 25.9; HRV 29.0; Saur 29.6; Spneu 31.6
20	HPIV1 100 PFU	HPIV1 29.1; HRV 28.7; Saur 29.4; Spneu 31.2
21	HPIV1 10 PFU	HPIV1 31.7; HRV 30.1; Saur 29.0; Spneu 31.4
22	HPIV2 1000 PFU	HPIV2 23.1; HRV 28.7; Saur 29.1; Spneu 31.3
23	HPIV2 100 PFU	HPIV2 26.5; HRV 26.6; Saur 29.1; Spneu 31.3
24	HPIV2 10 PFU	HPIV2 29.3; HRV 27.9; Saur 29.2; Spneu 31.8
25	HPIV3 1000 TCID50	HPIV3 25.1; HRV 26.7; Saur 29.6; Spneu 31.8
26	HPIV3 100 TCID50	HPIV3 28.1; HRV 29.0; Saur 29.8; Spneu 31.3
27	HPIV3 10 TCID50	HPIV3 29.8; HRV 28.1; Saur 29.2; Spneu 31.1
28	HPIV4A	HPIV4 25.65; HRV 28.68; Saur 29.8; Spneu 32.1
29	HPIV4B	HPIV4 28.1; HRV 27.19; Saur 29.4; Spneu 31.2
30	Cor229	Cor229 16.3; HRV 28.2; Saur 28.5; Spneu 30.5
31	Cor43	Cor43 15.1; HRV 29.0; Saur 29.1; Spneu 31.1
32	HRV A	HRV 23.1; Saur 29.7; Spneu 31.5
33	HRV B	HRV 30.2; Saur 29.0; Spneu 30.7
34	EV	EV 19.5; Saur 24.4
35	HAdV	HAdV 16.9; HRV 27.4; Saur 30.2; Spneu 31.0
36	HRSV A 100 PFU + FLUA 100 PFU	FLUA 29.5; HRSV 27.3; HRV 27.31; Saur 29.8
37	HRSV A 100 PFU + FLUA 1000 PFU	FLUA 27.4; HRSV 28.3; HRV 29.0; Saur 30.0; Spneu 31.6
38	HRSV A 100 PFU + HMPV 100 PFU	HMPV 27.2; HRSV 27.7; HRV 28.3; Saur 29.3; Spneu 32.0
39	HRSV A 100 PFU + HMPV 1000 PFU	HMPV 23.7; HRSV 30.2; HRV 33.5; Saur 29.3
40	HMPV 100 PFU + HHPIV3 100 PFU	HPIV3 27.9; HMPV 27.0; HRV 28.0; bacteria not tested
41	HHPIV1 100 PFU + HHPIV2 100 PFU	HPIV1 29.2; HPIV2 25.1; HRV 28.4; bacteria not tested
42	FLUA 100 PFU + HRV A 100 PFU	FLUA 28.8; HRV 24.5; Saur 29.6; Spneu 30.9
43	HRSV A 100 PFU + HRV A 100 PFU	HRSV 27.5; HRV 24.3; Saur 29.3; Spneu 31.2
44	HMPV 100 PFU + HRV A 100 PFU	HMPV 27.6; HRV 23.9; Saur 29.2; Spneu 30.9

(FLUA= Influenza A, FLUB= Influenza B, HRV= Human Rhinovirus, Cor229= Coronavirus 229E, Cor43= Coronavirus OC43, HPIV2= Human Parainfluenza virus 2, HPIV3= Human Parainfluenza virus 3, HPIV4= Human Parainfluenza virus 4, HRSV= Human Respiratory syncytial viruses A and B, HAdV= Human Adenovirus, EV= Enterovirus, HMPV= Human Metapneumovirus A and B, HPIV1= Human Parainfluenza virus 1, Spneu= *Streptococcus pneumoniae*, Saur= *Staphylococcus aureus*, PFU= plaque forming units, TCID= tissue culture infective dose)



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Table 17: Positive and negative samples determined with FTD Resp33 and John Hopkins in-house singleplex RT-PCR.

pathogen		FTD Resp33	
		pos	neg
HRSVA/B	pos	11	0
	neg	0	34
FLUA	pos	9	0
	neg	0	36
FLUB	pos	3	0
	neg	0	42
HMPV	pos	7	0
	neg	0	38
HPIV1	pos	4	0
	neg	0	41
HPIV2	pos	4	0
	neg	0	41
HPIV3	pos	4	0
	neg	0	41
HPIV4	pos	2	0
	neg	0	43
Cor229	pos	1	0
	neg	0	44
Cor43	pos	1	0
	neg	0	44
HRV	pos	45	0
	neg	0	0
EV	pos	1	0
	neg	0	44
HAdV	pos	1	0
	neg	0	44

(FLUA= Influenza A, FLUB= Influenza B, HRV= Human Rhinovirus, Cor229= Coronavirus 229E, Cor43= Coronavirus OC43, HPIV2= Human Parainfluenza virus 2, HPIV3= Human Parainfluenza virus 3, HPIV4= Human Parainfluenza virus 4, HRSV= Human Respiratory syncytial viruses A and B, HAdV= Human Adenovirus, EV= Enterovirus, HMPV= Human Metapneumovirus A and B, HPIV1= Human Parainfluenza virus 1)



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4.5 Study 5: External study 3 -NHS

57 samples (18 QCMD, 39 clinical samples) were analysed with FTD Resp33 (former version FTD 2P.2 Resp33) and a competitor test.

The competitor test detected 44 positive and 13 negative samples (Tables 18a and b). FTD Resp33 could confirm 43 positives and 10 negatives. The missed positive HPIV2 was a low positive sample and the 3 missed negatives came out as positives with FTD Resp33 (HAdV, HRV and H1N1, competitor test is not able to detect H1N1). Furthermore 2 co-infected samples were obtained with FTD Resp33 which were missed by the competitor test.

Table 18a: Positive and negative clinical samples determined with FTD Resp33 and competitor test (NHS).

Sample	Competitor	FTD Resp33
1	negative	negative
2	negative	negative
3	Cor43	Cor43
4	negative	negative
5	negative	negative
6	HPIV3	HPIV3, HAdV
7	EV	EV
8	HPIV4	HPIV4
9	HRSV	HRSV
10	HPIV2	negative
11	HRV	HRV
12	Mpneu	Mpneu
13	HAdV	HAdV
14	Mpneu	Mpneu
15	HRV	HRV
16	HRSV	HRSV
17	HBoV	HBoV
18	HPIV4	HPIV4
19	FLUB	FLUB
20	HAdV	HAdV
21	HPIV1	HPIV1
22	HPIV2	HPIV2
23	HRSV	HRSV
24	HPIV2	HPIV2, HRV
25	HRV	HRV
26	negative	negative
27	negative	negative
28	HRV	HRV
29	negative	negative
30	HRV	HRV
31	HRV	HRV
32	negative	negative
33	HRV	HRV
34	HRV	HRV
35	negative	HAdV
36	negative	HRV
37	negative	negative
38	HRV	HRV
39	negative	negative



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Table 18b: Positive and negative QCMD samples determined with FTD Resp33 and competitor test (NHS).

Sample	Competitor	FTD Resp33
1	negative	H1N1
2	FLUB	FLUB
3	FLUA	FLUA
4	FLUA	FLUA
5	FLUA	FLUA
6	FLUA	FLUA, H1N1
7	Cor43	Cor43
8	Cor63	Cor63
9	Cor229	Cor229
10	Cor63	Cor63
11	HRSV	HRSV
12	HMPV	HMPV
13	HMPV	HMPV
14	HMPV	HMPV
15	HPIV1	HPIV1
16	HPIV2	HPIV2
17	HPIV3	HPIV3
18	HPIV4	HPIV4



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5. Instand e.V. panels

External quality assurance (EQA) assessments of the Collaborating Centre for Quality Assurance and Standardisation in Laboratory Medicine were performed to verify the quality of FTD Resp33. Therefore, FTD Resp33 was evaluated with different Instand e.V. panels (Ringversuch panels, www.instandev.de) (Table 19). All samples were dissolved in accordance to the instructions of the supplier and analysed with FTD Resp33.

Table 19 shows an overview of Instand e.V. panels validated with FTD Resp33.

Table 19: List of the Instand e.V. panels tested with FTD Resp33.

pathogen	Instand 2011	Instand 2012	Instand 2013	Instand 2014	Instand 2015
HAdV	RV371 06/11, RV371 11/11	RV371 06/12, RV371 11/12	/	RV371 06/14	RV371 06/15, RV371 11/15
EV	RV372 06/11, RV372 11/11	RV372 06/12, RV372 11/12	RV372 06/13, RV372 11/13	RV372 06/14, RV372 11/14	RV372 06/15, RV372 11/15
FLUA/B	RV370 03/11, RV370 11/11	RV370 03/12, RV370 11/12	RV370 03/13, RV370 11/13	RV370 03/14, RV370 11/14	RV370 03/15, RV370 11/15
Mpneu	/	RV541 05/12, RV541 11/12	RV541 05/13, RV541 11/13	RV541 05/14, RV541 11/14	RV541 05/15, RV541 11/15
Cpneu	/	RV540 05/12, RV540 11/12	RV540 05/13, RV540 11/13	RV540 05/14, RV540 11/14	RV540 05/15, RV540 11/15
HRSV	/	RV359 06/12, RV359 11/12	RV359 06/13, RV359 11/13	RV359 06/14, RV359 11/14	/
Bord	/	/	RV532 05/13, RV532 11/13	RV532 05/14, RV532 11/14	RV532 06/15, RV532 11/15
Salm	/	RV537 05/12, RV537 11/12	/	RV537 05/14	RV537 05/15, RV537 11/15
HMPV	/	/	/	RV385 06/14, RV385 11/14	RV385 06/15
HPIV	/	/	/	RV388 09/14	RV388 09/15

(FLUA= influenza A, FLUB= influenza B, HRSV= respiratory syncytial viruses A and B, HAdV= adenovirus, EV= enterovirus, Mpneu= *Mycoplasma pneumoniae*, HMPV= human metapneumovirus, HPIV= parainfluenzavirus, Cpneu= *Chlamydia pneumoniae*, Bord= *Bordetella* spp., Salm= *Salmonella* spp.)

The results of the HAdV, EV, FLUA/B, *M. pneumoniae*, *C. pneumoniae* and, HRSV, *Bordetella* spp., *Salmonella* spp., HMPV and HPIV Instand e. V. panels are listed in Tables 20-30. All samples have been determined correctly with FTD Resp33.



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Table 20: Instand e. V. panels for adenovirus tested with FTD Resp33.

samples	sample content	sample status
Instand HAdV June 2011		
371009	negative	negative
371010	Adenovirus A type 31	detected
371011	Adenovirus D type 37	detected
371012	Adenovirus B type 11	detected
Instand HAdV November 2011		
371013	Adenovirus D type 37	detected
371014	Adenovirus B type 11	detected
371015	Adenovirus D type 37	detected
371016	Adenovirus B type 11	detected
Instand HAdV June 2012		
371017	Adenovirus D type 37	detected
371018	Adenovirus C type 2	detected
371019	Adenovirus A type 31	detected
371020	negative	negative
Instand HAdV November 2012		
371021	Adenovirus C type 2	detected
371022	negative	negative
371023	Adenovirus B type 11	detected
371024	Adenovirus D type 37	detected
Instand HAdV June 2014		
371033	negative	negative
371034	Adenovirus C type 2	detected
371035	Adenovirus D type 37	detected
371036	Adenovirus B type 11	detected
Instand HAdV June 2015		
371041	Adenovirus B type 11	detected
371042	negative	negative
371043	Adenovirus A type 31	detected
371044	Adenovirus B type 11	detected
Instand HAdV November 2015		
371045	Adenovirus D type 37	detected
371046	Adenovirus C type 2	detected
371047	negative	negative
371048	Adenovirus D type 37	detected



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Table 21: Instand e. V. panels for enterovirus tested with FTD Resp33.

samples	sample content	sample status
Instand EV June 2011		
372009	Coxsackievirus A21 (CVA21)	detected
372010	Echovirus 7 (EV7)	detected
372011	negative	negative
372012	Coxsackievirus B1 (CVB1)	detected
Instand EV November 2011		
372013	Echovirus 7 (EV7)	detected
372014	Coxsackievirus B6 (CVB6)	detected
372015	Echovirus 7 (EV7)	detected
372016	Coxsackievirus B6 (CVB6)	detected
Instand EV June 2012		
372017	Coxsackievirus B3 (CVB3)	detected
372018	negative	negative
372019	Echovirus 30 (EV30)	detected
372020	Coxsackievirus A21 (CVA21)	detected
Instand EV November 2012		
372021	negative	negative
372022	Echovirus 30 (EV30)	detected
372023	Coxsackievirus B4 (CVB4)	detected
372024	Coxsackievirus B3 (CVB3)	detected
Instand EV June 2013		
372025	Echovirus 30 (EV30)	detected
372026	Coxsackievirus B4 (CVB4)	detected
372027	Echovirus 7 (EV7)	detected
372028	negative	negative
Instand EV November 2013		
372029	Echovirus 7 (EV7)	detected
372030	Coxsackievirus B3 (CVB3)	detected
372031	negative	negative
372032	Coxsackievirus A21 (CVA21)	detected
Instand EV June 2014		
372033	Echovirus 30 (EV30)	detected
372034	negative	negative
372035	Coxsackievirus A21 (CVA21)	detected
372036	Coxsackievirus B3 (CVB3)	detected
Instand EV November 2014		
372037	Coxsackievirus B3 (CVB3)	detected
372038	Echovirus 30 (EV30)	detected
372039	Echovirus 7 (EV7)	detected
372040	Echovirus 7 (EV7)	detected
372041	Enterovirus 68 (Enterov68)	detected
Instand EV June 2015		
372042	negative	negative
372043	Enterovirus 68 (ENT68)	detected
372044	Coxsackievirus B3 (CVB3)	detected
372045	Coxsackievirus A21 (CVA21)	detected
Instand EV November 2015		
372046	Enterovirus 68 (ENT68)	detected
372047	Echovirus 30 (EV30)	detected
372048	negative	negative
372049	Echovirus 7 (EV7)	detected



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Table 22: Instand e. V. panels for influenza virus tested with FTD Resp33.

samples	sample content	sample status
Instand INF March 2011		
370007	Pandemic Influenza A (H1N1) pdm09	detected
370008	negative	negative
370009	Saisonal Influenza A H1N1	detected
370010	Saisonal Influenza B	detected
370011	Aviar Influenza A H5N1	detected
370012	Saisonal Influenza A H3N2	detected
Instand INF November 2011		
370013	Saisonal Influenza B	detected
370014	Saisonal Influenza A H1N1	detected
370015	Aviar Influenza A H5N1	detected
370016	Saisonal Influenza A H3N2	detected
370017	negative	negative
370018	Pandemic Influenza A (H1N1) pdm09	detected
Instand INF March 2012		
370019	Saisonal Influenza A H3N2	detected
370020	Aviar Influenza A H5N1	detected
370021	Pandemic Influenza A (H1N1) pdm09	detected
370022	negative	negative
370023	Saisonal Influenza A H1N1	detected
370024	Saisonal Influenza B	detected
Instand INF November 2012		
370025	Aviar Influenza A H5N1	detected
370026	Pandemic Influenza A (H1N1) pdm09	detected
370027	Saisonal Influenza A H3N2	detected
370028	Saisonal Influenza B	detected
370029	negative	negative
370030	Saisonal Influenza B	detected
Instand INF March 2013		
370031	negative	negative
370032	Saisonal Influenza A H3N2	detected
370033	Saisonal Influenza B	detected
370034	Saisonal Influenza A H1N1	detected
370035	Pandemic Influenza A (H1N1) pdm09	detected
370036	Aviar Influenza A H5N1	detected
Instand INF November 2013		
370037	Aviar Influenza A H5N1	detected
370038	Pandemic Influenza A (H1N1) pdm09	detected
370039	negative	negative
370040	Saisonal Influenza B	detected
370041	Aviar Influenza A H7N9	detected
370042	Saisonal Influenza A H3N2	detected



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Table 23: Instand e. V. panels for influenza virus tested with FTD Resp33.

samples	sample content	sample status
Instand INF March 2014		
370043	Saisonal Influenza A H3N2	detected
370044	Aviar Influenza A H7N9	detected
370045	Pandemic Influenza A (H1N1) pdm09	detected
370046	Aviar Influenza A H5N1	detected
370047	negative	negative
370048	Saisonal Influenza B	detected
Instand INF November 2014		
370049	Pandemic Influenza A (H1N1) pdm09	detected
370050	Saisonal Influenza B	detected
370051	negative	negative
370052	Aviar Influenza A H7N9	detected
370053	Aviar Influenza A H5N1	detected
370054	Saisonal Influenza A H3N2	detected
Instand INF March 2015		
370055	Saisonal Influenza B	detected
370056	Pandemic Influenza A (H1N1) pdm09	detected
370057	Saisonal Influenza A H3N2	detected
370058	negative	negative
370059	Saisonal Influenza B	detected
370060	Aviar Influenza A H7N9	detected
Instand INF November 2015		
370065	Saisonal Influenza B	detected
370066	Saisonal Influenza A H3N2	detected
370067	Saisonal Influenza B	detected
370068	Pandemic Influenza A (H1N1) pdm09	detected
370069	negative	negative
370070	Aviar Influenza A H5N8	detected



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Table 24: Instand e. V. panels for *M. pneumoniae* tested with FTD Resp33.

samples	sample content	sample status
Instand Mpneu May 2012		
1215411	<i>Mycoplasma pneumoniae</i>	detected
1215412	<i>Ureaplasma urealyticum</i>	negative
1215413	<i>Mycoplasma pneumoniae</i>	detected
1215414	<i>Escherichia coli</i> K12	negative
Instand Mpneu November 2012		
1225411	<i>Escherichia coli</i> K12	negative
1225412	<i>Mycoplasma pneumoniae</i>	detected
1225413	<i>Ureaplasma urealyticum</i>	negative
1225414	<i>Mycoplasma pneumoniae</i>	detected
Instand Mpneu May 2013		
1315411	<i>Escherichia coli</i> K12	negative
1315412	<i>Mycoplasma pneumoniae</i>	detected
1315413	<i>Mycoplasma pneumoniae</i>	detected
1315414	<i>Mycoplasma pneumoniae</i>	detected
Instand Mpneu November 2013		
1325411	<i>Mycoplasma genitalium</i>	negative
1325412	<i>Mycoplasma pneumoniae</i>	detected
1325413	<i>Mycoplasma pneumoniae</i>	detected
1325414	<i>Mycoplasma genitalium</i>	negative
Instand Mpneu May 2014		
1415411	<i>Escherichia coli</i> K12	negative
1415412	<i>Mycoplasma pneumoniae</i>	detected
1415413	<i>Mycoplasma genitalium</i>	negative
1415414	<i>Mycoplasma pneumoniae</i>	detected
Instand Mpneu November 2014		
1425411	<i>Mycoplasma pneumoniae</i>	detected
1425412	<i>Mycoplasma pneumoniae</i>	detected
1425413	<i>Mycoplasma pneumoniae</i>	detected
1425414	<i>Escherichia coli</i> K12	negative
Instand Mpneu May 2015		
1515411	<i>Mycoplasma pneumoniae</i>	detected
1515412	<i>Escherichia coli</i> K12	negative
1515413	<i>Mycoplasma pneumoniae</i>	detected
1515414	<i>Mycoplasma genitalium</i>	negative
Instand Mpneu November 2015		
1525411	<i>Mycoplasma pneumoniae</i>	detected
1525412	<i>Escherichia coli</i> K12	negative
1525413	<i>Mycoplasma salivarium</i>	negative
1525414	<i>Mycoplasma genitalium</i>	negative



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Table 25: Instand e. V. panels for *C. pneumoniae* tested with FTD Resp33.

samples	sample content	sample status
Instand Cpneu May 2012		
1215401	<i>Chlamydia pneumoniae</i>	detected
1215402	<i>Chlamydia pneumoniae</i>	detected
1215403	<i>Chlamydia pneumoniae</i>	detected
1215404	<i>Escherichia coli</i> K12	negative
Instand Cpneu November 2012		
1225401	<i>Chlamydia pneumoniae</i>	detected
1225402	<i>Chlamydia pneumoniae</i>	detected
1225403	<i>Escherichia coli</i> K12	negative
1225404	<i>Chlamydia pneumoniae</i>	detected
Instand Cpneu May 2013		
1315401	<i>Legionella pneumophila</i> SG3	negative
1315402	<i>Chlamydia pneumoniae</i>	detected
1315403	<i>Escherichia coli</i> K12	negative
1315404	<i>Chlamydia pneumoniae</i>	detected
Instand Cpneu November 2013		
1325401	<i>Chlamydia pneumoniae</i>	detected
1325402	<i>Mycoplasma pneumoniae</i>	negative
1325403	<i>Escherichia coli</i> K12	negative
1325404	<i>Chlamydia pneumoniae</i>	detected
Instand Cpneu May 2014		
1415401	<i>Chlamydia pneumoniae</i>	detected
1415402	<i>Chlamydia pneumoniae</i>	detected
1415403	<i>Chlamydia pneumoniae</i>	detected
1415404	<i>Escherichia coli</i> K12	negative
Instand Cpneu November 2014		
1425401	<i>Chlamydia pneumoniae</i>	detected
1425402	<i>Escherichia coli</i> K12	negative
1425403	<i>Chlamydia pneumoniae</i>	detected
1425404	<i>Chlamydia trachomatis</i>	negative
Instand Cpneu May 2015		
1515401	<i>Chlamydia pneumoniae</i>	detected
1515402	<i>Chlamydia pneumoniae</i>	detected
1515403	<i>Escherichia coli</i> K12	negative
1515404	<i>Escherichia coli</i> K12	negative
Instand Cpneu November 2015		
1525401	<i>Chlamydia pneumoniae</i>	detected
1525402	<i>Mycoplasma pneumoniae</i>	negative
1525403	<i>Chlamydia pneumoniae</i>	detected
1525404	<i>Chlamydia pneumoniae</i>	detected



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Table 26: Instand e. V. panels for respiratory syncytial virus tested with FTD Resp33.

samples	sample content	sample status
Instand HRSV June 2012		
359009	Respiratory syncytial virus B	detected
359010	Respiratory syncytial virus A	detected
Instand HRSV November 2012		
359011	Respiratory syncytial virus B	detected
359012	Respiratory syncytial virus B	detected
Instand HRSV June 2013		
359013	Respiratory syncytial virus B	detected
359014	negative	negative
Instand HRSV November 2013		
359015	Respiratory syncytial virus A	detected
359016	Respiratory syncytial virus A	detected
Instand HRSV June 2014		
359017	negative	negative
359018	Respiratory syncytial virus A	detected
Instand HRSV November 2014		
359019	Respiratory syncytial virus B	detected
359020	Respiratory syncytial virus A	detected

Table 27: Instand e. V. panels for *Bordetella* spp. validated with FTD Resp33.

samples	sample content	sample status
Instand Bord May 2013		
1315321	<i>Escherichia coli</i> K12	negative
1315322	<i>Bordetella pertussis</i>	detected
1315323	<i>Bordetella pertussis</i>	detected
1315324	<i>Escherichia coli</i> K12	negative
Instand Bord November 2013		
1325321	<i>Escherichia coli</i> K12	negative
1325322	<i>Bordetella pertussis</i>	detected
1325323	<i>Escherichia coli</i> K12	negative
1325324	<i>Bordetella parapertussis</i>	negative
Instand Bord May 2014		
1415321	<i>Bordetella pertussis</i>	detected
1415322	<i>Escherichia coli</i> K12	negative
1415323	<i>Bordetella pertussis</i>	detected
1415324	<i>Bordetella holmesii</i>	detected
Instand Bord November 2014		
1425321	<i>Bordetella homesii</i>	detected
1425322	<i>Escherichia coli</i> K12	negative
1425323	<i>Bordetella parapertussis</i>	negative
1425324	<i>Bordetella pertussis</i>	detected
Instand Bord May 2015		
1515321	<i>Bordetella pertussis</i>	detected
1515322	<i>Bordetella pertussis</i>	detected
1515323	<i>Bordetella pertussis</i>	detected
1515324	<i>Escherichia coli</i> K12	negative
Instand Bord November 2015		
1525321	<i>Bordetella bronchiseptica</i>	negative
1525322	<i>Bordetella pertussis</i>	detected
1525323	<i>Escherichia coli</i> K12	negative
1525324	<i>Bordetella parapertussis</i>	negative



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Table 28: Instand e. V. panels for *Salmonella* spp. validated with FTD Resp33.

samples	sample content	sample status
Instand Salm May 2011		
1215371	<i>Salmonella enterica</i> ser. Enteritidis	detected
1215372	<i>Escherichia coli</i> K12	negative
1215373	<i>Salmonella enterica</i> ser. Typhimurium	detected
1215374	<i>Escherichia coli</i> K12	negative
Instand Salm September 2011		
1225371	<i>Salmonella enterica</i> ser. Typhimurium	detected
1225372	<i>Salmonella enterica</i> ser. Typhimurium	detected
1225373	<i>Escherichia coli</i> K12	negative
1225374	<i>Salmonella enterica</i> ser. Typhimurium	detected
Instand Salm May 2014		
1415371	<i>Salmonella enterica</i> ser. Enteritidis	detected
1415372	<i>Escherichia coli</i> K12	negative
1415373	<i>Escherichia coli</i> K12	negative
1415374	<i>Salmonella enterica</i> ser. Enteritidis	detected
Instand Salm May 2015		
1515371	<i>Escherichia coli</i> K12	negative
1515372	<i>Salmonella enterica</i> ser. Typhimurium	detected
1515373	<i>Salmonella enterica</i> ser. Typhimurium	detected
1515374	<i>Salmonella enterica</i> ser. Typhimurium	detected
Instand Salm November 2015		
1525371	<i>Salmonella enterica</i> ser. Enteritidis	detected
1525372	<i>Salmonella enterica</i> ser. Grumpensis	detected
1525373	<i>Salmonella enterica</i> ser. Enteritidis	detected
1525374	<i>Escherichia coli</i> K12	negative

Table 29: Instand e. V. panels for human metapneumovirus validated with the FTD Resp33.

samples	sample content	sample status
Instand HMPV May 2014		
385001	HMPV TypA	detected
385002	HMPV TypA	detected
385003	negative	negative
385004	HMPV TypA	detected
Instand HMPV September 2014		
385005	HMPV TypA	detected
385006	HMPV TypA	detected
385007	HMPV TypA	detected
385008	HMPV TypA	detected
Instand HMPV June 2015		
385009	HMPV TypA	detected
385010	negative	negative
385011	HMPV TypA	detected
385012	HMPV TypA	detected



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Table 30: Instand e. V. panels for parainfluenzavirus validated with the FTD Resp33.

samples		sample content	sample status
Instand HPIV September 2014			
388005	HPIV3		detected
388006	HPIV2		detected
388007	negative		negative
388008	negative		negative
Instand HPIV September 2015			
388013	HPIV2		detected
388014	HPIV2		detected
388015	HPIV3		detected
388016	HPIV3		detected

6. QCMD panels

External quality control assessments of the Quality Control for Molecular Diagnostics were performed to verify the quality of FTD Resp33. (www.qcmd.org). All samples were dissolved in accordance to the instructions of the supplier and analysed with FTD Resp33. Table 31 shows an overview of QCMD panels validated with FTD Resp33.

Table 31: List of the QCMD panels tested with FTD Resp33.

Pathogen	QCMD 2012	QCMD 2013	QCMD 2014	QCMD 2015	QCMD 2016
HAdV	ADVDNA12 GastroV12	ADVDNA13 GastroV13	ADVDNA14 GastroV14	ADVDNA15 C1 ADVDNA15 C2 GastroV15	ADVDNA16 C1 / /
Cor43/63/229	CVRNA12	CVRNA13	CVRNA14	CVRNA15	CVRNA16
EV	EVRNA12	EVRNA13	EVRNA14	EVRNA15 C1 EVRNA15 C2	EVRNA16 C1 EVRNA16 C2
HMPV	MPVRNA12	MPVRNA13	MPVRNA14	MPVRNA15	MPVRNA16
FLU A/ B	INFRNA12	INFRNA13	INFRNA14	INFRNA15	INFRNA16
Mpneu/Cpneu	CP.MP12	CP.MP13	CP.MP14	CP.MP15	CP.MP16
HPeV	PeVRNA12	PeVRNA13	PeVRNA14	PeVRNA15C1 PeVRNA15C2	PeVRNA16C1 PeVRNA16C2
HRSV	RSVRNA12	RSVRNA13	RSVRNA14	RSVRNA15	RSVRNA15
HRV	/	RVRNA13	RVRNA14	RVRNA15	RVRNA15
HPIV	PINFRNA12	PINFRNA13	PINFRNA14	PINFRNA15	PINFRNA15
PCP	PCPDNA12	PCPDNA13	PCPDNA14	PCPDNA15	/
Salm	GastroB12	GastroB13	GastroB14	GastroB15	/
Lpneu/Long	/	/	LPDNA14	/	LPDNA16

(FLUA= influenza A, FLUB= influenza B, HRV= rhinovirus, Cor63= coronavirus NL63, Cor229= coronavirus 229E, Cor43= coronavirus OC43, HPIV2= parainfluenza virus 2, HPIV3= parainfluenza virus 3, HPIV4= parainfluenza virus 4, HRSV= respiratory syncytial viruses A and B, HAdV= adenovirus, EV= enterovirus, HPeV= parechovirus, HMPV= human metapneumovirus A and B, PCP= *Pneumocystis jirovecii*, Salm= *Salmonella* spp., HPIV1= parainfluenza virus 1, Mpneu= *Mycoplasma pneumoniae*, Cpneu= *Chlamydia pneumoniae*, Lpneu/Long= *Legionella pneumophila*/*Legionella longbeachae*)

The results of the QCMD 2012-2016 Adenovirus DNA EQA Program (ADVDNA12-16) are shown in Table 32. All HAdV samples could be detected except ADV12-07. This sample was described as infrequently detected and was negative in our test.



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Table 32: Overview results QCMD ADVDNA12-16 with FTD Resp33.

sample	sample content	sample status	FTD Resp 33
QCMD ADVDNA 2012			
ADVDNA12-01	Adenovirus type 34	detected	Positive (Ct 32.9)
ADVDNA12-02	Adenovirus type 1	detected	Positive (Ct 30.4)
ADVDNA12-03	negative	negative	negative
ADVDNA12-04	Adenovirus type 1	infrequently detected	Positive (Ct 32.4)
ADVDNA12-05	Adenovirus type 1	frequently detected	Positive (Ct 27.5)
ADVDNA12-06	Adenovirus type 4	frequently detected	Positive (Ct 27.6)
ADVDNA12-07	Adenovirus type 34	infrequently detected	negative
ADVDNA12-08	Adenovirus type 1	detected	Positive (Ct 30.2)
QCMD ADVDNA 2013			
ADVDNA13-01	Adenovirus type 14	frequently detected	Positive (Ct 21.0)
ADVDNA13-02	Adenovirus type 5	detected	Positive (Ct 33.5)
ADVDNA13-03	Adenovirus type 1	frequently detected	Positive (Ct 27.3)
ADVDNA13-04	negative	negative	negative
ADVDNA13-05	Adenovirus type 1	frequently detected	Positive (Ct 27.5)
ADVDNA13-06	Adenovirus type 4	detected	Positive (Ct 30.3)
ADVDNA13-07	Adenovirus type 1	frequently detected	Positive (Ct 28.8)
ADVDNA13-08	Adenovirus type 5	detected	Positive (Ct 30.4)
ADVDNA13-09	Adenovirus type 4	frequently detected	Positive (Ct 27.4)
ADVDNA13-10	Adenovirus type 1	detected	Positive (Ct 29.1)
QCMD ADVDNA 2014			
ADVDNA14-01	Adenovirus type 1	frequently detected	Positive (Ct 31.4)
ADVDNA14-02	Adenovirus type 1	frequently detected	Positive (Ct 30.3)
ADVDNA14-03	Adenovirus type 1	detected	Positive (Ct 32.1)
ADVDNA14-04	Adenovirus type 1	frequently detected	Positive (Ct 31.2)
ADVDNA14-05	Adenovirus type 4	detected	Positive (Ct 34.1)
ADVDNA14-06	Adenovirus type 4	frequently detected	Positive (Ct 31.3)
ADVDNA14-07	negative	negative	negative
ADVDNA14-08	Adenovirus type 14	frequently detected	Positive (Ct 21.0)
ADVDNA14-09	Adenovirus type 5	detected	Positive (Ct 37.5)
ADVDNA14-10	Adenovirus type 5	frequently detected	Positive (Ct 33.8)
QCMD ADVDNA 2015C1			
ADVDNA15C1-01	Adenovirus type 1	frequently detected	Positive (Ct 32.2)
ADVDNA15C1-02	negative	negative	Negative
ADVDNA15C1-03	Adenovirus type 1	frequently detected	Positive (Ct 31.6)
ADVDNA15C1-04	Adenovirus type 5	detected	Positive (Ct 33.1)
ADVDNA15C1-05	Adenovirus type 4	frequently detected	Positive (Ct 29.6)
QCMD ADVDNA 2015C2			
ADVDNA15C2-01	Adenovirus type 4	frequently detected	Positive (Ct 29.9)
ADVDNA15C2-02	negative	negative	negative
ADVDNA15C2-03	Adenovirus type 5	frequently detected	Positive (Ct 33.2)
ADVDNA15C2-04	Adenovirus type 1	detected	Positive (Ct 32.4)
ADVDNA15C2-05	Adenovirus type 1	frequently detected	Positive (Ct 32.0)
QCMD ADVDNA 2016C1			
ADVDNA16C1-01	Adenovirus type 1	frequently detected	Positive (Ct 34.2)
ADVDNA16C1-02	Adenovirus type 4	detected	Positive (Ct 33.7)
ADVDNA16C1-03	Adenovirus type 1	frequently detected	Positive (Ct 33.7)
ADVDNA16C1-04	negative	negative	negative
ADVDNA16C1-05	Adenovirus type 34	detected	Positive (Ct 37.5)



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The results of the QCMD 2012-2015 GastroV EQA Pilot Program (GastroV12-15) are shown in Table 33. All samples could be confirmed.

Table 33: Overview results QCMD GastroV12-15 with FTD Resp33.

sample	sample content	sample status	FTD Resp33
GastroV 2012			
GastroV12-01	Rotavirus	positive	negative
GastroV12-02	Sapovirus	positive	negative
GastroV12-03	Norovirus	positive	negative
GastroV12-04	Adenovirus	positive	Positive (Ct 32.0)
GastroV12-05	Negative	Negative	negative
GastroV12-06	Adenovirus	positive	Positive (Ct 27.4)
GastroV12-07	Norovirus	positive	negative
GastroV12-08	Rotavirus	positive	negative
GastroV12-09	Astrovirus	positive	negative
GastroV12-10	Astrovirus	positive	negative
GastroV12-11	Adenovirus	positive	Positive (Ct 26.3)
GastroV12-12	Rotavirus	positive	negative
GastroV12-13	Norovirus	positive	negative
GastroV12-14	Negative	Negative	negative
GastroV12-15	Adenovirus	positive	Positive (Ct 31.7)
GastroV12-16	Norovirus	positive	negative
GastroV12-17	Sapovirus	positive	negative
GastroV 2013			
GastroV13-01	Norovirus	positive	negative
GastroV13-02	Sapovirus	positive	negative
GastroV13-03	Astrovirus	positive	negative
GastroV13-04	Norovirus	positive	negative
GastroV13-05	Adenovirus	positive	Positive (Ct 40.0)
GastroV13-06	Rotavirus	positive	negative
GastroV13-07	Rotavirus	positive	negative
GastroV13-08	Negative	Negative	negative
GastroV13-09	Adenovirus	positive	Positive (Ct 30.8)
GastroV 2014			
GastroV14-01	Adenovirus	positive	Positive (Ct 30.5)
GastroV14-02	Norovirus	positive	negative
GastroV14-03	Rotavirus	positive	negative
GastroV14-04	Rotavirus	positive	negative
GastroV14-05	Negative	negative	negative
GastroV14-06	Norovirus	positive	negative
GastroV14-07	Adenovirus	positive	Positive (Ct 27.9)
GastroV14-08	Astrovirus	positive	negative
GastroV14-09	Norovirus	positive	negative
GastroV14-10	Sapovirus	positive	negative
GastroV 2015			
GastroV15-01	Norovirus	positive	negative
GastroV15-02	Astrovirus	positive	negative
GastroV15-03	negative	negative	negative
GastroV15-04	Norovirus	positive	negative
GastroV15-05	Adenovirus	positive	Positive (Ct 32.6)
GastroV15-06	Adenovirus	positive	Positive (Ct 29.7)
GastroV15-07	Sapovirus	positive	negative
GastroV15-08	Rotavirus	positive	negative
GastroV15-09	Astrovirus	positive	negative



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The results of QCMD 2012-2016 Coronavirus RNA EQA Program (CVRNA12-16) are shown in Tables 34 and 35. All samples could be confirmed.

Table 34: Overview results QCMD CVRNA12-16 with FTD Resp33.

sample	sample content	sample status	FTD Resp33
QCMD CV RNA 2012			
CVRNA 12-01	Coronavirus – OC43	frequently detected	Positive (Ct 26.3)
CVRNA 12-02	Coronavirus – NL63	detected	Positive (Ct 22.6)
CVRNA 12-03	Coronavirus – 229E	frequently detected	Positive (Ct 28.0)
CVRNA 12-04	Coronavirus – OC43	detected	Positive (Ct 32.4)
CVRNA 12-05	Coronavirus – NL63	detected	Positive (Ct 28.9)
CVRNA 12-06	negative	negative	negative
CVRNA 12-07	Coronavirus – 229E	detected	Positive (Ct 31.3)
CVRNA 12-08	Coronavirus – OC43	detected	Positive (Ct 29.7)
CVRNA 12-09	Coronavirus – NL63	detected	Positive (Ct 25.9)
CVRNA 12-10	Coronavirus – 229E	detected	Positive (Ct 34.5)
QCMD CV RNA 2013			
CVRNA 13-01	Coronavirus – OC43	detected	Positive (Ct 33.3)
CVRNA 13-02	Coronavirus – NL63	frequently detected	Positive (Ct 24.3)
CVRNA 13-03	Coronavirus – OC43	frequently detected	Positive (Ct 30.1)
CVRNA 13-04	negative	negative	negative
CVRNA 13-05	Coronavirus – NL63	detected	Positive (Ct 30.6)
CVRNA 13-06	Coronavirus – 229E	detected	Positive (Ct 34.5)
CVRNA 13-07	Coronavirus – NL63	frequently detected	Positive (Ct 27.1)
CVRNA 13-08	Coronavirus – 229E	frequently detected	Positive (Ct 31.1)
CVRNA 13-09	Coronavirus – OC43	frequently detected	Positive (Ct 27.2)
CVRNA 13-10	Coronavirus – 229E	frequently detected	Positive (Ct 28.4)
QCMD CV RNA 2014			
CVRNA 14-01	Coronavirus – 229E	detected	Positive (Ct 36.3)
CVRNA 14-02	Coronavirus – OC43	frequently detected	Positive (Ct 26.7)
CVRNA 14-03	Coronavirus – OC43	detected	Positive (Ct 33.5)
CVRNA 14-04	Coronavirus – OC43	detected	Positive (Ct 30.1)
CVRNA 14-05	Coronavirus – NL63	frequently detected	Positive (Ct 24.2)
CVRNA 14-06	Coronavirus – NL63	frequently detected	Positive (Ct 30.7)
CVRNA 14-07	Coronavirus – 229E	detected	Positive (Ct 33.4)
CVRNA 14-08	Coronavirus – NL63	frequently detected	Positive (Ct 27.3)
CVRNA 14-09	negative	negative	negative
CVRNA 14-10	Coronavirus – 229E	frequently detected	Positive (Ct 30.7)
QCMD CV RNA 2015			
CVRNA 15-01	Coronavirus – 229E	detected	Positive (Ct 31.6)
CVRNA 15-02	Coronavirus – NL63	detected	Positive (Ct 29.9)
CVRNA 15-03	Coronavirus – OC43	detected	Positive (Ct 32.9)
CVRNA 15-04	Coronavirus – NL63	detected	Positive (Ct 23.6)
CVRNA 15-05	Coronavirus – OC43	detected	Positive (Ct 26.6)
CVRNA 15-06	negative	negative	negative
CVRNA 15-07	Coronavirus – 229E	detected	Positive (Ct 33.8)
CVRNA 15-08	Coronavirus – OC43	detected	Positive (Ct 29.8)
CVRNA 15-09	Coronavirus – NL63	detected	Positive (Ct 27.0)
CVRNA 15-10	Coronavirus – 229E	detected	Positive (Ct 28.7)



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Table 35: Overview results QCMD CVRNA16 with FTD Resp33.

sample	sample content	sample status	FTD Resp33
QCMD CV RNA 2016			
CVRNA 16-01	Coronavirus – 229E	frequently detected	Positive (Ct 29.1)
CVRNA 16-02	Coronavirus – NL63	frequently detected	Positive (Ct 24.8)
CVRNA 16-03	Coronavirus – NL63	frequently detected	Positive (Ct 28.1)
CVRNA 16-04	Coronavirus – 229E	detected	Positive (Ct 31.4)
CVRNA 16-05	Coronavirus – 229E	detected	Positive (Ct 34.6)
CVRNA 16-06	Coronavirus – OC43	detected	Positive (Ct 34.2)
CVRNA 16-07	negative	negative	negative
CVRNA 16-08	Coronavirus – NL63	detected	Positive (Ct 30.7)
CVRNA 16-09	Coronavirus – OC43	frequently detected	Positive (Ct 30.5)
CVRNA 16-10	Coronavirus – OC43	frequently detected	Positive (Ct 27.3)

The results of the QCMD 2012-2016 Enterovirus RNA EQA Program (EVRNA12-16) are shown in Tables 36 and 37. All samples were confirmed except samples EVRNA 12-01 and EVRNA 12-10 in panel 2012, as well as the samples EVRNA 15C2-05, EVRNA 16C1-04 and EVRNA 16C2-04. These samples were described as infrequently detected or detected, but were negative in our test.

Table 36: Overview results QCMD EVRNA12-13 with FTD Resp33.

sample	sample content	sample status	FTD Resp33
QCMD EV RNA 2012			
EVRNA 12-01	Coxsackievirus B3	infrequently detected	negative
EVRNA 12-02	Coxsackievirus A24	detected	Positive (Ct 30.3)
EVRNA 12-03	Echovirus 30	frequently detected	Positive (Ct 32.8)
EVRNA 12-04	Coxsackievirus A9	detected	Positive (Ct 40.0)
EVRNA 12-05	Echovirus 11	detected	Positive (Ct 40.0)
EVRNA 12-06	Enterovirus 68	detected	Positive (Ct 25.8)
EVRNA 12-07	Enterovirus 71	detected	Positive (Ct 31.1)
EVRNA 12-08	Coxsackievirus A16	frequently detected	Positive (Ct 29.6)
EVRNA 12-09	Enterovirus 68	infrequently detected	Positive (Ct 37.3)
EVRNA 12-10	Echovirus 11	infrequently detected	negative
EVRNA 12-11	Coxsackievirus B3	detected	Positive (Ct 40.0)
EVRNA 12-12	negative	negative	negative
QCMD EV RNA 2013			
EVRNA 13-01	Coxsackievirus B3	frequently detected	Positive (Ct 30.4)
EVRNA 13-02	Echovirus 30	frequently detected	Positive (Ct 30.6)
EVRNA 13-03	Coxsackievirus A9	frequently detected	Positive (Ct 33.1)
EVRNA 13-04	Echovirus 11	frequently detected	Positive (Ct 28.3)
EVRNA 13-05	Coxsackievirus B3	detected	Positive (Ct 36.7)
EVRNA 13-06	Enterovirus 68	detected	Positive (Ct 26.1)
EVRNA 13-07	Coxsackievirus A16	frequently detected	Positive (Ct 31.5)
EVRNA 13-08	Enterovirus 68	detected	Positive (Ct 32.2)
EVRNA 13-09	negative	negative	negative
EVRNA 13-10	Enterovirus 71	frequently detected	positive (Ct 31.0)
EVRNA 13-11	Echovirus 11	detected	Positive (Ct 34.6)
EVRNA 13-12	Coxsackievirus A24	detected	Positive (Ct 30.4)



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Table 37: Overview results QCMD EVRNA14-16 with FTD Resp33.

sample	sample content	sample status	FTD Resp33
QCMD EV RNA 2014			
EVRNA 14-01	Enterovirus 71	frequently detected	Positive (Ct 27.8)
EVRNA 14-02	negative	negative	negative
EVRNA 14-03	Coxsackievirus A24	detected	Positive (Ct 28.4)
EVRNA 14-04	Enterovirus 71	detected	Positive (Ct 40.0)
EVRNA 14-05	Coxsackievirus B3	detected	Positive (Ct 40.0)
EVRNA 14-06	Echovirus 11	detected	Positive (Ct 40.0)
EVRNA 14-07	Coxsackievirus A9	detected	Positive (Ct 36.8)
EVRNA 14-08	negative	negative	negative
EVRNA 14-09	Coxsackievirus A16/ B5	frequently detected	Positive (Ct 32.2)
EVRNA 14-10	Enterovirus 68	detected	Positive (Ct 24.0)
EVRNA 14-11	Coxsackievirus B3	detected	Positive (Ct 28.0)
EVRNA 14-12	Echovirus 11	frequently detected	Positive (Ct 37.0)
QCMD EV RNA 2015 C1			
EVRNA 15C1-01	Coxsackievirus B3	frequently detected	Positive (Ct 28.4)
EVRNA 15C1-02	Enterovirus 68	frequently detected	Positive (Ct 23.6)
EVRNA 15C1-03	negative	negative	negative
EVRNA 15C1-04	Coxsackievirus B3	frequently detected	Positive (Ct 28.7)
EVRNA 15C1-05	Enterovirus 71	frequently detected	Positive (Ct 29.5)
QCMD EV RNA 2015 C2			
EVRNA 15C2-01	Coxsackievirus B3	frequently detected	Positive (Ct 32.2)
EVRNA 15C2-02	Echovirus 11	frequently detected	Positive (Ct 32.9)
EVRNA 15C2-03	Coxsackievirus B3	frequently detected	Positive (Ct 32.6)
EVRNA 15C2-04	Coxsackievirus A9	detected	Positive (Ct 35.5)
EVRNA 15C2-05	Coxsackievirus B3	detected	negative
QCMD EV RNA 2016 C1			
EVRNA 16C1-01	Echovirus 30	detected	Positive (Ct 35.7)
EVRNA 16C1-02	Echovirus 11	frequently detected	Positive (Ct 33.9)
EVRNA 16C1-03	Coxsackievirus B3	frequently detected	Positive (Ct 31.9)
EVRNA 16C1-04	Coxsackievirus A24	infrequently detected	negative
EVRNA 16C1-05	Coxsackievirus A9	frequently detected	Positive (Ct 30.7)
QCMD EV RNA 2016 C2			
EVRNA 16C2-01	negative	negative	negative
EVRNA 16C2-02	Coxsackievirus B3	frequently detected	Positive (Ct 34.6)
EVRNA 16C2-03	Enterovirus 71	frequently detected	Positive (Ct 32.9)
EVRNA 16C2-04	Enterovirus 71	infrequently detected	negative
EVRNA 16C2-05	Enterovirus 68	frequently detected	Positive (Ct 29.9)



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The results of the QCMD 2012-16 Metapneumovirus RNA EQA Program (MPVRNA12-16) are shown in Table 38. All samples could be confirmed.

Table 38: Overview results QCMD MPVRNA12-16 with FTD Resp33.

sample	sample content	sample status	FTD Resp33
QCMD MPV RNA 2012			
MPVRNA 12-01	Metapneumovirus Type B2	frequently detected	Positive (Ct 38.6)
MPVRNA 12-02	Metapneumovirus Type B2	detected	Positive (Ct 31.2)
MPVRNA 12-03	Metapneumovirus Type A1	detected	Positive (Ct 35.5)
MPVRNA 12-04	Metapneumovirus Type A1	frequently detected	Positive (Ct 29.1)
MPVRNA 12-05	Metapneumovirus Type B2	detected	Positive (Ct 33.8)
MPVRNA 12-06	negative	negative	negative
MPVRNA 12-07	Metapneumovirus Type A1	frequently detected	Positive (Ct 29.5)
MPVRNA 12-08	Metapneumovirus Type A1	frequently detected	Positive (Ct 32.6)
QCMD MPV RNA 2013			
MPVRNA 13-01	Metapneumovirus Type B2	frequently detected	Positive (Ct 25.7)
MPVRNA 13-02	Metapneumovirus Type A2	detected	Positive (Ct 28.3)
MPVRNA 13-03	Metapneumovirus Type A2	frequently detected	Positive (Ct 23.1)
MPVRNA 13-04	Metapneumovirus Type B2	frequently detected	Positive (Ct 22.5)
MPVRNA 13-05	negative	negative	negative
MPVRNA 13-06	Metapneumovirus Type B2	frequently detected	Positive (Ct 29.2)
MPVRNA 13-07	Metapneumovirus Type A2	frequently detected	Positive (Ct 24.6)
MPVRNA 13-08	Metapneumovirus Type A2	frequently detected	Positive (Ct 24.6)
QCMD MPV RNA 2014			
MPVRNA 14-01	Metapneumovirus Type A1	frequently detected	Positive (Ct 23.4)
MPVRNA 14-02	Metapneumovirus Type B2	frequently detected	Positive (Ct 22.4)
MPVRNA 14-03	Metapneumovirus Type A2	frequently detected	Positive (Ct 23.2)
MPVRNA 14-04	Metapneumovirus Type A2	frequently detected	Positive (Ct 24.9)
MPVRNA 14-05	Metapneumovirus Type A1	frequently detected	Positive (Ct 29.8)
MPVRNA 14-06	negative	negative	negative
MPVRNA 14-07	Metapneumovirus Type A1	frequently detected	Positive (Ct 26.5)
MPVRNA 14-08	Metapneumovirus Type A2	frequently detected	Positive (Ct 24.9)
QCMD MPV RNA 2015			
MPVRNA 15-01	Metapneumovirus Type A2	frequently detected	Positive (Ct 29.4)
MPVRNA 15-02	Metapneumovirus Type A2	frequently detected	Positive (Ct 29.5)
MPVRNA 15-03	Metapneumovirus Type A1	detected	Positive (Ct 33.3)
MPVRNA 15-04	Metapneumovirus Type B2	frequently detected	Positive (Ct 24.4)
MPVRNA 15-05	Metapneumovirus Type A1	infrequently detected	Positive (Ct 36.5)
MPVRNA 15-06	Metapneumovirus Type A1	frequently detected	Positive (Ct 30.4)
MPVRNA 15-07	negative	negative	negative
MPVRNA 15-08	Metapneumovirus Type A2	frequently detected	Positive (Ct 27.8)
QCMD MPV RNA 2016			
MPVRNA 16-01	Metapneumovirus Type A1	detected	Positive (Ct 33.4)
MPVRNA 16-02	negative	negative	negative
MPVRNA 16-03	Metapneumovirus Type B2	frequently detected	Positive (Ct 24.8)
MPVRNA 16-04	Metapneumovirus Type A1	detected	Positive (Ct 35.3)
MPVRNA 16-05	Metapneumovirus Type A2	frequently detected	Positive (Ct 27.5)
MPVRNA 16-06	Metapneumovirus Type A2	frequently detected	Positive (Ct 29.2)
MPVRNA 16-07	Metapneumovirus Type A1	frequently detected	Positive (Ct 29.9)
MPVRNA 16-08	Metapneumovirus Type A2	frequently detected	Positive (Ct 29.1)



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The results of the QCMD 2012-2016 Influenza virus A and B RNA EQA Program (INFRNA12-16) are shown in Tables 39 and 40. All samples could be confirmed.

Table 39: Overview results QCMD INFRNA12-15 with FTD Resp33.

sample	sample content	sample status	FTD Resp33
QCMD INF RNA 12			
INFRNA 12-01	Influenza virus B (Victoria)	frequently detected	Positive (Ct 25.7)
INFRNA 12-02	Influenza virus A (H3N2)	infrequently detected	Positive (Ct 34.3)
INFRNA 12-03	Influenza virus A (H1N1 pdm09)	frequently detected	Positive (Ct 26.1)
INFRNA 12-04	Influenza virus A (H1N1 pdm09)	detected	Positive (Ct 34.0)
INFRNA 12-05	Influenza virus A (H1N1 pdm09)	frequently detected	Positive (Ct 29.0)
INFRNA 12-06	Influenza virus B (Victoria)	detected	Positive (Ct 29.4)
INFRNA 12-07	Influenza virus A (H3N2)	frequently detected	Positive (Ct 28.0)
INFRNA 12-08	Influenza virus B (Yamagata)	frequently detected	Positive (Ct 24.0)
INFRNA 12-09	Influenza virus A (H3N2)	detected	Positive (Ct 31.1)
INFRNA 12-10	Influenza virus B (Victoria)	infrequently detected	Positive (Ct 35.2)
INFRNA 12-11	Influenza virus B (Yamagata)	frequently detected	Positive (Ct 27.3)
INFRNA 12-12	negative	negative	negative
QCMD INF RNA 13			
INFRNA 13-01	Influenza virus A (H5N1)	detected	Positive (Ct 27.9)
INFRNA 13-02	Influenza virus B (Yamagata)	frequently detected	Positive (Ct 28.5)
INFRNA 13-03	Influenza virus A (H3N2)	frequently detected	Positive (Ct 28.2)
INFRNA 13-04	Influenza virus B (Victoria)	frequently detected	Positive (Ct 26.6)
INFRNA 13-05	negative	negative	negative
INFRNA 13-06	Influenza virus A (H1N1 pdm09)	detected	Positive (Ct 31.7)
INFRNA 13-07	Influenza virus A (H3N2)	infrequently detected	Positive (Ct 37.8)
INFRNA 13-08	Influenza virus B (Yamagata)	detected	Positive (Ct 30.9)
INFRNA 13-09	Influenza virus A (H1N1 pdm09)	frequently detected	Positive (Ct 29.3)
INFRNA 13-10	Influenza virus B (Victoria)	detected	Positive (Ct 29.9)
INFRNA 13-11	Influenza virus B (Yamagata)	detected	Positive (Ct 28.0)
INFRNA 13-12	Influenza virus A (H3N2)	detected	Positive (Ct 32.0)
QCMD INF RNA 14			
INFRNA 14-01	Influenza virus A (H7N7)	frequently detected	Positive (Ct 28.8)
INFRNA 14-02	negative	negative	negative
INFRNA 14-03	Influenza virus A (H1N1 pdm09)	frequently detected	Positive (Ct 35.0)
INFRNA 14-04	Influenza virus A (H3N2)	frequently detected	Positive (Ct 31.8)
INFRNA 14-05	Influenza virus A (H3N2)	infrequently detected	Positive (Ct 40.0)
INFRNA 14-06	Influenza virus A (H3N2)	detected	Positive (Ct 36.0)
INFRNA 14-07	Influenza virus A (H1N1 pdm09)	detected	Positive (Ct 36.2)
INFRNA 14-08	Influenza virus B (Yamagata)	frequently detected	Positive (Ct 31.0)
INFRNA 14-09	Influenza virus B (Yamagata)	frequently detected	Positive (Ct 30.5)
INFRNA 14-10	Influenza virus B (Victoria)	detected	Positive (Ct 35.4)
INFRNA 14-11	Influenza virus B (Yamagata)	frequently detected	Positive (Ct 34.3)
INFRNA 14-12	Influenza virus B (Victoria)	frequently detected	Positive (Ct 30.5)
QCMD INF RNA 15			
INFRNA 15-01	Influenza virus B (Victoria)	frequently detected	Positive (Ct 28.6)
INFRNA 15-02	Influenza virus B (Victoria)	detected	Positive (Ct 32.2)
INFRNA 15-03	Influenza virus A (H1N1 pdm09)	frequently detected	Positive (Ct 32.6)
INFRNA 15-04	Influenza virus A (H3N2)	detected	Positive (Ct 33.8)
INFRNA 15-05	negative	negative	negative
INFRNA 15-06	Influenza virus A (H7N7)	frequently detected	Positive (Ct 28.6)
INFRNA 15-07	Influenza virus B (Yamagata)	frequently detected	Positive (Ct 30.5)
INFRNA 15-08	Influenza virus B (Yamagata)	detected	Positive (Ct 34.4)
INFRNA 15-09	Influenza virus A (H1N1 pdm09)	detected	Positive (Ct 34.6)
INFRNA 15-10	Influenza virus A (H3N2)	frequently detected	Positive (Ct 30.8)



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Table 40: Overview results QCMD INFRNA16 with FTD Resp33.

sample	sample content	sample status	FTD Resp33
QCMD INF RNA 16			
INFRNA 16-01	Influenza virus B (Yamagata)	detected	Positive (Ct 34.6)
INFRNA 16-02	Influenza virus A (H3N2)	frequently detected	Positive (Ct 30.6)
INFRNA 16-03	Influenza virus B (Yamagata)	frequently detected	Positive (Ct 30.9)
INFRNA 16-04	negative	negative	negative
INFRNA 16-05	Influenza virus A (H5N1)	frequently detected	Positive (Ct 31.9)
INFRNA 16-06	Influenza virus A (H3N2)	frequently detected	Positive (Ct 33.9)
INFRNA 16-07	Influenza virus A (H1N1 pdm09)	frequently detected	Positive (Ct 32.7)
INFRNA 16-08	Influenza virus B (Victoria)	detected	Positive (Ct 33.8)
INFRNA 16-09	Influenza virus A (H1N1 pdm09)	detected	Positive (Ct 36.2)
INFRNA 16-10	Influenza virus B (Victoria)	detected	Positive (Ct 32.7)

The results of the QCMD 2012-2016 *Chlamydomphila pneumoniae* & *Mycoplasma pneumoniae* EQA Program (CP.MP12-16) are shown in Tables 41 and 42. All samples could be confirmed except sample CP.MP 15-10 and CP.MP 16-06 which was described as an infrequently detected sample and was negative in our test.

Table 41: Overview results QCMD CP.MP12-13 with FTD Resp33.

sample	sample content	sample status	FTD Resp33
QCMD CP.MP 2012			
CP.MP 12-01	<i>Chlamydomphila pneumoniae</i>	frequently detected	Positive (Ct 31.7)
CP.MP 12-02	<i>Chlamydomphila pneumoniae</i>	frequently detected	Positive (Ct 28.5)
CP.MP 12-03	<i>Mycoplasma pneumoniae</i>	frequently detected	Positive (Ct 30.5)
CP.MP 12-04	CP/ MP negative	negative	negative
CP.MP 12-05	<i>Mycoplasma pneumoniae</i>	detected	Positive (Ct 31.6)
CP.MP 12-06	<i>Mycoplasma pneumoniae</i>	frequently detected	Positive (Ct 31.5)
CP.MP 12-07	<i>Mycoplasma pneumoniae</i>	detected	Positive (Ct 34.4)
CP.MP 12-08	<i>Mycoplasma pneumoniae</i>	infrequently detected	Positive (Ct 35.8)
CP.MP 12-09	<i>Chlamydomphila pneumoniae</i>	detected	Positive (Ct 34.0)
CP.MP 12-10	CP/ MP negative	negative	negative
CP.MP 12-11	<i>Chlamydomphila pneumoniae</i>	frequently detected	Positive (Ct 28.4)
QCMD CP.MP 2013			
CP.MP 13-01	<i>Mycoplasma pneumoniae</i>	infrequently detected	Positive (Ct 34.4)
CP.MP 13-02	<i>Chlamydomphila pneumoniae</i>	infrequently detected	Positive (Ct 37.9)
CP.MP 13-03	<i>Chlamydomphila pneumoniae</i>	frequently detected	Positive (Ct 31.8)
CP.MP 13-04	<i>Chlamydomphila pneumoniae</i>	frequently detected	Positive (Ct 29.2)
CP.MP 13-05	<i>Chlamydomphila pneumoniae</i>	detected	Positive (Ct 34.9)
CP.MP 13-06	<i>Mycoplasma pneumoniae</i>	infrequently detected	Positive (Ct 38.9)
CP.MP 13-07	<i>Mycoplasma pneumoniae</i>	infrequently detected	Positive (Ct 33.7)
CP.MP 13-08	CP/ MP negative	negative	negative
CP.MP 13-09	<i>Mycoplasma pneumoniae</i>	detected	Positive (Ct 31.5)
CP.MP 13-10	CP/ MP negative	negative	negative
CP.MP 13-11	<i>Mycoplasma pneumoniae</i>	infrequently detected	Positive (Ct 39.0)
CP.MP 13-12	<i>Chlamydomphila pneumoniae</i>	frequently detected	Positive (Ct 31.7)



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Table 42: Overview results QCMD CP.MP14-16 with FTD Resp33.

sample	sample content	sample status	FTD Resp33
QCMD CP.MP 2014			
CP.MP 14-01	<i>Chlamydophila pneumoniae</i>	frequently detected	Positive (Ct 33.0)
CP.MP 14-02	<i>Mycoplasma pneumoniae</i>	detected	Positive (Ct 25.9)
CP.MP 14-03	<i>Mycoplasma pneumoniae</i>	infrequently detected	Positive (Ct 36.5)
CP.MP 14-04	CP/ MP negative	negative	negative
CP.MP 14-05	<i>Chlamydophila pneumoniae</i>	frequently detected	Positive (Ct 33.2)
CP.MP 14-06	<i>Chlamydophila pneumoniae</i>	infrequently detected	Positive (Ct 40.0)
CP.MP 14-07	CP/ MP negative	negative	negative
CP.MP 14-08	<i>Mycoplasma pneumoniae</i>	detected	Positive (Ct 34.7)
CP.MP 14-09	<i>Chlamydophila pneumoniae</i>	detected	Positive (Ct 37.7)
CP.MP 14-10	<i>Chlamydophila pneumoniae</i>	infrequently detected	Positive (Ct 30.0)
CP.MP 14-11	<i>Mycoplasma pneumoniae</i>	detected	Positive (Ct 34.3)
CP.MP 14-12	<i>Mycoplasma pneumoniae</i>	frequently detected	Positive (Ct 36.3)
QCMD CP.MP 2015			
CP.MP 15-01	<i>Chlamydophila pneumoniae</i>	infrequently detected	Positive (Ct 36.1)
CP.MP 15-02	<i>Chlamydophila pneumoniae</i>	frequently detected	Positive (Ct 31.8)
CP.MP 15-03	<i>Mycoplasma pneumoniae</i>	detected	Positive (Ct 36.8)
CP.MP 15-04	CP/ MP negative	negative	negative
CP.MP 15-05	CP/ MP negative	negative	negative
CP.MP 15-06	<i>Mycoplasma pneumoniae</i>	frequently detected	Positive (Ct 33.2)
CP.MP 15-07	<i>Chlamydophila pneumoniae</i>	detected	Positive (Ct 35.5)
CP.MP 15-08	<i>Chlamydophila pneumoniae</i>	detected	Positive (Ct 35.6)
CP.MP 15-09	<i>Chlamydophila pneumoniae</i>	infrequently detected	Positive (Ct 36.5)
CP.MP 15-10	<i>Mycoplasma pneumoniae</i>	infrequently detected	negative
CP.MP 15-11	<i>Mycoplasma pneumoniae</i>	detected	Positive (Ct 35.9)
CP.MP 15-12	<i>Mycoplasma pneumoniae</i>	frequently detected	Positive (Ct 32.5)
QCMD CP.MP 2016			
CP.MP 16-01	<i>Chlamydophila pneumoniae</i>	infrequently detected	Positive (Ct 37.6)
CP.MP 16-02	<i>Mycoplasma pneumoniae</i>	infrequently detected	Positive (Ct 35.0)
CP.MP 16-03	<i>Mycoplasma pneumoniae</i>	detected	Positive (Ct 37.1)
CP.MP 16-04	<i>Chlamydophila pneumoniae</i>	detected	Positive (Ct 36.2)
CP.MP 16-05	<i>Mycoplasma pneumoniae</i>	frequently detected	Positive (Ct 29.9)
CP.MP 16-06	<i>Chlamydophila pneumoniae</i>	infrequently detected	negative
CP.MP 16-07	<i>Chlamydophila pneumoniae</i>	frequently detected	Positive (Ct 30.2)
CP.MP 16-08	<i>Mycoplasma pneumoniae</i>	frequently detected	Positive (Ct 30.4)
CP.MP 16-09	CP/ MP negative	negative	negative
CP.MP 16-10	<i>Mycoplasma pneumoniae</i>	detected	Positive (Ct 31.8)



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The results of the QCMD 2012-2016 Parechovirus RNA EQA Program (PeVRNA12-16) are shown in Table 43 and 44. All samples were confirmed except the sample PeVRNA 12-02, PeVRNA 12-10, PeVRNA 13-01, PeVRNA 13-09, PeVRNA 14-02 and PeVRNA 16C1-04. These samples were described as infrequently detected and were negative in our test.

Table 43: Overview results QCMD PeVRNA12-16 with FTD Resp33.

sample	sample content	sample status	FTD Resp33
QCMD PeV RNA 2012			
PeVRNA12-01	Parechovirus 1	detected	Positive (Ct 33.0)
PeVRNA12-02	Parechovirus 3	infrequently detected	negative
PeVRNA12-03	Parechovirus 1	detected	Positive (Ct 30.4)
PeVRNA12-04	Parechovirus 3	frequently detected	Positive (Ct 31.6)
PeVRNA12-05	Parechovirus 4	frequently detected	Positive (Ct 32.4)
PeVRNA12-06	Coxsackievirus A21	negative	negative
PeVRNA12-07	Parechovirus 2	frequently detected	Positive (Ct 32.0)
PeVRNA12-08	negative	negative	negative
PeVRNA12-09	Parechovirus 5	frequently detected	Positive (Ct 34.5)
PeVRNA12-10	Parechovirus 3	infrequently detected	negative
QCMD PeV RNA 13			
PeVRNA13-01	Parechovirus 3	infrequently detected	negative
PeVRNA13-02	Parechovirus 3	frequently detected	Positive (Ct 37.6)
PeVRNA13-03	Parechovirus 5	frequently detected	Positive (Ct 31.8)
PeVRNA13-04	Parechovirus 1	frequently detected	Positive (Ct 37.9)
PeVRNA13-05	negative	negative	negative
PeVRNA13-06	Coxsackievirus A21	negative	negative
PeVRNA13-07	Parechovirus 1	detected	Positive (Ct 31.2)
PeVRNA13-08	Parechovirus 4	frequently detected	Positive (Ct 33.4)
PeVRNA13-09	Parechovirus 3	infrequently detected	negative
PeVRNA13-10	Parechovirus 2	frequently detected	Positive (Ct 37.0)
QCMD PeV RNA 14			
PeVRNA14-01	Coxsackievirus A21	negative	negative
PeVRNA14-02	Parechovirus 3	infrequently detected	negative
PeVRNA14-03	Parechovirus 4	frequently detected	Positive (Ct 36.0)
PeVRNA14-04	negative	negative	Negative
PeVRNA14-05	Parechovirus 1	detected	Positive (Ct 40.0)
PeVRNA14-06	Parechovirus 3	infrequently detected	Positive (Ct 40.0)
PeVRNA14-07	Parechovirus 1	frequently detected	Positive (Ct 29.4)
PeVRNA14-08	Parechovirus 3	frequently detected	Positive (Ct 24.6)
PeVRNA14-09	Parechovirus 5	frequently detected	Positive (Ct 31.4)
PeVRNA14-10	Parechovirus 2	frequently detected	Positive (Ct 40.6)
QCMD PeV RNA 2015 C1			
PeVRNA15C1-01	negative	negative	negative
PeVRNA15C1-02	Parechovirus 1	frequently detected	Positive (Ct 27.6)
PeVRNA15C1-03	Parechovirus 1	frequently detected	Positive (Ct 27.3)
PeVRNA15C1-04	Parechovirus 5	frequently detected	Positive (Ct 27.4)
PeVRNA15C1-05	Parechovirus 4	detected	Positive (Ct 28.0)



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Table 44: Overview results QCMD PeVRNA15-16 with FTD Resp33.

sample	sample content	sample status	FTD Resp33
QCMD PeV RNA 2015 C2			
PeVRNA15C2-01	Parechovirus 1	frequently detected	Positive (Ct 30.5)
PeVRNA15C2-02	Parechovirus 3	infrequently detected	Positive (Ct 36.4)
PeVRNA15C2-03	Parechovirus 1	frequently detected	Positive (Ct 30.0)
PeVRNA15C2-04	Parechovirus 3	frequently detected	Positive (Ct 30.6)
PeVRNA15C2-05	Parechovirus 2	frequently detected	Positive (Ct 32.8)
QCMD PeV RNA 2016 C1			
PeVRNA16C1-01	Parechovirus 3	frequently detected	Positive (Ct 30.9)
PeVRNA16C1-02	Parechovirus 1	frequently detected	Positive (Ct 30.6)
PeVRNA16C1-03	Parechovirus 2	frequently detected	Positive (Ct 32.3)
PeVRNA16C1-04	Parechovirus 2	infrequently detected	negative
PeVRNA16C1-05	Parechovirus 3	detected	Positive (Ct 33.3)
QCMD PeV RNA 2016 C2			
PeVRNA16C2-01	Parechovirus 3	detected	Positive (Ct 34.3)
PeVRNA16C2-02	Parechovirus 4	frequently detected	Positive (Ct 30.9)
PeVRNA16C2-03	Parechovirus 3	frequently detected	Positive (Ct 31.5)
PeVRNA16C2-04	negative	negative	negative
PeVRNA16C2-05	Parechovirus 5	detected	Positive (Ct 34.7)

The results of the QCMD 2012-2016 Respiratory Syncytial Virus RNA EQA Program (RSVRNA12-16) are shown in Table 45. All samples could be confirmed.



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Table 45: Overview results QCMD RSV RNA 12-16 with FTD Resp33.

sample	sample content	sample status	FTD Resp33
QCMD RSV RNA 12			
RSV12-01	Respiratory Syncytial Virus Type A	detected	Positive (Ct 32.0)
RSV12-02	Respiratory Syncytial Virus Type B	frequently detected	Positive (Ct 30.0)
RSV12-03	Respiratory Syncytial Virus negative	negative	negative
RSV12-04	Respiratory Syncytial Virus Type B	detected	Positive (Ct 37.7)
RSV12-05	Respiratory Syncytial Virus Type B	frequently detected	Positive (Ct 30.1)
RSV12-06	Respiratory Syncytial Virus Type B	frequently detected	Positive (Ct 26.5)
RSV12-07	Respiratory Syncytial Virus Type A	detected	Positive (Ct 35.6)
RSV12-08	Respiratory Syncytial Virus Type A	frequently detected	Positive (Ct 28.8)
QCMD RSV RNA 13			
RSV13-01	Respiratory Syncytial Virus Type A	infrequently detected	Positive (Ct 38.1)
RSV13-02	Respiratory Syncytial Virus Type B	frequently detected	Positive (Ct 32.6)
RSV13-03	Respiratory Syncytial Virus Type B	frequently detected	Positive (Ct 29.6)
RSV13-04	Respiratory Syncytial Virus Type B	detected	Positive (Ct 36.4)
RSV13-05	Respiratory Syncytial Virus Type A	frequently detected	Positive (Ct 30.0)
RSV13-06	Respiratory Syncytial Virus negative	negative	negative
RSV13-07	Respiratory Syncytial Virus Type A	detected	Positive (Ct 32.9)
RSV13-08	Respiratory Syncytial Virus Type B	frequently detected	Positive (Ct 32.6)
QCMD RSV RNA 14			
RSV14-01	Respiratory Syncytial Virus negative	negative	negative
RSV14-02	Respiratory Syncytial Virus Type A	frequently detected	Positive (Ct 38.8)
RSV14-03	Respiratory Syncytial Virus Type B	frequently detected	Positive (Ct 34.8)
RSV14-04	Respiratory Syncytial Virus Type A	infrequently detected	Positive (Ct 39.1)
RSV14-05	Respiratory Syncytial Virus Type B	detected	Positive (Ct 37.0)
RSV14-06	Respiratory Syncytial Virus Type B	detected	Positive (Ct 38.4)
RSV14-07	Respiratory Syncytial Virus Type B	detected	Positive (Ct 39.2)
RSV14-08	Respiratory Syncytial Virus Type A	frequently detected	Positive (Ct 36.6)
QCMD RSV RNA 15			
RSV15-01	Respiratory Syncytial Virus Type B	frequently detected	Positive (Ct 29.3)
RSV15-02	Respiratory Syncytial Virus Type B	detected	Positive (Ct 37.3)
RSV15-03	Respiratory Syncytial Virus Type B	frequently detected	Positive (Ct 32.2)
RSV15-04	Respiratory Syncytial Virus Type A	frequently detected	Positive (Ct 29.4)
RSV15-05	Respiratory Syncytial Virus Type A	infrequently detected	Positive (Ct 37.4)
RSV15-06	Respiratory Syncytial Virus negative	negative	negative
RSV15-07	Respiratory Syncytial Virus Type B	frequently detected	Positive (Ct 32.2)
RSV15-08	Respiratory Syncytial Virus Type A	detected	Positive (Ct 32.9)
QCMD RSV RNA 16			
RSV16-01	Respiratory Syncytial Virus Type A	frequently detected	Positive (Ct 29.5)
RSV16-02	Respiratory Syncytial Virus Type A	detected	Positive (Ct 32.5)
RSV16-03	Respiratory Syncytial Virus Type B	frequently detected	Positive (Ct 31.0)
RSV16-04	Respiratory Syncytial Virus negative	negative	negative
RSV16-05	Respiratory Syncytial Virus Type A	frequently detected	Positive (Ct 29.5)
RSV16-06	Respiratory Syncytial Virus Type B	detected	Positive (Ct 34.8)
RSV16-07	Respiratory Syncytial Virus Type B	frequently detected	Positive (Ct 28.3)
RSV16-08	Metapneumovirus Type A2	negative	negative

The results of the QCMD 2013-2016 Rhinovirus RNA EQA Program (RVRNA13-16) are shown in Table 46. All samples were confirmed except the samples RVRNA 13-06, RVRNA 13-08, RVRNA 14-05, RVRNA 15-03 and RVRNA 15-09. These samples were described as infrequently detected and were negative in our test. Moreover, sample RV13-06 was false positive for Enterovirus 68, but with a high CT value of 38.7.



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Table 46: Overview results QCMD RVRNA13-16 with FTD Resp33.

sample	sample content	sample status	FTD Resp33
QCMD RV RNA 2013			
RV13-01	Rhinovirus (A) 90	detected	Positive (Ct 36.5)
RV13-02	Rhinovirus (B) 5	detected	Positive (Ct 43.5)
RV13-03	Rhinovirus (A) 90	frequently detected	Positive (Ct 34.6)
RV13-04	Rhinovirus (C)	detected	Positive (Ct 33.5)
RV13-05	Rhinovirus (A) 16	frequently detected	Positive (Ct 36.1)
RV13-06	Enterovirus 68	negative	Positive (Ct 38.7)
RV13-07	Rhinovirus (B) 5	detected	Positive (Ct 32.1)
RV13-08	Rhinovirus (A) 16	infrequently detected	negative
RV13-09	Rhinovirus (A) 8	detected	Positive (Ct 37.2)
RV13-10	negative	negative	negative
RV13-11	Rhinovirus (C)	detected	Positive (Ct 30.5)
QCMD RV RNA 2014			
RV14-01	Rhinovirus (A) 90	detected	Positive (Ct 34.7)
RV14-02	Rhinovirus (A) 16	detected	Positive (Ct 34.0)
RV14-03	Rhinovirus (C)	frequently detected	Positive (Ct 27.1)
RV14-04	Rhinovirus (A) 8	detected	Positive (Ct 34.3)
RV14-05	Rhinovirus (A) 16	infrequently detected	negative
RV14-06	Rhinovirus (A) 90	detected	Positive (Ct 31.4)
RV14-07	Rhinovirus (B) 42	detected	Positive (Ct 26.8)
RV14-08	Rhinovirus (B) 5	detected	Positive (Ct 33.5)
RV14-09	negative	negative	negative
RV14-10	Rhinovirus (B) 5	detected	Positive (Ct 26.4)
QCMD RV RNA 2015			
RV15-01	negative	negative	negative
RV15-02	Rhinovirus (B) 5	detected	Positive (Ct 29.5)
RV15-03	Enterovirus 68	negative	Positive (Ct 36.4)
RV15-04	Rhinovirus (C)	frequently detected	Positive (Ct 28.7)
RV15-05	Rhinovirus (A) 8	detected	Positive (Ct 34.9)
RV15-06	Rhinovirus (A) 90	frequently detected	Positive (Ct 34.2)
RV15-07	Rhinovirus (B) 5	detected	Positive (Ct 35.8)
RV15-08	Rhinovirus (A) 90	detected	Positive (Ct 37.1)
RV15-09	Rhinovirus (A) 16	infrequently detected	negative
RV15-10	Rhinovirus (A) 16	frequently detected	Positive (Ct 36.7)
QCMD RV RNA 2016			
RV16-01	Rhinovirus 8	frequently detected	Positive (Ct 28.7)
RV16-02	Rhinovirus 5	detected	Positive (Ct 28.9)
RV16-03	Rhinovirus 8	detected	Positive (Ct 36.6)
RV16-04	Enterovirus 68	negative	negative
RV16-05	Rhinovirus 16	detected	Positive (Ct 29.3)
RV16-06	Rhinovirus 90	frequently detected	Positive (Ct 30.2)
RV16-07	Rhinovirus 72	infrequently detected	Positive (Ct 36.4)
RV16-08	negative	negative	negative
RV16-09	Rhinovirus 72	detected	Positive (Ct 30.6)
RV16-10	Rhinovirus 42	detected	Positive (Ct 29.1)

The results of the QCMD 2012-2016 Parainfluenza virus RNA EQA Program (PINFRNA12-16) are shown in Table 47. All samples were confirmed except the sample PINFRNA 12-07, PINFRNA 13-10 and PINFRNA 14-08. These samples were described as infrequently detected and were negative in our test.



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Table 47: Overview results QCMD PINFRNA12-16 with FTD Resp33.

sample	sample content	sample status	FTD Resp33
QCMD PINF RNA 2012			
PINF12-01	Parainfluenza Type 1	detected	Positive (Ct 35.9)
PINF12-02	Parainfluenza Type 2	frequently detected	Positive (Ct 37.5)
PINF12-03	Parainfluenza Type 2	frequently detected	Positive (Ct 30.5)
PINF12-04	Parainfluenza Type 3	detected	Positive (Ct 21.2)
PINF12-05	Parainfluenza Type 1	detected	Positive (Ct 36.6)
PINF12-06	negative	negative	negative
PINF12-07	Parainfluenza Type 1	infrequently detected	negative
PINF12-08	Parainfluenza Type 4	infrequently detected	Positive (Ct 28.9)
PINF12-09	Parainfluenza Type 2	frequently detected	Positive (Ct 34.2)
PINF12-10	Parainfluenza Type 1	detected	Positive (Ct 35.8)
QCMD PINF RNA 2013			
PINF13-01	Parainfluenza Type 2	frequently detected	Positive (Ct 28.4)
PINF13-02	Parainfluenza Type 1	infrequently detected	Positive (Ct 35.3)
PINF13-03	Parainfluenza Type 3	frequently detected	Positive (Ct 25.0)
PINF13-04	Parainfluenza Type 1	infrequently detected	Positive (Ct 32.7)
PINF13-05	Parainfluenza Type 2	detected	Positive (Ct 30.7)
PINF13-06	Parainfluenza Type 1	detected	Positive (Ct 32.7)
PINF13-07	negative	negative	negative
PINF13-08	Parainfluenza Type 4	infrequently detected	Positive (Ct 31.8)
PINF13-09	Parainfluenza Type 2	frequently detected	Positive (Ct 24.4)
PINF13-10	Parainfluenza Type 1	infrequently detected	negative
QCMD PINF RNA 2014			
PINF14-01	Parainfluenza Type 1	detected	Positive (Ct 34.7)
PINF14-02	Parainfluenza Type 2	detected	Positive (Ct 27.2)
PINF14-03	Parainfluenza Type 3	detected	Positive (Ct 29.8)
PINF14-04	Parainfluenza Type 1	detected	Positive (Ct 38.6)
PINF14-05	Parainfluenza Type 4	detected	Positive (Ct 32.9)
PINF14-06	Parainfluenza Type 2	detected	Positive (Ct 30.7)
PINF14-07	Parainfluenza Type 2	detected	Positive (Ct 33.7)
PINF14-08	Parainfluenza Type 1	infrequently detected	negative
PINF14-09	negative	negative	negative
PINF14-10	Parainfluenza Type 1	detected	Positive (Ct 37.4)
QCMD PINF RNA 2015			
PINF15-01	Parainfluenza Type 1	detected	Positive (Ct 34.4)
PINF15-02	Parainfluenza Type 2	detected	Positive (Ct 31.8)
PINF15-03	Parainfluenza Type 1	frequently detected	Positive (Ct 26.3)
PINF15-04	Parainfluenza Type 1	frequently detected	Positive (Ct 28.7)
PINF15-05	Parainfluenza Type 3	detected	Positive (Ct 25.7)
PINF15-06	negative	negative	negative
PINF15-07	Parainfluenza Type 2	frequently detected	Positive (Ct 28.5)
PINF15-08	Parainfluenza Type 4	detected	Positive (Ct 27.1)
PINF15-09	Parainfluenza Type 1	detected	Positive (Ct 29.1)
PINF15-10	Parainfluenza Type 2	frequently detected	Positive (Ct 25.2)
QCMD PINF RNA 2016			
PINF16-01	Parainfluenza Type 1	frequently detected	Positive (Ct 26.0)
PINF16-02	Parainfluenza Type 4	detected	Positive (Ct 27.9)
PINF16-03	Parainfluenza Type 1	frequently detected	Positive (Ct 29.6)
PINF16-04	Parainfluenza Type 3	frequently detected	Positive (Ct 26.4)
PINF16-05	Parainfluenza Type 1	detected	Positive (Ct 32.9)
PINF16-06	negative	negative	negative
PINF16-07	Parainfluenza Type 2	detected	Positive (Ct 32.0)
PINF16-08	Parainfluenza Type 2	detected	Positive (Ct 29.0)
PINF16-09	Parainfluenza Type 2	detected	Positive (Ct 25.6)
PINF16-10	Parainfluenza Type 3	detected	Positive (Ct 29.7)



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To prove the received results we investigated a comparison study of the QCMD 2013 Parainfluenza virus RNA EQA Program (PINFRNA13) with FTD Resp33 and a competitor kit on the Applied Biosystems® 7500 (Thermo Fisher Scientific). The obtained results of FTD Resp33 and a competitor kit showed no difference. All results given bei QCMD were confirmed (Table 48).

Table 48: QCMD 2013 Parainfluenza virus RNA EQA Program tested with FTD Resp33 and a competitor kit.

sample	content	FTD Resp33	Competitor RT-PCR
QCMD PINF RNA 13			
PINF13-01	Parainfluenza Type 2	positive (HPIV2)	Positive (HPIV2 or 4)*
PINF13-02	Parainfluenza Type 1	positive (HPIV1)	Positive (HPIV1 or 3)*
PINF13-03	Parainfluenza Type 3	positive (HPIV3)	Positive (HPIV1 or 3)*
PINF13-04	Parainfluenza Type 1	positive (HPIV1)	Positive (HPIV1 or 3)*
PINF13-05	Parainfluenza Type 2	positive (HPIV2)	Positive (HPIV2 or 4)*
PINF13-06	Parainfluenza Type 1	positive (HPIV1)	Positive (HPIV1 or 3)*
PINF13-07	negative	negative	negative
PINF13-08	Parainfluenza Type 4	positive (HPIV4)	Positive (HPIV2 or 4)*
PINF13-09	Parainfluenza Type 2	positive (HPIV2)	Positive (HPIV2 or 4)*
PINF13-10	Parainfluenza Type 1	negative	negative

(HPIV1= parainfluenza virus 1, HPIV2= parainfluenza virus 2, HPIV3= parainfluenza virus 3, HPIV4= parainfluenza virus 4, * competitor kit can detect all 4 types of Parainfluenza, but it can not differentiate between type 2 and 4, and type 1 and 3)

In summary, we obtained no differences for the detection of HPIV1-4 with the competitor tests. 100% agreement could be obtained. We could exclude PCR inhibition and proved nucleic acid isolation by the detection of the IC in each extracted specimen.



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The results of the QCMD 2012-2015 *Pneumocystis jirovecii* DNA EQA Program (PCPDNA12-15) are shown in Table 49. All samples containing PCP could be confirmed except sample PCP 12-06. This sample was described as infrequently detected and was negative in our test.

Table 49: Overview results QCMD PCPDNA12-15 with FTD Resp33.

sample	content	Status	FTD Resp33
PCPDNA12			
PCPDNA 12-01	<i>Pneumocystis jirovecii</i> (Type: Ne)	detected	Positive (Ct 30.8)
PCPDNA 12-02	<i>Pneumocystis jirovecii</i> (Type: Ne)	detected	Positive (Ct 35.9)
PCPDNA 12-03	<i>Pneumocystis jirovecii</i> (Type: Ne)	frequently detected	Positive (Ct 28.2)
PCPDNA 12-04	<i>Pneumocystis jirovecii</i> (Type: A1)	detected	Positive (Ct 34.4)
PCPDNA 12-05	<i>Pneumocystis jirovecii</i> (Type: A1)	detected	Positive (Ct 30.6)
PCPDNA 12-06	<i>Pneumocystis jirovecii</i> (Type: Ne)	infrequently detected	negative
PCPDNA 12-07	<i>Pneumocystis jirovecii</i> (Type: Ne)	detected	Positive (Ct 31.8)
PCPDNA 12-08	negative	negative	negative
PCPDNA13			
PCPDNA 13-01	<i>Pneumocystis jirovecii</i> (Type: A1)	detected	Positive (Ct 28.6)
PCPDNA 13-02	<i>Pneumocystis jirovecii</i> (Type: Ne)	detected	Positive (Ct 31.0)
PCPDNA 13-03	<i>Pneumocystis jirovecii</i> (Type: Ne)	detected	Positive (Ct 30.2)
PCPDNA 13-04	<i>Pneumocystis jirovecii</i> (Type: Ne)	infrequently detected	Positive (Ct 33.9)
PCPDNA 13-05	negative	negative	negative
PCPDNA 13-06	<i>Pneumocystis jirovecii</i> (Type: Ne)	frequently detected	Positive (Ct 28.1)
PCPDNA 13-07	<i>Pneumocystis jirovecii</i> (Type: A1)	detected	Positive (Ct 31.7)
PCPDNA 13-08	<i>Pneumocystis jirovecii</i> (Type: Ne)	detected	Positive (Ct 30.3)
PCPDNA 13-09	<i>Pneumocystis jirovecii</i> (clinical sample)	infrequently detected	Positive (Ct 34.0)
PCPDNA 13-10	<i>Pneumocystis jirovecii</i> (clinical sample)	detected	Positive (Ct 34.8)
PCPDNA14			
PCPDNA 14-01	<i>Pneumocystis jirovecii</i> (Type: Ne)	detected	Positive (Ct 27.5)
PCPDNA 14-02	<i>Pneumocystis jirovecii</i> (Type: Ne)	detected	Positive (Ct 28.6)
PCPDNA 14-03	negative	negative	negative
PCPDNA 14-04	<i>Pneumocystis jirovecii</i> (clinical sample)	infrequently detected	Positive (Ct 38.4)
PCPDNA 14-05	<i>Pneumocystis jirovecii</i> (Type: Ne)	frequently detected	Positive (Ct 25.0)
PCPDNA 14-06	<i>Pneumocystis jirovecii</i> (clinical sample)	infrequently detected	Positive (Ct 36.6)
PCPDNA 14-07	<i>Pneumocystis jirovecii</i> (Type: Ne)	detected	Positive (Ct 27.4)
PCPDNA 14-08	<i>Pneumocystis jirovecii</i> (Type: Ne)	infrequently detected	Positive (Ct 35.6)
PCPDNA 14-09	<i>Pneumocystis jirovecii</i> (Type: A1)	detected	Positive (Ct 28.8)
PCPDNA 14-10	<i>Pneumocystis jirovecii</i> (Type: A1)	detected	Positive (Ct 26.4)
PCPDNA15			
PCPDNA 15-01	negative	negative	negative
PCPDNA 15-02	<i>Pneumocystis jirovecii</i> (Type: Ne)	frequently detected	Positive (Ct 29.2)
PCPDNA 15-03	<i>Pneumocystis jirovecii</i> (Type: Ne)	infrequently detected	Positive (Ct 32.1)
PCPDNA 15-04	<i>Pneumocystis jirovecii</i> (Type: A1)	frequently detected	Positive (Ct 28.5)
PCPDNA 15-05	<i>Pneumocystis jirovecii</i> (Type: Ne)	frequently detected	Positive (Ct 26.5)
PCPDNA 15-06	<i>Pneumocystis jirovecii</i> (Type: Ne)	detected	Positive (Ct 30.9)
PCPDNA 15-07	<i>Pneumocystis jirovecii</i> (Type: Ne)	frequently detected	Positive (Ct 29.6)
PCPDNA 15-08	<i>Pneumocystis jirovecii</i> (Type: A1)	detected	Positive (Ct 31.4)
PCPDNA 15-09	<i>Pneumocystis jirovecii</i> (clinical sample)	infrequently detected	Positive (Ct 33.9)
PCPDNA 15-10	<i>Pneumocystis jirovecii</i> (clinical sample)	infrequently detected	Positive (Ct 31.2)

The results of the QCMD 2012-2015 Bacterial Gastroenteritis EQA Pilot Study (GastroB12-15) are shown in Table 50. All samples containing *Salmonella* spp. could be confirmed.



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Table 50: Overview results QCMD GastroB12-15 with FTD Resp33.

sample	content	FTD Resp33
GastroB12		
GastroB 12-01	<i>Salmonella enteritidis</i>	Positive (Ct 21.8)
GastroB 12-02	<i>Campylobacter lari</i>	negative
GastroB 12-03	<i>Campylobacter jejuni</i>	negative
GastroB 12-04	<i>Yersinia enterocolytica</i>	negative
GastroB 12-05	<i>Salmonella enteritidis</i>	Positive (Ct 18.7)
GastroB 12-06	negative	negative
GastroB 12-07	<i>Campylobacter jejuni</i>	negative
GastroB 12-08	<i>Campylobacter ureolyticus</i>	negative
GastroB 12-09	<i>Campylobacter jejuni</i>	negative
GastroB 12-10	<i>Shigella flexnerii</i>	negative
GastroB 12-11	<i>Salmonella</i>	Positive (Ct 35.4)
GastroB 12-12	<i>Salmonella enteritidis</i>	Positive (Ct 22.1)
GastroB 12-13	<i>Campylobacter jejuni</i>	negative
GastroB 12-14	<i>Shigella flexnerii</i>	negative
GastroB 12-15	negative	negative
GastroB 12-16	<i>Campylobacter ureolyticus</i>	negative
GastroB 12-17	<i>Campylobacter jejuni</i>	negative
GastroB 12-18	<i>Salmonella enteritidis</i>	Positive (Ct 18.9)
GastroB 12-19	<i>Campylobacter jejuni</i>	negative
GastroB 12-20	<i>Yersinia enterocolytica</i>	negative
GastroB13		
GastroB 13-01	<i>Salmonella enteritidis</i>	Positive (Ct 22.8)
GastroB 13-02	<i>Campylobacter lari</i>	negative
GastroB 13-03	<i>Yersinia enterocolytica</i>	negative
GastroB 13-04	<i>Campylobacter jejuni</i>	negative
GastroB 13-05	negative	negative
GastroB 13-06	<i>Salmonella enteritidis</i>	Positive (Ct 25.8)
GastroB 13-07	<i>Campylobacter jejuni</i>	negative
GastroB 13-08	<i>Shigella flexnerii</i>	negative
GastroB 13-09	<i>Yersinia enterocolytica</i>	negative
GastroB 13-10	negative	negative
GastroB14		
GastroB 14-01	<i>Shigella flexnerii</i>	negative
GastroB 14-02	<i>Campylobacter jejuni</i>	negative
GastroB 14-03	<i>Campylobacter lari</i>	negative
GastroB 14-04	<i>Campylobacter jejuni</i>	negative
GastroB 14-05	negative	negative
GastroB 14-06	<i>Campylobacter jejuni</i>	negative
GastroB 14-07	<i>Yersinia enterocolytica</i>	negative
GastroB 14-08	<i>Salmonella enteritidis</i>	Positive (Ct 24.5)
GastroB 14-09	<i>Campylobacter coli</i>	negative
GastroB 14-10	<i>Plesiomonas shigelloides</i>	negative
GastroB15		
GastroB 15-01	<i>Plesiomonas shigelloides</i>	negative
GastroB 15-02	<i>Campylobacter coli</i>	negative
GastroB 15-03	<i>Salmonella enteritidis</i>	Positive (Ct 24.5)
GastroB 15-04	<i>Campylobacter lari</i>	negative
GastroB 15-05	<i>Campylobacter jejuni</i>	negative
GastroB 15-06	<i>Yersinia enterocolytica</i>	negative
GastroB 15-07	<i>Shigella flexnerii</i>	negative
GastroB 15-08	<i>Campylobacter jejuni</i>	negative
GastroB 15-09	negative	negative



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In order to assess the performance of FTD Resp33 detecting *Legionella pneumophila* and *Legionella longbeachae*, this assay was also evaluated with a QCMD panel for detection of *Legionella pneumophila* (LPDNA 14 and LPDNA 16, www.qcmd.org).

All samples were dissolved in accordance to the instructions of the supplier and analyzed with FTD 2P.3 Resp33 (Table 51). FTD 2P.3 Resp33 detected all positive samples and also samples LPDNA 14-04 and LPDNA 16-09 which contains *Legionella longbeachae* considered as negative in the *Legionella pneumophila* QCMD panel. Only one sample: LPDNA 16-02 was missed which was an educational sample.

Table 51: Overview results of QCMD LP.DNA14 and LP.DNA16 with FTD 2P.3 Resp33.

Sample	Sample content	Sample status	FTD 2P.3 Resp33
QCMD LP.DNA14			
LPDNA 14-01	L. pneumophila sg1	frequently detected	Positive (Ct 27.9)
LPDNA 14-02	L. pneumophila sg1	frequently detected	Positive (Ct 27.9)
LPDNA 14-03	L. pneumophila sg1	frequently detected	Positive (Ct 28.0)
LPDNA 14-04	L. longbeachae	negative	Positive (Ct 21.8)*
LPDNA 14-05	L. pneumophila sg3	frequently detected	Positive (Ct 29.4)
LPDNA 14-06	L. pneumophila sg1	frequently detected	Positive (Ct 31.0)
LPDNA 14-07	Clinical negative	negative	negative
LPDNA 14-08	Analytical negative	negative	negative
LPDNA 14-09	Analytical negative	negative	negative
LPDNA 14-10	L. pneumophila sg1	frequently detected	Positive (Ct 30.9)
QCMD LP.DNA16			
LPDNA 16-01	L. pneumophila sg6	frequently detected	Positive (Ct 29.8)
LPDNA 16-02	L. pneumophila sg1	detected	negative
LPDNA 16-03	L. pneumophila sg3	frequently detected	Positive (Ct 26.5)
LPDNA 16-04	L. pneumophila sg3	detected	Positive (Ct 33.9)
LPDNA 16-05	L. pneumophila sg1	frequently detected	Positive (Ct 27.9)
LPDNA 16-06	L. pneumophila sg6	frequently detected	Positive (Ct 27.9)
LPDNA 16-07	Analytical negative	negative	negative
LPDNA 16-08	L. pneumophila sg1	frequently detected	Positive (Ct 28.1)
LPDNA 16-09	L. longbeachae	negative	Positive (Ct 27.1)*
LPDNA 16-10	L. pneumophila sg1	frequently detected	Positive (Ct 31.8)

* as FTD Resp33 detects *L. pneumoniae* and *L. longbeachae*, samples LPDNA 14-04 and LPDNA 16-09 were positive for *L. longbeachae*



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7. Summary of clinical samples and quality control panels

Since 2011 68 QCMD panels and 65 Instand e. V. panels have been completed overall with FTD Resp33, only 24 samples were missed out of 903 in total. All of these were "infrequently detected" or "educational". Furthermore 1128 clinical samples were processed and compared to reference methods and all could be confirmed except 4 samples. 1 low positive HPIV2 sample which was missed by FTD Resp33. 3 of the false negative samples came out as positives (HRV, HAdV and FLUA) with FTD Resp33 (Table 52).

Furthermore 2 co-infected samples were detected which are not included in the calculation below see 5.5 study 5. Tabel 53 shows the overall agreement obtained for the analysis of QCMD, Instand e. V. and clinical samples.

Table 52: Summary of all samples, including QCMD, Instand e. V. and clinical samples validated with FTD Resp33.

pathogen	QCMD		Instand		specificity			sensitivity			precision			clinical samples					samples in total			
	conf.	total	conf.	total	A		B		C		D		E		F		G		H		conf.	total
					4.1	4.2	4.3	Study 1	Study 2	Study 3	Study 4	Study 5										
FLUA	28	28	37	37	1	1	1	1	2	2	23	23	/	/	10	10	9	9	4	4	115	115
FLUB	23	23	13	13	1	1	1	1	2	2	20	20	/	/	/	/	3	3	2	2	65	65
FLUC	/	/	/	/	/	/	1	1	2	2	24	24	/	/	/	/	/	/	/	/	27	27
Cor63	15	15	/	/	1	1	1	1	2	2	12	12	/	/	/	/	/	/	2	2	33	33
Cor43	15	15	/	/	1	1	1	1	2	2	23	23	/	/	/	/	1	1	2	2	45	45
Cor229	15	15	/	/	1	1	1	1	2	2	13	13	/	/	/	/	1	1	1	1	34	34
CorHKU1	/	/	/	/	/	/	1	1	2	2	6	6	/	/	/	/	/	/	/	/	9	9
HPIV1	16	19	/	/	1	1	1	1	2	2	18	18	/	/	/	/	4	4	2	2	44	47
HPIV2	15	15	3	3	1	1	1	1	2	2	19	19	/	/	/	/	4	4	3	4	48	49
HPIV3	6	6	3	3	1	1	1	1	2	2	18	18	/	/	/	/	4	4	2	2	37	37
HPIV4	5	5	/	/	1	1	1	1	2	2	16	16	/	/	/	/	2	2	3	3	30	30
HMPV	35	35	10	10	1	1	1	1	2	2	19	19	/	/	/	/	7	7	3	3	78	78
HRV	31	34	/	/	1	1	1	1	2	2	25	25	/	/	/	/	45	45	9	9	114	117
HRSV	34	34	10	10	2	2	1	1	2	2	22	22	/	/	/	/	11	11	4	4	86	86
HAdV	46	47	22	22	4	4	1	1	2	2	31	31	/	/	/	/	1	1	2	2	109	110
EV	45	50	33	33	2	2	1	1	2	2	16	16	/	/	/	/	1	1	1	1	101	106
HPeV	36	42	/	/	/	/	1	1	2	2	14	14	/	/	/	/	/	/	/	/	53	59
HBoV	/	/	/	/	/	/	1	1	2	2	7	7	/	/	/	/	/	/	1	1	11	11
Mpneu	24	25	17	17	1	1	1	1	2	2	16	16	/	/	/	/	/	/	2	2	63	64
Cpneu	22	23	20	20	/	/	1	1	2	2	6	6	125	125	/	/	/	/	/	/	176	177
Spneu	/	/	/	/	1	1	1	1	2	2	63	63	/	/	/	/	/	/	/	/	67	67
HIB	/	/	/	/	/	/	1	1	2	2	18	18	/	/	/	/	/	/	/	/	21	21
Saur	/	/	/	/	1	1	1	1	2	2	40	40	/	/	/	/	/	/	/	/	44	44
PCP	33	34	/	/	/	/	1	1	2	2	14	14	/	/	/	/	/	/	/	/	50	51
Kpneu	/	/	/	/	1	1	1	1	2	2	20	20	/	/	/	/	/	/	/	/	24	24
Haeinf	/	/	/	/	1	1	1	1	2	2	40	40	/	/	/	/	/	/	/	/	44	44
Morax	/	/	/	/	1	1	1	1	2	2	29	29	/	/	/	/	/	/	/	/	33	33
Salm	9	9	13	13	1	1	1	1	2	2	29	29	/	/	/	/	/	/	/	/	55	55
Bord	/	/	12	12	1	1	1	1	2	2	6	6	/	/	/	/	/	/	/	/	22	22
Lpneu	13	14	/	/	1	1	1	1	2	2	18	18	/	/	/	/	/	/	/	/	35	36
Llong	2	2	/	/	/	/	1	1	2	2	/	/	/	/	/	/	/	/	/	/	5	5
negative	143	145	75	75	49	49	0	0	30	30	0	0	18	18	0	0	0	0	10	13	325	330
total	611	635	268	268	77	77	31	31	92	92	625	625	143	143	10	10	93	93	53	57	2003	2031

(FLUA= influenza A, FLUB= influenza B, FLUC= influenza C, HRV= rhinovirus, Cor63= coronavirus NL63, Cor229= coronavirus 229E, Cor43= coronavirus OC43, HKU1= coronavirus HKU1, HPIV2= parainfluenza virus 2, HPIV3= parainfluenza virus 3, HPIV4= parainfluenza virus 4, HRV= rhinovirus, HRSV= respiratory syncytial viruses A and B, HAdV= adenovirus, EV= enterovirus, HPeV= parechovirus, HMPV= human metapneumovirus A and B, HPIV1= parainfluenza virus 1, HBoV= bocavirus, Mpneu= *Mycoplasma pneumoniae*, Cpneu= *Chlamydia pneumoniae*, Spneu= *Streptococcus pneumoniae*, Saur= *Staphylococcus aureus*, HIB= *Haemophilus influenzae* B, Salm= *Salmonella* spp., PCP= *Pneumocystis jirovecii*, Morax= *Moraxella catarrhalis*, Bord= *Bordetella* spp., Kpneu= *Klebsiella pneumoniae*, Lpneu/Llong= *Legionella pneumophila*/*Legionella longbeachae*, Haeinf= *Haemophilus influenzae*, A= clinical samples used for specificity analysis, B= clinical samples used for sensitivity analysis, C= clinical samples used for precision analysis, D-H= clinical samples used in clinical studies)



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Table 53: Overall agreement of QCMD, Instand e. V. and clinical samples validated with FTD Resp33.

	QCMD		Instand		clinical samples	
	detected	total	detected	total	detected	total
samples tested	611	635	268	268	1124	1128
overall % agreement	96.22		100		99.65	

8. Stability

The purpose of stability testing is to provide evidence on how the quality of FTD Resp33 varies with time and under the influence of different environmental factors such as temperature and freeze-thawing and to establish a re-test period for FTD Resp33 or a shelf-life under recommended storage conditions.

For liquid components of FTD Resp33 refer to stability studies which are listed in Table 54. Some of the studies are still ongoing.

Table 54: Overview of stability tests of FTD Resp33.

Stability studies									
Long term stability		Robustness							
long term stability (former kit size 24/48/96 patient kits)		in use stability (reflects 10x freeze/defreeze; new kit size 32/64 reactions kits)		simulation storage and transport conditions (7 days room temperature, further interval tests after storage at -20°C)		storage condition other countries (room temperature for 3 days, further interval tests after storage at -20°C)		storage condition other countries (climate chamber at 50°C for 1 hour, further interval tests after storage at -20°C)	
temperature	time	temperature	time	temperature	time	temperature	time	temperature	time
-20°C	0, 1, 2, 3, 6, 9, 12, 18 and 24 months	-20°C	0, 1, 2, 3, 6, 9, 12, 18 and 24 months	room temperature	7 days	room temperature	3 days	50°C	1 hour
				-20°C	0, 1, 2, 3, 6, 9, 12, 18 and 24 months	-20°C	0, 1, 2, 3, 6, 9, 12, 18 and 24 months	-20°C	0, 1, 2, 3, 6, 9, 12, 18 and 24 months

The results of the existing stability studies showed that no significant differences occurred. All data of the different measurements were within range of our quality system. This confirms that the liquid components are stable under the different conditions.

We recommend to strictly follow the instructions of the manual and to avoid unnecessary freeze-thawing of the kit.



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9. Legend of symbols

Symbol	Explanation
	Manufacturer
	Date Of Manufacture
	Catalogue Number
	Batch Code
	Use By
	Suffices For
	In vitro diagnostics
	Temperature Limits
	Caution
	Consult Instructions For Use