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IndiMag® Pathogen

Technical Data Report

For automated purification of viral RNA and DNA and bacterial DNA from animal samples using IndiMag® 2, IndiMag® 48/ 48s, KingFisher™ Apex, KingFisher™ Flex, KingFisher™ 96, BioSprint® 96 or equivalent workstation

INDICAL BIOSCIENCE

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1 Introduction

1.1 Intended use

The IndiMag Pathogen Kit and the IndiMag Pathogen Cartridges are intended for the automated extraction of pathogen nucleic acids (viral RNA and DNA and bacterial DNA) from animal whole blood, serum, plasma, other body fluids, swabs, washes and tissue homogenate using the IndiMag® 2, IndiMag® 48/ 48s, KingFisher™ Apex, KingFisher™ Flex, KingFisher™ 96, BioSprint® 96 or equivalent workstation.

For molecular biology application.

1.2 General information

Magnetic bead technology enables purification of high-quality nucleic acids that are free of proteins, nucleases and other impurities. The purified nucleic acids are ready for use in downstream applications, such as amplification or other enzymatic reactions.

The IndiMag Pathogen Kit and the IndiMag Pathogen Cartridges enable the rapid purification of viral RNA and DNA, as well as bacterial DNA, from a broad range of animal sample types using the IndiMag® 2 (INDICAL BIOSCIENCE GmbH, Leipzig, Germany), IndiMag® 48/ 48s (INDICAL BIOSCIENCE GmbH, Leipzig, Germany), KingFisher™ Apex (ThermoFisher Scientific Inc., Waltham, Massachusetts, USA), KingFisher™ Flex (ThermoFisher Scientific Inc., Waltham, Massachusetts, USA), KingFisher™ 96 (ThermoFisher Scientific Inc., Waltham, Massachusetts, USA), BioSprint® 96 (QIAGEN GmbH, Hilden, Germany) or equivalent workstation. However, specific combinations of sample types and pathogens should be validated by the user.

For details on the Protocols, Pretreatments and handling instructions for the kit, please see the applicable handbook available from INDICAL.

1.3 INDICAL product portfolio used for evaluation studies in this report

Product Name	Catalog Number
IndiMag 2 (110~240 V)	IN950048
IndiMag 48 (100~240 V)*	
IndiMag 48s (100~240 V)*	
IndiMag Pathogen Kit	SP947457
IndiMag Pathogen Kit w/o plastics	SP947257
IndiMag Pathogen IM2 Cartridge	SP957654C608
IndiMag Pathogen IM48 Cartridge	SP947654P224; SP947654P608
IndiMag Pathogen KF96 Cartridge	SP947855P196; SP947855P496
IndiSpin® Pathogen Kit	SP54104 / SP54106
IndiSpin QIAcube HT Pathogen Kit	SP54161
IndiMix™ JOE	MX299945 / MX299947
bactotype® C. burnetii PCR Kit	BT285885
bactotype MAP PCR Kit	BT285905
bactotype Mycoplasma Mg/Ms PCR Kit	BT288105
virotype® ASFV 2.0 PCR Kit	VT281925
virotype BVDV RT-PCR Kit	VT280373 / VT280375 / VT280377
virotype BVDV 2.0 Primers/Probes	PR280385
virotype BVDV 2.0 RT-PCR Kit	VT280385 / VT280387
virotype BTV pan/8 RT-PCR Kit*	
virotype BTV pan/8 2.0 RT-PCR Kit	VT280465
virotype CSFV RT-PCR Kit*	
virotype Influenza A 2.0 RT-PCR Kit	VT282625
virotype PCV2/PCV3 Primers/Probes	PR283685 / PR285687
virotype PRRSV RT-PCR Kit	VT282305
virotype SBV RT-PCR Kit*	

* products are not available anymore

2 Sample and pathogen overview IndiMag Pathogen / IndiMag Pathogen Cartridge extraction kits

		Pathogen tested	Specimens tested	Tests performed with (chapter)	
				IndiMag Pathogen Kit	IndiMag Pathogen Cartridge Kits
Swine	bacterial	*Brachyspira hyodysenteriae*	Feces	3.1.1.1 3.1.1.2 3.1.1.3	
		Haemophilus parasuis	Tissue	3.1.5	
		Salmonella Enterica	Cell culture	3.1.10.	
		Mycoplasma hyodysenteriae	Swabs	3.1.9	
	viral	*African Swine Fever Virus* (ASFV), dsDNA	Blood/ serum Tissue	3.2.1 3.2.2	
		Porcine Circovirus (PCV-2/3), ssDNA	Oral fluid (PCV-3) Oral fluid (PCV-2) Tissue	3.2.4 3.2.5 3.2.6.1 3.2.6.2 3.2.6.3	4.2.1 4.2.2
		Suid Herpesvirus (SHV-1), dsDNA	Tissue	3.2.7	
		Classical Swine Fever Virus (CSFV), ssRNA	Serum	3.3.8.1 3.3.8.2	
		Influenza A Virus, ssRNA	Oral fluid Tissue	3.3.10 3.3.11	
		Porcine Epidemic Diarrhea Virus (PEDV), ssRNA	Feces	3.3.14	
		Porcine Reproductive and Respiratory Syndrome Virus (PRRSV), ssRNA	Oral fluid Serum Tissue	3.3.15.1 3.3.15.2 3.3.15.3 3.3.16.1 3.3.16.2 3.3.17.1 3.3.17.2 3.3.17.3 3.3.17.4	4.3.4 4.3.5 4.3.6

ruminants	bacterial	*Chlamydia* spec.	Tissue	3.1.2	
		Clostridium chauvoei	Tissue	3.1.3	
		Coxiella burnetii	Milk	3.1.4	
		Mycobacterium avium ssp. *paratuberculosis* (MAP)	Feces Tissue	3.1.6.1 3.1.6.2 3.1.6.3 3.1.7.1 3.1.7.2	4.1.1 4.1.2
	viral	*Bovine Herpes Virus 1* (BHV-1), dsDNA	Tissue	3.2.3	
		Bovine Coronavirus, ssRNA	Feces	3.3.1	
		Bovine Respiratory Syncytial Virus (BRSV), ssRNA	Swabs	3.3.2	
		Bluetongue Virus (BTV), dsDNA	Blood Tissue	3.3.3.1 3.3.3.2 3.3.3.3 3.3.4	4.3.1
		Bovine Viral Diarrhea Virus (BVDV), ssRNA	Milk Blood/ Serum Tissue	3.3.5 3.3.6.1 3.3.6.2 3.3.6.3 3.3.7	 4.3.2
		Ovine Herpes Virus 2 (OvHV-2), dsDNA	Tissue	3.2.13	
		Schmallenberg Virus (SBV), ssRNA	Semen Blood/ Serum Tissue	3.3.18 3.3.19 3.3.20	
poultry	bact.	*Mycoplasma gallisepticum* (Mg) */ Mycoplasma synoviae* (Ms)	Cell culture Swabs	3.1.8.1 3.1.8.2	
	viral	*Influenza A Virus,* ssRNA	Swabs	3.3.12	4.3.3
comp. animals	bact.	*Taylorella equigenitalis*	Swabs	3.1.11.	
	viral	*Canine Distemper Virus* (CDV), ssRNA	Tissue	3.3.9	

3 IndiMag Pathogen Kit

3.1 Bacterial DNA extraction

3.1.1 ***Brachyspira hyodysenteriae*** (feces)

3.1.1.1 External field data 1 (Private German veterinary laboratory)

Procedure

DNA was extracted from *n* = 10 fecal samples, positive for *B. hyodysenteriae*, using the IndiMag Pathogen Kit and “Pretreatment F1”. The extraction step was performed with the KingFisher Flex device. Subsequently, eluates were further analyzed by an in-house PCR, performed on the Bio-Rad™ CFX™ 96 detection system (Bio-Rad Laboratories, Inc., Hercules, California, USA). Data were generated and kindly provided by a private German veterinary laboratory.

Results / Conclusion

Table 1 summarizes the data. All the fecal samples were tested positive for *B. hyodysenteriae* after being extracted with the IndiMag Pathogen Kit.

Table 1. Data summary for *B. hyodysenteriae* fecal samples extracted on the KingFisher Flex (KFF) device.

Sample	**Specimen**	**Status**	***Brachyspira hyodysenteriae*** **IndiMag Pathogen Kit KingFisher Flex** C_T
#1	feces	**positive**	25.65
#2	feces	**positive**	29.42
#3	feces	**positive**	34.86
#4	feces	**positive**	24.55
#5	feces	**positive**	29.49
#6	feces	**positive**	27.53
#7	feces	**positive**	28.24
#8	feces	**positive**	37.57
#9	feces	**positive**	36.95
#10	feces	**positive**	29.19

3.1.1.2 External field data 2 (German state eterinary laboratory)

Procedure

DNA was extracted from *n* = 13 fecal samples with the IndiMag Pathogen Kit and "Pretreatment F1" using the IndiMag 2 and the IndiMag 48s devices. The eluates were further analyzed by an in-house PCR for detection of *Brachyspira hyodysenteriae* on the Bio-Rad CFX 96 detection system. Data were generated and kindly provided by a German state veterinary laboratory.

Results / Conclusion

Results are presented in Table 2. All the fecal samples scored correctly, regardless of the automatic extraction instrument used. The IndiMag 2 and the IndiMag 48s performed to the same extent and extracted also the weak positive sample (#8) for *B. hyodysenteriae* with the IndiMag Pathogen Kit.

Table 2. Data summary for B*. hyodysenteriae* fecal samples extracted on the IndiMag 2 and IndiMag 48s devices.

Sample	**Specimen**	**Status**	***Brachyspira hyodysenteriae***	
			IndiMag Pathogen Kit IndiMag 2	**IndiMag Pathogen Kit IndiMag 48s**
			C_T	**C_T**
#1	feces	neg.	-	-
#2	feces	neg.	-	-
#3	feces	**positive**	25.50	25.70
#4	feces	**positive**	27.20	29.50
#5	feces	**positive**	25.90	26.20
#6	feces	**positive**	33.30	33.40
#7	feces	**positive**	33.20	33.20
#8	feces	**positive**	38.80	38.90
#9	feces	neg.	-	-
#10	feces	**positive**	23.10	23.10
#11	feces	**positive**	25.10	24.50
#12	feces	**positive**	35.00	33.00
#13	feces	**positive**	34.60	37.50

- = no C_T, neg. = negative

3.1.1.3 External field data 2 (Belgian testing laboratory, DGZ)

Procedure

DNA was extracted from *n* = 5 fecal samples using the IndiMag Pathogen Kit and "Pretreatment F1" on the IndiMag 2 and the KingFisher Flex devices. The eluates were further analyzed by an in-house PCR for detection of *Brachyspira hyodysenteriae* on the Bio-Rad CFX 96 detection system. Data were generated and kindly provided by Dierengezondheidszorg Vlaanderen (DGZ), Belgium.

Results / Conclusion

Table 3 summarizes the data. All the fecal samples scored correctly, regardless of the automatic extraction instrument used. The IndiMag 2 and the IndiMag 48s extracted the weak positive sample for *Brachyspira hyodysenteriae* with the IndiMag Pathogen Kit. Overall, DNA extracted with the IndiMag 2 performed better than with the IndiMag 48s device.

Table 3. Data summary for B*. hyodysenteriae* fecal samples extracted on the IndiMag 2 and KingFisher Flex devices.

Sample	**Specimen**	**Status**	***Brachyspira hyodysenteriae***	
			IndiMag Pathogen Kit IndiMag 2	**IndiMag Pathogen Kit IndiMag 48s**
			C_T	**C_T**
#1	feces	neg.	-	-
#2	feces	**positive**	25.13	26.16
#3	feces	**positive**	26.62	27.64
#4	feces	**positive**	26.34	32.02
#5	feces	**positive**	25.53	34.87

- = no C_T, neg. = negative

3.1.2 ***Chlamydia*** (tissue; external field data – German state veterinary laboratory)

Procedure

DNA was extracted from *n* = 9 tissue samples, positive for *Chlamydia* spec., using the IndiMag Pathogen Kit and "Pretreatment T1" on the IndiMag 2 and the IndiMag 48s devices. The eluates were further analyzed by an in-house PCR, performed on the Bio-Rad CFX 96 detection system. Data were generated and kindly provided by a German state veterinary laboratory.

Results / Conclusion

Results are presented in Table 4. All the tissue samples scored correctly, regardless of the automatic extraction instrument used. The IndiMag 2 and the IndiMag 48s performed to the same extent and extracted also the weak positive sample for *Chlamydia* spec. with the IndiMag Pathogen Kit.

Table 4. Data summary for *Chlamydia* tissue samples extracted on the IndiMag 2 and IndiMag 48s devices.

Sample	**Specimen**	**Status**	***Chlamydia* spec.**	
			IndiMag Pathogen Kit IndiMag 2	**IndiMag Pathogen Kit IndiMag 48s**
			C_T	**C_T**
#1	tissue	**positive**	20.60	20.50
#2	tissue	**positive**	19.80	19.70
#3	tissue	**positive**	23.50	23.80
#4	tissue	**positive**	33.50	34.20
#5	tissue	**positive**	21.40	23.20
#6	tissue	**positive**	20.10	20.40
#7	tissue	**positive**	32.50	32.80
#8	tissue	**positive**	38.00	38.80
#9	tissue	**positive**	35.10	35.70

3.1.3 ***Clostridium chauvoei*** (tissue; external field data – German state veterinary laboratory)

Procedure

DNA was extracted from *n* = 8 tissue samples using the IndiMag Pathogen Kit and "Pretreatment T1". The extraction was performed with the IndiMag 2 and the IndiMag 48s devices. Subsequently, the eluates were further analyzed by an in-house PCR for detection of *Clostridium chauvoei* (blackleg) on the Bio-Rad CFX 96 detection system. Data were generated and kindly provided by a German state veterinary laboratory.

Results / Conclusion

Table 5 summarizes the data. All the tissue samples scored correctly, regardless of the automatic extraction instrument used. The IndiMag 2 and the IndiMag 48s devices extracted also the weak positive sample for *Clostridium chauvoei* with the IndiMag Pathogen Kit.

Table 5. Data summary for *C. chauvoei* tissue samples extracted on the IndiMag 2 and IndiMag 48s devices.

Sample	**Specimen**	**Status**	***Clostridium chauvoei***	
			IndiMag Pathogen Kit IndiMag 2	**IndiMag Pathogen Kit IndiMag 48s**
			C_T	**C_T**
#1	tissue	**positive**	30.00	31.10
#2	tissue	**positive**	32.20	31.00
#3	tissue	**positive**	28.60	28.80
#4	tissue	neg.	-	-
#5	tissue	**positive**	32.00	33.70
#6	tissue	**positive**	34.00	33.70
#7	tissue	**positive**	31.00	28.10
#8	tissue	**positive**	34.50	33.60

- = no C_T, neg. = negative

3.1.4 ***Coxiella burnetii*** (milk; internal data – INDICAL BIOSCIENCE GmbH, Leipzig)

Procedure

DNA was extracted from *n* = 21 pooled milk samples positive for *Coxiella burnetii* using the IndiMag Pathogen Kit. The extraction was performed with the IndiMag 48 and the eluates were further analyzed using the bactotype C. burnetii PCR Kit (INDICAL BIOSCIENCE GmbH, Leipzig, Germany) on the Agilent Technologies AriaMx thermocycler (Agilent Technologies, Santa Clara, California, USA). Data were generated by INDICAL BIOSCIENCE GmbH, Leipzig, Germany.

Results / Conclusion

Results are presented in Table 6. All the samples scored positive for *Coxiella burnetii* after being extracted with the IndiMag Pathogen Kit using the IndiMag 48.

Table 6. Data summary for *C. burnetii* pooled milk samples extracted on the IndiMag 48 device.

Sample	Specimen	Status	*Coxiella burnetii* IndiMag Pathogen Kit IndiMag 48 C_T
#1	milk	positive	35.42
#2	milk	positive	33.95
#3	milk	positive	36.54
#4	milk	positive	35.33
#5	milk	positive	32.88
#6	milk	positive	35.35
#7	milk	positive	36.30
#8	milk	positive	32.09
#9	milk	positive	29.27
#10	milk	positive	32.71
#11	milk	positive	34.37
#12	milk	positive	33.15
#13	milk	positive	32.29
#14	milk	positive	31.76
#15	milk	positive	34.32
#16	milk	positive	33.94
#17	milk	positive	33.84
#18	milk	positive	33.13
#19	milk	positive	32.98
#20	milk	positive	33.23
#21	milk	positive	33.16

3.1.5 ***Haemophilus parasuis*** (tissue; external field data – German state veterinary laboratory

Procedure

DNA was extracted from *n* = 5 tissue samples (of which *n* = 4 had a positive and *n* = 1 had a negative status for *Haemophilus parasuis)*, using the IndiMag Pathogen Kit and "Pretreatment T1". The extraction was performed with the IndiMag 2 and the IndiMag 48s devices. Subsequently, the eluates were further analyzed by an in-house PCR on the Bio-Rad CFX 96 detection system. Data were generated and kindly provided by a German state veterinary laboratory.

Results / Conclusion

Table 7 summarizes the data. All the tissue samples were detected correctly, regardless of the automatic extraction instrument used. The IndiMag 2 and the IndiMag 48s performed to the same extent and extracted positive sample for *Haemophilus parasuis* with the IndiMag Pathogen Kit.

Table 7. Data summary for *H. parasuis* tissue samples extracted on the IndiMag 2 and IndiMag 48s devices.

Sample	**Specimen**	**Status**	***Haemophilus parasuis***	
			IndiMag Pathogen Kit IndiMag 2	**IndiMag Pathogen Kit IndiMag 48s**
			C_T	**C_T**
#1	tissue	**positive**	24.90	25.90
#2	tissue	neg.	-	-
#3	tissue	**positive**	29.00	30.51
#4	tissue	**positive**	19.50	19.50
#5	tissue	**positive**	29.50	29.10

- = no C_T, neg. = negative

3.1.6 ***Mycobacterium avium* subspecies *paratuberculosis*** (MAP/ Johnes' Disease; feces)

3.1.6.1 Internal data 1 (INDICAL BIOSCIENCE GmbH, Leipzig)

Procedure

DNA was extracted from *n* = 12 MAP-positive fecal samples, using the IndiMag Pathogen Kit, the IndiSpin QIAcube HT Pathogen and the IndiSpin Pathogen Kit (INDICAL BIOSCIENCE GmbH, Leipzig, Germany) and "Pretreatment F-MAP". The extraction was performed with the IndiMag 48, IndiMag 48s, KingFisher Flex, QIAcube HT device (QIAGEN GmbH, Hilden, Germany) or manually using IndiSpin columns. Subsequently, the eluates were further analyzed by using the bactotype MAP PCR Kit (INDICAL BIOSCIENCE GmbH, Leipzig, Germany). The PCR was performed on the Agilent Technologies Stratagene Mx3005P thermocycler. Data were generated by INDICAL BIOSCIENCE GmbH, Leipzig, Germany.

Results / Conclusion

Results are presented in Table 8. All fecal samples were positive for *Mycobacterium avium* subspecies *paratuberculosis* after extraction with the IndiMag Pathogen Kit, IndiSpin Pathogen Kit, and IndiSpin QIAcube HT Pathogen Kit und respective devices or after manual extraction.

Table 8. Data summary for *M. avium* ssp. *paratuberculosis*-positive feces samples extracted on the IndiMag 48 (IM 48), IndiMag 48s (IM 48s), KingFisher Flex (KFF), and QIAcube HT devices, and by manual extraction.

Sample	Specimen	Status	*Mycobacterium avium ssp. paratuberculosis* (MAP)				
			IndiMag Pathogen Kit			IndiSpin QIAcube HT Path. Kit	IndiSpin Pathogen Kit
			IM 48	IM 48s	KFF	QIAcube HT	manual
			C_T	C_T	C_T	C_T	C_T
#1	feces	pos.	27.86	27.86	28.32	27.30	28.13
#2	feces	pos.	26.62	26.78	26.69	25.70	27.06
#3	feces	pos.	21.88	22.12	21.73	21.57	22.64
#4	feces	pos.	21.65	21.70	22.16	21.15	21.88
#5	feces	pos.	31.51	31.29	32.13	30.16	30.89
#6	feces	pos.	31.66	31.57	33.26	31.45	32.12
#7	feces	pos.	28.52	26.73	27.19	26.83	27.16
#8	feces	pos.	24.40	22.22	22.37	21.14	22.31
#9	feces	pos.	24.81	22.63	23.26	21.66	22.65
#10	feces	pos.	29.58	27.32	27.45	26.29	27.00
#11	feces	pos.	21.15	18.17	18.25	17.74	18.51
#12	feces	pos.	20.49	18.73	17.86	17.59	17.97

Path. = Pathogen, pos. = positive

3.1.6.2 Internal data 2 (INDICAL BIOSCIENCE GmbH, Leipzig)

Procedure

DNA was extracted from *n* = 7 MAP-positive and *n* = 2 MAP-negative fecal samples, using the IndiMag Pathogen Kit and "Pretreatment F-MAP". The extraction was performed with the IndiMag 2, KingFisher Flex, KingFisher Apex and IndiMag 48 devices. Subsequently, the eluates were further analyzed using the bactotype MAP PCR Kit. The PCR was performed on the Agilent Technologies Stratagene Mx3005P thermocycler. Data were generated by INDICAL BIOSCIENCE GmbH, Leipzig, Germany.

Results / Conclusion

Table 9 summarizes the data. All fecal samples were detected correctly for *Mycobacterium avium* subspecies *paratuberculosis* after extraction with the IndiMag Pathogen Kit with all extraction devices. On average, samples were extracted more efficiently by the IndiMag 2 instrument.

Table 9. Data summary for *M. avium* ssp. *paratuberculosis* testing in feces samples extracted on the IndiMag 2 (IM 2), IndiMag 48 (IM 48), KingFisher Flex (KFF), and KingFisher Apex (KFA) devices.

Sample	**Specimen**	**Status**	***Mycobacterium avium ssp. paratuberculosis* (MAP)**			
			IndiMag Pathogen Kit			
			IM 2	**IM 48**	**KFF**	**KFA**
			C_T	**C_T**	**C_T**	**C_T**
#1	feces	**pos.**	21.41	21.72	21.59	21.72
#2	feces	**pos.**	31.30	31.52	31.90	32.39
#3	feces	**pos.**	21.12	21.66	20.67	21.17
#4	feces	**pos.**	32.94	33.20	33.27	32.96
#5	feces	**pos.**	18.32	19.79	18.52	18.95
#6	feces	**pos.**	26.63	27.02	27.10	27.16
#7	feces	neg.	-	-	-	-
#8	feces	**pos.**	28.45	28.79	28.61	28.77
#9	feces	neg.	-	-	-	-

pos. = positive, neg. = negative, - = no CT

3.1.6.3 External field data (Belgian testing laboratory, DGZ)

Procedure

DNA was extracted from *n* = 8 MAP-positive fecal samples using the IndiMag Pathogen Kit and "Pretreatment T1". The extraction was performed with the IndiMag 2 and the KingFisher Flex devices. The eluates were further analyzed by an in-house PCR, which was performed on the Bio-Rad CFX 96 detection system. Data were generated and kindly provided by Dierengezondheidszorg Vlaanderen (DGZ), Belgium.

Results / Conclusion

Results are presented in Table 10. All fecal samples were detected correctly, regardless of the automatic extraction instrument used. DNA from fecal samples was extracted more efficiently with the IndiMag 2 than with the KingFisher Flex.

Table 10. Data summary *M. avium* ssp. *paratuberculosis*-positive feces samples extracted on the IndiMag 2 and KingFisher Flex devices.

Sample	**Specimen**	**Status**	***Mycobacterium avium ssp. paratuberculosis* (MAP)**	
			IndiMag Pathogen Kit IndiMag 2	**IndiMag Pathogen Kit KingFisher Flex**
			C_T	**C_T**
#1	feces	**positive**	30.71	34.27
#2	feces	**positive**	33.30	35.97
#3	feces	**positive**	29.25	30.93
#4	feces	**positive**	30.30	37.90
#5	feces	**positive**	23.00	32.02
#6	feces	**positive**	30.51	34.22
#7	feces	**positive**	28.81	27.77
#8	feces	**positive**	31.89	32.61

3.1.7 ***Mycobacterium avium* subspecies *paratuberculosis*** (MAP/ Johnes' Disease; tissue)

3.1.7.1 Internal data 1 (INDICAL BIOSCIENCE GmbH, Leipzig)

Procedure

DNA was extracted from *n* = 7 tissue samples, using the IndiMag Pathogen Kit, the IndiSpin QIAcube HT Pathogen and the IndiSpin Pathogen Kit and "Pretreatment T1". The extraction was performed with the IndiMag 48, IndiMag 48s, KingFisher Flex, QIAcube HT device or manually with IndiSpin columns. Subsequently, the eluates were further analyzed by using the bactotype MAP PCR Kit. The PCR was performed on the Agilent Technologies Stratagene Mx3005P thermocycler. Data were generated by INDICAL BIOSCIENCE GmbH, Leipzig, Germany.

Results / Conclusion

Table 11 summarizes the data. All the tissue samples were detected correctly, regardless of the extraction setup used.

Table 11. Data summary for *M. avium* ssp. p*aratuberculosis* testing in tissue samples extracted on the IndiMag 48 (IM 48), IndiMag 48s (IM 48s), KingFisher Flex (KFF), and QIAcube HT devices, and by manual extraction.

Sample	**Specimen**	**Status**	***Mycobacterium avium ssp. paratuberculosis* (MAP)**				
			IndiMag Pathogen Kit			**IndiSpin QIAcube HT Path. Kit**	**IndiSpin Path. Kit**
			IM 48	**IM 48s**	**KFF**	**QIAcube HT**	**manual**
			C_T	**C_T**	**C_T**	**C_T**	**C_T**
#1	lymph node	**pos.**	22.70	22.11	23.31	22.09	23.26
#2	lymph node	**pos.**	18.52	16.69	16.86	16.43	16.63
#3	lymph node	**pos.**	22.79	21.68	22.33	21.67	22.43
#4	ileum	**pos.**	17.45	17.37	17.83	16.50	17.54
#5	ileum	**pos.**	33.18	24.69	24.84	24.26	25.02
#6	ileum	**pos.**	18.81	18.48	19.10	18.18	19.92
#7	liver	neg.	-	-	-	-	-

Path. = Pathogen, pos. = positive, neg. = negative, - = no C_T

3.1.7.2 Internal data 2 (INDICAL BIOSCIENCE GmbH, Leipzig)

Procedure

DNA was extracted from *n* = 7 tissue samples (of which *n* = 6 were positive for MAP and one was negative for MAP), using the IndiMag Pathogen Kit and "Pretreatment T1". The extraction was performed with the IndiMag 2, KingFisher Flex, KingFisher Apex and IndiMag 48 devices. Subsequently, the eluates were further analyzed by using the bactotype MAP PCR Kit. The PCR was performed on the Agilent Technologies Stratagene Mx3005P thermocycler. Data were generated by INDICAL BIOSCIENCE GmbH, Leipzig, Germany.

Results / Conclusion

Results are presented in Table 12. All the tissue samples were detected correctly, regardless of the automatic extraction instrument used. On average, samples were extracted equally by all extraction methods.

Table 12. Data summary for *M. avium* ssp. *paratuberculosis* testing in tissue samples extracted on the IndiMag 2 (IM 2), IndiMag 48 (IM 48), KingFisher Flex (KFF), and KingFisher Apex (KFA) devices.

Sample	**Specimen**	**Status**	***Mycobacterium avium ssp. paratuberculosis* (MAP)**			
			IndiMag Pathogen Kit			
			IM 2 C_T	**IM 48** C_T	**KFF** C_T	**KFA** C_T
#1	tissue	**pos.**	19.55	20.28	19.99	19.80
#2	tissue	**pos.**	19.43	19.98	19.80	19.62
#3	tissue	**pos.**	27.91	29.09	28.72	28.55
#4	tissue	**pos.**	24.97	25.04	24.98	24.97
#5	tissue	**pos.**	18.82	19.18	18.84	17.54
#6	tissue	**pos.**	23.11	23.40	23.28	23.47
#7	tissue	neg.	-	-	-	-

pos. = positive, neg. = negative, - = no C_T

3.1.8 ***Mycoplasma galliscepticum/ Mycoplasma synoviae*** (cell culture, swabs)

3.1.8.1 Internal data 1 (INDICAL BIOSCIENCE GmbH, Leipzig)

Procedure

DNA was extracted from *n* = 8 cell culture samples, of which *n* = 5 were positive for *Mycoplasma galliscepticum* and *n* = 5 were positive for *Mycoplasma synoviae*. Samples 2 and 7 were positive for both, *M. galliscepticum* and *M. synoviae*. DNA was extracted using the IndiMag Pathogen Kit and extraction was performed with the IndiMag 2, KingFisher Flex, KingFisher Apex and IndiMag 48 devices. The eluates were further analyzed by using the virotype Mg/Ms PCR Kit (INDICAL BIOSCIENCE GmbH, Leipzig, Germany) on the Agilent Technologies Stratagene Mx3005P thermocycler. Data were generated by INDICAL BIOSCIENCE GmbH, Leipzig, Germany.

Results / Conclusion

Table 13 summarizes the data for *Mycoplasma gallisepticum* testing and Table 14 shows the data for *Mycoplasma synoviae* testing. All the cell culture samples were detected correctly, regardless of the automatic extraction instrument used. On average, samples were extracted equally by all extraction methods.

Table 13. Data summary for *Mycoplasma gallisepticum* testing in cell culture samples extracted on the IndiMag 2 (IM 2), IndiMag 48 (IM 48), KingFisher Flex (KFF), and KingFisher Apex (KFA) devices.

Sample	**Specimen**	**Status**	***Mycoplasma gallisepticum***			
			IndiMag Pathogen Kit			
			IM 2	**IM 48**	**KFF**	**KFA**
			C_T	**C_T**	**C_T**	**C_T**
#1	culture	neg.	-	-	-	-
#2	culture	**pos.**	34.27	35.55	35.82	34.49
#3	culture	neg.	-	-	-	-
#4	culture	**pos.**	16.28	17.50	16.93	16.52
#5	culture	**pos.**	31.52	31.46	31.68	31.86
#6	culture	**pos.**	23.75	23.33	23.16	23.29
#7	culture	**pos.**	15.75	16.39	15.80	16.19
#8	culture	neg.	-	-	-	-

pos. = positive, neg. = negative, - = no C_T

Table 14. Data summary for *Mycoplasma synoviae* testing in cell culture samples extracted on the IndiMag 2 (IM 2), IndiMag 48 (IM 48), KingFisher Flex (KFF), and KingFisher Apex (KFA).

Sample	Specimen	Status	*Mycoplasma synoviae* IndiMag Pathogen Kit			
			IM 2 C_T	IM 48 C_T	KFF C_T	KFA C_T
#1	culture	**pos.**	20.59	21.36	22.38	21.51
#2	culture	**pos.**	23.31	23.32	23.91	23.35
#3	culture	**pos.**	33.16	33.43	33.38	33.70
#4	culture	neg.	-	-	-	-
#5	culture	neg.	-	-	-	-
#6	culture	neg.	-	-	-	-
#7	culture	**pos.**	32.73	32.59	32.15	33.37
#8	culture	**pos.**	16.82	18.17	17.82	17.65

pos. = positive, neg. = negative, - = no C_T

3.1.8.2 External field data (Belgian testing laboratory, DGZ)

Procedure

DNA was extracted from *n* = 8 *Mycoplasma synoviae*-positive swab samples using the IndiMag Pathogen Kit and an in-house pretreatment. The extraction was performed with the IndiMag 2 and the KingFisher Flex devices. The eluates were further analyzed by an in-house PCR, which was performed on the Applied Biosystems™ ABI 7500 thermocycler (ThermoFisher Scientific Inc., Waltham, Massachusetts, USA). Data were generated and kindly provided by Dierengezondheidszorg Vlaanderen (DGZ), Belgium.

Results / Conclusion

Results are presented in Table 15. All swab samples were detected correctly, regardless of the automatic extraction instrument used. DNA from swab samples was extracted more efficiently with the IndiMag 2 than with the KingFisher Flex.

Table 15. Data summary for *Mycoplasma synoviae*-positive swab samples extracted on the IndiMag 2 and KingFisher Flex devices.

Sample	**Specimen**	**Status**	***Mycoplasma synoviae***	
			IndiMag Pathogen Kit IndiMag 2	**IndiMag Pathogen Kit KingFisher Flex**
			C_T	**C_T**
#1	swab	**positive**	21.79	24.76
#2	swab	**positive**	23.14	25.64
#3	swab	**positive**	23.97	26.06
#4	swab	**positive**	23.85	25.37
#5	swab	**positive**	23.83	25.94
#6	swab	**positive**	23.82	25.01
#7	swab	**positive**	32.73	32.15
#8	swab	**positive**	16.82	17.82

3.1.9 ***Mycoplasma hyodysenteriae*** (swabs, external field data – Belgian testing laboratory, DGZ)

Procedure

DNA was extracted from *n* = 5 swab samples using the IndiMag Pathogen Kit and an in-house pretreatment. The extraction was performed with the IndiMag 2 and the KingFisher Flex devices. The eluates were further analyzed by an in-house PCR for detection of *Mycoplasma hyodysenteriae*, which was performed on the Applied Biosystems ABI 7500 thermocycler. Data were generated and kindly provided by Dierengezondheidszorg Vlaanderen (DGZ), Belgium.

Results / Conclusion

Results are presented in Table 16. All swab samples were detected correctly, regardless of the automatic extraction instrument used. DNA from swab samples was extracted more efficiently with the IndiMag 2 than with the KingFisher Flex.

Table 16. Data summary for *Mycoplasma hyodysenteriae* testing of swab samples extracted on the IndiMag 2 and KingFisher Flex device.

Sample	**Specimen**	**Status**	***Mycoplasma hyodysenteriae***	
			IndiMag Pathogen Kit IndiMag 2	**IndiMag Pathogen Kit KingFisher Flex**
			C_T	**C_T**
#1	swab	**positive**	29.94	30.94
#2	swab	**positive**	28.17	30.87
#3	swab	**positive**	24.68	28.32
#4	swab	**positive**	25.95	25.65
#5	swab	neg.	-	-

- = no C_T, neg. = negative

3.1.10 ***Salmonella* Enterica** (cell culture, external field data – Belgian testing laboratory, DGZ)

Procedure

DNA was extracted from *n* = 3 enriched cell culture samples for *Salmonella* Enterica using the IndiMag Pathogen Kit and an in-house pretreatment. The extraction was performed with the IndiMag 2 and the KingFisher Flex devices. The eluates were further analyzed by an in-house PCR, which was performed on the Applied Biosystems ABI 7500 thermocycler. Data were generated and kindly provided by Dierengezondheidszorg Vlaanderen (DGZ), Belgium.

Results / Conclusion

Table 17 depicts the results for testing *Salmonella* Enterica-enriched cell culture samples. All cell culture samples were detected correctly, regardless of the automatic extraction instrument used. DNA from enriched cell culture samples was extracted more efficiently with the IndiMag 2 than with the KingFisher Flex.

Table 17. Data summary for testing enriched *Salmonella Enterica*-positive cell culture samples extracted on the IndiMag 2 and KingFisher Flex device.

Sample	**Specimen**	**Status**	***Salmonella* Enterica**	
			IndiMag Pathogen Kit IndiMag 2	**IndiMag Pathogen Kit KingFisher Flex**
			C_T	**C_T**
#1	culture	**positive**	22.18	27.79
#2	culture	**positive**	26.34	30.95
#3	culture	**positive**	23.28	28.32

3.1.11 ***Taylorella equigenitalis*** (swab, external field data – Belgian testing laboratory, DGZ)

Procedure

DNA was extracted from *n* = 7 *Taylorella equigenitalis*-positive and n = 2 *T. equigenitalis*-negative swab samples using the IndiMag Pathogen Kit and "Pretreatment S1". The extraction was performed with the IndiMag 2 and the IndiMag 48s devices. The eluates were further analyzed by an in-house PCR, which was performed on the Bio-Rad CFX 96 thermocycler. Data were generated and kindly provided by Dierengezondheidszorg Vlaanderen (DGZ), Belgium.

Results / Conclusion

Results are presented in Table 18. All cell culture samples were detected correctly by the IndiMag 2, while one low positive sample was returned a negative result with the IndiMag 48s.

Table 18. Data summary for *Taylorella equigenitalis*-testing of swab samples extracted on the IndiMag 2 and IndiMag 48s device.

Sample	**Specimen**	**Status**	***Taylorella equigenitalis***	
			IndiMag Pathogen Kit IndiMag 2	**IndiMag Pathogen Kit IndiMag 48s**
			C_T	**C_T**
#1	swab	**positive**	35.90	35.60
#2	swab	**positive**	32.40	32.00
#3	swab	**positive**	33.40	32.80
#4	swab	neg.	-	-
#5	swab	neg.	-	-
#6	swab	**positive**	38.30	-
#7	swab	**positive**	33.10	32.70
#8	swab	**positive**	24.90	25.50
#9	swab	**positive**	24.90	25.50

- = no C_T, neg. = negative

3.2 Viral DNA extraction

3.2.1 ***African Swine Fever Virus*** (ASFV blood/serum; internal data – INDICAL BIOSCIENCE GmbH, Leipzig)

Procedure

All samples were provided inactivated and lyophilised by the European Reference Laboratory for ASFV (EURL-ASFV), INIA-CISA, Spain. DNA was extracted from *n* = 4 ASFV-positive and *n* = 3 ASFV-negative serum/blood samples, using the IndiMag Pathogen Kit. The extraction step was performed with the IndiMag 48 device. Subsequently, eluates were further analyzed by using the virotype ASFV 2.0 PCR Kit (INDICAL BIOSCIENCE GmbH, Leipzig, Germany), performed on the Bio-Rad CFX 96 detection system. Data were generated by INDICAL BIOSCIENCE GmbH, Leipzig, Germany.

Results / Conclusion

Table 19 summarizes the data. All samples were correctly identified for ASFV.

Table 19. Data summary for ASFV-positive and negative serum/blood samples extracted on the IndiMag 48 device.

Sample	Specimen	Status	*African Swine Fever Virus* **IndiMag Pathogen Kit IndiMag 48** **C_T**
#1	serum	**positive**	18.09
#2	serum	**positive**	21.03
#3	serum	**positive**	25.21
#4	serum	**positive**	24.10
#5	blood	neg.	-
#6	blood	neg.	-
#7	serum	neg.	-

- = no C_T, neg. = negative

3.2.2 ***African Swine Fever Virus*** (ASFV tissue; internal data – INDICAL BIOSCIENCE GmbH, Leipzig)

Procedure

All samples were provided inactivated and lyophilised by the European Reference Laboratory for ASFV (EURL-ASFV), INIA-CISA, Spain. DNA was extracted from *n* = 12 ASFV-positive and *n* = 3 ASFV ASFV-negative tissue samples, using the IndiMag Pathogen Kit and “Pretreatment T1”. The extraction step was performed with the IndiMag 48 device. Subsequently, eluates were further analyzed by using the virotype ASFV 2.0 PCR Kit, performed on the Bio-Rad CFX 96 detection system. Data were generated by INDICAL BIOSCIENCE GmbH, Leipzig, Germany.

Results / Conclusion

Test results are shown in Table 20. All samples were correctly identified for ASFV.

Table 20. Data summary for ASFV-positive and negative tissue samples extracted on the IndiMag 48 device.

Sample	Specimen	Status	*African Swine Fever Virus* IndiMag Pathogen Kit IndiMag 48 C_T
#1	liver	**positive**	26.73
#2	lung	**positive**	21.02
#3	lung	**positive**	21.18
#4	lung	**positive**	25.38
#5	lymph node	**positive**	21.65
#6	spleen	**positive**	22.19
#7	spleen	**positive**	33.47
#8	spleen	**positive**	20.28
#9	spleen	**positive**	29.91
#10	spleen	**positive**	28.12
#11	spleen	**positive**	27.81
#12	spleen	**positive**	34.81
#13	kidney	neg.	-
#14	lung	neg.	-
#15	tonsil	neg.	-

- = no C_T, neg. = negative

3.2.3 ***Bovine Herpes Virus* type 1** (BHV-1/ IBR; tissue; external field data – Belgian testing laboratory, Sciensano)

Procedure

DNA was extracted from *n* = 16 tissue samples (*n* = 13 positive and *n* = 3 negative for BHV-1). After an in-house pretreatment, extraction was performed either automated using the IndiMag Pathogen Kit combined with the IndiMag 2 device or manually with the QIAamp® DNA Mini Kit (QIAGEN GmbH, Hilden, Germany). The eluates were further analyzed by an in-house PCR, which was performed on the Roche Diagnostics LightCycler® 480 detection system (F. Hoffmann-La Roche Ltd., Basel, Switzerland). Data were generated and kindly provided by Sciensano, Belgium.

Results / Conclusion

Results are presented in Table 21. All the tissue samples were detected correctly, regardless of the extraction method used. The IndiMag 2 with the IndiMag Pathogen Kit performed better than the manual spin column method.

Table 21. Data summary for BHV-1 testing of tissue samples extracted on the IndiMag 2 (using the IndiMag Pathogen Kit) or manually (using the QIAamp DNA Mini Kit).

Sample	Specimen	Status	*Bovine Herpes Virus* type 1 (BHV-1)	
			IndiMag Pathogen Kit IndiMag 2	QIAamp DNA Mini Kit manual
			C_T	C_T
#1	tissue	positive	25.32	26.99
#2	tissue	positive	25.64	25.50
#3	tissue	positive	26.61	27.16
#4	tissue	positive	25.58	26.67
#5	tissue	positive	27.69	30.16
#6	tissue	positive	27.84	29.91
#7	tissue	positive	25.05	26.12
#8	tissue	positive	19.87	21.14
#9	tissue	positive	23.65	24.42
#10	tissue	positive	26.26	27.19
#11	tissue	positive	22.02	22.83
#12	tissue	positive	31.61	32.45
#13	tissue	positive	29.81	30.36
#14	tissue	neg.	-	-
#15	tissue	neg.	-	-
#16	tissue	neg.	-	-

- = no C_T, neg. = negative

3.2.4 ***Porcine Circovirus 3*** (PCV-3; oral fluid; internal data – INDICAL BIOSCIENCE GmbH, Leipzig)

Procedure

DNA was extracted from *n* = 16 PCV-3-positive oral fluid samples. After pretreating the samples according to “Pretreatment O2”, extraction was performed either using the IndiMag Pathogen Kit combined with the IndiMag 48s or the KingFisher Flex devices or using the MagMAX™ CORE Nucleic Acid Purification Kit (ThermoFisher Scientific, Waltham, Massachusetts, USA) combined with the KingFisher Flex device. The eluates were further analyzed by an in-house PCR, which was performed on the Agilent Technologies Stratagene Mx3005P detection system. Data were generated by INDICAL BIOSCIENCE GmbH, Leipzig, Germany.

Results / Conclusion

Table 22 summarizes the test results for the comparative PCV-3 tests. All the oral fluid samples were detected correctly, regardless of the extraction method used. The IndiMag Pathogen Kit performed as good as the MagMAX Core Nucleic Acid Purification Kit.

Table 22. Data summary for PCV-3-positive oral fluid samples extracted on the IndiMag 2 (using the IndiMag Pathogen Kit) or on the KingFisher Flex (using either the IndiMag Pathogen Kit or the MagMAX CORE Nucleic Acid Purification Kit).

Sample	Specimen	Status	*Porcine Circovirus 3* (PCV-3)		
			IndiMag Pathogen Kit IndiMag 48s	IndiMag Pathogen Kit KingFisher Flex	MagMAX CORE Kit KingFisher Flex
			C_T	C_T	C_T
#1	oral fluid	positive	32.24	32.62	32.99
#2	oral fluid	positive	28.10	28.68	28.54
#3	oral fluid	positive	36.12	35.79	36.40
#4	oral fluid	positive	36.78	36.96	35.73
#5	oral fluid	positive	36.75	36.90	36.22
#6	oral fluid	positive	33.22	33.59	33.15
#7	oral fluid	positive	36.37	34.70	34.17
#8	oral fluid	positive	34.47	34.07	33.22
#9	oral fluid	positive	33.71	33.23	32.98
#10	oral fluid	positive	29.39	30.06	29.04
#11	oral fluid	positive	37.45	38.41	38.06
#12	oral fluid	positive	30.81	31.11	31.12
#13	oral fluid	positive	25.98	25.91	25.03
#14	oral fluid	positive	29.06	29.47	29.60
#15	oral fluid	positive	36.23	36.55	36.09
#16	oral fluid	positive	35.27	34.88	35.25

3.2.5 ***Porcine Circovirus 2*** (PCV-2; oral fluid; internal data – INDICAL BIOSCIENCE GmbH, Leipzig)

Procedure

DNA was extracted from *n* = 7 PCV-2-positive serum samples from the 2017 PTS on *Porcine Circovirus* 2, organized by GD Deventer. DNA was extracted in duplicate. Extraction was performed either using the IndiMag Pathogen Kit combined with the IndiMag 48s or using the MagMAX CORE Nucleic Acid Purification Kit (ThermoFisher Scientific, Waltham, USA) combined with the KingFisher Flex device. The eluates were further analyzed by by using the virotype PCV2/PCV3 Primers/Probes (INDICAL BIOSCIENCE GmbH, Leipzig, Germany) and IndiMix JOE (INDICAL BIOSCIENCE GmbH, Leipzig, Germany). PCR was run on the Agilent Technologies Stratagene Mx3005P detection system. Data were generated by INDICAL BIOSCIENCE GmbH, Leipzig, Germany.

Results / Conclusion

The test results for the comparative PCV-2 tests are presented in Table 23. All serum samples from this ring trial were detected correctly, regardless of the extraction method used. The IndiMag Pathogen Kit performed as good as the MagMAX Core Nucleic Acid Purification Kit.

Table 23. Data summary for PCV-2-positive serum ring trial samples extracted on the IndiMag 48s (using the IndiMag Pathogen Kit) or on the KingFisher Flex (using the MagMAX CORE Nucleic Acid Purification Kit).

Sample	Specimen	Status	*Porcine Circovirus 2* (PCV-2)	
			IndiMag Pathogen Kit IndiMag 48s	MagMAX CORE Kit KingFisher Flex
			C_T	C_T
PCV-2 vaccine	serum	positive	21.37 21.25	20.94 20.94
PCV-2 field sample	serum	positive	33.25 33.79	33.96 34.93
PCV-2 wildtype virus	serum	positive	26.35 26.42	27.22 26.75
PCV-2 wildtype virus	serum	positive	37.60 -	37.15 -
PCV-2 field sample	serum	positive	36.13 34.64	35.08 35.35
PCV-2 wildtype virus	serum	positive	33.21 33.25	33.76 35.10
PCV-2 wildtype virus	serum	positive	22.84 22.81	23.61 23.62

- = no C_T

3.2.6 ***Porcine Circovirus*** (PCV; tissue)

3.2.6.1 Internal data 1 (INDICAL BIOSCIENCE GmbH, Leipzig)

Procedure

DNA was extracted from *n* = 7 PCV-2-positive tissue samples, using the IndiMag Pathogen Kit, the IndiSpin QIAcube HT Pathogen and the IndiSpin Pathogen Kit and “Pretreatment T1”. The extraction was performed on the IndiMag 48, IndiMag 48s, KingFisher Flex or QIAcube HT device or manually with IndiSpin columns. Subsequently, the eluates were further analyzed by using the virotype PCV2/PCV3 Primers/Probes and IndiMix JOE. The PCR was performed on the Agilent Technologies Stratagene Mx3005P thermocycler. Data were generated by INDICAL BIOSCIENCE GmbH, Leipzig, Germany.

Results / Conclusion

Table 24 summarizes the data. All the tissue samples were detected correctly, regardless of the extraction setup used.

Table 24. Data summary for PCV-2 testing in tissue samples extracted on the IndiMag 48 (IM 48), IndiMag 48s (IM 48s), KingFisher Flex (KFF), and QIAcube HT devices, and by manual extraction.

Sample	**Specimen**	**Status**	***Porcine Circovirus 2* (PCV-2)**				
			IndiMag Pathogen Kit			**IndiSpin QIAcube HT Path. Kit**	**IndiSpin Pathogen Kit**
			IM 48	**IM 48s**	**KFF**	**QIAcube HT**	**manual**
			C_T	**C_T**	**C_T**	**C_T**	**C_T**
#1	tissue	**pos.**	16.07	15.68	15.40	16.35	16.52
#2	tissue	**pos.**	19.36	19.16	19.28	19.47	19.83
#3	tissue	**pos.**	25.17	24.74	24.67	26.02	25.35
#4	tissue	**pos.**	17.38	16.89	16.54	17.84	18.44
#5	tissue	**pos.**	19.94	19.34	19.21	20.56	20.69
#6	tissue	**pos.**	8.90	8.46	8.76	8.78	9.38
#7	tissue	**pos.**	17.62	15.05	14.92	15.01	15.34
#8	tissue	**pos.**	8.84	8.07	9.84	9.11	8.72

pos. = positive

3.2.6.2 Internal data 2 (INDICAL BIOSCIENCE GmbH, Leipzig)

Procedure

DNA was extracted from *n* = 16 PCV-2-positive tissue samples DNA was extracted using the IndiMag Pathogen Kit and “Pretreatment T1” and extraction was performed with the IndiMag 2, KingFisher Flex, KingFisher Apex and IndiMag 48 devices. The eluates were further analyzed with the virotype PCV2/PCV3 Primers/Probes and IndiMix JOE using the Agilent Technologies Stratagene Mx3005P thermocycler. Data were generated by INDICAL BIOSCIENCE GmbH, Leipzig, Germany.

Results / Conclusion

Table 25 summarizes the data for comparative PCV-2 testing. All tissue samples were detected correctly, regardless of the automatic extraction instrument used. Overall, the IndiMag 2 performed better than all other tested extraction methods.

Table 25. Data summary for testing PCV-2 positive tissue samples extracted on the IndiMag 2 (IM 2), IndiMag 48 (IM 48), KingFisher Flex (KFF), and KingFisher Apex (KFA) devices.

Sample	Specimen	Status	*Porcine Circovirus 2* (PCV-2) IndiMag Pathogen Kit			
			IM 2 C_T	IM 48 C_T	KFF C_T	KFA C_T
#1	tissue	pos.	18.04	18.59	19.25	18.67
#2	tissue	pos.	13.41	14.05	14.09	13.90
#3	tissue	pos.	28.99	30.78	30.64	29.74
#4	tissue	pos.	30.64	33.80	31.55	32.93
#5	tissue	pos.	18.13	18.45	18.74	18.31
#6	tissue	pos.	28.59	29.54	27.52	29.26
#7	tissue	pos.	18.00	18.81	18.96	18.63
#8	tissue	pos.	20.84	21.94	22.13	21.68
#9	tissue	pos.	13.47	13.83	13.56	13.76
#10	tissue	pos.	17.04	18.08	17.78	17.47
#11	tissue	pos.	23.32	23.44	23.62	23.57
#12	tissue	pos.	14.92	15.14	15.07	14.95
#13	tissue	pos.	17.59	17.89	17.95	17.79
#14	tissue	pos.	5.27	6.31	6.37	5.90
#15	tissue	pos.	12.48	13.90	13.32	13.31
#16	tissue	pos.	5.89	7.00	7.01	6.69

pos. = positive

3.2.6.3 External field data (German state veterinary laboratory)

Procedure

DNA was extracted from *n* = 3 PCV-2 and PCV-2- double positive tissue samples DNA was extracted using the IndiMag Pathogen Kit and “Pretreatment T1” and extraction was performed with the IndiMag 2 and IndiMag 48s devices. The eluates were further analyzed by using the virotype PCV2/PCV3 Primers/Probes and IndiMix JOE on the Bio-Rad CFX 96 thermocycler. Data were generated and kindly provided by a German state veterinary laboratory.

Results / Conclusion

Comparative results of PCV-2 and PCV-3 testing is presented in Table 26. All tissue samples were detected correctly, regardless of the automatic extraction instrument used. The IndiMag 2 was able to extract DNA more efficiently from tissue samples.

Table 26. Data summary for testing PCV-2 / PCV-3 double positive tissue samples extracted on the IndiMag 2 (IM 2) and the IndiMag 48s (IM 48s) devices.

Sample	Specimen	PCV-2 / PCV-3 status	PCV-2 IndiMag Pathogen Kit		PCV-3 IndiMag Pathogen Kit	
			IM 2 C_T	IM 48s C_T	IM 2 C_T	IM 48s C_T
#1	tissue	**pos./ pos.**	23.70	24.00	20.30	34.20
#2	tissue	**pos./ pos.**	36.80	38.20	31.30	31.40
#3	tissue	**pos./ pos.**	30.50	33.00	31.20	30.90

pos. = positive

3.2.7 ***Suid Herpesvirus* 1** (SHV-1, Aujeszky's Disease; Tissue; external field data – Belgian testing laboratory, Sciensano)

Procedure

DNA was extracted from *n* = 12 tissue samples (*n* = 10 positive and *n* = 2 negative for SHV-1). After an in-house pretreatment, extraction was performed either automated using the IndiMag Pathogen Kit combined with the IndiMag 2 device or manually with the QIAamp DNA Mini Kit (QIAGEN GmbH, Hilden, Germany). The eluates were further analyzed by an in-house PCR, which was performed on the Roche Diagnostics LightCycler 480 detection system. Data were generated and kindly provided by Sciensano, Belgium.

Results / Conclusion

Results are presented in Table 27. Almost all tissue samples were detected correctly, regardless of the extraction method used. DNA from sample 12 was correctly identified as positive by the IndiMag 2 extraction method only. Overall, the IndiMag 2 with the IndiMag Pathogen Kit performed better than the manual spin column method.

Table 27. Data summary for SHV-1 testing of tissue samples extracted on the IndiMag 2 (using the IndiMag Pathogen Kit) or manually (using the QIAamp DNA Mini Kit).

Sample	Specimen	Status	*Suid Herpesvirus 1* (SHV-1)	
			IndiMag Pathogen Kit IndiMag 2	QIAamp DNA Mini Kit manual
			C_T	C_T
#1	tissue	**positive**	22.82	24.22
#2	tissue	**positive**	26.34	27.73
#3	tissue	**positive**	29.69	31.12
#4	tissue	**positive**	20.72	21.52
#5	tissue	neg.	-	-
#6	tissue	neg.	-	-
#7	tissue	**positive**	25.33	27.01
#8	tissue	**positive**	28.79	30.54
#9	tissue	**positive**	32.19	33.69
#10	tissue	**positive**	23.24	24.37
#11	tissue	neg.	-	-
#12	tissue	**positive**	33.82	-

- = no C_T, neg. = negative

3.3 Viral RNA extraction

3.3.1 ***Bovine Coronavirus*** (feces; external field data – German state veterinary laboratory)

Procedure

RNA was extracted from *n* = 5 *Bovine Coronavirus*-positive fecal samples using the IndiMag Pathogen Kit and "Pretreatment F1". The extraction was performed with the IndiMag 2 and the IndiMag 48s devices. The eluates were further analyzed by an in-house RT-PCR, which was performed on the Bio-Rad CFX 96 thermocycler. Data were generated and kindly provided by a German state veterinary laboratory.

Results / Conclusion

Results are presented in Table 28. All fecal samples were detected correctly by both setups with comparable performances.

Table 28. Data summary for testing *Bovine Coronavirus*-positive fecal samples extracted on the IndiMag 2 and IndiMag 48s device.

Sample	**Specimen**	**Status**	***Bovine Coronavirus***	
			IndiMag Pathogen Kit IndiMag 2	**IndiMag Pathogen Kit IndiMag 48s**
			C_T	**C_T**
#1	feces	**positive**	21.50	21.70
#2	feces	**positive**	13.90	14.20
#3	feces	**positive**	27.40	28.00
#4	feces	**positive**	34.20	33.30
#5	feces	**positive**	38.20	37.60

3.3.2 ***Bovine Respiratory Syncytial Virus*** (BRSV; swabs; external field data – German state veterinary laboratory)

Procedure

RNA was extracted from *n* = 13 nasal swabs positive for *Bovine Respiratory Syncytial Virus* (BRSV) using the IndiMag Pathogen Kit and "Pretreatment S1". The extraction was performed with the IndiMag 2 and the IndiMag 48s devices. The eluates were further analyzed by an in-house RT-PCR, which was performed on the Bio-Rad CFX 96 thermocycler. Data were generated and kindly provided by a German state veterinary laboratory.

Results / Conclusion

Table 29 presents the obtained results for this comparative study. All swab samples were detected correctly by both setups.

Table 29. Data summary for testing *Bovine Coronavirus*-positive fecal samples extracted on the IndiMag 2 and IndiMag 48s device.

Sample	Specimen	Status	***Bovine Respiratory Syncytial Virus*** **(BRSV)**	
			IndiMag Pathogen Kit IndiMag 2	**IndiMag Pathogen Kit IndiMag 48s**
			C_T	**C_T**
#1	swab	**positive**	27.70	26.00
#2	swab	**positive**	26.40	25.30
#3	swab	**positive**	26.10	25.40
#4	swab	**positive**	32.30	31.20
#5	swab	**positive**	23.00	22.60
#6	swab	**positive**	23.10	23.00
#7	swab	**positive**	33.20	34.40
#8	swab	**positive**	38.20	37.30
#9	swab	**positive**	37.70	35.90
#10	swab	**positive**	27.60	27.20
#11	swab	**positive**	24.80	24.70
#12	swab	**positive**	34.40	32.00
#13	swab	**positive**	35.40	34.80

3.3.3 ***Bluetongue Virus*** (BTV; blood)

3.3.3.1 Internal data 1 (INDICAL BIOSCIENCE GmbH, Leipzig)

Procedure

RNA was extracted from *n* = 10 BTV-positive blood samples, using the IndiMag Pathogen Kit, the IndiSpin Pathogen Kit or the Indispin QIAcube HT Pathogen Kit. The extraction was performed with the KingFisher Flex, the QIAcube HT device or manually using IndiSpin columns. Subsequently, the eluates were further analyzed with the virotype BTV pan/8 RT-PCR Kit. The RT-PCR was performed on the Agilent Technologies Stratagene Mx3005P thermocycler. Data were generated by INDICAL BIOSCIENCE GmbH, Leipzig, Germany.

Results / Conclusion

Table 30 summarizes the data. All samples were detected correctly, regardless of the extraction method used.

Table 30. Data summary for testing BTV-positive blood samples extracted on the KingFisher Flex (KFF) or QIAcube HT device or by manual extraction.

Sample	Specimen	Status	Bluetongue Virus (BTV)		
			IndiMag Pathogen Kit KFF C_T	IndiSpin QIAcube HT Path. Kit QIAcube HT C_T	IndiSpin Pathogen Kit manual C_T
#1	blood	pos.	23.14	23.27	23.07
#2	blood	pos.	19.92	22.41	20.77
#3	blood	pos.	21.81	22.09	21.53
#4	blood	pos.	30.23	29.91	28.32
#5	blood	pos.	24.18	23.97	22.21
#6	blood	pos.	22.35	22.76	23.15
#7	blood	pos.	26.08	26.72	26.65
#8	blood	pos.	29.84	31.52	29.94
#9	blood	pos.	36.66	36.95	35.16
#10	blood	pos.	29.40	29.63	27.18

pos. = positive, Path. = Pathogen

3.3.3.2 Internal data 2 (INDICAL BIOSCIENCE GmbH, Leipzig)

Procedure

RNA was extracted from *n* = 8 BTV-positive blood samples, using the IndiMag Pathogen Kit, the IndiSpin QIAcube HT Pathogen, and the IndiSpin Pathogen Kit. The extraction was performed with the IndiMag 48, IndiMag 48s, KingFisher Flex, QIAcube HT device or manually using IndiSpin columns. Subsequently, the eluates were further analyzed by using the virotype BTV pan/8 RT-PCR Kit. The RT-PCR was performed on the Agilent Technologies Stratagene Mx3005P thermocycler. Data were generated by INDICAL BIOSCIENCE GmbH, Leipzig, Germany.

Results / Conclusion

Table 31 summarizes the data. All blood samples were detected correctly, regardless of the extraction method used. Automated extraction performed better compared to the manual extraction methods.

Table 31. Data summary for testing BTV-positive blood samples extracted on the IndiMag 48 (IM 48), IndiMag 48s (IM 48s), KingFisher Flex (KFF), and QIAcube HT devices, and by manual extraction.

Sample	**Specimen**	**Status**	***Bluetongue Virus* (BTV)**				
			IndiMag Pathogen Kit			**IndiSpin QIAcube HT Path. Kit**	**IndiSpin Pathogen Kit**
			IM 48	**IM 48s**	**KFF**	**QIAcube HT**	**manual**
			C_T	**C_T**	**C_T**	**C_T**	**C_T**
#1	blood	**pos.**	27.64	26.25	26.16	26.88	27.70
#2	blood	**pos.**	22.94	22.35	23.52	24.91	25.56
#3	blood	**pos.**	25.51	24.32	24.92	25.97	26.69
#4	blood	**pos.**	24.23	23.35	24.32	24.74	25.24
#5	blood	**pos.**	24.28	23.46	24.54	24.92	25.60
#6	blood	**pos.**	29.66	29.37	30.11	30.90	31.91
#7	blood	**pos.**	25.25	23.64	23.88	24.00	23.95
#8	blood	**pos.**	26.13	25.65	25.91	25.01	25.70

Path. = Pathogen, pos. = positive

3.3.3.3 Internal data 3 (INDICAL BIOSCIENCE GmbH, Leipzig)

Procedure

RNA was extracted from *n* = 6 BTV-positive serum samples. These samples were either part of a ring test trial 2009 or were obtained as a BTV-8 reference sample from Friedrich Loeffler-Institut, FLI, Insel Riems, Germany. RNA was extracted in duplicate using the IndiMag Pathogen Kit and the MagMAX CORE Nucleic Acid Purification Kit. The extraction was performed with the IndiMag 48 or KingFisher Flex devices. The eluates were further analyzed with the virotype BTV pan/8 RT-PCR Kit, which was performed on the Agilent Technologies Stratagene Mx3005P thermocycler. Data were generated by INDICAL BIOSCIENCE GmbH, Leipzig, Germany.

Results / Conclusion

Table 32 presents the obtained results for this comparative study. All the samples were detected correctly, regardless of the extraction instrument used. Both automatic extraction methods performed equally well.

Table 32. Data summary for BTV-positive blood ring trial samples extracted on the IndiMag 48 (using the IndiMag Pathogen Kit) or on the KingFisher Flex (using the MagMAX CORE Nucleic Acid Purification Kit).

Sample	**Specimen**	**Status**	***Bluetongue Virus* (BTV)**	
			IndiMag Pathogen Kit IndiMag 48	**MagMAX CORE Kit KingFisher Flex**
			C_T	**C_T**
BTV positive #6	serum	**positive**	23.09 22.94	23.00 23.01
BTV positive #7	serum	**positive**	24.08 23.92	23.45 23.61
BTV positive #8	serum	**positive**	27.01 26.96	28.97 26.78
BTV positive #9	serum	**positive**	28.10 28.36	28.30 27.37
BTV positive #11	serum	**positive**	23.25 23.37	22.99 22.50
Reference blood FLI BTV-8	serum	**positive**	29.07 29.20	27.62 27.53

3.3.4 ***Bluetongue Virus*** (BTV; tissue; internal data – INDICAL BIOSCIENCE GmbH, Leipzig)

Procedure

RNA was extracted from *n* = 2 BTV-positive tissue samples, using the IndiMag Pathogen Kit, the IndiSpin Pathogen Kit or the Indispin QIAcube HT Pathogen Kit. The extraction was performed with the KingFisher Flex, the QIAcube HT device or manually using IndiSpin columns. Subsequently, the eluates were further analyzed by using the virotype BTV pan/8 RT-PCR Kit (INDICAL BIOSCIENCE GmbH, Leipzig, Germany). The RT-PCR was performed on the Agilent Technologies Stratagene Mx3005P thermocycler. Data were generated by INDICAL BIOSCIENCE GmbH, Leipzig, Germany.

Results / Conclusion

Table 33 summarizes the data. All samples were detected correctly, regardless of the extraction method used. Anyway, automated extraction methods performed better than manual extraction.

Table 33. Data summary for testing BTV-positive tissue samples extracted on the KingFisher Flex (KFF) or QIAcube HT device or by manual extraction.

Sample	**Specimen**	**Status**	***Bluetongue Virus* (BTV)**		
			IndiMag Pathogen Kit	**IndiSpin QIAcube HT Path. Kit**	**IndiSpin Pathogen Kit**
			KFF	**QIAcube HT**	**manual**
			C_T	**C_T**	**C_T**
#1	lymph node	**pos.**	28.87	29.59	29.80
#2	spleen	**pos.**	32.29	32.65	32.87

pos. = positive, Path. = Pathogen

3.3.5 ***Bovine Viral Diarrhea Virus*** *(BVDV*; milk; internal data – INDICAL BIOSCIENCE GmbH, Leipzig)

Procedure

Samples from *n* = 2 BVDV-positive milk samples were generated by serial dilution. RNA from a total of seven different dilutions was extracted using the IndiMag Pathogen Kit. The extraction was performed with the BioSprint 96 device. The eluates were further analyzed using the virotype BVDV RT-PCR Kit (INDICAL BIOSCIENCE GmbH, Leipzig, Germany), which was performed on the Agilent Technologies Stratagene Mx3005P thermocycler. Data were generated by INDICAL BIOSCIENCE GmbH, Leipzig, Germany.

Results / Conclusion

Table 34 presents the obtained results for this comparative study. All milk samples were detected correctly.

Table 34. Data summary for BVDV-positive milk samples extracted on the BioSprint 96 device.

Sample	Specimen	Dilution	Status	BVDV IndiMag Pathogen Kit BioSprint 96 C_T
1-1	milk	1:10	**positive**	25.27
1-2	milk	1:100	**positive**	27.80
1-3	milk	1:1,000	**positive**	30.47
1-4	milk	1:10,000	**positive**	33.82
1-5	milk	1:100,000	**positive**	35.91
2-1	milk	1:10	**positive**	27.94
2-2	milk	1:100	**positive**	33.98

3.3.6 ***Bovine Viral Diarrhea Virus*** *(BVDV*; blood)

3.3.6.1 Internal data 1 (INDICAL BIOSCIENCE GmbH, Leipzig)

Procedure

RNA was extracted from *n* = 2 BVDV-positive blood samples dilutions (1:100 and 1:1,000) in *n* = 20 replicates using the IndiMag Pathogen Kit. The extraction was performed with the IndiMag 2 and the KingFisher Flex devices. The eluates were further analyzed using the virotype BVDV RT-PCR Kit, which was performed on the Agilent Technologies Stratagene Mx3005P thermocycler. Data were generated by INDICAL BIOSCIENCE GmbH, Leipzig, Germany.

Results / Conclusion

Table 35 depicts the results for testing BVDV-positive blood samples in a 1:100 dilution and Table 36 presents results for the 1:1,000 diluted blood samples. All samples were detected correctly, regardless of the automatic extraction instrument used. The RNA extraction using the IM 2 device performed better than with the KingFisher Flex device.

Table 35. Data summary for BVDV-testing of 1:100 diluted blood samples in 20 replicates extracted on the IndiMag 2 and KingFisher Flex devices.

Sample	Specimen	Status	*Bovine Viral Diarrhea Virus* (BVDV)	
			IndiMag Pathogen Kit IndiMag 2	IndiMag Pathogen Kit KingFisher Flex
			C_T	C_T
#1	blood	positive	26.01	26.46
#2	blood	positive	26.30	26.76
#3	blood	positive	26.03	26.85
#4	blood	positive	26.36	26.98
#5	blood	positive	26.13	26.49
#6	blood	positive	25.62	26.85
#7	blood	positive	25.88	26.36
#8	blood	positive	25.76	26.60
#9	blood	positive	25.83	26.29
#10	blood	positive	25.87	26.33
#11	blood	positive	26.11	26.74
#12	blood	positive	25.84	26.46
#13	blood	positive	26.14	26.43
#14	blood	positive	25.95	26.46
#15	blood	positive	25.93	26.28
#16	blood	positive	26.40	26.83
#17	blood	positive	26.48	26.43
#18	blood	positive	25.78	26.53
#19	blood	positive	26.06	26.54
#20	blood	positive	26.24	26.96

Table 36. Data summary for BVDV-testing of 1:1,000 diluted blood samples in 20 replicates extracted on the IndiMag 2 and KingFisher Flex devices.

Sample	Specimen	Status	*Bovine Viral Diarrhea Virus* (BVDV)	
			IndiMag Pathogen Kit IndiMag 2	IndiMag Pathogen Kit KingFisher Flex
			C_T	C_T
#1	blood	positive	29.17	30.04
#2	blood	positive	28.89	29.46
#3	blood	positive	28.93	30.06
#4	blood	positive	29.06	30.43
#5	blood	positive	29.27	30.65
#6	blood	positive	29.10	30.03
#7	blood	positive	29.35	30.16
#8	blood	positive	29.24	29.83
#9	blood	positive	29.53	29.50
#10	blood	positive	29.79	29.43
#11	blood	positive	29.30	29.86
#12	blood	positive	29.20	30.01
#13	blood	positive	29.26	30.27
#14	blood	positive	29.44	29.81
#15	blood	positive	29.18	29.80
#16	blood	positive	29.30	29.81
#17	blood	positive	28.84	29.69
#18	blood	positive	29.90	30.40
#19	blood	positive	29.34	30.00
#20	blood	positive	29.25	29.71

3.3.6.2 External field data (German state veterinary laboratory)

Procedure

RNA was extracted from *n* = 10 BVDV-positive blood samples. Sample derived from a BVDV negative bovine blood sample spiked with BVDV derived from cell culture. A serial dilution was prepared, and RNA was extracted in duplicate using the IndiMag Pathogen Kit either on the IndiMag 2 or on the IndiMag 48. The eluates were further analyzed with the virotype BVDV RT-PCR Kit, which was performed on the Bio-Rad CFX 96 detection system. Data were generated and kindly provided by a German state veterinary laboratory.

Results / Conclusion

Table 37 depicts the results the comparative study. All samples were detected correctly, regardless of the automatic extraction instrument used. Extraction using the IndiMag 2 performed better compared to the IndiMag 48 device.

Table 37. Data summary for BVDV-testing of spiked blood samples extracted on the IndiMag 2 and IndiMag 48 devices.

Sample	**Specimen**	**Status**	***Bovine Viral Diarrhea Virus* (BVDV)**	
			IndiMag Pathogen Kit IndiMag 2	**IndiMag Pathogen Kit IndiMag 48**
			C_T	**C_T**
#1	blood	**positive**	23.22	26.63
#2	blood	**positive**	23.14	25.78
#3	blood	**positive**	26.93	29.39
#4	blood	**positive**	26.79	29.82
#5	blood	**positive**	30.08	33.62
#6	blood	**positive**	30.36	33.43
#7	blood	**positive**	33.81	36.76
#8	blood	**positive**	34.08	36.70
#9	blood	**positive**	37.03	39.96
#10	blood	**positive**	37.38	38.24

3.3.6.3 External field data (Belgian testing laboratory, Sciensano)

Procedure

RNA was extracted from *n* = 5 BVDV-positive and one BVDV-negative blood samples either automated using the IndiMag Pathogen Kit on the IndiMag 2 or manually with the QIAamp Viral RNA Mini Kit (QIAGEN GmbH, Hilden, Germany). The eluates were further analyzed by an in-house RT-PCR, which was performed on the Roche Diagnostics LightCycler 480 detection system. Data were generated and kindly provided by Sciensano, Belgium.

Results / Conclusion

Test results are presented in Table 38. All samples were detected correctly, regardless of the extraction method used. Extraction using the IndiMag 2 performed better compared to manual extraction via silica spin columns.

Table 38. Data summary for BVDV-testing of blood samples extracted on the IndiMag 2 device and manually.

Sample	**Specimen**	**Status**	***Bovine Viral Diarrhea Virus* (BVDV)**	
			IndiMag Pathogen Kit IndiMag 2	**QIAamp RNA Viral Mini Kit manual**
			C_T	**C_T**
#1	blood	**positive**	25.91	27.17
#2	blood	**positive**	28.20	29.25
#3	blood	**positive**	27.40	28.36
#4	blood	**positive**	28.79	29.10
#5	blood	neg.	-	-

- = no C_T, neg. = negative

3.3.7 ***Bovine Viral Diarrhea Virus*** *(BVDV*; tissue; external field data – Belgian testing laboratory, Sciensano)

Procedure

RNA was extracted from *n* = 3 BVDV-positive ear notches. After an in-house pretreatment, extraction was performed either automated using the IndiMag Pathogen Kit on the IndiMag 2 or manually with the RNeasy® Mini Kit (QIAGEN GmbH, Hilden, Germany). The eluates were further analyzed by an in-house RT- PCR, which was performed on the Roche LightCycler 480 detection system. Data were generated and kindly provided by Sciensano, Belgium.

Results / Conclusion

Test results are presented in Table 39. All samples were detected correctly, regardless of the extraction method used. Extraction using the IndiMag 2 performed better compared to manual extraction via silica spin columns.

Table 39. Data summary for BVDV-testing of ear notch samples extracted on the IndiMag 2 device and manually.

Sample	**Specimen**	**Status**	***Bovine Viral Diarrhea Virus* (BVDV)**	
			IndiMag Pathogen Kit IndiMag 2	**RNeasy Mini Kit manual**
			C_T	**C_T**
#1	ear notch	**positive**	29.46	31.23
#2	ear notch	**positive**	33.11	34.58
#3	ear notch	neg.	-	-

- = no C_T, neg. = negative

3.3.8 ***Classical Swine Fever Virus*** (CSFV; serum)

3.3.8.1 Internal data (INDICAL BIOSCIENCE GmbH, Leipzig)

Procedure

RNA was extracted from *n* = 8 CSFV-positive serum samples (derived from several ring trial tests). RNA was extracted in duplicate using the IndiMag Pathogen Kit and the MagMAX CORE Nucleic Acid Purification Kit. The extraction was performed with the IndiMag 48 or KingFisher Flex devices. The eluates were further analyzed using the virotype CSFV RT-PCR Kit (INDICAL BIOSCIENCE GmbH, Leipzig, Germany), which was performed on the Agilent Technologies Stratagene Mx3005P thermocycler. Data were generated by INDICAL BIOSCIENCE GmbH, Leipzig, Germany.

Results / Conclusion

Table 40 presents the obtained results for this comparative study. All the samples were detected correctly, regardless of the extraction instrument used. Extraction using the IndiMag Pathogen Kit on the IndiMag 48 performed better compared to extraction with the MagMAX CORE Nucleic Acid Purification Kit on the KingFisher Flex device.

Table 40. Data summary for CSFV-positive serum ring trial samples extracted on the IndiMag 48 (using the IndiMag Pathogen Kit) or on the KingFisher Flex (using the MagMAX CORE Nucleic Acid Purification Kit).

Sample	Specimen	Status	*Classical Swine Fever Virus* (CSFV)	
			IndiMag Pathogen Kit IndiMag 48	MagMAX CORE Kit KingFisher Flex
			C_T	C_T
CSF1045 (GE 2009, gt 2.3) (C/D - RV 2009)	serum	positive	21.51 21.29	20.73 20.89
CSF0104 (GE1994, gt 2.3) (#14 - RV 2010)	serum	positive	37.65 35.09	26.31 35.76
CSF0104 (GE1994 gt 2.3) (#21 - RV 2010)	serum	positive	36.41 36.33	40.00 39.08
CSF0634 (gt 2.3) (L/M - RV 2009)	serum	positive	26.40 26.16	25.53 25.95
CSF0849 (gt 2.1) (G/R - RV 2009)	serum	positive	29.37 29.42	29.52 29.70
CSF0849 (gt 2.1) (T/Y - RV 2009)	serum	positive	26.49 26.44	26.07 25.91
CSF0634 (gt 2.3) (F/P - RV 2009)	serum	positive	30.17 30.44	29.05 29.31
CSF0849 (gt 2.1) (S/N - RV 2009)	serum	positive	26.64 26.59	25.94 26.15

3.3.8.2 External field data (Belgian testing laboratory, Sciensano)

Procedure

RNA was extracted from *n* = 6 CSFV-positive and one CSFV-negative serum samples. After an in-house pretreatment, extraction was performed either automated using the IndiMag Pathogen Kit on the IndiMag 2 or manually with the RNeasy Mini Kit. The eluates were further analyzed by an in-house RT-PCR, which was performed on the Roche Diagnostics LightCycler 480 detection system. Data were generated and kindly provided by Sciensano, Belgium.

Results / Conclusion

Test results are shown in Table 41. All samples were detected correctly, regardless of the extraction method used. Extraction using the IndiMag 2 performed better compared to manual extraction via silica spin columns.

Table 41. Data summary for CSFV-testing of serum samples extracted on the IndiMag 2 device and manually.

Sample	**Specimen**	**Status**	***Classical Swine Fever Virus* (CSFV)**	
			IndiMag Pathogen Kit IndiMag 2	**RNeasy Mini Kit manual**
			C_T	**C_T**
#1	serum	**positive**	32.89	36.51
#2	serum	neg.	-	-
#3	serum	**positive**	36.01	38.04
#4	serum	**positive**	27.24	28.51
#5	serum	**positive**	29.48	32.71
#6	serum	**positive**	32.95	36.53
#7	serum	**positive**	32.95	35.78

- = no C_T, neg. = negative

3.3.9 ***Canine Distemper Virus*** (CDV; tissue; external field data – German state veterinary laboratory)

Procedure

RNA was extracted from *n* = 8 CDV-positive and one CDV-negative brain tissue samples. After pre-treating the samples according to "Pretreatment T1", RNA was extracted using the IndiMag Pathogen Kit either on the IndiMag 2 or on the IndiMag 48s. The eluates were further analyzed by an in-house RT-PCR, which was performed on the Bio-Rad CFX 96 detection system. Data were generated and kindly provided by a German state veterinary laboratory.

Results / Conclusion

Table 42 depicts the results the comparative study. All samples were detected correctly, regardless of the automatic extraction instrument used. Overall, RNA extracted with the IndiMag 2 performed better than with the IndiMag 48s device.

Table 42. Data summary for CDV-testing of brain tissue samples extracted on the IndiMag 2 and IndiMag 48s devices.

Sample	**Specimen**	**Status**	***Canine Distemper Virus* (CDV)**	
			IndiMag Pathogen Kit IndiMag 2	**IndiMag Pathogen Kit IndiMag 48s**
			C_T	**C_T**
#1	tissue	**positive**	21.30	21.00
#2	tissue	**positive**	20.30	21.50
#3	tissue	**positive**	21.20	22.10
#4	tissue	**positive**	27.30	29.30
#5	tissue	**positive**	31.50	32.10
#6	tissue	**positive**	22.40	23.40
#7	tissue	**positive**	17.60	18.30
#8	tissue	**positive**	20.50	21.10

3.3.10 **Influenza A Virus** (oral fluid; internal data – INDICAL BIOSCIENCE GmbH, Leipzig)

Procedure

RNA was extracted from *n* = 10 Influenza A Virus-positive pig oral fluid samples. After pre-treating the samples according to “Pretreatment O2”, extraction was performed either using the IndiMag Pathogen Kit combined with the IndiMag 48s or the KingFisher Flex devices or using the MagMAX CORE Nucleic Acid Purification Kit combined with the KingFisher Flex device. The eluates were further analyzed using the virotype Influenza A 2.0 RT-PCR Kit (INDICAL BIOSCIENCE GmbH, Leipzig, Germany), which was performed on the Agilent Technologies Stratagene Mx3005P detection system. Data were generated by INDICAL BIOSCIENCE GmbH, Leipzig, Germany.

Results / Conclusion

Table 43 summarizes the test results for the comparative Avian Influenza A tests. All the oral fluid samples were detected correctly, regardless of the extraction method used.

Table 43. Data summary for Influenza A-positive oral fluid samples extracted on the IndiMag 2 (using the IndiMag Pathogen Kit) or on the KingFisher Flex (using either the IndiMag Pathogen Kit or the MagMAX CORE Nucleic Acid Purification Kit).

Sample	Specimen	Status	*Porcine Circovirus 3* (PCV-3)		
			IndiMag Pathogen Kit IndiMag 48s	IndiMag Pathogen Kit KingFisher Flex	MagMAX CORE Kit KingFisher Flex
			C_T	C_T	C_T
#1	oral fluid	positive	38.45	37.31	-
#2	oral fluid	positive	26.68	26.39	27.22
#3	oral fluid	positive	30.50	28.83	34.27
#4	oral fluid	positive	27.68	27.21	27.48
#5	oral fluid	positive	34.38	33.79	34.47
#6	oral fluid	positive	36.94	37.27	-
#7	oral fluid	positive	27.99	27.54	27.87
#8	oral fluid	positive	23.33	22.91	23.21
#9	oral fluid	positive	28.64	28.44	27.45
#10	oral fluid	positive	31.92	31.72	33.40

3.3.11 **Influenza A Virus** (tissue; internal data – INDICAL BIOSCIENCE GmbH, Leipzig)

Procedure

RNA was extracted from *n* = 8 Influenza A Virus-positive pig tissue samples, using the IndiMag Pathogen Kit and "Pretreatment T1" and extraction was performed on the IndiMag 2, KingFisher Flex, KingFisher Apex and IndiMag 48 devices. The eluates were further analyzed with the virotype Influenza A 2.0 RT-PCR Kit using the Agilent Technologies Stratagene Mx3005P thermocycler. Data were generated by INDICAL BIOSCIENCE GmbH, Leipzig, Germany.

Results / Conclusion

Table 44 summarizes the data for comparative Influenza A Virus testing. All tissue samples were detected correctly, regardless of the automatic extraction instrument used. Automatic RNA extraction with the IM 2 performed better than using other devices.

Table 44. Data summary for testing Influenza A Virus-spiked tissue samples extracted on the IndiMag 2 (IM 2), IndiMag 48 (IM 48), KingFisher Flex (KFF), and KingFisher Apex (KFA) devices.

Sample	**Specimen**	**Status**	**Influenza A Virus**			
			IndiMag Pathogen Kit			
			IM 2	**IM 48**	**KFF**	**KFA**
			C_T	**C_T**	**C_T**	**C_T**
#1	tissue	**pos.**	32.63	34.12	34.11	33.44
#2	tissue	**pos.**	31.34	32.62	33.24	33.23
#3	tissue	**pos.**	33.76	33.44	33.52	33.53
#4	tissue	**pos.**	30.62	33.90	31.58	33.77
#5	tissue	**pos.**	32.73	33.07	32.75	33.22
#6	tissue	**pos.**	32.35	33.27	33.42	33.02
#7	tissue	**pos.**	32.33	33.40	33.16	32.76
#8	tissue	**pos.**	33.00	33.45	33.27	33.57

pos. = positive

3.3.12 **Influenza A Virus** (swabs; internal data – INDICAL BIOSCIENCE GmbH, Leipzig)

Procedure

RNA was extracted from *n* = 8 Influenza A Virus-spiked chicken swab samples, using the IndiMag Pathogen Kit, the IndiSpin QIAcube HT Pathogen and the IndiSpin Pathogen Kit and "Pretreatment S1". The extraction was performed with the IndiMag 48, IndiMag 48s, KingFisher Flex, QIAcube HT device or manually with IndiSpin columns. Subsequently, the eluates were further analyzed using the virotype Influenza A 2.0 RT-PCR Kit. The RT-PCR was performed on the Agilent Technologies Stratagene Mx3005P thermocycler. Data were generated by INDICAL BIOSCIENCE GmbH, Leipzig, Germany.

Results / Conclusion

Table 45 summarizes the data. All the spiked swab samples were detected correctly, regardless of the extraction setup used. Automatic RNA extraction performed better than when using the manual silica spin columns. Furthermore, extraction with the IndiMag Pathogen Kit performed best when using the IndiMag 48 or the IndiMag 48s.

Table 45. Data summary for Influenza A Virus-testing in spiked swab samples extracted on the IndiMag 48 (IM 48), IndiMag 48s (IM 48s), KingFisher Flex (KFF), and QIAcube HT devices, and by manual extraction.

Sample	Specimen	Status	Influenza A Virus				
			IndiMag Pathogen Kit			IndiSpin QIAcube HT Path. Kit	IndiSpin Pathogen Kit
			IM 48	IM 48s	KFF	QIAcube HT	manual
			C_T	C_T	C_T	C_T	C_T
#1	swab (H1)	pos.	22.95	22.00	23.16	23.41	25.10
#2	swab (H3N3)	pos.	26.58	26.26	27.32	26.76	27.83
#3	swab (H5)	pos.	23.90	23.00	24.19	24.39	25.48
#4	swab (H6N6)	pos.	21.77	21.34	22.20	22.24	24.12
#5	swab (H7N1)	pos.	23.11	23.11	23.76	23.47	25.86
#6	swab (H9)	pos.	23.70	23.50	24.51	28.75	24.80
#7	swab (P1645)	pos.	19.40	18.91	20.27	20.25	25.04
#8	swab (H9)	pos.	19.25	18.29	19.46	19.84	22.44

Path. = Pathogen, pos. = positive

3.3.13 ***Ovine Herpes Virus 2*** (OvHV-2, causing Bösartiges Katarrhalfieber, BFK / Malignant catarrhal fever, MCF; tissue; external field data – German state veterinary laboratory)

Procedure

RNA was extracted from *n* = 4 OvHV-2-positive tissue samples. After pre-treating the samples according to "Pretreatment T1", RNA was extracted using the IndiMag Pathogen Kit either on the IndiMag 2 or on the IndiMag 48s device. The eluates were further analyzed with an in-house RT-PCR, which was performed on the Bio-Rad CFX 96 detection system. Data were generated and kindly provided by a German state veterinary laboratory.

Results / Conclusion

Table 46 depicts the results the comparative study. All samples were detected correctly, regardless of the automatic extraction instrument used. Extraction using the IM2 device performed better compared IM 48s.

Table 46. Data summary for OvHV-2-testing of spiked tissue samples extracted on the IndiMag 2 and IndiMag 48s devices.

Sample	**Specimen**	**Status**	***Ovine Herpes Virus 2*** **(OvHV-2)**	
			IndiMag Pathogen Kit IndiMag 2	**IndiMag Pathogen Kit IndiMag 48s**
			C_T	**C_T**
#1	tissue	**positive**	25.30	25.20
#2	tissue	**positive**	29.60	30.50
#3	tissue	**positive**	33.60	35.40
#4	tissue	**positive**	35.70	36.10

3.3.14 ***Porcine Epidemic Diarrhea Virus*** (PEDV; feces; external field data – German state veterinary laboratory)

Procedure

RNA was extracted from *n* = 8 PEDV-positive fecal samples. After pre-treating the samples according to "Pretreatment F1", RNA was extracted using the IndiMag Pathogen Kit either on the IndiMag 2 or on the IndiMag 48s device. The eluates were further analyzed with an in-house RT-PCR, which was performed on the Bio-Rad CFX 96 detection system. Data were generated and kindly provided by a German state veterinary laboratory.

Results / Conclusion

Table 47 presents the results the comparative study. All samples were detected correctly, regardless of the automatic extraction instrument used. Overall, RNA extracted with the IndiMag 2 performed better than with the IndiMag 48s device.

Table 47. Data summary for PEDV-testing of spiked tissue samples extracted on the IndiMag 2 and IndiMag 48s devices.

Sample	**Specimen**	**Status**	***Porcine Epidemic Diarrhea Virus* (PEDV)**	
			IndiMag Pathogen Kit IndiMag 2	**IndiMag Pathogen Kit IndiMag 48s**
			C_T	**C_T**
#1	feces	**positive**	16.20	15.90
#2	feces	**positive**	18.00	19.00
#3	feces	**positive**	19.00	18.30
#4	feces	**positive**	35.10	37.50
#5	feces	**positive**	15.20	16.60
#6	feces	**positive**	29.20	30.21
#7	feces	**positive**	20.90	18.75
#8	feces	**positive**	29.20	30.20

3.3.15 ***Porcine Reproductive and Respiratory Syndrome Virus*** (PRRSV; oral fluid)

3.3.15.1 Internal data 1: PRRSV EU oral fluid samples (INDICAL BIOSCIENCE GmbH, Leipzig)

Procedure

RNA was extracted from *n* = 34 oral fluid samples, positive for PRRSV European (EU) strain. After pre-treating the samples according to "Pretreatment O2", extraction was performed either using the IndiMag Pathogen Kit combined with the IndiMag 48s or the KingFisher Flex devices or using the MagMAX CORE Nucleic Acid Purification Kit combined with the KingFisher Flex device. The eluates were further analyzed with the virotype PRRSV RT-PCR Kit, which was performed on the Agilent Technologies Stratagene Mx3005P detection system. Data were generated by INDICAL BIOSCIENCE GmbH, Leipzig.

Results / Conclusion

Test results are presented in Table 48. All the oral fluid samples were detected correctly, regardless of the extraction method used. Overall, RNA extraction using the IndiMag Pathogen Kit performed equal to slightly better than with the MagMAX Core Nucleic Acid Purification Kit.

Table 48. Data summary for PRRSV EU-positive oral fluid samples extracted on the IndiMag 2 (using the IndiMag Pathogen Kit) or on the KingFisher Flex (using either the IndiMag Pathogen Kit or the MagMAX CORE Nucleic Acid Purification Kit) device.

Sample	Specimen	Status	PRRSV EU strain		
			IndiMag Pathogen Kit IndiMag 48s	IndiMag Pathogen Kit KingFisher Flex	MagMAX CORE Kit KingFisher Flex
			C_T	C_T	C_T
#1	oral fluid	positive	35.14	35.95	35.92
#2	oral fluid	positive	30.34	30.88	32.02
#3	oral fluid	positive	35.93	34.42	34.91
#4	oral fluid	positive	33.79	34.42	35.66
#5	oral fluid	positive	33.94	36.13	36.19
#6	oral fluid	positive	36.48	34.86	34.90
#7	oral fluid	positive	38.82	36.60	37.43
#8	oral fluid	positive	31.42	31.75	33.03
#9	oral fluid	positive	31.00	31.94	32.13

#10	oral fluid	**positive**	30.96	31.69	31.02
#11	oral fluid	**positive**	33.04	33.12	32.75
#12	oral fluid	**positive**	31.01	31.21	30.80
#13	oral fluid	**positive**	34.60	35.67	35.03
#14	oral fluid	**positive**	32.31	33.60	36.88
#15	oral fluid	**positive**	32.28	31.60	31.35
#16	oral fluid	**positive**	38.23	37.83	36.68
#17	oral fluid	**positive**	32.94	33.13	32.59
#18	oral fluid	**positive**	33.66	34.22	33.52
#19	oral fluid	**positive**	34.77	32.46	32.63
#20	oral fluid	**positive**	30.27	29.62	29.60
#21	oral fluid	**positive**	34.97	33.48	34.15
#22	oral fluid	**positive**	38.09	38.33	40.00
#23	oral fluid	**positive**	32.44	32.75	32.86
#24	oral fluid	**positive**	34.04	34.14	36.65
#25	oral fluid	**positive**	32.27	32.84	34.70
#26	oral fluid	**positive**	33.56	34.48	34.68
#27	oral fluid	**positive**	37.31	36.76	36.43
#28	oral fluid	**positive**	32.58	31.89	32.26
#29	oral fluid	**positive**	29.33	28.90	29.45
#30	oral fluid	**positive**	29.52	29.14	30.35
#31	oral fluid	**positive**	36.23	36.67	35.92
#32	oral fluid	**positive**	34.22	35.39	34.30
#33	oral fluid	**positive**	32.12	32.10	32.34
#34	oral fluid	**positive**	33.99	33.63	33.27

3.3.15.2 Internal data 2: PRRSV NA oral fluid samples (INDICAL BIOSCIENCE GmbH, Leipzig)

Procedure

RNA was extracted from *n* = 8 oral fluid samples, of which *n* = 7 were positive and *n* = 1 was negative for PRRSV EU strain. Samples were first pre-treated according to “Pretreatment O2” and then RNA extraction was performed using the IndiMag Pathogen Kit on the IndiMag 2, KingFisher Flex, KingFisher Apex and IndiMag 48 devices. The eluates were further analyzed with the virotype PRRSV RT-PCR Kit on the Agilent Technologies Stratagene Mx3005P thermocycler. Data were generated by INDICAL BIOSCIENCE GmbH, Leipzig.

Results / Conclusion

Table 49 summarizes the data for comparative PRRSV EU testing. All tissue samples were detected correctly with comparative quantitative results, regardless of the automatic extraction instrument used.

Table 49. Data summary for testing PRRSV EU-positive oral fluid samples extracted on the IndiMag 2 (IM 2), IndiMag 48 (IM 48), KingFisher Flex (KFF), and KingFisher Apex (KFA) devices.

Sample	**Specimen**	**Status**	**PRRSV EU**			
			IndiMag Pathogen Kit			
			IM 2	**IM 48**	**KFF**	**KFA**
			C_T	**C_T**	**C_T**	**C_T**
#1	oral fluid	**pos.**	31.73	32.35	31.86	31.36
#2	oral fluid	**pos.**	37.20	36.39	36.43	36.82
#3	oral fluid	**pos.**	27.33	33.77	27.15	26.56
#4	oral fluid	**pos.**	35.91	34.33	36.90	35.82
#5	oral fluid	**pos.**	27.11	27.65	27.30	26.53
#6	oral fluid	**pos.**	28.50	29.36	28.47	27.98
#7	oral fluid	**pos.**	30.41	33.02	30.79	30.23
#8	oral fluid	neg.	-	-	-	-

pos. = positive, neg. = negative, - = no C_T

3.3.15.3 Internal data 3: PRRSV NA oral fluid samples (INDICAL BIOSCIENCE GmbH, Leipzig)

Procedure

RNA was extracted from *n* = 40 oral fluid samples, positive for PRRSV North American (NA) strain. After pre-treating the samples according to "Pretreatment O2", extraction was performed either using the IndiMag Pathogen Kit combined with the IndiMag 48s or the KingFisher Flex devices or using the MagMAX CORE Nucleic Acid Purification Kit combined with the KingFisher Flex device. The eluates were further analyzed with the virotype PRRSV RT-PCR Kit, which was performed on the Agilent Technologies Stratagene Mx3005P detection system. Data were generated by INDICAL BIOSCIENCE GmbH, Leipzig.

Results / Conclusion

Table 50 shows an overview of the obtained test results. All the oral fluid samples were detected correctly with comparable quantitative results, regardless of the extraction method used.

Table 50. Data summary for PRRSV NA-positive oral fluid samples extracted on the IndiMag 2 (using the IndiMag Pathogen Kit) or on the KingFisher Flex (using either the IndiMag Pathogen Kit or the MagMAX CORE Nucleic Acid Purification Kit) device.

Sample	Specimen	Status	PRRSV NA strain		
			IndiMag Pathogen Kit IndiMag 48s	IndiMag Pathogen Kit KingFisher Flex	MagMAX CORE Kit KingFisher Flex
			C_T	C_T	C_T
#1	oral fluid	positive	32.46	32.53	32.25
#2	oral fluid	positive	32.87	31.11	31.02
#3	oral fluid	positive	32.01	30.74	29.84
#4	oral fluid	positive	30.38	29.45	30.03
#5	oral fluid	positive	29.64	28.57	28.19
#6	oral fluid	positive	31.74	30.47	31.50
#7	oral fluid	positive	29.10	27.20	28.20
#8	oral fluid	positive	27.98	27.00	27.29
#9	oral fluid	positive	30.55	30.16	28.89
#10	oral fluid	positive	34.78	35.63	35.47
#11	oral fluid	positive	28.36	27.20	37.38

#12	oral fluid	**positive**	30.30	28.90	28.56
#13	oral fluid	**positive**	28.37	28.60	27.91
#14	oral fluid	**positive**	29.59	30.96	30.31
#15	oral fluid	**positive**	30.85	30.72	29.89
#16	oral fluid	**positive**	31.71	30.46	30.55
#17	oral fluid	**positive**	27.64	25.56	25.42
#18	oral fluid	**positive**	29.90	28.74	28.60
#19	oral fluid	**positive**	32.05	30.11	31.29
#20	oral fluid	**positive**	28.85	29.54	27.58
#21	oral fluid	**positive**	30.30	29.34	28.57
#22	oral fluid	**positive**	29.83	28.62	27.51
#23	oral fluid	**positive**	34.31	32.85	32.79
#24	oral fluid	**positive**	30.31	29.07	32.31
#25	oral fluid	**positive**	31.46	28.32	28.46
#26	oral fluid	**positive**	34.96	32.50	32.21
#27	oral fluid	**positive**	28.51	28.58	27.33
#28	oral fluid	**positive**	26.38	26.72	25.89
#29	oral fluid	**positive**	31.36	31.53	30.40
#30	oral fluid	**positive**	27.74	28.54	27.23
#31	oral fluid	**positive**	28.79	29.55	29.99
#32	oral fluid	**positive**	30.34	30.75	30.60
#33	oral fluid	**positive**	30.63	31.34	29.10
#34	oral fluid	**positive**	33.11	32.74	32.20
#35	oral fluid	**positive**	26.75	27.51	27.36
#36	oral fluid	**positive**	31.53	33.90	32.54
#37	oral fluid	**positive**	33.57	35.91	40.00
#38	oral fluid	**positive**	32.47	32.54	31.71
#39	oral fluid	**positive**	31.82	31.48	29.23
#40	oral fluid	**positive**	33.64	33.34	33.24

3.3.16 ***Porcine Reproductive and Respiratory Syndrome Virus*** (PRRSV; serum)

3.3.16.1 Internal data 1: PRRSV EU serum samples (INDICAL BIOSCIENCE GmbH, Leipzig)

Procedure

RNA was extracted from *n* = 28 PRRSV EU strain-positive serum samples. Extraction was performed either using the IndiMag Pathogen Kit combined with the IndiMag 48s or the KingFisher Flex devices or using the MagMAX CORE Nucleic Acid Purification Kit combined with the KingFisher Flex device. The eluates were further analyzed with the virotype PRRSV RT-PCR Kit, which was performed on the Agilent Technologies Stratagene Mx3005P detection system. Data were generated by INDICAL BIOSCIENCE GmbH, Leipzig.

Results / Conclusion

Test results are presented in Table 51. All the serum samples were detected correctly with comparable quantitative results, regardless of the extraction method used.

Table 51. Data summary for PRRSV EU-positive serum samples extracted on the IndiMag 2 (using the IndiMag Pathogen Kit) or on the KingFisher Flex (using either the IndiMag Pathogen Kit or the MagMAX CORE Nucleic Acid Purification Kit) device.

Sample	Specimen	Status	PRRSV EU strain		
			IndiMag Pathogen Kit IndiMag 48s	IndiMag Pathogen Kit KingFisher Flex	MagMAX CORE Kit KingFisher Flex
			C_T	C_T	C_T
#1	serum	positive	35.14	35.95	35.92
#2	serum	positive	30.34	30.88	32.02
#3	serum	positive	35.93	34.42	34.91
#4	serum	positive	33.79	34.42	35.66
#5	serum	positive	33.94	36.13	36.19
#6	serum	positive	36.48	34.86	34.90
#7	serum	positive	38.82	36.60	37.43
#8	serum	positive	31.42	31.75	33.03
#9	serum	positive	31.00	31.94	32.13
#10	serum	positive	30.96	31.69	31.02

#11	serum	**positive**	33.04	33.12	32.75
#12	serum	**positive**	31.01	31.21	30.80
#13	serum	**positive**	34.60	35.67	35.03
#14	serum	**positive**	32.31	33.60	36.88
#15	serum	**positive**	32.28	31.60	31.35
#16	serum	**positive**	38.23	37.83	36.68
#17	serum	**positive**	32.94	33.13	32.59
#18	serum	**positive**	33.66	34.22	33.52
#19	serum	**positive**	34.77	32.46	32.63
#20	serum	**positive**	30.27	29.62	29.60
#21	serum	**positive**	34.97	33.48	34.15
#22	serum	**positive**	38.09	38.33	40.00
#23	serum	**positive**	32.44	32.75	32.86
#24	serum	**positive**	34.04	34.14	36.65
#25	serum	**positive**	32.27	32.84	34.70
#26	serum	**positive**	33.56	34.48	34.68
#27	serum	**positive**	37.31	36.76	36.43
#28	serum	**positive**	32.58	31.89	32.26

3.3.16.2 External field data: PRRSV EU/NA strain serum samples (Private German veterinary laboratory)

Procedure

RNA was extracted from *n* = 15 pig serum samples. Sample #1 was PRRSV EU and NA strain-negative and *n* = 11 samples were PRRSV EU or NA strain.positive (samples #2-#4; #7-#14 were PRRSV EU strain-positive and samples #5, #6 and #15 were PRRSV NA strain-positive). Extraction was performed either using the IndiMag Pathogen Kit combined with the IndiMag 2, the IndiMag 48 or the BioSprint 96 devices. The eluates were further analyzed with the virotype PRRSV RT-PCR Kit (INDICAL BIOSCIENCE GmbH, Leipzig, Germany), which was performed on the Bio-Rad CFX 96 detection system. Data were generated and kindly provided by a private German veterinary laboratory.

Results / Conclusion

Table 52 shows an overview of the obtained test results for PRRSV EU testing and Table 53 presents data for PRRSV NA testing. All samples were detected correctly, regardless of the extraction method used. Overall, extraction using the IndiMag 2 device performed better compared with the BioSprint 96 or IndiMag 48 device.

Table 52. Data summary for PRRSV EU-positive serum samples extracted on the IndiMag 2, the IndiMag 48 or the BioSprint 96 devices (all using the IndiMag Pathogen Kit) device.

Sample	Specimen	PRRSV EU status	PRRSV EU strain		
			IndiMag Pathogen Kit IndiMag 2	IndiMag Pathogen Kit IndiMag 48	IndiMag Pathogen Kit BioSprint 96
			C_T	C_T	C_T
#1	serum	neg.	-	-	-
#2	serum	**positive**	23.27	23.72	24.20
#3	serum	**positive**	22.03	21.76	22.29
#4	serum	**positive**	24.43	25.08	25.22
#5	serum	neg.	-	-	-
#6	serum	neg.	-	-	-
#7	serum	**positive**	24.05	24.02	25.38
#8	serum	**positive**	22.36	22.83	22.68
#9	serum	**positive**	27.83	27.76	28.68
#10	serum	**positive**	21.57	21.63	22.51
#11	serum	**positive**	25.02	24.87	25.71
#12	serum	**positive**	27.52	27.88	28.09
#13	serum	**positive**	27.13	27.41	27.87
#14	serum	**positive**	24.28	25.13	24.61
#15	serum	neg.	-	-	-

neg. = negative, - = no C_T

Table 53. Data summary for PRRSV NA-positive serum samples extracted on the IndiMag 2, the IndiMag 48 or the BioSprint 96 devices (all using the IndiMag Pathogen Kit).

Sample	Specimen	PRRSV NA status	PRRSV NA strain		
			IndiMag Pathogen Kit IndiMag 2	IndiMag Pathogen Kit IndiMag 48	IndiMag Pathogen Kit BioSprint 96
			C_T	C_T	C_T
#1	serum	neg.	-	-	-
#2	serum	neg.	-	-	-
#3	serum	neg.	-	-	-
#4	serum	neg.	-	-	-
#5	serum	positive	27.12	27.25	28.23
#6	serum	positive	26.03	26.41	26.98
#7	serum	neg.	-	-	-
#8	serum	neg.	-	-	-
#9	serum	neg.	-	-	-
#10	serum	neg.	-	-	-
#11	serum	neg.	-	-	-
#12	serum	neg.	-	-	-
#13	serum	neg.	-	-	-
#14	serum	neg.	-	-	-
#15	serum	positive	20.33	21.07	20.58

- = no C_T, neg. = negative

3.3.17 ***Porcine Reproductive and Respiratory Syndrome Virus*** (PRRSV; tissue)

3.3.17.1 Internal data 1: PRRSV EU tissue samples (INDICAL BIOSCIENCE GmbH, Leipzig)

Procedure

RNA was extracted from *n* = 16 PRRSV EU strain-positive tissue samples using the IndiMag Pathogen Kit and “Pretreatment T1”. The extraction step was performed with the IndiMag 48 device. Subsequently, eluates were further analyzed using the virotype PRRSV RT-PCR Kit, performed on the Agilent Technologies Stratagene Mx3005P thermocycler. Data were generated by INDICAL BIOSCIENCE GmbH, Leipzig, Germany.

Results / Conclusion

Table 54 summarizes the data. All the tissue samples when using the IndiMag Pathogen Kit on the IndiMag 48 device.

Table 54. Data summary for testing PRRSV EU strain in tissue samples extracted on the IndiMag 48 device.

Sample	Specimen	Status	*Brachyspira hyodysenteriae* IndiMag Pathogen Kit IndiMag 48 C_T
#1	colon	positive	27.45
#2	lung	positive	28.76
#3	lung	positive	20.75
#4	lung	positive	27.27
#5	lung	positive	24.35
#6	lung	positive	23.42
#7	lung	positive	25.90
#8	lung	positive	26.32
#9	lung	positive	34.17
#10	lung	positive	34.83
#11	lung	positive	32.11
#12	lung	positive	25.74
#13	lung	positive	29.65
#14	lung	positive	26.20
#15	lung	positive	31.16

3.3.17.2 Internal data 2: PRRSV EU/NA tissue samples (INDICAL BIOSCIENCE GmbH, Leipzig)

Procedure

RNA was extracted from *n* = 7 PRRSV-positive tissue samples. Samples #1, #3, #5 and #7 were PRRSV EU strain positive and PRRSV NA strain-negative, whereas samples #2 and #6 were PRRSV NA strain positive and PRRSV EU strain-negative. Sample #4 was double-positive for PRRSV EU and NA strains. The samples were extracted using the IndiMag Pathogen Kit, the IndiSpin QIAcube HT Pathogen Kit, the IndiSpin Pathogen Kit and "Pretreatment T1". The extraction was performed with the IndiMag 48, IndiMag 48s, KingFisher Flex, QIAcube HT device or manually with IndiSpin columns. Subsequently, the eluates were further analyzed using the virotype PRRSV RT-PCR Kit. The RT-PCR was performed on the Agilent Technologies Stratagene Mx3005P thermocycler. Data were generated by INDICAL BIOSCIENCE GmbH, Leipzig, Germany.

Results / Conclusion

Table 55 (PRRSV EU) and Table 56 (PRRSV NA) summarize the data. Almost all lung tissue samples were detected correctly when using the IndiMag Pathogen Kit or the IndiSpin Kits. Differences were only observed when dealing with weak positive samples. All samples were correctly detected when using the IndiMag Pathogen Kit on the IndiMag 48 and IndiMag 48s devices.

Table 55. Data summary for PRRSV EU-testing in lung tissue samples extracted on the IndiMag 48 (IM 48), IndiMag 48s (IM 48s), KingFisher Flex (KFF), and QIAcube HT devices, and by manual extraction.

Sample	Specimen	PRRSV EU status	PRRSV EU				
			IndiMag Pathogen Kit			IndiSpin QIAcube HT Path. Kit	IndiSpin Pathogen Kit
			IM 48 C_T	IM 48s C_T	KFF C_T	QIAcube HT C_T	manual C_T
#1	lung	pos.	30.66	30.76	31.61	28.56	30.74
#2	lung	neg.	-	-	-	-	-
#3	lung	pos.	36.67	36.51	34.20	33.98	36.59
#4	lung	pos.	30.64	29.48	28.73	27.64	29.75
#5	lung	pos.	34.50	33.72	32.21	31.62	33.70
#6	lung	neg.	-	-	-	-	-
#7	lung	pos.	34.32	35.47	35.61	33.01	-

Path. = Pathogen, pos. = positive, neg = negative, - = no C_T

Table 56. Data summary for PRRSV NA-testing in lung tissue samples extracted on the IndiMag 48 (IM 48), IndiMag 48s (IM 48s), KingFisher Flex (KFF), and QIAcube HT devices, and by manual extraction.

Sample	Specimen	Status	PRRSV NA				
			IndiMag Pathogen Kit			IndiSpin QIAcube HT Path. Kit	IndiSpin Pathogen Kit
			IM 48 C_T	IM 48s C_T	KFF C_T	QIAcube HT C_T	manual C_T
#1	lung	neg.	-	-	-	-	-
#2	lung	pos.	26.91	27.10	26.22	25.25	27.02
#3	lung	neg.	-	-	-	-	-
#4	lung	pos.	27.83	27.02	26.22	25.10	27.20
#5	lung	neg.	-	-	-	-	-
#6	lung	pos.	24.45	23.22	22.27	21.28	23.44
#7	lung	neg.	-	-	-	-	-

Path. = Pathogen, pos. = positive, neg = negative, - = no C_T

3.3.17.3 Internal data 3: PRRSV EU/NA tissue samples (INDICAL BIOSCIENCE GmbH, Leipzig)

Procedure

RNA was extracted from *n* = 8 tissue samples. Samples #4, #5, #6 and #7 were PRRSV EU strain-positive and PRRSV NA strain-negative, whereas samples #2, #3 and #8 were PRRSV NA-strain positive and PRRSV EU strain-negative. Sample #1 was double-positive for PRRSV EU and NA-strains. Samples were first pre-treated according to "Pretreatment T1" and then RNA extraction was performed using the IndiMag Pathogen Kit on the IndiMag 2, KingFisher Flex, KingFisher Apex and IndiMag 48 devices. The eluates were further analyzed with the virotype PRRSV RT-PCR Kit on the Agilent Technologies Stratagene Mx3005P thermocycler. Data were generated by INDICAL BIOSCIENCE GmbH, Leipzig, Germany.

Results / Conclusion

Table 57 (PRRSV EU) and Table 58 (PRRSV NA) summarize the data for comparative PRRSV EU and NA testing of tissue samples. All tissue samples were detected correctly with comparable quantitative results when using the IndiMag Pathogen Kit on all automatic extraction devices.

Table 57. Data summary for testing PRRSV EU-positive tissue samples extracted on the IndiMag 2 (IM 2), IndiMag 48 (IM 48), KingFisher Flex (KFF), and KingFisher Apex (KFA) devices.

Sample	**Specimen**	**Status**	**PRRSV EU**			
			IndiMag Pathogen Kit			
			IM 2	**IM 48**	**KFF**	**KFA**
			C_T	**C_T**	**C_T**	**C_T**
#1	tissue	**pos.**	22.63	22.88	23.93	22.87
#2	tissue	neg.	-	-	-	-
#3	tissue	neg.	-	-	-	-
#4	tissue	**pos.**	25.81	26.03	26.20	25.76
#5	tissue	**pos.**	33.64	35.37	33.92	33.38
#6	tissue	**pos.**	35.17	37.09	35.04	34.18
#7	tissue	**pos.**	29.78	29.82	29.51	29.66
#8	tissue	neg.	-	-	-	-

pos. = positive, neg. = negative, - = no C_T

Table 58. Data summary for testing PRRSV NA-positive tissue samples extracted on the IndiMag 2 (IM 2), IndiMag 48 (IM 48), KingFisher Flex (KFF), and KingFisher Apex (KFA) devices.

Sample	Specimen	Status	PRRSV NA			
			IndiMag Pathogen Kit			
			IM 2	IM 48	KFF	KFA
			C_T	C_T	C_T	C_T
#1	tissue	pos.	22.26	22.05	23.26	22.72
#2	tissue	neg.	22.07	21.49	22.15	21.94
#3	tissue	neg.	20.19	20.10	20.15	19.98
#4	tissue	pos.	-	-	-	-
#5	tissue	pos.	-	-	-	-
#6	tissue	pos.	-	-	-	-
#7	tissue	pos.	-	-	-	-
#8	tissue	neg.	32.00	32.43	32.82	31.29

pos. = positive, neg. = negative, - = no C_T

3.3.17.4 External field data: PRRSV EU tissue samples (German state veterinary laboratory)

Procedure

RNA was extracted from *n* = 5 PRRSV EU-positive and *n* = 3 PRRSV-EU-negative tissue samples. After pre-treating the samples according to “Pretreatment T1”, RNA was extracted using the IndiMag Pathogen Kit either on the IndiMag 2 or on the IndiMag 48s device. The eluates were further analyzed with the virotype PRRSV RT-PCR Kit. RT-PCR was performed on the Bio-Rad CFX 96 detection system. Data were generated and kindly provided by a German state veterinary laboratory.

Results / Conclusion

Table 59 presents the results the comparative study. All samples were detected correctly when using the IndiMag Pathogen Kit on all automatic extraction devices. Overall, RNA extracted with the IndiMag 2 performed better than with the IndiMag 48s device.

Table 59. Data summary for PRRSV EU-testing of tissue samples extracted on the IndiMag 2 and IndiMag 48s devices.

Sample	Specimen	Status	PRRSV EU	
			IndiMag Pathogen Kit IndiMag 2	IndiMag Pathogen Kit IndiMag 48s
			C_T	C_T
#1	tissue	neg.	-	-
#2	tissue	neg.	-	-
#3	tissue	**positive**	20.90	22.50
#4	tissue	**positive**	26.50	28.00
#5	tissue	**positive**	25.60	26.50
#6	tissue	**positive**	26.10	28.00
#7	tissue	**positive**	26.40	27.90
#8	tissue	neg.	-	-

neg. = negative, - = no C_T

3.3.18 ***Schmallenberg Virus*** (SBV; semen; internal data – INDICAL BIOSCIENCE GmbH, Leipzig)

Procedure

RNA was extracted from *n* = 14 SBV-positive and *n* = 3 SBV-negative semen samples using the IndiMag Pathogen Kit and "Pretreatment SE-SBV" (for samples #1-#11 and #15-#17) or pretreatment using TRIzol™ Reagent (ThermoFisher Scientific, Waltham, Massachusetts, USA; sample #12), QIAzol™ Lysis Reagent (QIAGEN GmbH, Hilden, Germany; sample #13) or peqGOLD RNA Pure™ FL (VWR International GmbH, Darmstadt, Germany; sample #14). The extraction step was performed with the KingFisher Flex device. Subsequently, eluates were further analyzed using the virotype SBV RT-PCR Kit (INDICAL BIOSCIENCE GmbH, Leipzig, Germany), performed on the Agilent Technologies Stratagene Mx3005P thermocycler. Data were generated by INDICAL BIOSCIENCE GmbH, Leipzig, Germany.

Results / Conclusion

Test results are presented in Table 60. All the semen samples were detected correctly when using the IndiMag Pathogen Kit on the KingFisher Flex device.

Table 60. Data summary for *Schmallenberg Virus*- testing of semen samples extracted on the KingFisher Flex device.

Sample	Specimen	Status	*Schmallenberg Virus* IndiMag Pathogen Kit KingFisher Flex C_T
#1	semen	**positive**	30.31
#2	semen	**positive**	32.95
#3	semen	**positive**	35.20
#4	semen	**positive**	31.43
#5	semen	**positive**	34.91
#6	semen	**positive**	33.82
#7	semen	**positive**	31.21
#8	semen	**positive**	34.59
#9	semen	**positive**	32.07
#10	semen	**positive**	30.86
#11	semen	**positive**	28.57
#12	semen*	**positive**	37.22
#13	semen**	**positive**	37.03
#14	semen***	**positive**	35.81
#15	semen	neg.	-
#16	semen	neg.	-
#17	semen	neg.	-

pos. = positive, neg. = negative, - = no C_T
*TRIzol™ Reagent, **QIAzol Lysis Reagent, *** peqGOLD RNA Pure™ FL

3.3.19 ***Schmallenberg Virus*** (SBV; blood, serum; internal data – INDICAL BIOSCIENCE GmbH, Leipzig)

Procedure

RNA was extracted from *n* = 5 SBV-positive blood and *n* = 5 SBV-positive serum samples with the IndiMag Pathogen Kit using the KingFisher Flex device. Subsequently, eluates were further analyzed with the virotype SBV RT-PCR Kit, performed on the Agilent Technologies Stratagene Mx3005P thermocycler. Data were generated by INDICAL BIOSCIENCE GmbH, Leipzig.

Results / Conclusion

Table 61 shows the test results for testing of SBV-positive serum and blood samples. All samples were correctly detected when using the IndiMag Pathogen Kit on the KingFisher Flex device.

Table 61. Data summary for testing *Schmallenberg Virus*-positive blood and serum samples extracted on the KingFisher Flex device.

Sample	Specimen	Status	***Schmallenberg Virus*** **IndiMag Pathogen Kit KingFisher Flex** C_T
#1	blood	**positive**	28.01
#2	blood	**positive**	25.64
#3	blood	**positive**	26.91
#4	blood	**positive**	32.24
#5	blood	**positive**	35.18
#6	serum	**positive**	29.59
#7	serum	**positive**	30.73
#8	serum	**positive**	33.21
#9	serum	**positive**	34.06
#10	serum	**positive**	33.07

3.3.20 ***Schmallenberg Virus*** (SBV; tissue; internal data – INDICAL BIOSCIENCE GmbH, Leipzig)

Procedure

RNA was extracted from *n* = 5 SBV-positive brain and *n* = 5 SBV-positive spleen tissue samples with the IndiMag Pathogen Kit with the KingFisher Flex device. Subsequently, eluates were further analyzed using the virotype SBV RT-PCR Kit, performed on the Agilent Technologies Stratagene Mx3005P thermocycler. Data were generated by INDICAL BIOSCIENCE GmbH, Leipzig, Germany.

Results / Conclusion

Test results are shown in Table 62. All samples were detected correctly when using the IndiMag Pathogen Kit on the KingFisher Flex device.

Table 62. Data summary for testing SBV-positive tissue samples extracted on the KingFisher Flex device.

Sample	**Specimen**	**Status**	***Schmallenberg Virus*** **IndiMag Pathogen Kit KingFisher Flex** C_T
#1	brain	**positive**	22.20
#2	brain	**positive**	30.56
#3	brain	**positive**	29.70
#4	brain	**positive**	30.90
#5	brain	**positive**	31.92
#6	spleen	**positive**	31.29
#7	spleen	**positive**	37.29
#8	spleen	**positive**	37.54
#9	spleen	**positive**	30.12
#10	spleen	**positive**	36.13

4 IndiMag Pathogen Cartridge Kits

4.1 Bacterial DNA extraction

4.1.1 ***Mycobacterium avium* subspecies *paratuberculosis*** (MAP/ Johnes' Disease; feces; internal data – INDICAL BIOSCIENCE GmbH, Leipzig)

Procedure

DNA was extracted from *n* = 14 fecal samples of which *n* = 11 were MAP-positive and *n* = 3 MAP-negative. Extraction was performed after pre-treating the samples according to "Pretreatment F-MAP". Subsequently, samples were extracted using either the IndiMag Pathogen IM48 Cartridge, the IndiMag Pathogen KF96 Cartridge, the IndiMag Pathogen Kit, or the IndiSpin Pathogen Kit. The extraction was performed with the IndiMag 48s, KingFisher Flex devices, or manually with IndiSpin columns. Subsequently, the eluates were further analyzed using the bactotype MAP PCR Kit. The PCR was performed on the Agilent Technologies Stratagene Mx3005P thermocycler. Data were generated by INDICAL BIOSCIENCE GmbH, Leipzig, Germany.

Results / Conclusion

Results are presented in Table 63. All fecal samples were detected correctly for *Mycobacterium avium* subspecies *paratuberculosis* after extraction using the IndiMag Pathogen KF96 Cartridge, the IndiMag Pathogen IM48 Cartridge, the IndiMag Pathogen Kit and the IndiSpin Pathogen Kit. The IndiMag Pathogen Cartridge Kits performed better or equal to the IndiMag Pathogen Kit or the IndiSpin Pathogen Kit.

Table 63. Data summary for *M. avium* ssp. *paratuberculosis*-testing in feces samples extracted on the IndiMag 48s (IM 48s) and KingFisher Flex (KFF) devices, and by manual extraction.

Sample	Specimen	Status	*Mycobacterium avium* ssp. *paratuberculosis* (MAP)			
			IM Pathogen IM48 Cartridge	IM Path. KF96 Cartr.	IM Path. Kit	IndiSpin Path. Kit
			IM 48s	KFF	KFF	manual
			C_T	C_T	C_T	C_T
#1	feces	**pos.**	27.46	27.63	28.32	28.13
#2	feces	**pos.**	26.58	26.65	26.69	27.06
#3	feces	**pos.**	22.36	22.35	21.73	22.64
#4	feces	neg.	-	-	-	-
#5	feces	**pos.**	21.67	21.56	22.16	21.88
#6	feces	**pos.**	30.66	31.36	32.13	30.89
#7	feces	**pos.**	31.65	33.58	33.26	32.12
#8	feces	**pos.**	26.94	27.54	27.19	27.16
#9	feces	**pos.**	21.76	22.73	22.37	22.31
#10	feces	neg.	-	-	-	-
#11	feces	**pos.**	22.66	23.74	23.26	22.65
#12	feces	**pos.**	27.19	28.97	27.45	27.00
#13	feces	neg.	-	-	-	-
#14	feces	**pos.**	18.28	18.34	18.25	18.51

IM = IndiMag, Path. = Pathogen, Cartr. = Cartridge, pos. = positive, neg. = negative, - = no C_T

4.1.2 ***Mycobacterium avium* subspecies *paratuberculosis*** (MAP/ Johnes' Disease; tissue; internal data – INDICAL BIOSCIENCE GmbH, Leipzig)

Procedure

DNA was extracted from *n* = 7 tissue samples of which *n* = 6 were MAP-positive and *n* = 1 MAP-negative. Extraction was performed after pre-treating the samples according to "Pretreatment T1". Subsequently, samples were extracted using either the IndiMag Pathogen IM48 Cartridge, the IndiMag Pathogen KF96 Cartridge, the IndiMag Pathogen Kit, or the IndiSpin Pathogen Kit. The extraction was performed with the IndiMag 48s, KingFisher Flex devices, or manually with IndiSpin columns. Subsequently, the eluates were further analyzed using the bactotype MAP PCR Kit. The PCR was performed on the Agilent Technologies Stratagene Mx3005P thermocycler. Data were generated by INDICAL BIOSCIENCE GmbH, Leipzig, Germany.

Results / Conclusion

Table 64 presents the comparative data. All tissue samples were detected correctly for *Mycobacterium avium* subspecies *paratuberculosis* after extraction using the IndiMag Pathogen KF96 Cartridge, the IndiMag Pathogen IM48 Cartridge, the IndiMag Pathogen Kit and the IndiSpin Pathogen Kit. The IndiMag Pathogen Cartridge Kits performed better or equal to the IndiMag Pathogen Kit or the IndiSpin Pathogen Kit.

Table 64. Data summary for *M. avium* ssp. *paratuberculosis*-testing in tissue samples extracted on the IndiMag 48s (IM 48s) and KingFisher Flex (KFF) devices, and by manual extraction.

Sample	**Specimen**	**Status**	***Mycobacterium avium* ssp. *paratuberculosis* (MAP)**			
			IM Pathogen IM48 Cartridge	**IM Path. KF96 Cartr.**	**IM Path. Kit**	**IndiSpin Path. Kit**
			IM 48s	**KFF**	**KFF**	**manual**
			C_T	**C_T**	**C_T**	**C_T**
#1	lymph node	**pos.**	22.44	22.84	23.31	23.26
#2	lymph node	**pos.**	16.47	16.66	16.86	16.63
#3	lymph node	**pos.**	22.16	21.85	22.33	22.43
#4	ileum	**pos.**	17.49	17.66	17.83	17.54
#5	ileum	**pos.**	24.88	24.85	24.84	25.02
#6	ileum	**pos.**	18.68	18.43	19.10	19.92
#7	liver	neg.	-	-	-	-

IM = IndiMag, Path. = Pathogen, Cartr. = Cartridge, pos. = positive, neg. = negative, - = no C_T

4.2 Viral DNA extraction

4.2.1 ***Porcine Circovirus-3*** (PCV-3; oral fluid; internal data – INDICAL BIOSCIENCE GmbH, Leipzig)

Procedure

DNA was extracted from *n* = 16 PCV-3-positive oral fluid samples. Extraction was performed after pre-treating the samples according to “Pretreatment O2”. Subsequently, samples were extracted using either the IndiMag Pathogen IM48 Cartridge, the IndiMag Pathogen KF96 Cartridge, the IndiMag Pathogen Kit, or the MagMAX CORE Nucleic Acid Purification Kit. The extraction was performed on the IndiMag 48s or the KingFisher Flex devices, respectively. Subsequently, the eluates were further analyzed using the virotype PCV2/PCV3 Primers/Probes and IndiMix JOE. The PCR was performed on the Agilent Technologies Stratagene Mx3005P thermocycler. Data were generated by INDICAL BIOSCIENCE GmbH, Leipzig, Germany.

Results / Conclusion

Table 65 presents the comparative data. All oral fluid samples were detected correctly, regardless of the extraction method used.

Table 65. Data summary for testing PCV-3-positive oral fluid samples extracted on the IndiMag 48s (IM 48s) or KingFisher Flex (KFF) devices.

Sample	Specimen	Status	*Porcine Circovirus*-3 (PCV-3)			
			IM Path. IM48 Cartr.	IM Path. KF96 Cartr.	IndiMag Path. Kit	MagMAX CORE Kit
			IM 48s	KFF	IM 48s	KFF
			C_T	C_T	C_T	C_T
#1	oral fluid	pos.	31.31	32.30	32.62	32.99
#2	oral fluid	pos.	27.11	27.93	28.68	28.54
#3	oral fluid	pos.	35.68	35.75	35.79	36.40
#4	oral fluid	pos.	37.89	37.91	36.96	35.73
#5	oral fluid	pos.	35.33	34.99	36.90	36.22
#6	oral fluid	pos.	32.33	33.24	33.59	33.15
#7	oral fluid	pos.	34.14	33.90	34.70	34.17
#8	oral fluid	pos.	33.72	33.77	34.07	33.22
#9	oral fluid	pos.	32.40	32.81	33.23	32.98
#10	oral fluid	pos.	29.10	29.66	30.06	29.04
#11	oral fluid	pos.	37.20	37.51	38.41	38.06
#12	oral fluid	pos.	30.29	30.20	31.11	31.12
#13	oral fluid	pos.	25.75	25.80	25.91	25.03
#14	oral fluid	pos.	28.45	28.98	29.47	29.60
#15	oral fluid	pos.	35.02	35.51	36.55	36.09
#16	oral fluid	pos.	34.35	34.51	34.88	35.25

IM = IndiMag, Path. = Pathogen, Cartr. = Cartridge, pos. = positive

4.2.2 ***Porcine Circovirus-2*** *(PCV-2*; tissue; internal data – INDICAL BIOSCIENCE GmbH, Leipzig)

Procedure

DNA was extracted from *n* = 8 PCV-2-positive tissue samples. Extraction was performed after pre-treating the samples according to "Pretreatment T1". Subsequently, samples were extracted using either the IndiMag Pathogen IM48 Cartridge, the IndiMag Pathogen KF96 Cartridge, the IndiMag Pathogen Kit, or the IndiSpin Pathogen Kit. The extraction was performed on the IndiMag 48, IndiMag 48s, KingFisher Flex devices, or manually with IndiSpin columns. Subsequently, the eluates were further analyzed using the virotype PCV2/PCV3 Primers/Probes and IndiMix JOE. The PCR was performed on the Agilent Technologies Stratagene Mx3005P thermocycler. Data were generated by INDICAL BIOSCIENCE GmbH, Leipzig, Germany.

Results / Conclusion

Table 66 presents the comparative data. All tissue samples were detected correctly, regardless of the extraction method used. Overall, the IndiMag Pathogen Cartridge kits and the IndiMag Pathogen Kit performed better than the IndiSpin Pathogen Kit.

Table 66. Data summary for testing PCV-2-positive tissue samples extracted on the IndiMag 48 (IM 48), IndiMag 48s (IM 48s) and KingFisher Flex (KFF) devices, and by manual extraction.

Sam-ple	**Specimen**	**Status**	***Porcine Circovirus*-2 (PCV-2)**				
			IM Pathogen IM48 Cartridge		**IM Path. KF96 Cartr.**	**IM Path. Kit**	**IndiSpin Path. Kit**
			IM 48	**IM 48s**	**KFF**	**KFF**	**manual**
			C_T	**C_T**	**C_T**	**C_T**	**C_T**
#1	tissue	**pos.**	15.32	15.17	14.84	15.40	16.52
#2	tissue	**pos.**	20.04	19.11	18.86	19.28	19.83
#3	tissue	**pos.**	25.13	24.62	24.41	24.67	25.35
#4	tissue	**pos.**	16.93	16.37	16.14	16.54	18.44
#5	tissue	**pos.**	20.11	19.01	20.40	19.21	20.69
#6	tissue	**pos.**	9.69	9.37	8.96	8.76	9.38
#7	tissue	**pos.**	15.07	15.87	15.11	14.92	15.34
#8	tissue	**pos.**	10.03	8.96	9.19	9.84	8.72

IM = IndiMag, Path. = Pathogen, Cartr. = Cartridge, pos. = positive

4.3 Viral RNA extraction

4.3.1 ***Bluetongue Virus*** (BTV; blood; internal data – INDICAL BIOSCIENCE GmbH, Leipzig)

Procedure

RNA was extracted from *n* = 8 BTV-positive blood samples using either the IndiMag Pathogen IM48 Cartridge, the IndiMag Pathogen KF96 Cartridge, the IndiMag Pathogen Kit, or the IndiSpin Pathogen Kit. The extraction was performed on the IndiMag 48, IndiMag 48s, KingFisher Flex devices, or manually with IndiSpin columns. Subsequently, the eluates were further analyzed using the virotype BTV pan/8 2.0 RT-PCR Kit. The RT-PCR was performed on the Agilent Technologies Stratagene Mx3005P thermocycler. Data were generated by INDICAL BIOSCIENCE GmbH, Leipzig, Germany.

Results / Conclusion

Results are presented in Table 67. All blood samples were detected correctly, regardless of the extraction method used. The IndiMag Pathogen Cartridge kits and the IndiMag Pathogen Kit performed better than the IndiSpin Pathogen Kit.

Table 67. Data summary for testing BTV-positive blood samples extracted on the IndiMag 48 (IM 48), IndiMag 48s (IM 48s) and KingFisher Flex (KFF) devices, and by manual extraction.

Sample	**Specimen**	**Status**	**Bluetongue Virus (BTV)**				
			IM Pathogen IM48 Cartridge		**IM Path. KF96 Cartr.**	**IM Path. Kit**	**IndiSpin Path. Kit**
			IM 48	**IM 48s**	**KFF**	**KFF**	**manual**
			C_T	**C_T**	**C_T**	**C_T**	**C_T**
#1	blood	**pos.**	26.17	26.25	25.41	26.16	27.70
#2	blood	**pos.**	23.34	22.35	23.06	23.52	25.56
#3	blood	**pos.**	24.47	24.32	24.66	24.92	26.69
#4	blood	**pos.**	23.85	23.35	23.49	24.32	25.24
#5	blood	**pos.**	23.94	23.46	23.70	24.54	25.60
#6	blood	**pos.**	29.66	29.37	29.83	30.11	31.91
#7	blood	**pos.**	23.26	23.64	22.95	23.88	23.95
#8	blood	**pos.**	24.59	25.65	25.24	25.91	25.70

IM = IndiMag, Path. = Pathogen, Cartr. = Cartridge, pos. = positive

4.3.2 ***Bovine Viral Diarrhea Virus*** *(BVDV*; serum; internal data – INDICAL BIOSCIENCE GmbH, Leipzig)

Procedure

RNA was extracted from *n* = 6 BVDV-positive and *n* = 2 BVDV-negative serum samples using the IndiMag Pathogen Kit or the IndiMag Pathogen KF96 Cartridge. The extraction was performed on the KingFisher Flex device. The eluates were further analyzed using the virotype BVDV 2.0 RT-PCR Kit, which was performed on the Agilent Technologies Stratagene Mx3005P thermocycler. Data were generated by INDICAL BIOSCIENCE GmbH, Leipzig, Germany.

Results / Conclusion

Table 68 depicts the results for BVDV-testing of serum samples. All tissue samples were detected correctly, regardless of the extraction method used. The IndiMag Pathogen KF96 Cartridge Kit performed equally to the IndiMag Pathogen Kit.

Table 68. Data summary for BVDV-testing serum samples extracted on the KingFisher Flex device using the IndiMag Pathogen Kit and the IndiMag Pathogen KF96 Cartridge.

Sample	**Specimen**	**Status**	***Bovine Viral Diarrhea Virus* (BVDV)**	
			IndiMag Pathogen Kit **KingFisher Flex**	**IndiMag Pathogen KF96 Cartridge** **KingFisher Flex**
			C_T	**C_T**
#1	serum	**positive**	21.17	21.35
#2	serum	**positive**	24.94	24.70
#3	serum	**positive**	28.08	28.35
#4	serum	**positive**	31.37	31.25
#5	serum	**positive**	35.27	35.13
#6	serum	**positive**	38.52	39.34
#7	serum	neg.	-	-
#8	serum	neg.	-	-

neg. = negative, - = no C_T

4.3.3 **Influenza A Virus** (swabs; internal data – INDICAL BIOSCIENCE GmbH, Leipzig)

Procedure

RNA was extracted from *n* = 8 Influenza A Virus-spiked swab samples using either the IndiMag Pathogen IM48 Cartridge, the IndiMag Pathogen KF96 Cartridge, the IndiMag Pathogen Kit, or the IndiSpin Pathogen Kit. The extraction was performed on the IndiMag 48, IndiMag 48s, KingFisher Flex devices, or manually with IndiSpin columns. Subsequently, the eluates were further analyzed using the virotype Influenza A 2.0 RT-PCR Kit. The RT-PCR was performed on the Agilent Technologies Stratagene Mx3005P thermocycler. Data were generated by INDICAL BIOSCIENCE GmbH, Leipzig, Germany.

Results / Conclusion

Results are presented in Table 69. All swab samples were detected correctly, regardless of the extraction method used. The IndiMag Pathogen Cartridge kits and the IndiMag Pathogen Kit performed better than the IndiSpin Pathogen.

Table 69. Data summary for testing Influenza Virus-spiked swab samples extracted on the IndiMag 48 (IM 48), IndiMag 48s (IM 48s) and KingFisher Flex (KFF) devices, and by manual extraction.

Sample	**Specimen**	**Status**	**Influenza A Virus**				
			IM Pathogen IM48 Cartridge		**IM Path. KF96 Cartr.**	**IM Path. Kit**	**IndiSpin Path. Kit**
			IM 48	**IM 48s**	**KFF**	**KFF**	**manual**
			C_T	**C_T**	**C_T**	**C_T**	**C_T**
#1	swab (H1)	**pos.**	25.96	22.97	23.05	23.16	25.10
#2	swab (H3N3)	**pos.**	28.92	26.64	27.23	27.32	27.83
#3	swab (H5)	**pos.**	25.47	23.78	24.10	24.19	25.48
#4	swab (H6N6)	**pos.**	22.94	22.06	22.29	22.20	24.12
#5	swab (H7N1)	**pos.**	25.28	23.75	24.28	23.76	25.86
#6	swab (H9)	**pos.**	25.02	23.93	25.17	24.51	24.80
#7	swab (P1645)	**pos.**	20.90	19.48	20.23	20.27	25.04
#8	swab (H9)	**pos.**	20.15	19.23	19.66	19.46	22.44

IM = IndiMag, Path. = Pathogen, Cartr. = Cartridge, pos. = positive

4.3.4 ***Porcine Reproductive and Respiratory Syndrome Virus*** (PRRSV; oral fluid; internal data – INDICAL BIOSCIENCE GmbH, Leipzig)

Procedure

RNA was extracted from *n* = 34 oral fluid samples, positive for PRRSV European (EU) strain and *n* = 40 oral fluid samples, positive for PRRSV North American (NA) strain. After pre-treating the samples according to "Pretreatment O2", extraction was performed using either the IndiMag Pathogen IM48 Cartridge, the IndiMag Pathogen KF96 Cartridge, the IndiMag Pathogen Kit, or the MagMAX CORE Nucleic Acid Purification Kit. The extraction was performed on the IndiMag 48s or the KingFisher Flex devices, respectively. Subsequently, the eluates were further analyzed using the virotype PRRSV RT-PCR Kit. RT-PCR was performed on the Agilent Technologies Stratagene Mx3005P thermocycler. Data were generated by INDICAL BIOSCIENCE GmbH, Leipzig, Germany.

Results / Conclusion

Table 70 summarizes the results for PRRSV EU testing and Table 71 presents the data for PRRSV NA testing. All the oral fluid samples were detected correctly, regardless of the extraction method used. Overall, RNA extraction using the IndiMag Pathogen Kit and the IndiMag Pathogen Cartridge kits performed equal to the MagMAX Core Nucleic Acid Purification Kit.

Table 70. Data summary for PRRSV EU-positive oral fluid samples extracted on the IndiMag 48s (using the IndiMag Pathogen IM48 Cartridge) or on the KingFisher Flex (using either the IndiMag Pathogen Kit, the IndiMag Pathogen KF96 Cartridge or the MagMAX CORE Nucleic Acid Purification Kit) device.

Sample	**Specimen**	**Status**	**PRRSV (EU strain)**			
			IM Path. IM48 Cartr.	**IM Path. KF96 Cartr.**	**IndiMag Path. Kit**	**MagMAX CORE Kit**
			IM 48s	**KFF**	**KFF**	**KFF**
			C_T	**C_T**	**C_T**	**C_T**
#1	oral fluid	**pos.**	34.30	34.46	35.95	35.92
#2	oral fluid	**pos.**	30.95	32.38	30.88	32.02
#3	oral fluid	**pos.**	36.77	36.34	34.42	34.91
#4	oral fluid	**pos.**	35.75	34.76	34.42	35.66
#5	oral fluid	**pos.**	34.13	35.33	36.13	36.19
#6	oral fluid	**pos.**	35.55	36.15	34.86	34.90
#7	oral fluid	**pos.**	35.56	39.48	36.60	37.43
#8	oral fluid	**pos.**	31.44	32.18	31.75	33.03

#9	oral fluid	**pos.**	31.69	31.98	31.94	32.13
#10	oral fluid	**pos.**	31.43	32.25	31.69	31.02
#11	oral fluid	**pos.**	31.97	32.60	33.12	32.75
#12	oral fluid	**pos.**	31.07	31.33	31.21	30.80
#13	oral fluid	**pos.**	35.69	36.26	35.67	35.03
#14	oral fluid	**pos.**	34.83	36.54	33.60	36.88
#15	oral fluid	**pos.**	30.58	32.66	31.60	31.35
#16	oral fluid	**pos.**	37.90	35.88	37.83	36.68
#17	oral fluid	**pos.**	32.77	33.94	33.13	32.59
#18	oral fluid	**pos.**	32.61	33.38	34.22	33.52
#19	oral fluid	**pos.**	33.80	32.53	32.46	32.63
#20	oral fluid	**pos.**	30.06	30.52	29.62	29.60
#21	oral fluid	**pos.**	33.97	34.15	33.48	34.15
#22	oral fluid	**pos.**	40.00	38.23	38.33	40.00
#23	oral fluid	**pos.**	33.54	32.58	32.75	32.86
#24	oral fluid	**pos.**	34.34	35.18	34.14	36.65
#25	oral fluid	**pos.**	33.82	34.78	32.84	34.70
#26	oral fluid	**pos.**	34.11	34.56	34.48	34.68
#27	oral fluid	**pos.**	34.67	36.60	36.76	36.43
#28	oral fluid	**pos.**	32.14	32.86	31.89	32.26
#29	oral fluid	**pos.**	29.12	30.03	28.90	29.45
#30	oral fluid	**pos.**	29.68	31.63	29.14	30.35
#31	oral fluid	**pos.**	36.57	37.79	36.67	35.92
#32	oral fluid	**pos.**	35.16	36.54	35.39	34.30
#33	oral fluid	**pos.**	32.95	33.63	32.10	32.34
#34	oral fluid	**pos.**	33.64	33.64	33.63	33.27

IM = IndiMag, Path. = Pathogen, Cartr. = Cartridge, pos. = positive

Table 71. Data summary for PRRSV NA-positive oral fluid samples extracted on the IndiMag 48s (using the IndiMag Pathogen IM48 Cartridge) or on the KingFisher Flex (using either the IndiMag Pathogen Kit, the IndiMag Pathogen KF96 Cartridge or the MagMAX CORE Nucleic Acid Purification Kit).

Sample	Specimen	Status	PRRSV (NA strain)			
			IM Path. IM48 Cartr.	IM Path. KF96 Cartr.	IndiMag Path. Kit	MagMAX CORE Kit
			IM 48s	KFF	KFF	KFF
			C_T	C_T	C_T	C_T
#1	oral fluid	pos.	32.77	34.38	32.53	32.25
#2	oral fluid	pos.	30.40	31.58	31.11	31.02
#3	oral fluid	pos.	29.64	29.64	30.74	29.84
#4	oral fluid	pos.	28.51	28.51	29.45	30.03
#5	oral fluid	pos.	27.97	27.97	28.57	28.19
#6	oral fluid	pos.	29.66	29.66	30.47	31.50
#7	oral fluid	pos.	25.60	25.60	27.20	28.20
#8	oral fluid	pos.	27.01	27.01	27.00	27.29
#9	oral fluid	pos.	29.66	29.66	30.16	28.89
#10	oral fluid	pos.	34.59	34.59	35.63	35.47
#11	oral fluid	pos.	27.49	27.49	27.20	37.38
#12	oral fluid	pos.	28.57	28.57	28.90	28.56
#13	oral fluid	pos.	27.58	27.58	28.60	27.91
#14	oral fluid	pos.	30.64	30.64	30.96	30.31
#15	oral fluid	pos.	30.52	30.52	30.72	29.89
#16	oral fluid	pos.	30.12	30.12	30.46	30.55
#17	oral fluid	pos.	25.83	25.83	25.56	25.42
#18	oral fluid	pos.	27.84	27.84	28.74	28.60
#19	oral fluid	pos.	29.50	29.50	30.11	31.29
#20	oral fluid	pos.	28.74	28.74	29.54	27.58
#21	oral fluid	pos.	28.72	28.72	29.34	28.57
#22	oral fluid	pos.	27.49	27.49	28.62	27.51
#23	oral fluid	pos.	32.86	32.86	32.85	32.79
#24	oral fluid	pos.	27.70	27.70	29.07	32.31

#25	oral fluid	**pos.**	28.09	28.09	28.32	28.46
#26	oral fluid	**pos.**	32.22	32.22	32.50	32.21
#27	oral fluid	**pos.**	27.62	27.62	28.58	27.33
#28	oral fluid	**pos.**	24.73	24.73	26.72	25.89
#29	oral fluid	**pos.**	30.54	30.54	31.53	30.40
#30	oral fluid	**pos.**	27.28	27.28	28.54	27.23
#31	oral fluid	**pos.**	27.49	27.49	29.55	29.99
#32	oral fluid	**pos.**	29.64	29.64	30.75	30.60
#33	oral fluid	**pos.**	29.31	29.31	31.34	29.10
#34	oral fluid	**pos.**	32.15	32.15	32.74	32.20
#35	oral fluid	**pos.**	27.54	27.54	27.51	27.36
#36	oral fluid	**pos.**	32.40	32.40	33.90	32.54
#37	oral fluid	**pos.**	33.74	33.74	35.91	40.00
#38	oral fluid	**pos.**	31.05	31.05	32.54	31.71
#39	oral fluid	**pos.**	29.85	29.85	31.48	29.23
#40	oral fluid	**pos.**	32.86	32.86	33.34	33.24

IM = IndiMag, Path. = Pathogen, Cartr. = Cartridge, pos. = positive

4.3.5 ***Porcine Reproductive and Respiratory Syndrome Virus*** (PRRSV; serum; internal data – INDICAL BIOSCIENCE GmbH, Leipzig)

Procedure

RNA was extracted from *n* = 28 PRRSV European (EU) strain-positive serum samples. Extraction was performed using either the IndiMag Pathogen IM48 Cartridge, the IndiMag Pathogen KF96 Cartridge, or the IndiMag Pathogen Kit. The extraction was performed with the IndiMag 48s or the KingFisher Flex devices, respectively. Subsequently, the eluates were further analyzed using the virotype PRRSV RT-PCR Kit. RT-PCR was performed on the Agilent Technologies Stratagene Mx3005P thermocycler. Data were generated by INDICAL BIOSCIENCE GmbH, Leipzig, Germany.

Results / Conclusion

Table 72 presents the results for PRRSV EU testing. All the serum samples were detected correctly, regardless of the extraction method used. The IndiMag Pathogen Cartridge kits and the IndiMag Pathogen Kit performed equally well.

Table 72. Data summary for PRRSV EU-positive serum samples extracted on the IndiMag 48s (either using the IndiMag Pathogen IM48 Cartridge or the IndiMag Pathogen Kit) or on the KingFisher Flex (using the IndiMag Pathogen KF96 Cartridge) device.

Sample	**Specimen**	**Status**	**PRRSV (EU strain)**		
			IM Path. IM48 Cartridge	**IM Path. KF96 Cartridge**	**IndiMag Path. Kit**
			IM 48s	**KFF**	**IM48s**
			C_T	**C_T**	**C_T**
#1	serum	**pos.**	31.63	31.84	31.39
#2	serum	**pos.**	33.33	33.44	33.50
#3	serum	**pos.**	27.60	27.57	26.94
#4	serum	**pos.**	33.97	33.75	33.64
#5	serum	**pos.**	31.11	31.33	31.77
#6	serum	**pos.**	31.17	32.12	31.39
#7	serum	**pos.**	28.32	29.38	28.60
#8	serum	**pos.**	32.90	32.30	32.18
#9	serum	**pos.**	27.17	27.63	27.02
#10	serum	**pos.**	28.44	28.25	28.05
#11	serum	**pos.**	25.13	25.76	24.97

#12	serum	**pos.**	28.47	28.83	28.37
#13	serum	**pos.**	32.84	32.28	31.96
#14	serum	**pos.**	31.29	31.14	30.81
#15	serum	**pos.**	29.22	30.15	28.74
#16	serum	**pos.**	33.39	33.93	33.28
#17	serum	**pos.**	26.77	28.33	26.62
#18	serum	**pos.**	25.98	26.22	25.85
#19	serum	**pos.**	34.99	34.08	31.69
#20	serum	**pos.**	26.05	26.08	25.89
#21	serum	**pos.**	29.30	29.83	30.09
#22	serum	**pos.**	29.91	30.15	30.28
#23	serum	**pos.**	32.40	33.08	32.89
#24	serum	**pos.**	32.62	32.61	32.74
#25	serum	**pos.**	30.55	32.48	30.63
#26	serum	**pos.**	28.82	28.71	29.86
#27	serum	**pos.**	29.28	29.40	29.33
#28	serum	**pos.**	31.25	30.94	31.27

IM = IndiMag, Path. = Pathogen, Cartr. = Cartridge, pos. = positive

4.3.6 ***Porcine Reproductive and Respiratory Syndrome Virus*** (PRRSV; tissue; internal data – INDICAL BIOSCIENCE GmbH, Leipzig)

Procedure

RNA was extracted from *n* = 7 lung tissue samples. Samples #1, #3, #5 and #7 were PRRSV EU strain positive and PRRSV NA strain-negative, whereas samples #2 and #6 were PRRSV NA strain positive and PRRSV EU strain-negative. Sample #4 was double-positive for PRRSV EU and NA strains. Following pre-treating the samples according to "Pretreatment T1", the samples were extracted using the IndiMag Pathogen IM48 Cartridge, the IndiMag Pathogen KF96 Cartridge the IndiMag Pathogen Kit or the IndiSpin Pathogen Kit. The extraction was performed on the IndiMag 48s, KingFisher Flex devices or manually with IndiSpin columns. Subsequently, the eluates were further analyzed using the virotype PRRSV RT-PCR Kit. The RT-PCR was performed on the Agilent Technologies Stratagene Mx3005P thermocycler. Data were generated by INDICAL BIOSCIENCE GmbH, Leipzig, Germany.

Results / Conclusion

Results are presented in Table 73 for PRRSV EU testing and in Table 74 for PRRSV NA testing. All lung tissue samples were detected correctly with the IndiMag Pathogen Cartridge kits and the IndiMag Pathogen Kit. Overall, the IndiMag Pathogen Cartridge kits and the IndiMag Pathogen Kit performed equally well or better that the IndiSpin Pathogen Kit.

Table 73. Data summary for testing PRRSV EU-positive lung tissue samples extracted on the IndiMag 48s (IM 48s) and KingFisher Flex (KFF) devices, and by manual extraction.

Sample	Specimen	PRRSV EU status	PRRSV (EU strain)			
			IM Pathogen IM48 Cartridge	IM Path. KF96 Cartr.	IM Path. Kit	IndiSpin Path. Kit
			IM 48s	KFF	KFF	manual
			C_T	C_T	C_T	C_T
#1	lung	**pos.**	30.32	29.10	31.61	30.74
#2	lung	neg.	-	-	-	-
#3	lung	**pos.**	35.67	34.09	34.20	36.59
#4	lung	**pos.**	29.01	28.42	28.73	29.75
#5	lung	**pos.**	32.23	32.81	32.21	33.70
#6	lung	neg.	-	-	-	-
#7	lung	**pos.**	34.36	34.46	35.61	-

IM = IndiMag, Path. = Pathogen, Cartr. = Cartridge, pos. = positive, neg. = negative, - = no C_T

Table 74. Data summary for testing PRRSV NA-positive lung tissue samples extracted on the IndiMag 48s (IM 48s) and KingFisher Flex (KFF) devices, and by manual extraction.

Sample	Specimen	PRRSV NA status	PRRSV (NA strain)			
			IM Pathogen IM48 Cartridge	IM Path. KF96 Cartr.	IM Path. Kit	IndiSpin Path. Kit
			IM 48s	KFF	KFF	manual
			C_T	C_T	C_T	C_T
#1	lung	neg.	-	-	-	-
#2	lung	**pos.**	26.14	25.40	26.22	27.02
#3	lung	neg.	-	-	-	-
#4	lung	**pos.**	26.87	26.00	26.22	27.20
#5	lung	neg.	-	-	-	-
#6	lung	**pos.**	22.88	21.72	22.27	23.44
#7	lung	neg.	-	-	-	-

IM = IndiMag, Path. = Pathogen, Cartr. = Cartridge, pos. = positive, neg. = negative, - = no C_T

5 Robustness

5.1 Cross-contamination

5.1.1 IndiMag 2

Procedure

To test for cross-contamination when using the IndiMag 2 instrument, two blood samples of cows were used. Sample one originated from a *Bovine Viral Diarrhea Virus* (BVDV)-positive cow (C_T value ~ 15) and sample two from a BVDV-negative cow. Each sample was pipetted next to each other to test for any cross-contamination and extracted using the IndiMag Pathogen Kit and the respective consumables. To further investigate any bias in the position of positive and negative samples in the extraction procedure, the position of positive and negative samples was changed between the different layouts (Figure 1):

1. Lysis/Block/Elution (L/B/E) = three washes
   (Lysis was performed in the lysis strip, three washes were performed in the 32-well block and nucleic acid was eluted in the elution strip)

2. -/Block/- (-/B/-) = two washes (IM48 Cartridge format)
   (Lysis, two washing steps and nucleic acid elution were performed in the 32-well block)

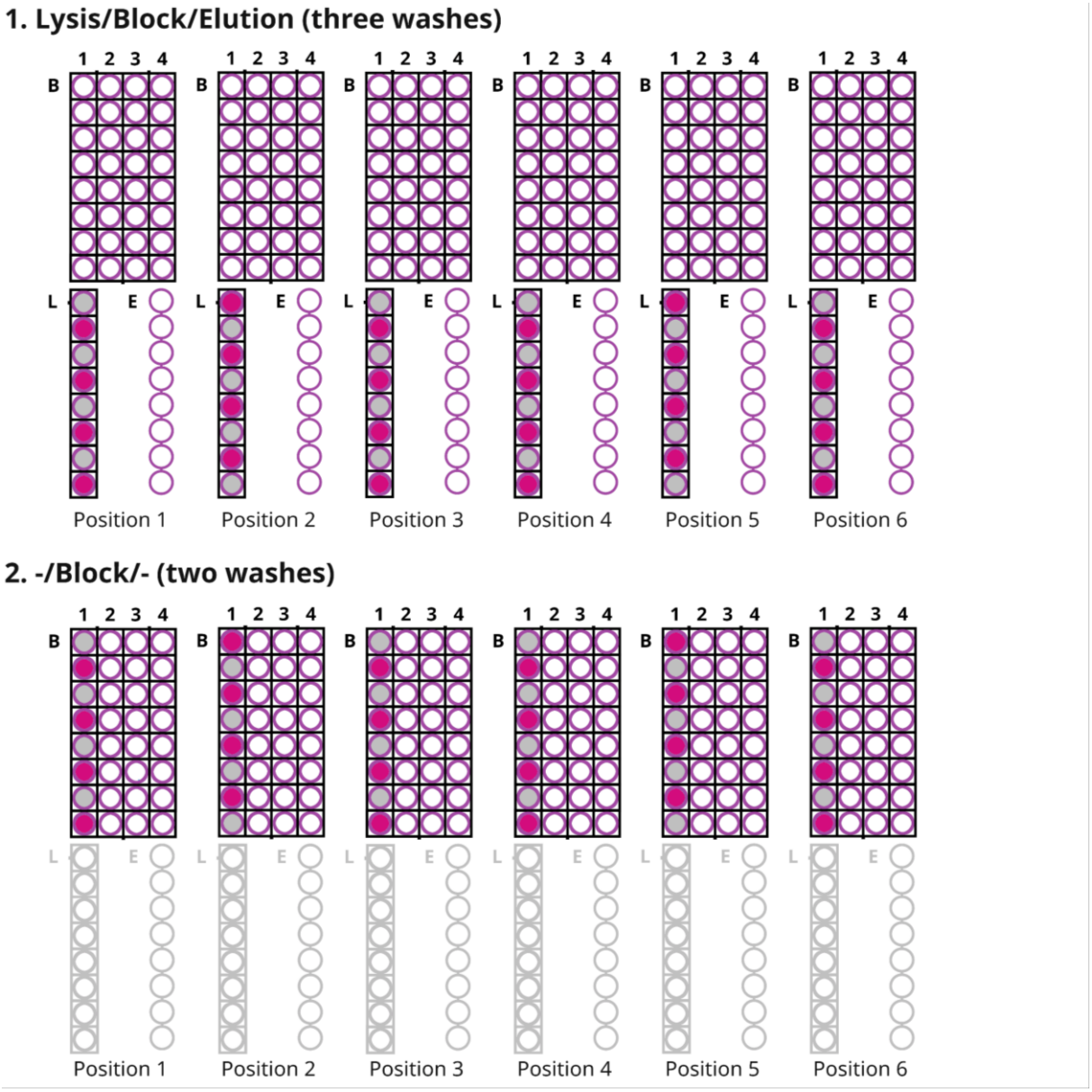

Figure 1. Alternating pattern setup for cross-contamination evaluation on the IndiMag 2 device for layout 1 (Lysis/Block/Elution (L/B/E) = three washes) and 2 (-/Block/- (-/B/-) = two washes, IM48 Cartridge format).
Pink filling = BVDV-positive, Grey filling = BVDV-negative

In total, *n* = 48 samples were extracted per IndiMag 2 extraction layout with all six positions of the instrument being used. Extracted nucleic acids were tested with the IndiMix JOE in combination with the virotype BVDV 2.0 Primers/Probes and were analysed on the Agilent Technologies Stratagene Mx3005P thermocycler. Both, the BVDV (FAM) and the exogenous control (HEX) signals (data not shown) were analyzed. Combined C_T values for positive and negative sample groups per block for each extraction method were compared to each other and statistically analyzed by one-way ANOVA (Tukey's multiple comparison test; GraphPad Prism 10).

Results / Conclusion

No cross-contamination in the BVDV-negative samples was observed when using the IndiMag 2 instrument (Table 75, Table 76). Furthermore, the position of the BVDV-positive and BVDV-negative sample was irrelevant with regards to cross-contamination. There was no statistical difference observed within the sample groups (i.e., BVDV-positive and BVDV-negative sample), nor between the two different extraction layouts being used ($P > 0.05$).

Table 75. Data summary for cross-contamination testing on the IM 2 (Lysis/Block/Elution format – three washes).

	BVDV-positive/negative bovine blood					
	C_T (BVDV, FAM)					
	Position 1	**Position 2**	**Position 3**	**Position 4**	**Position 5**	**Position 6**
A	-	15.78	-	-	15.68	-
B	15.38	-	15.58	15.80	-	15.74
C	-	15.70	-	-	15.76	-
D	15.77	-	15.72	15.97	-	16.10
E	-	15.80	-	-	15.65	-
F	15.51	-	15.77	16.11	-	15.75
G	-	15.74	-	-	15.93	-
H	15.74	-	15.86	15.81	-	15.75

- = no C_T

Table 76. Data summary for cross-contamination testing on the IM 2 (-/Block/- format – two washes).

	BVDV-positive/negative bovine blood C_T (BVDV, FAM)					
	Position 1	**Position 2**	**Position 3**	**Position 4**	**Position 5**	**Position 6**
A	-	15.34	-	-	15.10	-
B	15.40	-	15.40	15.13	-	15.50
C	-	15.30	-	-	14.99	-
D	15.26	-	15.13	15.23	-	15.60
E	-	15.22	-	-	15.68	-
F	15.43	-	15.50	15.42	-	15.40
G	-	15.97	-	-	15.49	-
H	15.71	-	15.66	15.69	-	15.43

- = no C_T

5.1.2 IndiMag 48/48s

Procedure

To confirm the absence of cross-contamination of the automated extraction protocol, alternating patterns of a strong *Reproductive and Respiratory Syndrome Virus* (PRRSV)-positive and a PRRSV-negative sample were run on the IndiMag 48. The positions of positive and negative samples were switched within each of the six positions in the lysis well (Figure 2). In total, *n* = 48 samples were extracted using the IndiMag Pathogen Kit on the IndiMag 48 instrument and the RT-PCR performed using the virotype PRRSV RT-PCR Kit on the Agilent Technologies Stratagene Mx3005P thermocycler. Combined C_T values for positive and negative sample groups per position were compared to each other and statistically analyzed by one-way ANOVA (Tukey's multiple comparison test; GraphPad Prism 10).

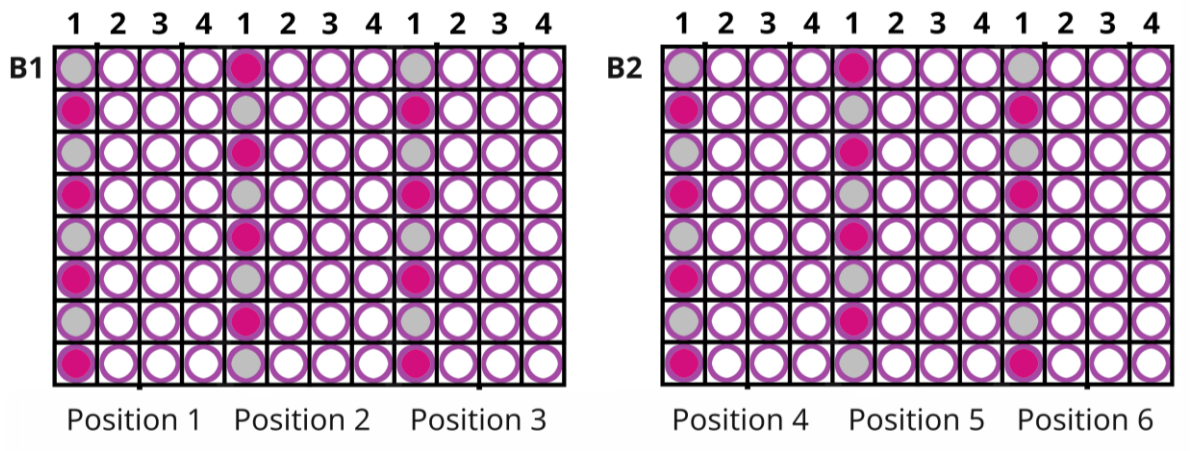

Figure 2. Alternating pattern setup for cross-contamination evaluation on the IndiMag 48 device.
Pink filling = PRRSV-positive sample; grey filling= PRRSV-negative sample

Results / Conclusion

The data are summarized in Table 77. No cross-contamination from one well to another was detected using the IndiMag Pathogen kit on the IndiMag 48 device. There was no statistical difference observed within the sample groups (i.e., PRRSV-positive and PRRSV-negative sample), nor between the positions being used (P > 0.05).

Table 77. Data summary for cross-contamination testing on the IndiMag 48.

	PRRSV-positive/negative porcine blood					
	C_T (PRRSV EU, FAM)					
	Position 1	**Position 2**	**Position 3**	**Position 4**	**Position 5**	**Position 6**
A	-	19.06	-	-	17.93	-
B	17.43	-	18.45	18.45	-	18.04
C	-	17.88	-	-	17.82	-
D	18.33	-	18.51	18.45	-	18.80
E	-	18.18	-	-	17.91	-
F	17.88	-	18.20	17.87	-	18.19
G	-	18.38	-	-	17.85	-
H	18.38	-	17.62	18.10	-	18.39

- = no C_T

5.1.3 KingFisher Flex

Procedure

To confirm the absence of cross-contamination of the automated extraction protocol, alternating patterns of a strong Influenza A Virus-positive and an Influenza A Virus-negative sample were run on the KingFisher Flex. The positions of positive and negative samples were switched within each of the 12 individual columns. In total, *n* = 96 samples were extracted using the IndiMag Pathogen Kit on the KingFisher Flex instrument (see Figure 3) and the RT-PCR performed using the virotype Influenza A RT-PCR Kit on the Agilent Technologies Stratagene Mx3005P thermocycler. Combined C_T values for positive and negative sample groups per column (1-12) were compared to each other and statistically analyzed by one-way ANOVA (Tukey's multiple comparison test; GraphPad Prism 10).

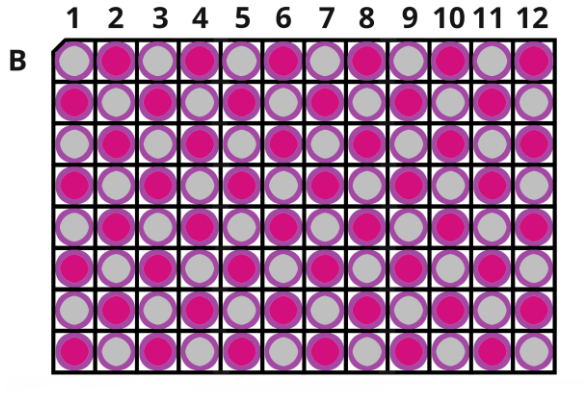

Figure 3. Alternating pattern setup for cross-contamination evaluation on the KingFisher Flex device.
Pink filling = Influenza A Virus- positive sample; grey filling = Influenza Virus A-negative sample.

Results / Conclusion

The data are summarized in Table 78. No cross-contamination from one well to another was detected using the IndiMag Pathogen kit on the KingFisher Flex device. There was no statistical difference observed within the sample groups (i.e., Influenza A Virus-positive and Influenza A Virus-negative sample), nor between the columns being used (P > 0.05).

Table 78. Data summary for cross-contamination testing on the KingFisher Flex.

	Influenza A Virus-positive culture media spiked in bovine blood - no pre-treatment											
	C_T (Influenza A Virus, FAM)											
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	-	22.47	-	22.23	-	22.31	-	22.37	-	22.27	-	21.97
B	22.00	-	21.86	-	22.08	-	22.45	-	21.99	-	22.11	-
C	-	21.79	-	21.73	-	21.97	-	21.73	-	21.56	-	22.49
D	21.91	-	21.78	-	21.79	-	21.97	-	21.77	-	21.64	-
E	-	21.94	-	21.44	-	21.86	-	21.95	-	21.62	-	22.37
F	22.13	-	21.60	-	20.69	-	21.82	-	21.73	-	21.46	-
G	-	21.62	-	21.84	-	21.94	-	21.91	-	21.86	-	22.13
H	22.09	-	22.00	-	22.57	-	22.34	-	22.31	-	21.94	-

- = no C_T

5.2 Homogeneity

5.2.1 Homogenic processing on the IndiMag 2

Procedure

In order to test if the position or the layout of extraction of the sample showed any influence on the performance of the nucleic acid extraction, one blood sample (diluted 1:100) of a BVDV-positive cow was extracted 48 times using the IndiMag Pathogen Kit and the respective consumables. The following extraction layouts were used:

1. Lysis/Block/Elution (L/B/E) = three washes
   (Lysis was performed in the lysis strip, three washes were performed in the 32-well block and nucleic acid was eluted in the elution strip)
2. -/Block/Elution (-/B/E) = three washes
   (Lysis and three washes were performed in the 32-well block and nucleic acid was eluted in the elution strip)
3. Lysis/Block/- (L/B/-) = three washes
   (Lysis was performed in the lysis strip, three washes and nucleic acid elution was performed in the 32-well block)
4. -/Block/- (-/B/-) = two washes (IM48 Cartridge format)
   (Lysis, two washing steps and nucleic acid elution were performed in the 32-well block)

All six positions of the instrument were used (Figure 4).

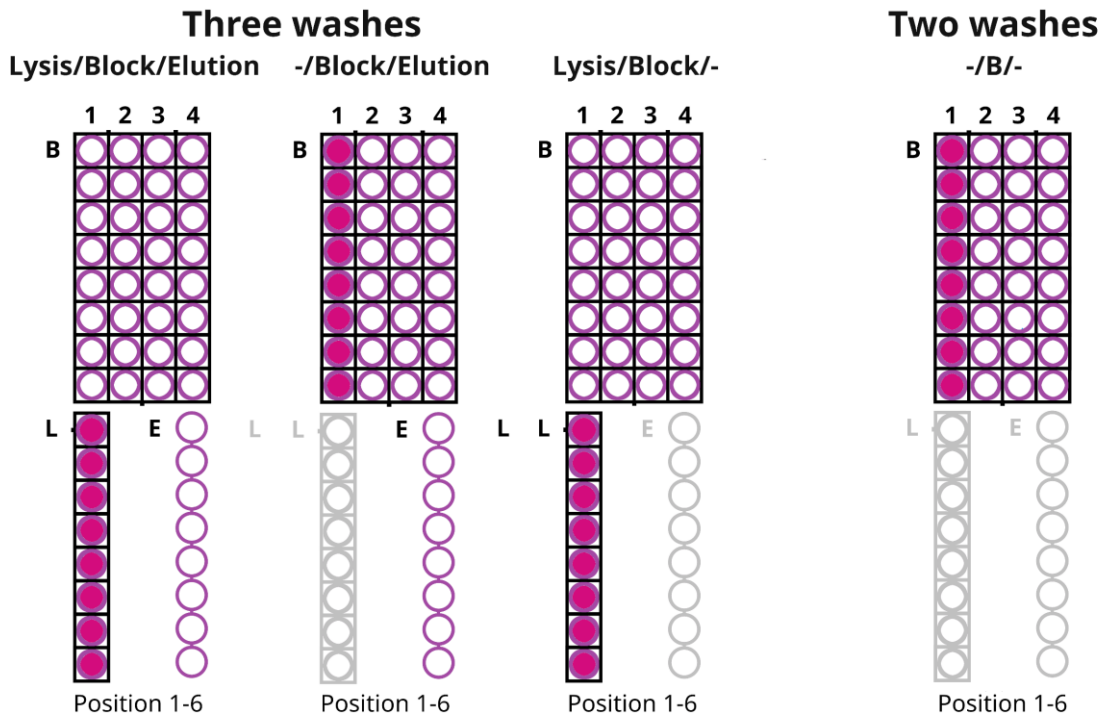

Figure 4. Extraction layouts for homogeneity evaluation on the IndiMag 2 device.

Extracted nucleic acids were tested with the IndiMix JOE in combination with the virotype BVDV 2.0 Primers/Probes and were analyzed on the Agilent Technologies Stratagene Mx3005P thermocycler.

Combined C_T values for each extraction method were compared to each other and statistically analyzed by one-way ANOVA (Tukey's multiple comparison test; GraphPad Prism 10).

When comparing the different positions (1-6) in the instrument, the data from the L/B/E, -/B/E and L/B/- format were used to analyze the inter-position performance by one-way ANOVA (Tukey's multiple comparison test; GraphPad Prism 10).

Results/ Conclusion

All extraction methods using three washes (L/B/E, -/B/E) and L/B/-) showed improved performances that were statistically significantly different to the method only using two washes (IM48 setup, -/B/- as shown in Figure 5. The highest mean C_T value difference (mean dC_T) was observed between the L/B/E method (mean dC_T=-0.475) and the method using two washes (IM48; -/B/-), followed by -/B/E (mean dC_T=-0.388) and L/B/- (mean dC_T=-0.332). Less differences were observed between the methods using three washes (L/B/E; -/B/E and L/B/-).

The inter-position comparison between lysis strips, 32-well block and elution strips did not reveal any statistically significant differences (P > 0.05, Figure 6) between the positions nor between the positions within the different formats. Thus, it can be concluded that neither the position nor the format (three washes only) used for performing the extraction should influence the outcome of the procedure. All positions of the IndiMag 2 perform the nucleic acid extraction equally well.

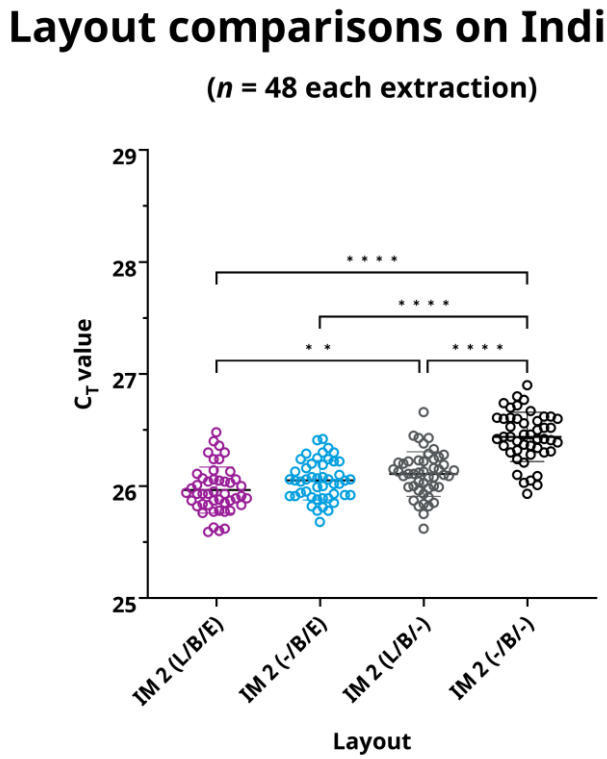

Figure 5. Comparison of different extraction layouts on the IndiMag 2 instrument.
(**** P < 0.0001; ** P = 0.035)

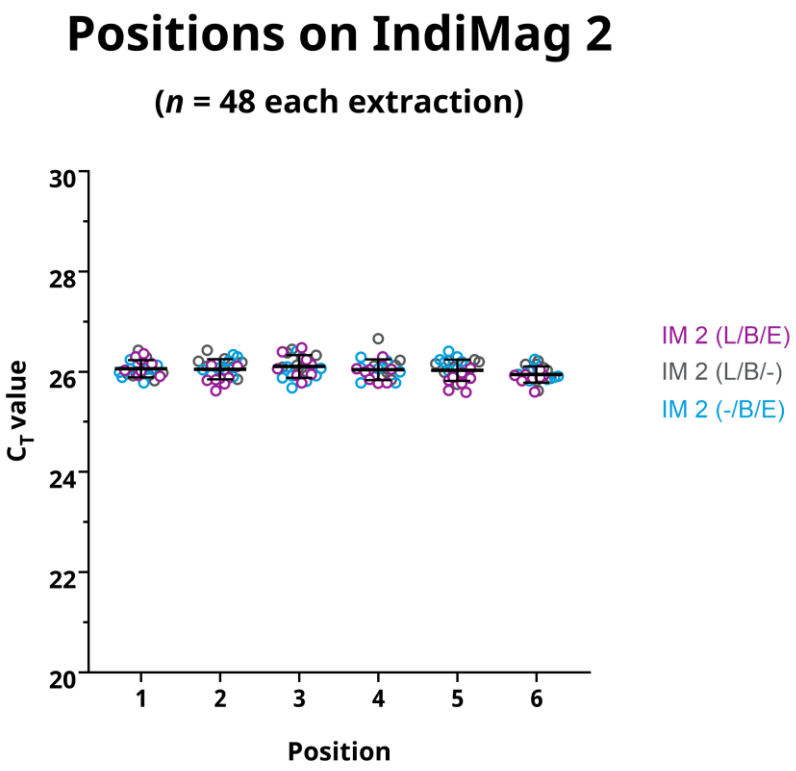

Figure 6. Comparison of different positions on the IndiMag 2 instrument. No statistically significant differences were observed (P > 0.05).

5.2.2 Homogeneity of the IndiMag Pathogen IM48 Cartridges

Procedure

For analysis, one sample was distributed in multiple replicates across several plates of the same produced batch of IndiMag Pathogen IM48 Cartridge. The extracts were subsequently tested in different PCR and RT-PCR runs. Both intype IC-RNA and blood were used for the amplification of the internal control signal (DNA and RNA).

Figure 7 shows the plate set up for the extraction using the IndiMag 48s (using 4x 8-sample blocks for simultaneous extraction of *n* = 32 replicates and using 2x 24-sample blocks for simultaneous extraction of *n* = 48 replicates).

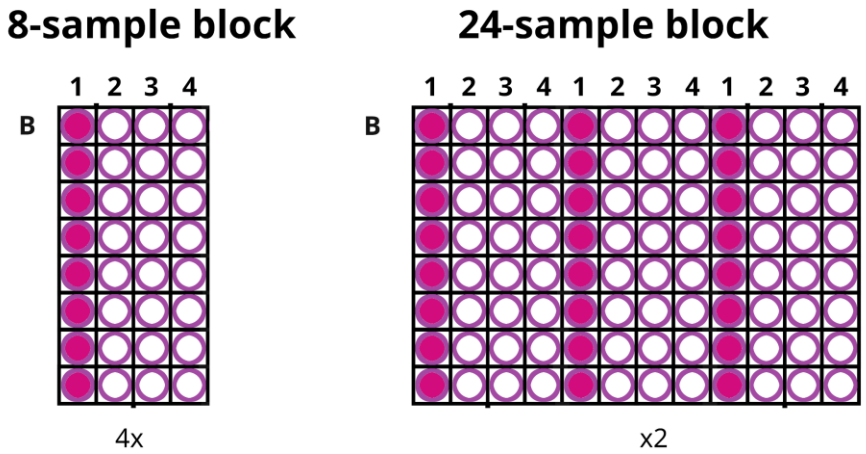

Figure 7. Sample set-up for homogeneity tests (8-sample block; 24-sample block).
Pink filling = sample 1

Results/ Conclusion

Table 79 summarizes both the values for mean and standard deviation as well as coefficient of variation for the 8-sample blocks (*n* = 32 replicates) and the 24-sample blocks (*n* = 48 replicates) for each test system and for different tests. The intra-run variance per parameter was very low with a range from 0.97 % to 3.07 %.

Table 79. Results of the homogeneity tests for the IndiMag Pathogen IM48 Cartridges tested on the IndiMag 48s.

	8-sample blocks (*n* = 32)			**24-sample blocks (*n* = 48)**		
	intype IC-RNA	**blood (RNA)**	**blood (DNA)**	**intype IC-RNA**	**blood (RNA)**	**blood (DNA)**
C_T mean value	26.88	20.07	24.86	27.08	20.22	24.78
SD	0.26	0.23	0.71	0.30	0.36	0.76
CV	0.97 %	1.15 %	2.86 %	1.11 %	1.78 %	3.07 %

SD = standard deviation, CV = coefficient of variation