MACHEREY-NAGEL

NucleoMag® VET / Kylt®

Summary of International Proficiency Testing Scheme reports for veterinary pathogens – an interplay of NucleoMag® VET and Kylt® technology

Introduction

AniCon Labor GmbH in Höltinghausen, Germany, is a leading lab service provider for livestock and known as a laboratory centre for molecular, microbiological, immunological as well as post mortem veterinary diagnostics. AniCon was founded in 2005 and in the year 2009 the company established the new brand Kylt®, providing mainly Real-Time PCR Kits to simplify the detection of a large variety of relevant livestock and foodsafety associated pathogens.

AniCon established a diagnostic workflow to accomplish the ongoing challenge to improve current standards and to cover future veterinary diagnostic requirements. To satisfy the rising demand for high quality diagnostics methods, AniCon is using the NucleoMag® VET kit from MACHEREY-NAGEL to extract nucleic acids from bacterial and viral pathogens originating from diverse veterinary samples, such as tissues, swabs, and feces, processing up to 96 samples within 2 hours. Subsequent DNA/RNA analysis is performed with AniCon's continuously expanding range of Kylt® products.

Reliable veterinary sample analysis plays an essential role in animal and human welfare and strongly depends on products of high performance and quality. Therefore, certified veterinary service providers participate in Proficiency Testing Schemes (also known as "Intercomparisons") to ensure the effectiveness of each laboratory's diagnostic process.

The established workflow for nucleic acid extraction with the NucleoMag® VET kit processed on two Hamilton® Microlab® Starlets in combination with diverse Kylt® products, was used to participate at several International Proficiency Testing Schemes.

GD Animal Health, Deventer, Netherlands, provided all mentioned Proficiency Testing Schemes and generously granted the permission to use the data in this application note.

The following results are referring to:

ViralRNA

Figure 2. Report of the 2017 International Proficiency Testing Scheme (PTS) for Infectious Bronchitis Virus (IBV) RNA detection in medium and chicken kidney tissue.

Bacterial dsDNA

Figure 3. Report of the 2017 International Proficiency Testing Scheme (PTS) for *Mycoplasma gallisepticum / synoviae*

Viral ssDNA

Figure 4. Report of the 2017 International Proficiency Testing Scheme (PTS) for Porcine Circovirus type 2 (PCV-2) DNA detection in serum samples

Viral dsRNA

Figure 5. Report of the 2017 International Proficiency Testing Scheme (PTS) for Infectious Bursal Disease Virus (IBDV) RNA detection in medium and chicken kidney tissue samples

Materials and methods

Exctraction kit:

NucleoMag® VET (MACHEREY-NAGEL) for magnetic bead based purification of viral nucleic acids and bacterial DNA

Automation system:

Hamilton® Microlab® Starlet „Mseries“ with Autoload, 8 Channels, CORE-Grip; Hamilton® Heater Shaker (HHS) 3mm, flat bottom; NucleoMag® Sep (Magnetic separator); Venus 4; Liquid/Solid Waste Kit; Multiflex Cooling Unit

Detection system:

Kylt® IB-aCoV; Kylt® IBV-Variant 4/91; Kylt® IBV-Variant Massachusetts; Kylt® IBV-Variant QX 100; Kylt® IBV-Variant Q1; Kylt® IBV-Variant O2; Kylt® IBV-Variant D1466 (Avian Coronaviruses including Infectious Bronchitis Virus)

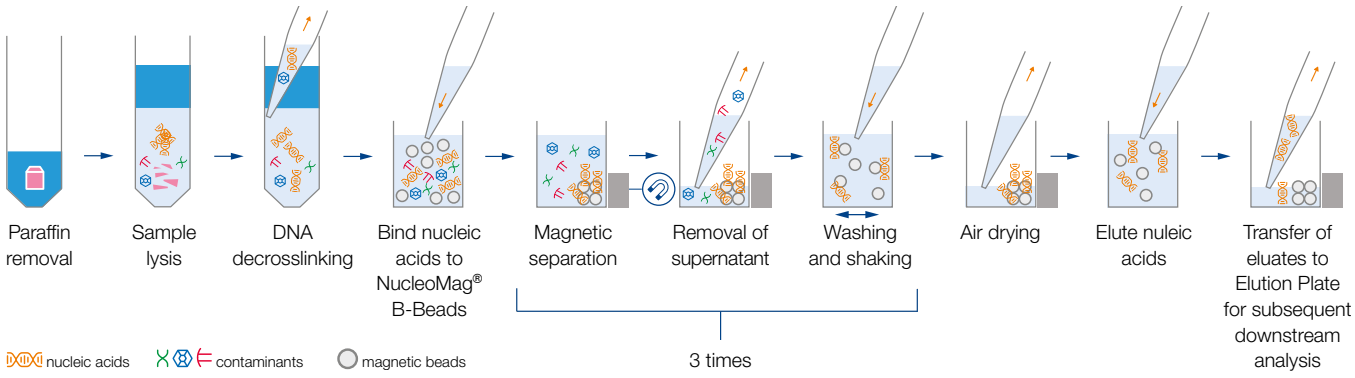
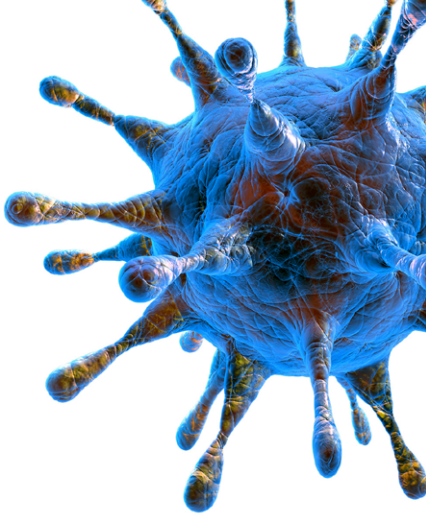


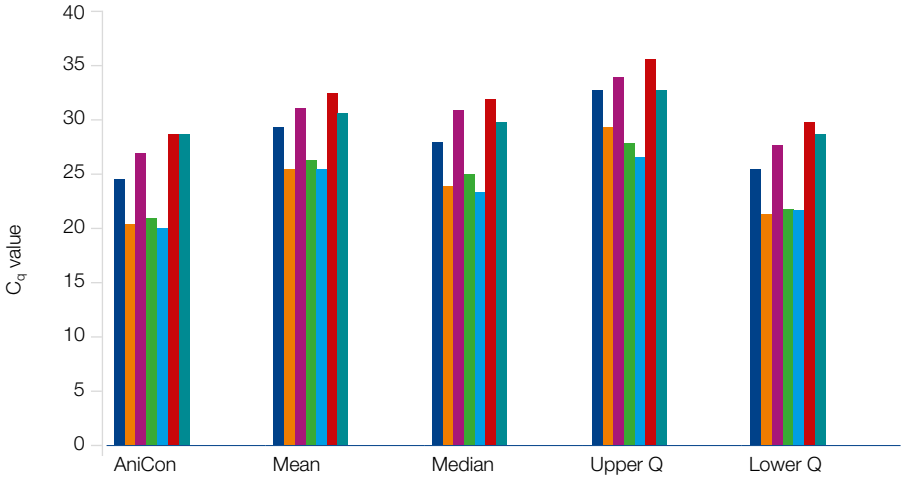
Fig 1. Workflow on automation platform Hamilton® Microlab® Starlet

Provided test sample material such as serum, suspension or medium (200 µL) was lysed with Lysis Buffer VL1 (180 µL) and Liquid Proteinase K (20 µL) for 10 minutes at 70 °C. After the addition of a synthetic Internal Control RNA fragment (Kylt® IC-RNA) reversible adsorption of nucleic acids to paramagnetic beads (20 µL NucleoMag® B-Beads) was enabled by adjustment of appropriate buffer conditions (600 µL Binding Buffer VEB). Subsequent to the magnetic separation, the NucleoMag® B-Beads are washed to remove contaminants and salts using Wash Buffer VEW1, VEW2, and 80 % ethanol (600 µL each) respectively. After air drying the NucleoMag® B-Beads for 15 min at 70°C, highly pure nucleic acids are finally eluted under low ionic strength conditions in the slightly alkaline Elution Buffer VEL (150 µL).



Application data

Infectious Bronchitis Virus (IBV) is an economically significant pathogen with high impact on the global commercial egg and chicken meat industry (Cavanagh 2007). The IBV produces pathological effects on body tissues, accompanied by a significant reduction in egg production, reduced shell quality and albumen quality. The initial infection usually occurs in the respiratory system, but as the disease progresses IBV is capable to multiply in different tissue epithelia e.g., in the Harderian gland, gastrointestinal tract, oviduct and in kidney tissue. Some nephrotropic strains infecting the kidney epithelium can cause kidney failure (Bande et al., 2016). The dataset below presents the results from the Report of the 2017 International Proficiency Testing Scheme (PTS) for Infectious Bronchitis Virus (IBV) RNA detection in medium and chicken kidney tissue.



Infectious Bronchitis Virus (IBV)	AniCon	Mean	Median	Upper Q	Lower Q
Infectious bronchitis strain Q1	24.5	29.4	28.0	32.7	25.5
Infectious bronchitis strain D1466	20.4	25.5	23.9	29.4	21.3
Infectious bronchitis strain CR88121 serotype 4/91 CR88 793B	26.9	31.1	30.9	34.0	27.7
Infectious bronchitis strain CR88121 serotype 4/91 CR88 793B	20.9	26.3	25.0	27.9	21.8
Infectious bronchitis strain D388 (QX)	20.0	25.5	23.3	26.6	21.7
Infectious bronchitis strain Var2 (1494 V2)	28.7	32.5	31.9	35.6	29.8
Infectious bronchitis strain Massachusetts 41	28.7	30.7	29.8	32.8	28.8

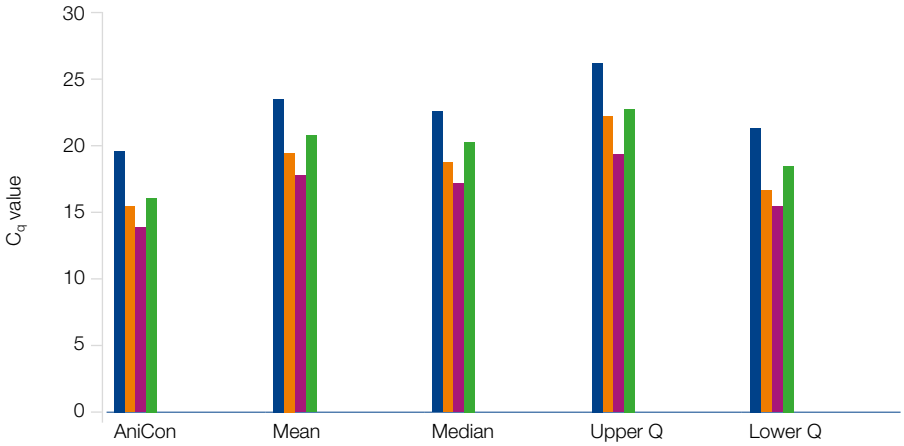
Fig 2. RT-qPCR detection of infectious bronchitis virus (IBV) in medium and chicken kidney tissue

RNA was isolated from the sample material provided for the International Proficiency Testing Scheme (PTS) for Infectious Bronchitis Virus (IBV) in medium and chicken kidney tissue. Infectious Bronchitis strain material: Q1 in 10-3 medium, D1466 in 10-2 medium, CR88121 of serotype 4/91 CR88 793B in 10-2 medium, CR88121 of serotype 4/91 CR88 793B in 10-2 kidney suspension, Var2 (1494 V2) in 10-2 kidney suspension and Massachusetts 41 in 10-5 medium. Mean/Median = Mean/Median Cq values for each sample material calculated for all participants of the PTS. Upper Q/Lower Q = Values of the 1st and 4th quartile for each sample material detected from participants of the PTS. Lower Cq-Values indicate a higher sensitivity of the diagnostic process. Infectious samples were processed using NucleoMag® VET on a Hamilton Microlab Starlet and subjected to a subsequent RT-qPCR analysis with the Kylt® IB-aCoV (Avian Coronaviruses including Infectious Bronchitis Virus) kit on a BioRad CFX96 Real-Time PCR system.

Positive samples were then tested with all of the Kylt® IBV Variant kits, including: Kylt® IBV-Variant Q1, Kylt® IBV-Variant D1466, Kylt® IBV-Variant 4/91, Kylt® IBV-Variant QX, Kylt® IBV-Variant 02, Kylt® IBV-Variant Massachusetts. Only the results from the separate positively tested Variant PCRs are listed above.

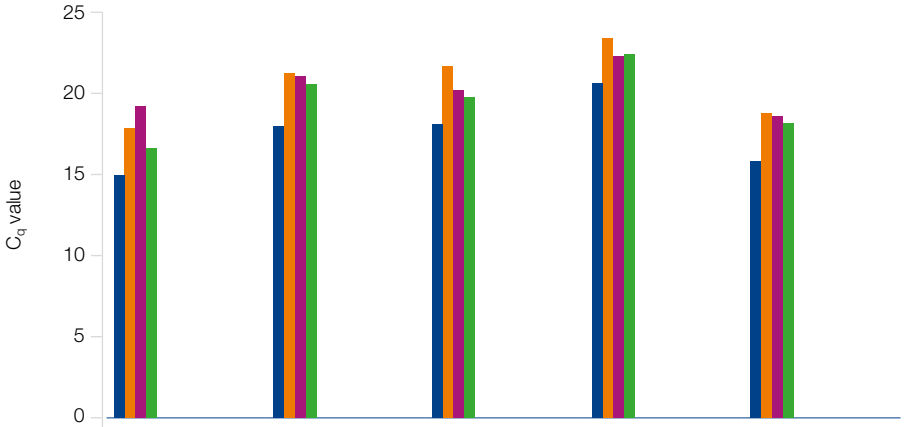
The bacterium *Mycoplasma gallisepticum* is responsible for chronic respiratory disease (CRD) in poultry and infectious sinusitis in turkeys, chickens and various birds, estimated to cause annual global losses exceeding several hundred million dollars (Hennigan *et al*., 2012). *M. gallisepticum* infections lead to reduced egg production, stunted growth with high mortality rates among young birds and impairs the function of the immune system leading to a high vulnerability to any common disease (Levison and Kleven. 2000).

A



Mycoplasma gallisepticum	AniCon	Mean	Median	Upper Q	Lower Q
Ms ATCC® 25204TM / Mg ATCC® 15302TM	19.6	23.5	22.6	26.2	21.4
Mg F vaccine 1200786 MS-H vaccine 11253A	15.5	19.5	18.8	22.2	16.7
Nobilis Mg 6/85	13.9	17.8	17.2	19.4	15.5
Mg field strain	16.1	20.8	20.3	22.8	18.5

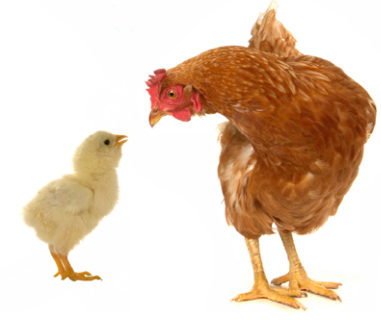
B



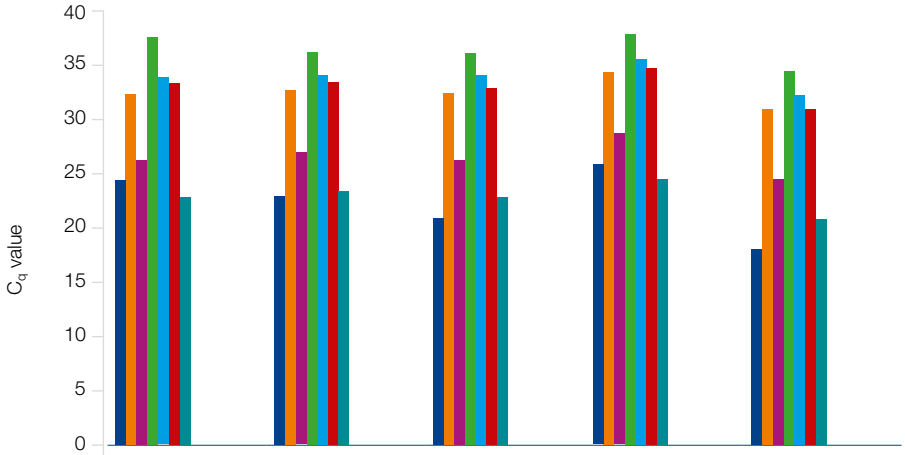
Mycoplasma synoviae	AniCon	Mean	Median	Upper Q	Lower Q
Ms ATCC® 25204TM / Mg ATCC® 15302TM	15.0	18.0	18.1	20.6	15.8
Mg F vaccine 1200786 MS-H vaccine 11253A	17.9	21.3	21.7	23.4	18.8
Ms field strain	19.2	21.1	20.2	22.3	18.6
MS-H vaccine 11253A/1e 33C	16.6	20.6	19.8	22.4	18.2

Fig 3. Detection of *Mycoplasma gallisepticum* (A) and *Mycoplasma synoviae* (B) via qPCR analysis

DNA was isolated from different sample material provided for the International Proficiency Testing Scheme (PTS) for *Mycoplasma gallisepticum/synoviae*. Provided PTS sample material was "Avian Mycoplasma Liquid Medium" (Mycoplasma Experience, Reigate, UK) comprised of one or more different spiked avian mycoplasma species. A negative control sample containing no mycoplasma was included as well (data not shown). Mean/Median = Mean/Median C_q values for each sample material calculated for all participants of the PTS. Upper Q/Lower Q = Values of the 1st and 4th quartile for each sample material detected from participants of the PTS. Lower C_q values indicate a higher sensitivity of the diagnostic process. Samples were processed using NucleoMag® VET on a Hamilton® Microlab® Starlet and subjected to a subsequent qPCR analysis with the Kylt® MGS Triplex (*Mycoplasma gallisepticum/synoviae*) kit on a BioRad® CFX96 Real-Time PCR system.



The small non-enveloped ssDNA Porcine Circovirus type 2 (PCV-2) is one of the most important porcine pathogens, and presently is subjected to the highest volume of prophylactic intervention in the form of vaccines in global swine production (Karuppannan and Opriessnig, 2017). The virus preferentially targets the lymphoid tissues, which leads to lymphoid depletion and immunosuppression in pigs. PCV-2 associated respiratory symptoms, congenital tremors, enteritis, dermatitis, nephropathy and reproductive issues were described and later grouped as Porcine Circovirus-associated diseases (PCV-AD) in North America (Opriessnig et al., 2007) and Porcine Circovirus diseases (PCVD) in Europe (Segales et al., 2005).

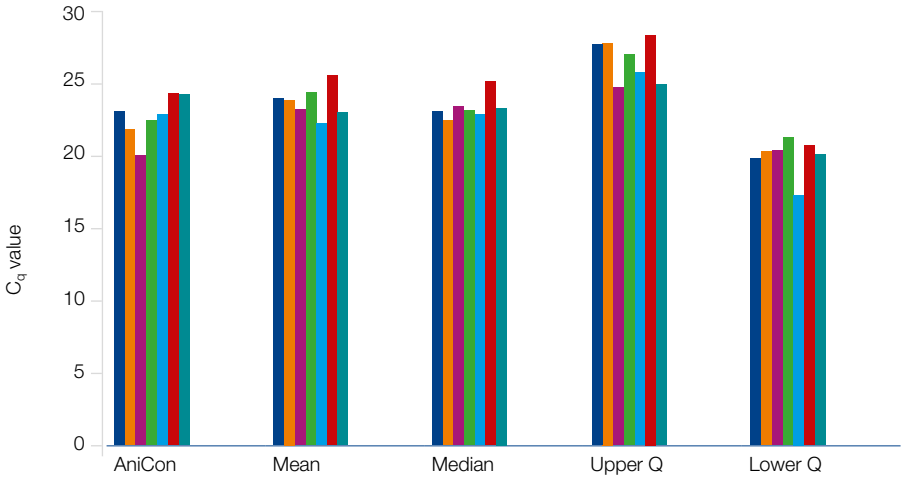


Porcine Circovirus type 2 (PCV-2)	AniCon	Mean	Median	Upper Q	Lower Q
PCV-2 vaccine 1:10 diluted	24.4	22.9	21.0	25.9	18.1
Field sample PCV-2	32.4	32.7	32.5	34.4	31.0
PCV-2 wildtype virus 1:10^3 diluted	26.3	27.0	26.3	28.7	24.5
PCV-2 wildtype virus 1:10^6 diluted	37.6	36.2	36.1	37.9	34.5
Field sample PCV-22	33.9	34.1	34.1	35.6	32.3
PCV-2 wildtype virus 1:10^5 diluted	33.4	33.5	32.9	34.8	31.0
PCV-2 wildtype virus 1:10^2 diluted	22.8	23.4	22.8	24.5	20.9

Fig 4. Detection of Porcine Circovirus type 2 (PCV-2) in serum samples

DNA of Porcine Circovirus type 2 was extracted and analyzed in the course of the International Proficiency Testing Scheme (PTS) for Porcine Circovirus type 2 (PCV-2) DNA detection in serum samples. Mean/Median = Mean/Median C_q values for each sample material calculated for all participants of the PTS. Upper Q/Lower Q = Values of the 1st and 4th quartile for each sample material detected from participants of the PTS. Lower C_q values indicate a higher sensitivity of the diagnostic process. All samples were processed using NucleoMag® VET on a Hamilton® Microlab® Starlet and subjected to a subsequent qPCR analysis with the Kylt® PCV-2 (Porcine Circovirus Type 2) kit on a BioRad® CFX96 Real-Time PCR system.

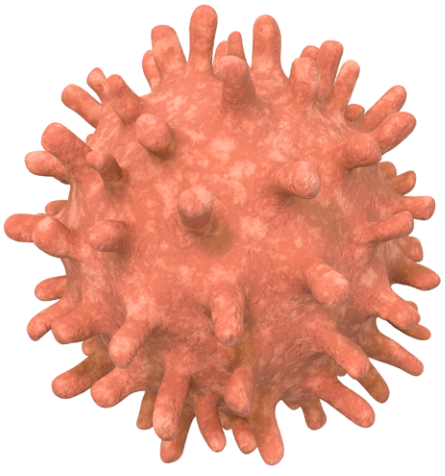
The Infectious Bursal Disease (IBD), also called Gumboro disease, is a highly contagious immunosuppressive disease affecting young chickens worldwide. The Infectious Bursal Disease Virus (IBDV) destroys the bursa of Fabricius (BF), the central immune organ of chicken. Replication of the dsRNA IBDV within immature B-lymphocytes causes a depletion of these cells within the BF. This leads to immunosuppression and reduced responses of chickens to vaccines, which increases their susceptibility to other pathogens (Rautenschlein et al., 2002). The broiler chicken industry e.g., in Saskatchewan, Canada loses approximately 3.9 million kg per year due to "variant" IBDV infection. In 2014, this amount of chicken had a wholesale market value of over $13 million according to the 2014 Annual Report of the Chicken Farmers of Canada (Zachar et al., 2016).



Infectious Bursal Disease Virus (IBDV)	AniCon	Mean	Median	Upper Q	Lower Q
5-week-old spf layers infected with Poulvac Bursine 2	23.1	23.95	23.1	27.78	19.9
4-week-old spf layers infected with Hipra GM97	21.85	23.88	22.5	27.83	20.38
6-week-old spf layers infected with vv IBDV D6948	20.05	23.29	23.4	24.78	20.43
6-week-old spf layers infected with CEVAC IBDL	22.45	24.42	23.2	27.03	21.28
6-week-old spf layers infected with European variant IBD 10191 field isolate	22.85	22.25	22.85	25.75	17.35
5-week-old spf layers infected with Nobilis 228E	24.4	25.58	25.15	28.4	20.78
PCV-2 wildtype virus 1:10^2 diluted	24.25	23.03	23.35	24.98	20.08

Fig 5. RT-qPCR detection of Infectious Bursal Disease Virus (IBDV) in medium and chicken kidney tissue.

RNA was isolated from sample material provided for the International Proficiency Testing Scheme (PTS) for Infectious Bursal Disease Virus (IBDV) in medium and chicken kidney tissue. Young aged (4-6 week-old) specific pathogen free (SPF) layer chicken were infected with various IBDV strains and a sample 4 days post infection was analyzed within the PTS. Mean / Median = Mean / Median C_q values for each sample material calculated for all participants of the PTS. Lower Q = Values of the 1st and 4th quartile for each sample material detected from participants of the PTS. Lower C_q values indicate a higher sensitivity of the diagnostic process. Infectious samples were processed using NucleoMag® VET on a Hamilton® Microlab® Starlet and subjected to a subsequent RT-qPCR analysis with the Kylt® IBDV Serotype 1 (Infectious Bursal Disease Virus – Gumboro) kit on a BioRad® CFX96 Real-Time PCR system.



Summary

A reliable and sensitive pathogen detection is essential for a successful veterinary diagnostic service. AniCon established a diagnostic workflow for pathogen related nucleic acid extraction with the NucleoMag® VET kit from MACHEREY-NAGEL. All veterinary samples are automatically processed on two Hamilton® Microlab® Starlets and subsequently analyzed with diverse Kylt® PCR products covering all relevant pathogen species. The International Proficiency Testing Scheme data set generated with the above mentioned workflow, demonstrates a highly competitive detection of various pathogens such as *Mycoplasma gallisepticum* and *Mycoplasma synoviae* (bacterial DNA), PCV-2 (viral ssDNA), IBV (viral ssRNA) or IBDV (viral dsRNA). The C_q values for nearly all tested samples are in the range between the mean- and lower quartile value of all participants of the testing scheme.

Acknowledgements

MACHEREY-NAGEL would like to thank the AniCon Labor GmbH team members Dr. Jennifer Haneke and Florian Werner for their contribution to this application note. Furthermore we would like to thank the organizer of the Proficiency Testing Schemes (PTS), GD Animal Health for providing the test results of all reported inter-laboratory studies in this application note.

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Segalés et al., 2005 “Porcine circovirus diseases.” Animal Health Research Reviews

Zachar et al., 2016 “A 5-year study of the incidence and economic impact of variant infectious bursal disease viruses on broiler production in Saskatchewan, Canada” Canadian Journal of Veterinary Research

Ordering information

Product	Specifications	Preps	REF
NucleoMag® VET	Magnetic bead based kit for extraction of RNA and DNA from veterinary samples, containing NucleoMag® B-Beads, buffers, Carrier RNA, Proteinase K	1 x 96 / 4 x 96	744200.1 / .4
Kylt® IB-aCoV	Real-Time RT-PCR Detection Kit for Infectious Bronchitis Virus and further Avian Coronaviruses	100 / 25	31193 / 31194 *
Kylt® IBV-Variant 4/91	IBV-Variant-specific Real-Time RT-PCR Detection Kit for Variant 4/91 / 793B / CR88	100 / 25	31082 / 31083 *
Kylt® IBV-Variant Massachusetts	IBV-Variant-specific Real-Time RT-PCR Detection Kit for Variant Massachusetts	100 / 25	31084 / 31085 *
Kylt® IBV-Variant QX	IBV-Variant-specific Real-Time RT-PCR Detection Kit for Variant QX	100 / 25	31094 / 31095 *
Kylt® IBV-Variant Q1	IBV-Variant-specific Real-Time RT-PCR Detection Kit for Variant Q1	100 / 25	31179 / 31180 *
Kylt® IBV-Variant O2	IBV-Variant-specific Real-Time RT-PCR Detection Kit for Variant O2	100 / 25	31187 / 31188 *
Kylt® IBV-Variant D1466	IBV-Variant-specific Real-Time RT-PCR Detection Kit for Variant D1466	100 / 25	31092 / 31093 *
Kylt® MGS Triplex	Triplex Real-Time PCR Detection Kit Mycoplasma gallisepticum and Mycoplasma synoviae	100 / 25	31020 / 31021 *
Kylt® PCV-2	Real-Time PCR Detection Kit for Porcine Circovirus Type 2	100 / 25	31394 / 31395 *
Kylt® IBDV Pathotyping	Real-Time PCR Pathotyping Kit for Infectious Bursal Disease Virus (Gumboro), differentiation of very virulent and intermediate plus strains vs. intermediate and non-virulent strains	100 / 25	31443 / 31444 *

*These products are available at www.kylt.eu

Partner: Kylt® AniCon Labor GmbH

KATEN300192 Application Note NucleoMag VET PTS-Kylt-Hamilton Starlet en1 / 0 / 4 / 04.2023 DD · Printed in Germany

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