REPUBLICA MOLDOVA

CERTIFICAT DE ÎNREGISTRARE

PRIN PREZENTUL SE CERTIFICĂ . CĂ **ÎNTREPRINDEREA MIXTĂ "GBG-MLD" S.R.L.** ESTE ÎNREGISTRATĂ LA CAMERA ÎNREGISTRĂRII DE STAT

Numărul de indentificare de stat - codul fiscal

1003600117582

Data înregistrării **06.01.1995**

Data eliberării **21.12.2004**

Iovu Galina, registrator de stat
Funcția, numele, prenumele persoanei care a eliberat certificatul

semnătura

CAMERA ÎNREGISTRĂRII DE STAT 103

MD 0006733

AGENŢIA SERVICII PUBLICE

Departamentul înregistrare şi licenţiere a unităţilor de drept

EXTRAS

din Registrul de stat al persoanelor juridice

Nr. **399048** data **03.12.2018**

Denumirea completă: **Societatea cu Răspundere Limitată "GBG-MLD"**
Denumirea prescurtată: **"GBG-MLD" S.R.L.**
Forma juridică de organizare: **Societate cu răspundere limitată,**
Numărul de identificare de stat şi codul fiscal (IDNO): **1003600117582**
Data înregistrării de stat: **06.01.1995**
Modul de constituire: **nou creată.**
Sediul: **MD-2001, str. Tighina, 65, mun. Chişinău, Republica Moldova.**
Obiectul principal de activitate:

1. **Comerţul cu ridicata al produselor farmaceutice**
2. **Cercetare şi dezvoltare în ştiinţe fizice şi naturale**
3. **Comerţul cu amănuntul al produselor farmaceutice şi de parfumerie**
4. **Producţia echipamentului de control pentru procesele industriale**
5. **Practica medicală**
6. **Fabricarea utilajului medical şi chirurgical şi a dispozitivelor ortopedice**
7. **Producţia de aparatura şi instrumente de măsură, verificare şi control**
8. **Transporturi rutiere de mărfuri**

Capitalul social: **5400** lei,

Administrator: **CEAICOVSCHI TUDOR, IDNP 0971601546960**

Asociaţii:
1. COLEVA VERA, IDNP 2000048101473, cota 108 lei, ce constituie 2%
2. CEAICOVSCHI TUDOR, IDNP 0971601546960, cota 5292 lei, ce constituie 98%

Beneficiar efectiv:
2.1.CEAICOVSCHI TUDOR, IDNP 0971601546960, cota - 98%

Prezentul extras este eliberat în temeiul art.34 al Legii nr.220-XVI din 19 octombrie 2007 privind înregistrarea de stat a persoanelor juridice şi a întreprinzătorilor individuali şi confirmă datele din Registrul de stat la data de: **03.12.2018**.

Registrator **Lozovanu Constantin**

EB 0248571

Specificaţii tehnice (F4.1)

Numărul procedurii de achiziție 21009958 din 18 iulie 2019

Denumirea procedurii de achiziție: Seturilor diagnostice pentru detectia virușilor bolilor de Influența Aviară, Newcastle, Pesta Porcină Clasică, Bluetongue la rumegătoare

Cod CPV	Denumirea bunurilor	Modelul articolului	Ţara de origine	Produ-cătorul	Specificarea tehnică deplină solicitată de către autoritatea contractantă	Specificarea tehnică deplină propusă de către ofertant	Standarde de referinţă
1	2	3	4	5	7	6	8
33141625-7	Poz.1 Detecția ARN, Avian Influenza A	4485261, VetMAX™-Gold AIV Detection Kit	SUA	ThermoFisher Scientific	vezi anunţul de participare	vezi anexa tehnica	ISO
33141625-7	Poz.2 Detecția ARN, Newcastle disease virus	NDV, VetMAX™ NDV Kit	Franta	LSI(parte ThermoFisher Scientific)	vezi anunţul de participare	vezi anexa tehnica	ISO

Semnat:_____ Numele, prenumele: Ceaicovschi Tudor În calitate de: Director general

Specificaţii de preţ (F4.2)

Numărul procedurii de achiziție 21009958 din 18 iulie 2019

Denumirea procedurii de achiziție: Seturilor diagnostice pentru detectia virușilor bolilor de Influența Aviară, Newcastle, Pesta Porcină Clasică, Bluetongue la rumegătoare

Cod CPV	Denumirea bunurilor	Unitatea de măsură	Canti-tatea	Preţ unitar (fără TVA)	Preţ unitar (cu TVA)	Suma fără TVA	Suma cu TVA	Termenul de livrare
33141625-7	Poz.1Detecția ARN, Avian Influenza A	set	4	9835,00	11802,000	39340,00	47208,00	conform cerintelor
33141625-7	Poz.2Detecția ARN, Newcastle disease virus	set	1	18185,00	21822,000	18185,00	21822,00	conform cerintelor
	Suma totala					**57525,00**	**69030,00**	

Semnat:_____ Numele, prenumele: Ceaicovschi Tudor În calitate de: Director general

Ofertantul: „GBG-MLD" SRL Adresa: mun. Chişinău, str. Tighina, 65, of. 607

Anexa tehnica

Parmetri ceruti	Parametri oferiti	Referinta
Poz 1. Detecția ARN, Avian Influenza A		
	4485261, VetMAX™-Gold AIV Detection Kit, ThermoFisherScientific, SUA	Pag 1 manual
Set (nu mai mic de 100 de doze)	Set (100 de doze)	
Tip reacție : RT-PCR, de tip "one step, single tube" (reacțiile de reverstranscripție, amplificare și detecție să aibă loc într-un singur tub, într-o etapa)	Tip reacție : RT-PCR, de tip "one step, single tube" (reacțiile de reverstranscripție, amplificare și detecție au loc într-un singur tub, într-o etapa)	Pag 1 manual
Obligatoriu: Setul va conține toate cele necesare pentru reverstranscripție, amplificare și detecție.	Obligatoriu: Setul conține toate cele necesare pentru reverstranscripție, amplificare și detecție: 2X Multiplex RT-PCR Buffer Multiplex RT-PCR Enzyme Mix Influenza Virus Primer Probe Mix Xeno™ RNA Control (10,000 copies/µL) Influenza Virus-Xeno™ RNA Control Mix (1000 copies/µL) Nuclease-free Water	Pag 1 manual
Setul să conțină control pozitiv extern și control intern de extracție.	Setul conține control pozitiv extern(Influenza Virus-Xeno™ RNA Control Mix) și control intern de extracție(Xeno™ RNA Control).	Pag 3 manual, PCR setup
Setul să fie compatibil cu instrumentele Corbet Researche Rotor Gene 3000 și ThermoFisher Quant Studio 5.	Setul este compatibil cu oricare tip de instrument RT PCR care dispune de canale de citire FAM si VIC, inclusiv si cu instrumentele Corbet Researche Rotor Gene 3000 și ThermoFisher Quant Studio 5.	Da, pag 2 manual, proposed RNA isolation method
Posibilitatea de detecție a ARN-ui obținut prin metoda de extracție prin particole magnetice*.	Posibilitatea de detecție a ARN-ui obținut prin metoda de extracție prin particole magnetice.	
Furnizorul este obligat la livrare să asigure demonstrarea parametrilor setului livrat în condițiile de laborator(sediul bineficiarului)	Furnizorul se obliga la livrare să asigure demonstrarea parametrilor setului livrat în condițiile de laborator(sediul bineficiarului)	Pag 1 si 4 manual
Marcat de producător pentru uz veterinar	Marcat de producător pentru uz veterinar	
Poz 2. Detecția ARN, Newcastle disease virus		
	NDV, VetMAX™ NDV Kit, LSI(parte ThermoFisher Scientific), Franta	
Set (nu mai mic de 100 de doze)	Set (100 de doze)	Pag 1 manual
Tip reacție :	Tip reacție :	

RT-PCR, de tip "one step, single tube" (reacţiile de reverstranscripţie, amplificare şi detecţie să aibă loc într-un singur tub, într-o etapa) Obligatoriu:	RT-PCR, de tip "one step, single tube" (reacţiile de reverstranscripţie, amplificare şi detecţie au loc într-un singur tub, într-o etapa) Obligatoriu:	Pag 1, 2 manual
Setul va conţine toate cele necesare pentru reverstranscripţie, amplificare şi detecţie.	Setul conţine toate cele necesare pentru reverstranscripţie, amplificare şi detecţie: 25X NDV Primer Probe Mix 2X qRT-PCR Buffer 25X qRT-PCR Enzyme Mix Nuclease-free Water 25X NDV Control RNA Xeno™ RNA Control(10,000 copies/µL) Nucleic Acid Dilution Solution	Pag 1 manual, kit contens
Setul să conţină control pozitiv extern şi control intern de extracţie.	Setul conţine control pozitiv extern(NDV Control RNA) şi control intern de extracţie(Xeno™ RNA Control).	Pag 1 manual, pagina web NDV
Setul să fie compatibil cu instrumentele Corbet Researche Rotor Gene 3000 şi ThermoFisher Quant Studio 5.	Setul este compatibil cu oricare tip de instrument RT PCR care dispune de canale de citire FAM si CAL Fluor Orange, inclusiv si cu cu instrumentele Corbet Researche Rotor Gene 3000 şi ThermoFisher Quant Studio 5.	Pag 1, 2 manual
Posibilitatea de detecţie a ARN-ui obţinut prin metoda de extracţie prin particole magnetice*. Furnizorul este obligat la livrare să asigure demonstrarea parametrilor setului livrat în condiţiile de laborator(sediul bineficiarului)	Posibilitatea de detecţie a ARN-ui obţinut prin metoda de extracţie prin particole magnetice*. Furnizorul se oblige la livrare să asigure demonstrarea parametrilor setului livrat în condiţiile de laborator(sediul bineficiarului)	Pag 1 manual, proposed RNA isolation methods
Marcat de producător pentru uz veterinar	Marcat de producător pentru uz veterinar	Manual

Anexa tehnica

Parmetri ceruti	Parametri oferiti	Referinta
Poz 1. Detecția ARN, Avian Influenza A		
	4485261, VetMAX™-Gold AIV Detection Kit, ThermoFisherScientific, SUA	Pag 1 manual
Set (nu mai mic de 100 de doze)	Set (100 de doze)	
Tip reacție : RT-PCR, de tip "one step, single tube" (reacțiile de reverstranscripție, amplificare și detecție să aibă loc într-un singur tub, într-o etapa)	Tip reacție : RT-PCR, de tip "one step, single tube" (reacțiile de reverstranscripție, amplificare și detecție au loc într-un singur tub, într-o etapa)	Pag 1 manual
Obligatoriu: Setul va conține toate cele necesare pentru reverstranscripție, amplificare și detecție.	Obligatoriu: Setul conține toate cele necesare pentru reverstranscripție, amplificare și detecție: 2X Multiplex RT-PCR Buffer Multiplex RT-PCR Enzyme Mix Influenza Virus Primer Probe Mix Xeno™ RNA Control (10,000 copies/µL) Influenza Virus-Xeno™ RNA Control Mix (1000 copies/µL) Nuclease-free Water	Pag 1 manual
Setul să conțină control pozitiv extern și control intern de extracție.	Setul conține control pozitiv extern(Influenza Virus-Xeno™ RNA Control Mix) și control intern de extracție(Xeno™ RNA Control).	Pag 3 manual, PCR setup
Setul să fie compatibil cu instrumentele Corbet Researche Rotor Gene 3000 și ThermoFisher Quant Studio 5.	Setul este compatibil cu oricare tip de instrument RT PCR care dispune de canale de citire FAM si VIC, inclusiv si cu instrumentele Corbet Researche Rotor Gene 3000 și ThermoFisher Quant Studio 5.	Da, pag 2 manual, proposed RNA isolation method
Posibilitatea de detecție a ARN-ui obținut prin metoda de extracție prin particole magnetice*.	Posibilitatea de detecție a ARN-ui obținut prin metoda de extracție prin particole magnetice.	
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Marcat de producător pentru uz veterinar	Marcat de producător pentru uz veterinar	
Poz 2. Detecția ARN, Newcastle disease virus		
	NDV, VetMAX™ NDV Kit, LSI(parte ThermoFisher Scientific), Franta	
Set (nu mai mic de 100 de doze)	Set (100 de doze)	Pag 1 manual
Tip reacție :	Tip reacție :	

RT-PCR, de tip "one step, single tube" (reacțiile de reverstranscripție, amplificare și detecție să aibă loc într-un singur tub, într-o etapa) Obligatoriu:	RT-PCR, de tip "one step, single tube" (reacțiile de reverstranscripție, amplificare și detecție au loc într-un singur tub, într-o etapa) Obligatoriu:	Pag 1, 2 manual
Setul va conține toate cele necesare pentru reverstranscripție, amplificare și detecție.	Setul conține toate cele necesare pentru reverstranscripție, amplificare și detecție: 25X NDV Primer Probe Mix 2X qRT-PCR Buffer 25X qRT-PCR Enzyme Mix Nuclease-free Water 25X NDV Control RNA Xeno™ RNA Control(10,000 copies/µL) Nucleic Acid Dilution Solution	Pag 1 manual, kit contens
Setul să conțină control pozitiv extern și control intern de extracție.	Setul conține control pozitiv extern(NDV Control RNA) și control intern de extracție(Xeno™ RNA Control).	Pag 1 manual, pagina web NDV
Setul să fie compatibil cu instrumentele Corbet Researche Rotor Gene 3000 și ThermoFisher Quant Studio 5.	Setul este compatibil cu oricare tip de instrument RT PCR care dispune de canale de citire FAM si CAL Fluor Orange, inclusiv si cu cu instrumentele Corbet Researche Rotor Gene 3000 și ThermoFisher Quant Studio 5.	Pag 1, 2 manual
Posibilitatea de detecție a ARN-ui obținut prin metoda de extracție prin particole magnetice*. Furnizorul este obligat la livrare să asigure demonstrarea parametrilor setului livrat în condițiile de laborator(sediul bineficiarului)	Posibilitatea de detecție a ARN-ui obținut prin metoda de extracție prin particole magnetice*. Furnizorul se oblige la livrare să asigure demonstrarea parametrilor setului livrat în condițiile de laborator(sediul bineficiarului)	Pag 1 manual, proposed RNA isolation methods
Marcat de producător pentru uz veterinar	Marcat de producător pentru uz veterinar	Manual

„Secret comercial, confidențial"

MAIB
Moldova Agroindbank

Dlui Tudor Ceaicovschi,
Administrator al S.R.L. „GBG-MLD"
MD-2001, mun. Chișinău, str. Tighina, 65,

C180/E00214
05 martie 2019

CERTIFICAT

Prin prezenta, BC "Moldova-Agroindbank" S.A. confirmă că „GBG-MLD" S.R.L. (IDNO 1003600117582) dispune de următoarele conturile curente:

Numărul contului curent, cod IBAN	Valuta
MD14AG0000002251848O1542	MDL
MD64AG0000002251448O7542	EUR
MD81AG0000000225116779935	CHF
MD70AG0000000225113932O4	UAH
MD17AG0000002251148O4542	RUB
MD62AG0000002251948O2542	USD
MD39AG0000000225130595S3	GBP
MD81AG0000000225820801O7	MDL

Certificatul este eliberat pentru a fi prezentat la destinație.

Cu respect,

Victor Iuraș
Vicepreședinte al Comitetului de Conducere
al BC"Moldova-Agroindbank"S.A.

Ex.: Ivan Buga
Tel.: 022-30-33-64

life technologies™

Avian Influenza Virus RNA Test Kit

VetMAX™-Gold AIV Detection Kit

Catalog Number 4485261

Pub. No. 4486415 **Rev.** A

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **www.lifetechnologies.com/support.**

Product information

Name, intended use, and principle of the procedure

The Avian Influenza Virus RNA Test Kit is a highly sensitive, qualitative, one-step, real-time reverse transcription PCR (real-time RT-PCR) assay to detect Avian Influenza Virus (AIV) RNA isolated from poultry (chicken/turkey) oropharyngeal/tracheal swab samples.

AIV is an enveloped, negative-sense RNA virus of the genus *Influenzavirus A* and family *Orthomyxoviridae*. AIV subtypes are defined by the surface glycoproteins hemagglutinin and neuraminidase. Low pathogenic avian influenza (LPAI) strains exist in avian reservoir hosts and can be transmitted to poultry. H5 and H7 subtypes of LPAI strains are unique in their capability to undergo host adaptation and to evolve into highly pathogenic avian influenza (HPAI). HPAI viral infections arise *de novo* in poultry infected with LPAI H5 and H7 subtypes and become rapidly fatal due to an overwhelming systemic collapse. The VetMAX™-Gold AIV Detection Kit enables diagnosis of AIV in poultry populations.

The assay consists of a single-well/tube, real-time RT-PCR assay in which RNA is reverse-transcribed into cDNA; two viral matrix targets and one nucleoprotein target are amplified and detected in real time using fluorescent TaqMan® probes (hydrolysis probe chemistry). The assay detects sequences that are common to all AIV subtypes. The kit includes:

- Influenza Virus-Xeno™ RNA Control Mix: serves as a positive control for the real-time RT-PCR components and it is also used to set the cycle threshold (C_T) for evaluating test results.
- Xeno™ RNA Control: serves as an internal positive control for the RNA purification process and monitors for the presence of PCR inhibitors.
- Influenza Virus Primer Probe Mix for optimized multiplex real-time RT-PCR amplification of Xeno™ RNA Control and AIV RNA targets.

Limitations

- The kit is not intended for differentiating AIV subtypes.
- Handle samples as recommended in Table 1 to prevent degradation of any AIV RNA that is present.
- RNA extraction methods should yield RNA free of RT-PCR inhibitors, which can prevent amplification of target RNA.
- Follow "Good laboratory practices for PCR and RT-PCR" on page 5 to prevent false positive amplifications due to contamination of test samples with PCR products.

For Veterinary Use Only.

Kit contents and storage conditions

Reagents for 100 25-µL real-time RT-PCR tests are supplied.

Component	Volume	Storage
2X Multiplex RT-PCR Buffer	1375 µL	–30°C to –10°C
Multiplex RT-PCR Enzyme Mix	280 µL	–30°C to –10°C
Influenza Virus Primer Probe Mix	110 µL	–30°C to –10°C
Xeno™ RNA Control (10,000 copies/µL)	250 µL	–30°C to –10°C
Influenza Virus-Xeno™ RNA Control Mix (1000 copies/µL)	80 µL	–30°C to –10°C
Nuclease-free Water	1.75 mL	–30°C to +25°C

Required materials not supplied

Item	Source
Plates or tubes appropriate for the Applied Biosystems® 7500 Fast Real-Time PCR System (96-well)	• MicroAmp® Optical 8-Cap Strip (Cat. no. 4323032), or equivalent • MicroAmp® Fast Optical 96-Well Reaction Plate with Barcode, 0.1-mL (Cat. nos. 4366932, 4346906), or equivalent. • MicroAmp® Optical Adhesive Film (Cat. nos. 4311971, 4360954), or equivalent • MicroAmp® Fast 8-Tube Strip, 0.1-mL (Cat. no. 4358293), or equivalent • Precision Plate Holder for 0.1-mL tube strips (Cat. no. 4403809), or equivalent
Nuclease-free pipettes and filtered pipette tips	Major laboratory supplier (MLS)
Nuclease-free reagent tubes for preparing master mixes	MLS
Real-time PCR thermal cycler	Applied Biosystems® 7500 Fast Real-Time PCR System (96-well), running SDS software v1.4
1X Phosphate Buffered Saline (PBS), pH 7.4	MLS
Viral Transport Media	MLS
2 ice buckets: • One for the PCR setup area where the master mix is prepared • One for the area where RNA may be present	MLS

Isolate RNA from samples

Table 1 Sample handling recommendations

Step or process	Recommendation
Transport/storage of samples	Transport oropharyngeal/tracheal swab samples between 4°C and 25°C or in accordance with manufacturer's instructions.
Preparation of swab samples	1. Place one oropharyngeal/tracheal swab sample into a 1.5-mL tube or deep-well 96-well plate, then add 0.75 mL of Viral Transport Media. 2. Vortex vigorously for 3 minutes, then pulse-spin to remove debris from the tube cap. 3. Remove 50 µL of supernatant for RNA isolation.
Preparation of mock-purified samples (for use in extraction control PCRs)	Prepare duplicate mock-purified samples, using 1X PBS as the starting material. Process with the same RNA isolation method that is used for test samples.
Proposed RNA isolation method	MagMAX™-96 Viral RNA Isolation Kit (Cat. nos. AM1836, AMB1836-5) or an equivalent RNA isolation method.
Required modifications to the RNA isolation method	• Add 2 µL of undiluted Xeno™ RNA Control per isolation to the lysis solution used for RNA isolation. • Add carrier RNA to the lysis solution according to the manufacturer recommendations. Carrier RNA is provided in the MagMAX™-96 Viral RNA Isolation Kit (Cat. nos. AM1836, AMB1836-5)

Perform real-time RT-PCR

1 Determine the quantity of reactions and thaw the reagents

a. On each plate, include the following control reactions (for step 4 of this procedure):
   - Positive control (prepare duplicate reactions); use 8 µL of the Influenza Virus-Xeno™ RNA Control Mix (1000 copies/µL).
   - No-template control (NTC) (prepare duplicate reactions); use Nuclease-free Water in place of sample RNA.

b. Plan the plate layout so that the wells containing NTCs are located as far as possible from positive controls and test samples to prevent accidental cross-contamination.

c. Thaw RT-PCR master mix reagents in one ice bucket and controls and samples in a separate ice bucket, gently vortex each tube to mix the contents thoroughly, then briefly centrifuge to collect the solution at the bottom of the tube. Keep the reagents on ice.

2 Prepare the RT-PCR master mix on ice

Combine the following components for the number of reactions required plus 10% overage.

Component	Volume per reaction
2X Multiplex RT-PCR Buffer	12.5 µL
Multiplex RT-PCR Enzyme Mix	2.5 µL
Influenza Virus Primer Probe Mix	1.0 µL
Nuclease-free Water	1.0 µL
Total volume of RT-PCR master mix	**17.0 µL**

3 Set up the RT-PCR reactions

a. Dispense 17 µL of RT-PCR master mix to the appropriate wells of a PCR plate or PCR tubes on ice.

b. Add the appropriate component for the reaction type, according to the following table:

Reaction type	Component	Volume per reaction
Test sample	Sample RNA	8.0 µL
NTC	Nuclease-free Water	8.0 µL
Positive control	Influenza Virus-Xeno™-RNA Control Mix (1000 copies/µL)	8.0 µL
Extraction control	Mock-purified sample	8.0 µL

c. Seal each reaction vessel, mix, then centrifuge briefly to bring the contents to the bottom.

4 Set up and run the real-time PCR instrument

a. Following the manufacturer's instructions, set up the run using the following parameters:
- Run mode: Standard 7500
- Reaction volume: 25 µL
- ROX™ passive reference dye: Included in the RT-PCR Buffer
- TaqMan® probe reporter dyes and quenchers:

Target	Reporter	Quencher
AIV RNA	FAM™ dye[1]	Eclipse® Q
Xeno™ RNA Control	VIC® dye[2]	Eclipse® Q

[1] Absorbance maximum of 495 nm; emission maximum of 520 nm.
[2] Absorbance maximum of 540 nm; emission maximum of 552 nm.

b. Run the thermal cycler program and collect real-time amplification data during stage 3. Use the following thermal cycler settings:

Stage		Reps.	Temp.	Time
Reverse transcription	1	1	48°C	10 minutes
RT inactivation/initial denaturation	2	1	95°C	10 minutes
Amplification	3	40	95°C 60°C	15 seconds 45 seconds

Data analysis

Refer to your real-time PCR instrument user guide for instructions on how to analyze your data, using the following method.

Table 2 Data analysis

Method	Details
Use the **Control-Based Threshold** setting for data analysis.	1. Select **Manual C_T**. 2. Export ΔRn values for the positive control samples (Influenza Virus-Xeno™ RNA Control, 1000 copies/µL). 3. Average the FAM™ and VIC® values (separately) for the ΔRn at cycle 40 for all replicates of the positive control reaction. 4. Set the threshold for the AIV RNA reactions at 5% of the average maximum fluorescence value of the AIV RNA amplification signal in the positive control reactions. Example: If the average maximum fluorescence value for the AIV RNA target in the positive control reactions is 3.0, set the AIV RNA threshold at 0.15. 5. Repeat step 4 for the Xeno™ RNA Control target using a 5% threshold. Example: If the average maximum fluorescence value for the Xeno™ RNA target in the positive control reactions is 2.0, set the Xeno™ RNA threshold at 0.1.
Check the raw fluorescence data.	Verify that increased fluorescence seen in the normalized data is also evident without mathematical data processing.

Interpretation of test results

Table 3 Criteria for a valid real-time RT-PCR run

Reaction type	C_T value for AIV RNA	C_T value for Xeno™ RNA Control
Positive control	25–29	25–29
NTC	40 (undetermined)[1]	40 (undetermined)[1]
Extraction control	40 (undetermined)[1]	27.5–34

[1] The run is invalid if the C_T values for either AIV or Xeno™ RNA Control targets in the NTC are <40, see "Troubleshooting" on page 4.

Table 4 Interpretation of sample test results

C_T value for AIV RNA	C_T value for Xeno™ RNA Control	Interpretation
<38	27.5–34[1]	AIV-positive sample
40 (undetermined)	27.5–34	AIV-negative sample
38–40	27.5–34[2]	Suspect result

[1] If the C_T value falls outside of this range, see "Troubleshooting" on page 4.
[2] If the C_T value falls outside of this range, see Table 5.

Table 5 Assessment of suspect results

Result	Action	
Suspect result: The sample AIV C_T value is 38–40.	Analyze suspect RNA samples for the presence/absence of RT-PCR inhibitors by calculating the Xeno™ RNA C_T shift: **Xeno™ RNA C_T Shift = SS – XEC**, where: **SS** = C_T of Xeno™ RNA in the suspect sample **XEC** = Average C_T of Xeno™ RNA in the extraction controls	
	Workflow A **Xeno™ RNA C_T shift ≥1.5**	**Workflow B** **Xeno™ RNA C_T shift <1.5**
	1. Repeat the real-time RT-PCR with 2 µL of the suspect RNA sample. (RT-PCR inhibitors may be present in the RNA.) If the AIV C_T value is: • <38—The sample is AIV positive. No further testing is required. • ≥38—Continue with steps 2 through 5 of this procedure. 2. Dilute the original diagnostic sample 1:4. 3. Repeat the RNA purification on triplicate aliquots of the diluted sample. 4. Repeat the real-time RT-PCR with 8 µL of purified RNA from step 3. 5. Determine the number of samples with a AIV C_T value <40: • 0 of 3: AIV negative • 1 of 3: Presumptive positive; confirm with secondary test method • ≥2 of 3: AIV positive	1. Repeat the RNA purification on triplicate aliquots of the original diagnostic sample. 2. Repeat the real-time RT-PCR with 8 µL of purified RNA from step 1. 3. Determine the number of samples with a AIV C_T value <40: • 0 of 3: AIV negative • 1 of 3: Presumptive positive; confirm with secondary test method • ≥2 of 3: AIV positive

Confirmatory testing

All samples generating a positive or presumptive positive test result with the VetMAX™-Gold AIV Detection Kit should be submitted to a national laboratory (or equivalent authorized laboratory) for confirmatory testing.

Troubleshooting

Observation	Possible cause	Recommended action
Positive control reaction Influenza Virus-Xeno™Control RNA—no signal Xeno™ RNA Control—no signal	The Influenza Virus-Xeno™ RNA Control Mix was improperly handled, resulting in RNA degradation.	Use appropriate precautions against RNase contamination when handling the control RNAs. For example, wear clean gloves and use nuclease-free barrier pipette tips.
	The Multiplex RT-PCR Enzyme Mix was stored or handled improperly, and it lost activity.	Repeat the RT-PCR with fresh reagents.
	The thermal cycler was not properly set up.	Check the thermal cycler settings. See "Set up and run the real-time PCR instrument" on page 3.
	The RT-PCR master mix was prepared incorrectly.	Repeat the test with correctly prepared RT-PCR master mix.

Observation	Possible cause	Recommended action
NTC or extraction control reaction C_T value is <40	There was contamination during the RNA extraction or PCR.	• Repeat the RNA isolation or real-time RT-PCR with fresh reagents and freshly decontaminated pipettes. • Set up the real-time RT-PCR in an area separate from areas used for RNA isolation and PCR product analysis.
Test samples Xeno™ RNA Control—no or low signal AIV RNA—high signal	The Xeno™ RNA Control primers and probe are at limiting concentrations in the RT-PCR. High levels of AIV RNA in a sample can reduce amplification of Xeno™ RNA Control.	No or low signal from Xeno™ RNA Control is expected in a reaction that has a strong signal for AIV RNA.
Test samples Xeno™ RNA Control—no signal AIV RNA—no signal or inconclusive-range signal	Poor RNA recovery.	Check the C_T values of Xeno™ RNA Control in the mock-purified samples. $C_T \geq 38$: indicates that Xeno™ RNA Control was omitted or that RNA recovery was poor. Repeat the RNA purification of the original diagnostic sample.
	The RNA samples contain inhibitors of RT-PCR.	See Table 5.

Explanation of symbols

The symbols present on the product label are explained in the following table.

	MANUFACTURER		USE BY
REF	CATALOG NUMBER		CONSULT INSTRUCTIONS FOR USE
LOT	BATCH CODE		CAUTION, CONSULT ACCOMPANYING DOCUMENTS
SN	SERIAL NUMBER		UPPER AND LOWER LIMITS OF TEMPERATURE

Good laboratory practices for PCR and RT-PCR

When preparing samples for PCR or RT-PCR amplification:

- Wear clean gloves and a clean lab coat (not previously worn while handling amplified products or used during sample preparation).
- Change gloves whenever you suspect that they are contaminated.
- Maintain separate areas and dedicated equipment and supplies for:
  - Sample preparation and reaction setup.
  - Amplification and analysis of products.
- Do not bring amplified products into the reaction setup area.
- Open and close all sample tubes carefully. Avoid splashing or spraying samples.
- Keep reactions and components capped as much as possible.
- Use a positive-displacement pipettor or aerosol-resistant barrier pipette tips.
- Clean lab benches and equipment periodically with 10% bleach solution or DNA*Zap*™ Solutions (Cat. no. AM9890).

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

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For support visit **lifetechnologies.com/support** or email **techsupport@lifetech.com**

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25 March 2014

life technologies™

VetMAX™ NDV Kit

TaqMan® real-time RT-PCR for the detection of paramyxovirus type 1

Catalog Number NDV

Doc. Part No. 100020401 **Pub. No.** MAN0008826 **Rev.** B.0

Technology	Species	Samples	Test type
Real-time RT-PCR (RNA) • Duplex assay • Xeno™ RNA Control	Poultry	Tracheal, cloacal swabs Feces Tissues	Individual

⚠ **WARNING!** Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support.**

⚠ **WARNING! POTENTIAL BIOHAZARD.** Read the biological hazard safety information at this product's page at **thermofisher.com.** Wear appropriate protective eyewear, clothing, and gloves.

Product information

Name, intended use, and principle of the procedure

The **Applied Biosystems™ VetMAX™ NDV Kit** (Cat. No. NDV) enables real-time reverse transcription PCR (RT-PCR) detection of paramyxovirus type 1 extracted from swabs, feces, or tissues.

NDV is an enveloped, single-stranded RNA type 1 paramyxovirus responsible for Newcastle Disease, an infectious and contagious disease which affects domestic and wild birds.

The VetMAX™ NDV Kit provides assays and reagents required for single-well, real-time RT-PCR in which RNA is transcribed to cDNA, and NDV and Xeno™ RNA targets are amplified and detected using fluorescent TaqMan® probes (hydrolysis probe chemistry). It contains:

- 25X NDV Primer Probe Mix: primers and TaqMan® probes for optimized duplex real-time PCR amplification of NDV target and Xeno™ RNA target.
- 2X qRT-PCR Buffer: RT-PCR buffer.
- 25X qRT-PCR Enzyme Mix: RT-PCR enzyme.
- 25X NDV Control RNA: serves as an external positive control for the real-time RT-PCR components and it is also used to set the cycle threshold (C_t) for evaluating test results.
- Xeno™ RNA Control: the internal positive control is added to each sample and control at the lysis step of the RNA extraction procedure. It serves as an internal positive control for the RNA purification process and monitors for the presence of RT-PCR inhibitors.

Kit contents and storage conditions

Reagents for 100 25-µL real-time RT-PCR tests are supplied.

Component	Volume	Storage
25X NDV Primer Probe Mix	110 µL	–30°C to –10°C
2X qRT-PCR Buffer	1375 µL	–30°C to –10°C
25X qRT-PCR Enzyme Mix	110 µL	–30°C to –10°C
Nuclease-free Water	1750 µL	–30°C to +25°C
25X NDV Control RNA	15 µL	–25°C to –15°C
Xeno™ RNA Control (10,000 copies/µL)	110 µL	–25°C to –15°C
Nucleic Acid Dilution Solution	500 µL	–30°C to –10°C

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. MLS: Fisher Scientific (**fisherscientific.com**) or other major laboratory supplier.

Item	Source
Real-time PCR thermal cycler capable of detecting: • FAM™ (maximum emission: λ515 nm) • CAL Fluor™ Orange 560 (maximum emission: λ561 nm)	MLS
96-well plate, PCR strips (8- or 12-wells), microtubes or capillaries compatible with thermal cycler used	MLS
Suitable plate covers or caps	MLS
Nuclease-free pipettes and filtered pipette tips	MLS
Nuclease-free reagent tubes for preparing master mix	MLS
2 ice buckets or refrigerated racks: • One for the PCR setup area where the RT-PCR master mix is prepared • One for the area where RNA may be present	MLS
1X TE buffer	MLS
DNase/RNase-free water	MLS

Isolate RNA from samples

RNA extraction from initial samples is required prior to real-time PCR analysis.

Step or process	Recommendation
Preparation of mock-purified samples for use as extraction control	Prepare mock-purified samples, using nuclease-free water as the starting material. Process with the same RNA isolation method that is used for test samples.
Proposed RNA isolation methods	MagMAX™-96 Viral RNA Isolation Kit (Cat. Nos. AM1836, AMB18365) or an equivalent RNA isolation method.
Required modifications to the RNA isolation method	• Add 1 µL of undiluted Xeno™ RNA Control per isolation to the lysis solution used for RNA isolation. • Add carrier RNA to the lysis solution according to the manufacturer recommendations. Carrier RNA is provided in the MagMAX™-96 Viral RNA Isolation Kit (Cat. Nos. AM1836, AMB18365)

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Perform real-time RT-PCR

1 Determine the quantity of reactions and thaw the reagents.

a. For each real-time RT-PCR run, include the following reactions:
- Positive control; use 10.5 µL of diluted NDV Positive Control.
- Extraction control; use 10.5 µL of mock-purified samples.
- No-template control; use nuclease-free water in place of sample RNA.

b. Thaw PCR master mix reagents in one ice bucket or refrigerated rack and controls and samples in a separate ice bucket or refrigerated rack, gently vortex each tube to mix the contents thoroughly, then briefly centrifuge to collect the solution at the bottom of the tube. Keep the reagents on ice or on a refrigerated rack.

2 Prepare the diluted NDV Positive Control.

Combine the following components and place the mixture on ice between 2°C and 8°C for immediate use, or below –16°C for later use.

Component	Volume
Nucleic Acid Dilution Solution	9.5 µL
25X NDV Control RNA	1.0 µL
Total volume of diluted NDV Positive Control	10.5 µL

3 Prepare the real-time RT-PCR master mix on ice.

Combine the following components for the number of reactions required plus 10% overage.

Component	Volume per reaction
2X qRT-PCR Buffer	12.5 µL
25X qRT-PCR Enzyme Mix	1.0 µL
25X NDV Primer Probe Mix	1.0 µL
Total volume of real-time RT-PCR master mix	14.5 µL

4 Set up the real-time RT-PCR reactions.

a. Dispense 14.5 µL of real-time RT-PCR master mix to the appropriate PCR plate well, PCR strips, or capillaries.

b. Add the appropriate component for the sample type, according to the following table:

Sample type	Component	Volume per reaction
Test sample	Sample RNA	10.5 µL
Positive control	Diluted NDV Positive Control	10.5 µL
Extraction control	Mock-purified sample	10.5 µL
No-template control	Nuclease-free water	10.5 µL

c. Seal each reaction vessel.

5 Set up and run the real-time RT-PCR instrument.

a. Following the manufacturer's instructions, set up the following parameters for the real-time RT-PCR run.
- Reaction volume: 25 µL
- ROX™ passive reference dye: Included in 2X qRT-PCR Buffer (required if the thermal cycler is capable of ROX™ detection)

b. Set up and assign TaqMan® probe reporter dyes and quenchers for each well, tube, or capillary used in the analysis:

Target	Reporter	Quencher
NDV	FAM™ dye	BHQ™-1 dye
Xeno™ RNA Control	CAL Fluor™ Orange 560 dye	BHQ™-1 dye

Note: Use CAL Fluor™ Orange 560 dye to calibrate the thermal cycler, if possible. Otherwise use equivalent dye detectors such as VIC™.

c. Run the thermal cycler program and collect real-time amplification data during the elongation step (45 seconds at 60°C). Use the following thermal cycler settings:

Stage	Repetitions	Temperature	Time
1	1×	48°C	10 minutes
2	1×	95°C	10 minutes
3	40×	95°C	15 seconds
		60°C	45 seconds

Data analysis

Refer to the recommendations of the thermal cycler manufacturer for raw data analysis.

1. Set thresholds separately for each real-time RT-PCR target.
2. Interpret the results for each control and samples according to the obtained C_t values as indicated in the following section.

Validation

The run is validated if the following criteria are met.

Control reaction	C_t value for NDV RNA	C_t Value for Xeno™ RNA Control
Positive control	24–27	>40 (no signal detected)
Extraction control	>40 (no signal detected)	29–34
No-template control	>40 (no signal detected)	>40 (no signal detected)

Interpretation

C_t value for NDV RNA	C_t Value for Xeno™ RNA Control	Interpretation
<40	<40	Sample positive for NDV
>40 (no signal detected)	< C_t Extraction control ± 3C_t	Sample negative for NDV
>40 (no signal detected)	>40 (no signal detected)	Not validated[1]

[1] See "Troubleshooting".

Troubleshooting

Observation	Possible cause	Recommended action
Test samples Xeno™ RNA Control—no signal NDV RNA—high signal	The Xeno™ RNA Control primers and probes are present at limiting concentrations in the RT-PCR reactions. High levels of NDV RNA in a sample can reduce amplification of Xeno™ RNA Control.	No or low signal from the Xeno™ RNA Control is expected in a reaction that has a strong signal for NDV RNA.
Test samples NDV RNA—no signal Xeno™ RNA Control—no signal	Xeno™ RNA Control was omitted	Verify that Xeno™ RNA Control was added to the lysis solution during RNA extraction.
	Poor RNA recovery	Check the recovery of RNA carrier used in RNA extraction.
	The RNA samples contain inhibitors of RT-PCR	Reduce the quantity of sample added to the RT-PCR reactions. For example: • Add 1–2 µL of sample (add nuclease-free water to bring the reaction to the proper volume) • Dilute the RNA sample 1:10 in Nucleic Acid Dilution Solution before adding it to the reaction. • The C_t values for Xeno™ RNA Control and NDV amplifications are expected to decrease proportionally to the decrease in sample quantity (~2-3C_t).
	Poor RNA recovery Or The RNA samples contain inhibitors of RT-PCR	Compare the results of amplifying sample RNA using RT-PCR master mix with and without 1 µL of Xeno™ RNA Control: • If the reactions amplified using the RT-PCR master mix with addition of Xeno™ RNA Control return a signal, but the reactions amplified using the RT-PCR master mix without addition of Xeno™ RNA Control return no signal, Xeno™ RNA Control was not recovered. • If Xeno™ RNA Control signal is not detected in either sample, the RNA sample contains inhibitors of real-time RT-PCR.

Observation	Possible cause	Recommended action
Positive control reaction NDV Positive Control—no signal Xeno™ RNA Control—no signal	The NDV Positive Control was improperly handled, resulting in RNA degradation.	Use appropriate precautions against RNase contamination when handling the control RNAs. For example, wear clean gloves and use nuclease-free barrier pipette tips.
	The 25X qRT-PCR Enzyme Mix was stored or handled improperly, and it lost activity.	Repeat the RT-PCR with fresh reagents.
	The thermal cycler was not properly set up.	Check the thermal cycler settings. See "Set up and run the real-time PCR instrument." on page 5.
	The RT-PCR master mix was prepared incorrectly.	Repeat the test with correctly prepared RT-PCR master mix.
No-template control reaction NDV RNA—signal detected or Xeno™ RNA Control—signal detected	Contamination during the PCR.	• Repeat the real-time RT-PCR with fresh reagents and freshly decontaminated pipettes. • Set up the real-time RT-PCR in an area separate from areas used for RNA isolation and PCR product analysis.
Extraction control reaction NDV RNA—signal detected	Contamination during RNA extraction or the PCR.	• Repeat the RNA isolation or real-time RT-PCR with fresh reagents and freshly decontaminated pipettes. • Set up the real-time RT-PCR in an area separate from areas used for RNA isolation and PCR product analysis.

Documentation and support

Customer and technical support

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Visit **thermofisher.com/support** for the latest in services and support, including:

- Worldwide contact telephone numbers
- Order and web support
- User guides, manuals, and protocols
- Certificates of Analysis
- Safety Data Sheets (SDSs; also known as MSDSs)
  NOTE: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **thermofisher.com/support**.

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Revision history of Pub. No. MAN0008826 (English)

Revision	Date	Description
B.0	9 May 2017	Updated to the current document template, with associated updates to the warranty, trademarks, and logos.
A.0	10 April 2014	Baseline for revision history