FOR VETERINARY USE ONLY

Vcheck M
Canine Diarrhea 8 Panel

For use with Vcheck M10 system

BIONOTE

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REF VCM107

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IVD

1. Intended Use

Vcheck M Canine Diarrhea 8 Panel test is a multiplex real-time PCR test intended for use with Vcheck M10 system for the qualitative detection of nucleic acids from Canine parvovirus, Canine coronavirus, Canine distempervirus, *Giardia lamblia*, *Cryptosporidium* spp., *Salmonella* spp., *Clostridium perfringens, Campylobacter* spp. (Hereinafter, 8 target pathogens) in feces collected from dogs.

Results include the identification of nucleic acids from 8 target pathogens. Positive results are indicative of the presence of 8 target pathogens nucleic acids; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out viral infection or co-infection with other bacteria or protozoa. The agent detected may not be the definite cause of the disease.

Negative results do not preclude 8 target pathogens infection and should not be used as the sole basis for treatment or other patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

Vcheck M Canine Diarrhea 8 Panel test is intended to be performed by trained users in both laboratory and animal hospitals.

2. Summary and Explanation

Diarrhea is a condition in which there is an increased amount or more frequent occurrence of loose bowel movements compared to usual. Diarrhea is not a disease in itself but rather a clinical symptom of various underlying conditions. Mild cases of diarrhea can often be resolved with simple treatments, but they can also result in more serious diseases. In such cases, it is important to seek prompt medical attention to identify and treat the underlying cause.
This kit is supportive for the diagnosis of infection of 8 target pathogens. The test results are only for clinical reference and cannot be used as a basis for confirming or excluding cases by itself.

Vcheck M Canine Diarrhea 8 Panel test is a molecular *in vitro* diagnostic test that aids in the detection and diagnosis of 8 target pathogens and is based on widely used nucleic acid amplification technology. Vcheck M Canine Diarrhea 8 Panel test contains primers, probes and internal control (IC) used in PCR for the qualitative detection of 8 target pathogens nucleic acids in fecal samples.

Cartridge Description

Vcheck M Canine Diarrhea 8 Panel cartridge is a disposable plastic device that allows performance of fully automated molecular assays by containing all reagents required for the test.
Within the cartridge, multiple steps are automatically performed in sequence using pneumatic pressure to transfer samples and fluids via the part to their intended destinations.

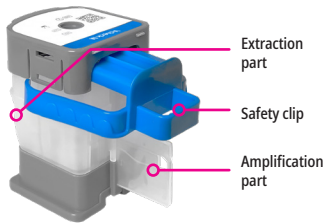

Figure 1. Layout of Vcheck M Canine Diarrhea 8 Panel cartridge

3. Principle of the Procedure

Vcheck M Canine Diarrhea 8 Panel test is an automated *in vitro* diagnostic test for qualitative detection of nucleic acid from 8 target pathogens. This test is performed on Vcheck M10 system.

Vcheck M10 system automate and integrate sample preparation, nucleic acid extraction and amplification, and detection of the target sequences in various samples using molecular diagnostic assays. The system consists of the Vcheck M10 Module and the Vcheck M10 Console with preloaded software for running tests and viewing the results. The system requires the use of single-use cartridges that hold the PCR reagents and host the PCR process. Because the cartridges are self-contained, cross contamination between samples is minimized. For a full description of the systems, check the user manual of Vcheck M10 system.

Vcheck M Canine Diarrhea 8 Panel test includes reagents for the detection of nucleic acids from 8 target pathogens in fecal samples. The cartridge is present to control for adequate processing of the sample and PCR reaction.
The table below indicates which target is designed to be detected by which channel.

Table 1. Fluorescent channel of each target gene pathogen

Well	Target	Channel
1	*Campylobacter* spp. *(C. coli, C. jejuni)*	FAM
	Salmonella spp. *(S. enteritidis, S. typhimurium)*	HEX
	Internal control (IC)	Cy5
2	Canine coronavirus	FAM
	Canine distempervirus	HEX
	Internal control (IC)	Cy5
3	*Cryptosporidium* spp. *(C. canis)*	FAM
	*Clostridium perfringens (*alpha, entero, netE, netF toxin gene*)*	HEX
	Internal control (IC)	Cy5
4	Canine parvovirus type 2	FAM
	*Giardia lamblia (*Assemblages A, B, C, D*)*	HEX
	Internal control (IC)	Cy5

4. Materials Provided

The Vcheck M Canine Diarrhea 8 Panel kit contains sufficient reagents to process 5 samples or quality control samples.

Table 2. Contents of the Vcheck M Canine Diarrhea 8 Panel kit

Contents	Quantity	Usage in each reaction	Note
Cartridge	5	1 ea	
Disposable swab	5	1 ea	
Stool pretreatment solution	5	1 ea	
Tube cap	5	1 ea	
Disposable dropper	5	1 ea	1.4 ml line
Stool pretreatment tool	5	1 ea	
Quick reference instructions (QRI)	1	-	
Instructions for use (IFU)	1	-	

5. Storage and Handling

Store the Vcheck M Canine Diarrhea 8 Panel kit at 2~28 °C (36~82 °F). If the cartridge has been refrigerated, perform the test after stabilizing it for 30 minutes at room temperature. Do not remove the safety clip of the cartridge and do not press the cartridge until actual use. Do not use a cartridge that has leaked or is wet. Under these conditions, cartridges can be stored until the expiration date printed on the packaging.

6. Materials Required but Not Provided

- Vcheck M10 system with User Manual
  At least one Vcheck M10 Console and one Vcheck M10 Module
- PPE (Personal Protective Equipment)
- Biohazard container
- Quality control samples (Positive control, Negative control)

7. Warnings and Precautions

1. This kit is only for *in vitro* diagnostics for dogs.
2. If the test is not performed according to this IFU, inaccurate results may be obtained.
3. Do not use this kit with any product other than the Vcheck M10 suggested by the manufacturer.
4. Samples can contain an unknown virus, bacteria, or protozoa, so be careful when handling them. If contamination is suspected, replace all tools and discard used reagents immediately.
5. It is recommended that the solid waste used in the experiment be sterilized by autoclaving at 121 °C for at least 15 minutes, and must be safely disposed of by national or regional regulations.

6. These cartridges must be used after the pretreatment of the fecal sample with supplied pretreatment tools.
7. If the fecal sample is not properly pretreated with the pretreatment tools provided by the supplier, an error may occur with the test result.
8. Use the supplied materials for the test.
9. Minimize exposure of the cartridge to light.
10. Do not remove the safety clip of the cartridge before use.
11. Do not press the cartridge until actual use. If the cartridge is exposed to moisture, the performance may deteriorate.
12. Do not use a cartridge that has leaked or is wet.
13. Do not shake the cartridge as much as possible and be careful not to spill the reagent by turning over an opened cartridge.
14. Direct contact, such as touching the amplification part of the cartridge, may affect the test result, so do not touch it with your hands.
15. Test within 10 minutes after dispensing the sample into the cartridge.
16. Cartridges are for single use only, so do not reuse processed cartridges.
17. Do not use a cartridge with a damaged barcode label.
18. Do not use reagents whose expiration date has passed.
19. A professional veterinarian must make a final diagnosis based on the results of this kit and other test results and clinical findings.

8. Sample Collection, Transport, and Storage

Proper sample collection, transportation, and storage are critical to the performance of the test. Improper sample collection, inappropriate sample handling and/or transportation can lead to false results.

In order to obtain an adequate sample, follow the test procedure provided by the supplier.

1. Collect and transfer the liquid or soft feces into the commercially available sterilized container.
2. Samples should be tested immediately after collection.
3. If samples are not tested immediately, they should be stored at 2~8 ℃ for 24 hours.
4. For longer storage of samples, samples should be kept frozen at -20 ℃ or below. Frozen samples should be brought to room temperature prior to use.

9. Procedure

Preparing the Sample

1. Stabilize all reagents and samples at room temperature for 30 minutes before testing.
2. Open carefully the lid of the sample collection container and avoid solid particles that may affect the test.

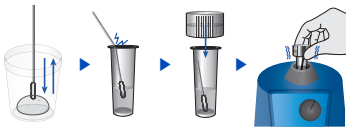

Figure 2. Sample preparation

3. Using the disposable swab, collect 0.1 g of the feces and put it in the stool pretreatment solution.
4. After closing the Tube cap, vortex the stool pretreatment solution for 10 seconds to ensure that the sample and buffer are thoroughly mixed.
5. The diluted sample is used in the process.

Starting the Vcheck M10 system

Note

For the detailed instructions, refer to the Vcheck M10 system User Manual.

If you have scanned the cartridge barcode in the Vcheck M10 and the software version is not compatible, a 'Not Supported Device' error message appears. Update the software before proceeding the test.

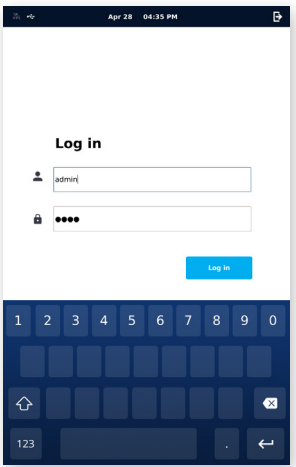

Figure 3. Log In screen

1. Turn on the Vcheck M10 system.
2. Check the Vcheck M10 Console and the Vcheck M10 Module is connected and functional.
3. Enter the ID and Password on the Log In screen of the Vcheck M10 Console and click the Log in button.

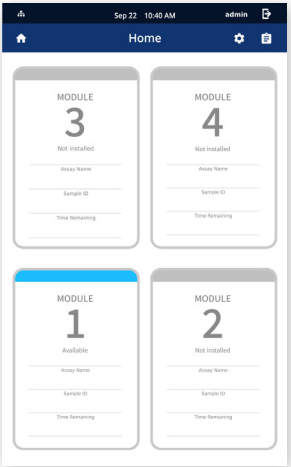

Figure 4. Home screen

4. Touch the Vcheck M10 Module to run on the Home screen.

   (The door of the selected Vcheck M10 Module will automatically open for cartridge loading.)

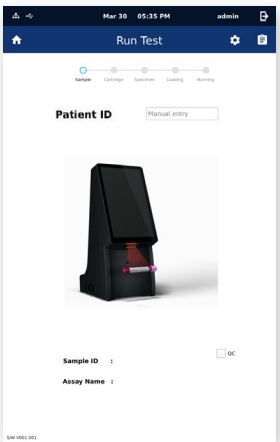

Figure 5. Entering Patient ID

5. Enter a Patient ID by scanning the barcode or using virtual keyboard on the M10 Console screen.

   (Patient ID is optional. You can turn off the Patient ID option from the 'Settings'.)

Figure 6. Entering Sample ID

6. Enter a Sample ID by scanning the barcode of the sample or using virtual keyboard on the M10 Console screen.

   (For quality control test, tick the QC check box.)

Figure 7. Scanning a cartridge

7. Scan the Vcheck M Canine Diarrhea 8 Panel cartridge to be used the Vcheck M10 Module automatically recognizes the assay to be run based on the cartridge barcode.

Loading a sample into the Vcheck M Canine Diarrhea 8 Panel cartridge

If the cartridge has been refrigerated, perform the test after stabilizing it for 30 minutes at room temperature.

Start the test within 10 minutes of loading the sample into the Vcheck M Canine Diarrhea 8 Panel cartridge.

Note

False negative results may occur if insufficient sample is added into the cartridge.

Figure 8. Sample Guide screen

1. Remove the safety clip located underneath the lid of the cartridge.
2. Pierce the sealed cartridge by pressing down the lid until fully engaged into the cartridge groove.

   *Caution: Incomplete engaging may cause a driving error.
3. Open the cartridge lid and check that the seal is completely punctured before loading a sample.
4. After separating the stool pretreatment tool, insert the lower part into the sample hole of the cartridge.
5. After collecting 1.4 mL of the diluted sample using the disposable dropper, transfer it to the lower part of the stool pretreatment tool.
6. Load the entire amount of sample into the cartridge using the upper part of the stool pretreatment tool.

   *Test within 10 minutes after dispensing the sample into the cartridge.
7. After a few seconds, Sample Guide screen will automatically change to the Insert Cartridge screen. Touch the Sample Guide screen if you want to skip the guide.
8. Close the cartridge lid.

Figure 9. Loading a sample

Running a test

Figure 10. Insert Cartridge screen

1. Load the cartridge on the selected Vcheck M10 Module with the Amplification part facing the inside of the module.

   (The status indicator of the selected module will blink green.)

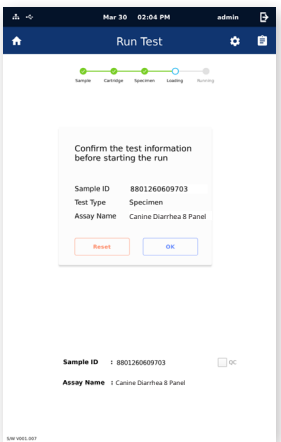

Figure 11. Confirm the test screen

2. Close the door completely.
3. After confirm the sample and cartridge information, touch the OK button on the screen.

   (Touch the Reset button to re-input the information.)

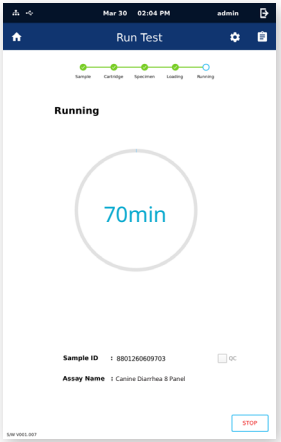

Figure 12. Running screen

4. Assay starts automatically, and remaining time will appear on the screen.

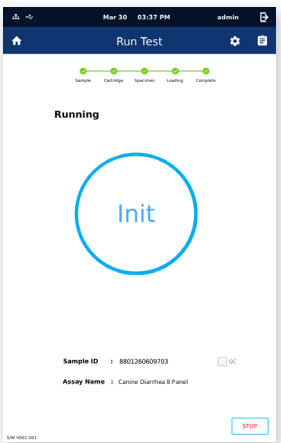

Figure 13. Test complete

5. When the run is finished, it switches to the Review screen and the result is displayed.
6. Dispose of used cartridges in the appropriate sample waste containers according to your institution's standard practices.
7. To run another test, touch the Home icon and repeat the process.

   (If another Vcheck M10 Module connected to the Vcheck M10 Console is available, you can start a new test while another test is running.)

10. Interpretation of Results

The results are interpreted automatically by the Vcheck M10 Console and are clearly shown in the Review screen. The interpretation of the Vcheck M Canine Diarrhea 8 Panel test results is determined based on Table 3. If an invalid result is obtained, perform a retest.

Table 3. Interpretation of results

Result*	Pathogen	Internal Control
Positive	+ ct value	V
Negative	− N/A	V
Invalid / Re-test	+ / −	!

* Canine Diarrhea 8 panel : Canine parvovirus, Canine coronavirus, Canine distempervirus, *Giardia lamblia*, *Cryptosporidium* spp., *Salmonella* spp., *Clostridium perfringens*, *Campylobacter* spp.

11. Performance

1. Analytical sensitivity
   [LOD, Limit of Detection]

Target	Subtype	LOD (Copies/mL)
Campylobacter spp.	*C. coli, C. jejuni*	$2.7x10^3$ Copies/mL
Salmonella spp.	*S. Enteritidis, S. Typhimurium*	$2.7x10^3$ Copies/mL
Canine coronavirus	-	$2.7x10^3$ Copies/mL
Canine distempervirus	-	$2.7x10^3$ Copies/mL
Cryptosporidium spp.	*C. canis*	$2.7x10^3$ Copies/mL
Clostridium perfringens	alpha, entero, netE, netF toxin gene	$2.7x10^3$ Copies/mL
Canine parvovirus type 2	-	$2.7x10^3$ Copies/mL
Giardia lamblia	Assemblages A,B,C,D	$9x10^2$ Copies/mL

2. Analytical Specificity

- Interference

There was no interference for potential interfering substances listed below.

Substance	Concentration
Hemoglobin	50 g/dL
Bilirubin	5 mg/dL
Mineral oil	10 % (w/v)
Mucin from porcine stomach	60 ug/mL
Amoxicillin trihydrate	22 mg/kg
Loperamide hydrochloride	0.2 mg/kg
Canine Whole Blood	1,000 mg/dL

- Cross-reactivity

There was no cross-reaction with pathogens associated with canine diseases in the stool samples for the following high-concentration substances, as listed below.

No	Substance	Result
1	*Campylobacter coli*	No cross-reaction
2	*Salmonella enterica*	No cross-reaction
3	Canine coronavirus	No cross-reaction
4	Canine distempervirus	No cross-reaction
5	*Clostridium perfringens*	No cross-reaction
6	*Giardia intestinalis*	No cross-reaction
7	Canine parvovirus type 2	No cross-reaction
8	Canine adenovirus 1	No cross-reaction
9	*Escherichia coli*	No cross-reaction
10	*Ehrlichia canis*	No cross-reaction
11	*Anaplasma phagocytophilum*	No cross-reaction
12	*Bartonella henselae*	No cross-reaction
13	*Leishmania infantum*	No cross-reaction
14	*Borrelia burgdorferi*	No cross-reaction
15	*Leptospira interrogans*	No cross-reaction

3. Precision

In the repeatability and reproducibility tests using standard materials, it was confirmed that all of the negative and 3 positive samples met the test standards for finished products.

12. Limitations

1. This kit must use feces from dogs.
2. This kit can detect nucleic acids of *Salmonella* spp. (*S. typhimurium, S. enteritidis*), but cannot distinguish them.
3. This kit can detect nucleic acids of *Campylobacter* spp. *(C. coli, C. jejuni)*, but cannot distinguish them.
4. This kit can detect nucleic acids of *Clostridium perfringens* (alpha, entero, netE, netF toxin gene), but cannot distinguish them.
5. This kit can detect nucleic acids of Canine parvovirus type 2 but cannot distinguish the subtypes.
6. This kit can detect nucleic acids of *Giardia lamblia* (Assemblages A,B,C,D), but cannot distinguish them.
7. Failure to follow the procedures in this IFU may result in inaccurate results.
8. Contamination of the laboratory environment, contamination of cartridges, and cross-contamination of samples can lead to false-positive results.
9. Incorrect handling of the kit during movement, storage and use may reduce the sensitivity of the reagent detection results and lead to erroneous results.
10. This kit is designed to automatically perform nucleic acids extraction, amplification and detection of 8 target pathogens but if a mutation occurs in the detection target sequence, it may not be detected.

13. References

1. Aline Baumann da Rocha Gizzi et al., Presence of infectious agents and co-infections in diarrheic dogs determined with a real-time polymerase chain reaction-based panel, BMC Veterinary Research volume 10, Article number: 23 (2014)
2. MW Kim, et al., Faecal PCR panel results and clinical findings in Western Australian dogs with diarrhoea, Australian Veterinary Journal (2020)

14. Symbols

Symbols	Description	Symbols	Description
	Manufacturer		Note
	Consult instructions for use		Use by date
	Reference number		Temperature limit
	Batch code		Do not re use
	Date of manufacture Indicates the date of manufacture		Indicates to keep the analyzer dry that you should keep the analyzer dry
	Do not use if packaging is damaged		Keep away from sunlight
	Contains Sufficient for <n> Tests		Do not use if packaging is damaged

Vcheck M
Canine Diarrhea 8 Panel

For use with Vcheck M10 system

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