

[Reagent for food testing]

NH IMMUNOCHROMATO VT 1/2 <<Instruction Manual>>

Please read this manual before using this kit.

Cat# NPH-999700000

[Introduction] Enterohemorrhagic Escherichia coli (E. coli), which has been reported in association with extensive food poisoning or deaths, induces symptoms by producing verotoxins (VTs). The verotoxins are broadly classified into verotoxin type 1 (VT1) and verotoxin type 2 (VT2), which are known to have very strong toxicity.

In Japan, a number of serotypes have been detected such as O157 and O26¹⁾. For these serotypes, test methods²⁾ have already been provided through notification from the reguratory authority. For other E. coli. serotypes, examination of verotoxins in food is effective in preventing enterohemorrhagic food poisoning. Furthermore, a verotoxin test should be performed if enterohemorrhagic E. coli is detected because verotoxin production is used to confirm enterohemorrhagic E. coli.

This product is a kit for detecting verotoxin using immunochromatography. With this kit, simple tests can be conducted rapidly to differentiate VT1 and VT2.

[Product Features]

- 1) The simple one-step operation of the kit.
- The test gives rapid results. 2)
- 3) There is no need for special test equipment.

[Kit Contents]

1) Components

1)

- A: Test strip 2-test × 10 packs
- B: Instruction manual 1 sheet 1 bag
- C: Plastic pouched bag

Illustration of Test strip

2)	Ingredients
(1)	Reagent-containing section
	Gold colloid-labeled anti-VT1 antibody (rabbits)
	Gold colloid-labeled anti-VT2 antibody (rabbits)
(2)	Detecting section
	Anti-VT1 antibody (rabbits)
	Anti-VT2 antibody (rabbits)
	Anti-rabbit immunoglobulin antibody (goat)

[Application]

- Detection of VT1 and VT2 in Foods 1)
- 2) Examination of VT1 and VT2 production from isolated enterohemorrhagic E. coli.

[Illustration of Test strip and the Principle of assay]



Principle of assay



- a. Sample solution drop section (Be careful not to touch this section with your finger.))
- b. Reagent-containing section
- с. Detecting section (Be careful not to scratch this section and touch this section with your finger.))
- d. Absorbent pad
- Measurement items listing position e.
- Test line for detection VT1 appearance position f. (Approx.28mm from the sample solution drop section.)
- g. Test line for detection VT2 appearance position (Approx.30mm from the sample solution drop section.)
- h. Control line appearance position (Approx.40mm from the sample solution drop section)

When a sample solution is dropped onto the sample solution drop section, gold colloid-labeled anti-VT1 antibodies and gold colloid-labeled anti-VT2 antibodies (2) in the Reagent-containing section dissolve and form complexes with verotoxins (1). These complexes move to the detecting section by capillary attraction and are trapped by anti-VT1 or anti-VT2 antibodies (3) that is fixed in the test line appearance position. This results in the appearance of a reddish purple line of gold colloid. .This reddish purple line can be detected by visual inspection and used to judge the presence or absence of VT1 and VT2 in the sample solution.

The excess gold-labeled antibodies, regardless of the presence or absence of verotoxin in the sample solution, travel further through the detecting section and are trapped by the anti-rabbit immunoglobulin antibodies (4) fixed at the control line appearance position, where they form a second reddish purple line. The presence of this line indicates that the sample solution has reached the detecting section.



[Preparation of the sample solution 1 (detection in food)]

1) Required Equipment and Instruments

Stomacher bag (preferably with a filter), stomacher, incubator, autoclave, micropipettes and tips, centrifuge, constant temperature water bath, mEC broth with novobiocin and polymyxin B solution, etc.

2) **Preparation of Test Samples**

- (1) Weigh out 25 g of test food in stomacher bag to use as specimen.
- (2) Add 225 mL of mEC broth with novobiocin to the 25g specimen in stomacher bag and homogenize with a stomacher for 1 minute.
- (3) Incubate the specimen in the stomacher bag at 42° C for 18–24 hours.
- (4) Remove the stomacher bag from the incubator after 18-24 hours. Gently mix the contents of the stomacher bag, taking care not to splash it.
- (5) Dispense 1 mL of the culture medium into sterilized vessels using a sterilized pipette.
- (6) Add polymyxin B solution to the dispensed culture medium so that the final concentration is 0.5 mg/mL.
- (7) Warm the culture medium supplemented with polymyxin B solution in a constant temperature water bath set at 37 °C for 30 minuts with shaking every 5 to 10 minutes.
- (8) After 30 minutes, centrifuge the culture medium at $1,500 \times \text{g}$ for 15 minutes and use the supernatant as the sample solution.
- Note 1: When performing the test, take preventive measures against infection such as wearing protective groves and glasses, because Infectious samples solutions are used.
- Note 2: Because the remainder of the culture solution might be required for use in confirmatory tests following those conducted with the kit, do not sterilize it and retain it until all the tests have been completed.

[Preparation of Sample Solution 2 (detection in isolated colony)]

1) **Required Equipment and Instruments**

Incubator, autoclave, micropipettes and tips, centrifuge, constant temperature water bath, CAYE culture medium, CAYE broth supplement, culture medium for enterohemorrhagic E. coli, and polymyxin B solution, etc.

2) Preparation of Test Samples

2)-1 If isolation medium for enterohemorrhagic E. coli is 2)-2 If CAYE medium is used: used:

- (1) Inoculate the test colony onto the isolation medium for enterohemorrhagic E. coli and incubate it at 37 °C for 19 to 24 hours.
- (2) After the incubation, collect colonies on the medium surface (one third to all surface of the plate medium) and suspend them in 1 mL of 0.5 mg/mL polymyxin B solution.
- (3) Warm the suspension in a constant temperature water bath set at 37 °C for 30 minutes with shaking every 5 to 10 minutes.
- (4) After 30 minutes, centrifuge the suspension at $1,500 \times g$ for 15 minutes to use the supernatant as the sample solution.

- (1) Inoculate the test colony into 1 mL of CAYE medium (containing CAYE broth supplement) and incubate at 37 °C for 6 to 24 hours.
- After the incubation, add polymyxin B solution so that the (2)final concentration is 0.5 mg/mL.
- (3) Warm the medium supplemented with polymyxin B solution in a constant temperature water bath set at 37 °C for 30 minutes with shaking every 5 to 10 minutes.
- (4) After 30 minutes, centrifuge the culture medium at $1,500 \times$ g for 15 minutes to use the supernatant as the sample solution.
- Note 1: When performing the test, take preventive measures against infection such as wearing protective groves and glasses, because infectious samples solutions are used.
- Note 2: Because the remainder of the culture solution might be required for use in confirmatory tests following those conducted with the kit, do not sterilize it and retain it until all the tests have been completed.

[Operating Procedures for Testing]

1) NH Immunochromato VT 1/2 Test Procedures

- (1) Bring the test strip contained in the aluminum pouch to room temperature and remove from the pouch immediately before use.
- (2) With an oil-based marker pen, write the name of the test sample or the number of the subject under test on the absorbent pad of the test strip removed from the pouch.
- (3) Place the test strip carefully on the flat stand and drop a 100 μ L-portion of the sample solution onto the sample solution drop section (see the figure on the left). Otherwise, dispense a 150 µL-portion of the sample solution into a test tube and attach the test strip to the test tube so that the test sample drop section of the test strip is immersed in the sample solution (see the figure on the right).
- (4) Allow the test strip to stand undisturbed for 15 minutes and then visually judge the presence or absence of VT 1/2 in the solution.





- Note 1: Do not remove the test strip from the aluminum pouch until it has returned to room temperature, otherwise incorrect test results may be obtained as a result of moisture absorption. Test strips which are not used should be placed in the plastic pouch bag again together with a desiccant, and should be preserved in a refrigerator.
- Note 2: Be careful not to scratch the sample solution drop section or detecting section and do not touch them with your fingers. When handling the test strip, make sure that you hold the absorbent pad.
- Note 3: Make sure that you use a sterilized pipette or chip to drop or dispense the sample solution. Change the pipette or chip for every sample solution.
- Note 4: Make sure that the 100-µL portion of sample solution does not overflow the test strip when dropping it. If necessary, drop the solution in two or more portions.
- Note 5: It is recommended that a wrap etc. should be placed under the test strip when dropping the sample solution.

3) Judgment of the Test Result

(Use the Reference Sheet at the end of this Instruction Manual)

- (1)-1 The test results is judged as VT1 positive when a reddish purple line is observed at the VT1 test line appearance position and at the control line appearance position 15 minutes after the start of the test.
- (1)-2 The test results is judged as VT2 positive when a reddish purple line is observed at the VT2 test line appearance position and at the control line appearance position 15 minutes after the start of the test.
- (1)-3 The test results is judged as VT1 positive and VT2 positive when a reddish purple line is observed at the VT1 test line appearance position, the VT2 test line appearance position , and control line appearance position 15 minutes after the start of the test.
- (2) Judge the test results as negative when no reddish purple line is observed at the test line appearance position, but a line is observed at the control line appearance position.
- (3) Retest in cases where no reddish purple line is observed at the control appearance position, regardless of the presence or absence of a line at the test line appearance position. It is likely that there is something abnormal in the development of the sample solution in such cases.

Note 1: Samples judged as positive using this kit must be subject to identification test by other method such as PCR.

Note 2: When concentrated VT1 or VT2 exists in the sample solution, it may be observed VT1 test line and VT2 test line together.

[Performance]

1) Sensitivity Test

The results of tests conducted in accordance with instructions for the preparation of the sample solution and the operating procedures for testing described in this manual will be positive when the concentration of both VT1 and VT2 is more than 2.5 ng/mL.

2) Repeatability Tests

When VT1 and/or VT2 sample solution and negative sample solutions (mEC broth with novobiocin and CAYE medium) were simultaneously tested three times each, all positive sample solutions exhibited positive results and all negative sample solutions showed negative results.

3) Minimum Detection Sensitivity

The results of testing of purified VT1 and VT2 confirmed that the minimum detection sensitivity is 2.5 ng/mL for both VT1 and VT2.

Note 1:The minimum detection sensitivity of this kit could vary depending on the effects of the components of the sample solution.

4) Cross-reactivity

(1) Cross-reactivity with the following bacterial strains has not been observed.

	Strain No.	Results
Escherichia coli	ATCC 43888, 700728, 25922, 11775	—
Escherichia hermanii	JCM 1473	—
Citrobacter freundii	ATCC 8090	—
Enterobacter aerogenes	ATCC 13048	—
Enterobacter cloacae	ATCC 13047, 49141	—
Enterobacter sakazakii	ATCC 51329	—
Klebsiella oxytoca	ATCC 8724	—
Serratia liquefaciens	ATCC 27592	—
Serratia marcescens	ATCC 8100	—
Serratia odorifera	ATCC 33077	—
Proteus vulgaris	ATCC 6380	_
Pseudomonas aeruginosa	ATCC 9027	—





[Precautions in Using the Kit]

1) Precautions in Handling the Kit

- (1) Read the instruction manual carefully before use. Use the kit in accordance with the test method described in this manual.
- (2) This instruction manual is intended as a guideline for those in charge of testing. Verify your own operating procedures for the kit and the appropriateness of its use for each particular food.
- (3) Do not use a kit whose use-by date has passed. The expiry date is indicated on the label on the external package of the kit and on the aluminum pouch of the test strip.
- (4) This kit is a reagent designed to detect VT1 and VT2 in food or isolated colony. It is not be used for clinical diagnosis.
- (5) Tests may give false-positive results as a result the effects of the ingredients present in the specimen and concentration of verotoxin. Positive test results from the kit should be confirmed by other test methods or procedures.
- (6) Confirm with the manufacturers or distributors that and any instruments and reagents (including culture media) used for preparation of sample solutions are suitable for the purpose.
- (7) Product specifications may be changed without notice.

2) Precautions Regarding Risk Prevention

- (1) Even minute amounts of Verotoxin, which the kit is designed to detect, presents strong toxicity. Infection with microorganisms such as *E. coli* may occur. Thus, pay full attention in conducting tests by wearing protective gloves and safety glasses.
- (2) Tests should be performed only where appropriate equipment and facilities are available. Follow standard microorganism testing procedures under the guidance of responsible supervisors.
- (3) If you accidentally get any sample solution in your eyes or mouth, adopt emergency measures, such as immediately washing away the solution with tap water, and then seek medical attention.
- (4) If you feel unwell after performing a test with the kit, obtain immediate treatment from a physician.

3) Precautions Regarding Disposal of Waste Materials

- (1) Note that surplus sample solutions and used test strips, culture media, and test samples could carry contagious microorganisms. Therefore, make sure that waste materials are subject to appropriate sterilization, for example by autoclave treatment for 20 minutes at 121 °C or immersion of the materials in a sodium chlorite solution for more than 1 hour.
- (2) Discard the kits, test samples, and surplus sample solutions in strict compliance with your local waste-disposal regulations and with full consideration of environmental sanitation.

[Storage Method and Use-By Date]

- 1) Storage method: Refrigerate at 2–8 °C and shade from the light. Avoid freezing.
- 2) Use-by date: 12 months from the date of manufacture.

[Packaging Unit]

NH Immunochromato VT 1/220 tests

[References]

- 1) Akemi Kai, et al., Clinical Diagnosis and Microorganism, 23, pp 827–834, 1996.
- 2) Ministry of Health, Labour and Welfare, Inspection Method for Intestinal hemorrhagic *E. coli* O157 and O26 (Shokuankanhatsu No. 1102004, November 2, 2006)





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NHイムノクロマト VT1/2 説明資料



人輝く、食の未来

日本ハム株式会社 中 央 研 究 所



ベロ毒素について

NHイムノクロマト VT1/2の検出対象であるベロ毒素(Verotoxin)は、O157 をはじめとする腸管出血性大腸菌が産生する毒素で食中毒の原因物質。

ベロ毒素は、ベロ毒素1型(VT1)と2型(VT2)に分類される。

<ベロ毒素検査の必要性>

- ●腸管出血性大腸菌の確定は、ベロ毒素産生性確認により実施される。
- 通知法*では大腸菌O157、O26の分離に先立ち、ベロ毒素産生遺伝子によりスクリーニングを行うことが認められている。

※(「腸管出血性大腸菌0157及び026の検査法について(平成18年11月2日付け食安監発第1102004号通知)」

●腸管出血性大腸菌は多種類あり、O抗原だけで130種類以上報告されている。

	症例数	0157	O26	0111	その他
2006年	3, 922例	68. 6%	22. 1%	2.8%	6. 5%
2007年	4, 617例	74. 3%	11. 5%	5.5%	8.7%
2008年	4, 321例	64.0%	21. 2%	3.9%	10. 9%

参考資料:腸管出血性大腸菌感染症における血清型検出頻度

感染症情報センターホームページより抜粋

血清型が多数存在する腸管出血大腸菌による食中毒を防止するためには、 大腸菌O157、O26、O111の検査に加え、 ベロ毒素の検査を実施することが望ましい



NHイムノクロマトVT1/2





く特徴>

低コスト

1テスト700円と現在市販されているキットの中で最も安価です。

簡便な操作と容易な判定

試料溶液をテストプレートに滴下し、15分後に赤紫色のラインを 確認するだけです。

VT1とVT2の識別が可能

1本のテストストリップでベロ毒素の有無だけでなく、VT1とVT2の 識別が可能です。

大腸菌O157、O26、O111と同時に検査可能 大腸菌O157、O26、O111検査用に増菌した培養液から検査可 能です。

<NHイムノクロマトシリーズラインナップ>

品名	容量	希望納入価
NHイムノクロマト O157	20回用	10, 000円
NHイムノクロマト O26	20回用	10, 000円
NHイムノクロマト 0111	20回用	10, 000円
NHイムノクロマトサルモネラ	20回用	10, 000円
NHイムノクロマトリステリア	20回用	14, 000円
NHイムノクロマトカンピロバクター	20回用	14, 000円
NHイムノクロマト VT1/2	20回用	14, 000円

●貯法:冷蔵(2~8℃)保存 ●使用期限:製造日より12ヶ月



NHイムノクロマトVT1/2試験方法NH Nippon Ham Group





NHイムノクロマトVT1/2試験方法NH Nippon Ham Group



コスモ・バイオ株式会社

NHイムノクロマトVT1/2試験方法NH Nippon Ham Group



- ①-1: 試験開始後15分後にVT1テストライン出現位置 およびコントロールライン出現位置に赤紫色のライ ンが観察された場合には、VT1陽性と判定。
- ①-2: 試験開始後15分後にVT2テストライン出現位置 およびコントロールライン出現位置に赤紫色のライ ンが観察された場合には、VT2陽性と判定。
- ①-3: 試験開始後15分後にVT1テストライン出現位置、 VT2テストライン出現位置、およびコントロールラ イン出現位置に赤紫色のラインが観察された場合 には、VT1およびVT2陽性と判定。
 - テストライン出現位置に赤紫色のラインが観察さ れず、コントロールライン出現位置にのみ赤紫色 のラインが観察された場合には陰性と判定。
 - コントロールライン出現位置に赤紫色のラインが観 察されない場合には、テストラインの有無に関わら
- 注1:赤紫色のラインの濃淡に関わらず、ラインが観察された場合には陽性と判
- 注2: 陽性を示した検体については、他の方法(PCR法等)にて必ず確認試験を

注3: 高濃度のVT1もしくはVT2が試料溶液中に存在する場合、VT1テストライン およびVT2テストラインともに観察される可能性あり。



性能~精製ベロ毒素での感度試験~



【試験方法】

- ① VT1およびVT2の精製品をmEC培地にて希釈
- ② 希釈したVT1、VT2溶液、およびノボビオシン加mEC培地、CAYE培地100µLを滴下
- 3 15分後に目視判定

	ベロ毒素濃度							
	5ng∕mL	5ng/mL 2.5ng/mL ノボビオシン加 CAYE培地						
VT1	陽性	弱陽性	陰性	陰性				
VT2	陽性	陽性	陰性	陰性				

NHイムノクロマトVT1/2の検出感度は、 VT1、VT2ともに2.5ng/mL

性能~腸管出血性大腸菌培養液での確認~ NH Nippon Ham Group

【試験方法】

- ① PCR法にてベロ毒素産生性を確認した被検菌株をノボビオシン加mEC培地にて42℃、 22時間培養
- ② 培養液をポリミキシンB処理
- ③ 培養液100µLを滴下し、15分後に目視判定

血清型	また	本キット試験結果 菌株		PCR法試験結果	
山川空	困怀	VT1	VT2	VT1	VT2
0157	ATCC43888	陰性	陰性	陰性	陰性
0157	分離株1	陽性	陽性	陽性	陽性
0121	RIMD05091859	陽性	陽性	陽性	陽性
0111	RIMD05091865	陽性	陰性	陽性	陰性
0111	分離株2	陽性	陽性	陽性	陽性
0111	分離株3	陽性	陰性	陽性	陰性
0111	分離株4	陽性	陽性	陽性	陽性
026	IID3005	陽性	陰性	陽性	陰性

PCR法での試験結果と良好な一致率を示した。

性能~特異性~



【試験方法】

- ① ベロ毒素非産生の被検菌株をトリプトソーヤブイヨンにて37℃、22時間培養
- ② 培養液をポリミキシンB処理
- ③ 培養液ならびに100µLを滴下し、15分後に目視判定

菌名	ᄷᇴᆸ	本キット試験結果		
困石	株番号	VT1	VT2	
Escherichia coli	ATCC 43888	陰性	陰性	
Escherichia coli	ATCC 700728	陰性	陰性	
Escherichia coli	ATCC 25922	陰性	陰性	
Escherichia coli	ATCC 11775	陰性	陰性	
Escherichia hermanii	JCM 1473	陰性	陰性	
Citrobacter freundii	ATCC 8090	陰性	陰性	
Enterobacter aerogenes	ATCC 13048	陰性	陰性	
Enterobacter cloacae	ATCC 13047	陰性	陰性	
Enterobacter cloacae	ATCC 49141	陰性	陰性	
Enterobacter sakazakii	ATCC 51329	陰性	陰性	

菌名	ᄷᇴᆸ	本キット試験結果		
困石	株番号	VT1	VT2	
Klebsiella oxytoca	ATCC 8724	陰性	陰性	
Serratia liquefaciens	ATCC 27592	陰性	陰性	
Serratia marcescens	ATCC 8100	陰性	陰性	
Serratia odorifera	ATCC 33077	陰性	陰性	
Proteus vulgaris	ATCC 6380	陰性	陰性	
Pseudomonas aeruginosa	ATCC 9027	陰性	陰性	

増菌培地および被検菌株に 対する偽陽性は 認められなかった。



性能~食品検体での確認~



【試験方法】

- ② 42℃、22時間培養後、培養液をポリミキシンB処理
- ③ 培養液100µLをテストストリップに滴下し、15分後に目視判定

被検食品	ベロ毒素大	腸菌未接種	ベロ毒素大腸菌接種		
恢 快良吅	VT1試験結果	VT2試験結果	VT1試験結果	VT2試験結果	
ほうれん草	陰性	陰性	陽性	陽性	
カイワレダイコン	陰性	陰性	陽性	陽性	
牛肉(こま切れ)	陰性	陰性	陽性	陽性	
豚肉(ひき肉)	陰性	陰性	陽性	陽性	
豚肉(こま切れ)	陰性	陰性	陽性	陽性	
鶏肉(ひき肉)	陰性	陰性	弱陽性	陽性	
鶏肉(モモ肉)	陰性	陰性	陽性	陽性	

●ベロ毒素産生大腸菌未接種の被検食品はすべて陰性を示した。
●ベロ毒素産生大腸菌を接種した被検食品はすべて陽性を示した。



性能~食品検体での検出感度~

【試験方法】

- ① 被検食品25gに対し、ノボビオシン加mEC培地225mL、およびVT1、VT2産生大腸菌を下記菌数接種し、 1分間ストマッキング
- ② 42℃、22時間培養後、培養液をポリミキシンB処理
- ③ 培養液100µLをテストストリップに滴下し、15分後に目視判定

	未接種		0.03250	cfu接種	0.325c	fu接種	3.25cf	u接種
	VT1	VT2	VT1	VT2	VT1	VT2	VT1	VT2
ほうれん草	陰性	陰性	陰性	陰性	陰性	陰性	陽性	陽性
カイワレ	陰性	陰性	陰性	陰性	陰性	陰性	陽性	陽性
もやし	陰性	陰性	陰性	陰性	陰性	陰性	陽性	陽性
牛肉	陰性	陰性	陰性	陰性	陰性	陰性	陽性	陽性
豚肉	陰性	陰性	陰性	陰性	陰性	陰性	陽性	陽性
鶏肉	陰性	陰性	陰性	陰性	陰性	陰性	陽性	陽性

いずれの被検食品においても、 検体25gに対し、3.25cfu接種した場合に陽性を示した。

注:ベロ毒素の産生量は個々の菌株により異なるため、菌株によっては本試験の結果と異なる結果になる場合があります。