

VITASSAY

Yersinia enterocolitica O:3+O:9

Rapid test for the simultaneous qualitative detection of
Yersinia enterocolitica O:3 and Yersinia enterocolitica O:9
in human stool samples.

IUE-7455032-67 Ed00 October 2018



For professional *in vitro* diagnostic use only.

INTENDED USE

Vitassay Yersinia enterocolitica O:3+O:9 is a rapid, immunochromatographic, one step assay for the simultaneous qualitative detection of Yersinia enterocolitica O:3 and Yersinia enterocolitica O:9 in human stool samples.

Simple, non-invasive and highly sensitivity immunoassay to make a presumptive diagnosis of a Yersinia enterocolitica O:3 and/or Yersinia enterocolitica O:9 infection.

INTRODUCTION

Yersinia enterocolitica is a food-borne pathogen that causes human yersiniosis. Yersiniosis is the third most commonly reported food-borne zoonosis and often occurs in young children. The most frequent symptom is gastroenteritis with diarrhea, but additional sequelae such as reactive arthritis and erythema nodosum may occur, especially in adults. Y. enterocolitica is a heterogeneous species that is divided into several biotypes and serotypes. Bioserotypes 1B/O:8, 2/O:5, 27, 2/O:9, 3/O:3, and 4/O:3 are associated commonly with human disease. Of these, bioserotype 4/O:3 strains have been responsible for yersiniosis cases in Europe, the United States, Canada, and Japan. Recently, bioserotype 2/O:9 infections have been on the rise. Besides humans, bioserotype 4/O:3 frequently is isolated from samples of pig origin.

The most common reported serotype of Y. enterocolitica strains isolated from human cases in Europe is O:3 and less commonly O:9.

Y. enterocolitica is considered to be an important food-borne pathogen. Infection is most often acquired by eating raw or undercooked pork. Rarely, this organism is transmitted through contaminated blood during a transfusion. Common symptoms of food-borne infections are diarrhoea, abdominal pain and fever, but sequelae, such as joint pain (reactive arthritis) and skin rash (erythema nodosus), are not uncommon. Infection with Y. enterocolitica occurs most often in young children.

Uncomplicated yersiniosis usually resolves on its own without antimicrobial treatment. However, in more severe cases, like septicaemia or focal extra-intestinal infection, and in immune-compromised patients, medication may be required.

PRINCIPLE

Vitassay Yersinia enterocolitica O:3+O:9 is a qualitative immunochromatographic assay for the detection of Yersinia enterocolitica O:3 and Yersinia enterocolitica O:9 in human stool samples.

Strip A: The test line zone of the nitrocellulose membrane is pre-coated with monoclonal antibodies against Yersinia enterocolitica O:3.

Strip B: The test line zone of the nitrocellulose membrane is pre-coated with monoclonal antibodies against Yersinia enterocolitica O:9.

During the process, the sample reacts with the antibodies against Yersinia enterocolitica O:3 (strip A) and/or Yersinia enterocolitica O:9 (strip B), forming conjugates. The mixture moves upward on the membrane by capillary action. If the sample is Yersinia enterocolitica O:3 positive, antibodies present on the membrane (test line) capture the conjugate complex and a **red** line will be visible in the strip A, and if the sample is Yersinia enterocolitica O:9 positive, antibodies present on the membrane (test line) capture the conjugate complex and a **red** line will be visible in strip B. Although the sample is positive or negative, the mixture continues to move across the membranes and the **green** control line always appears (for both strips).

The presence of these **green** lines (in the control zone (C)) indicates that sufficient volume is added; proper flow is obtained and serves as an internal control for the reagents.

PRECAUTIONS

- For professional *in vitro* use only.
- Do not use after expiration date.
- Do not use the test if its pouch is damaged.
- Specimens should be considered as potentially hazardous and handle in the same manner as an infectious agent. A new test must be used for each sample to avoid contaminations errors.
- Tests should be discarded in a proper biohazard container after testing.
- Reagents contain preservatives. Avoid any contact with the skin or mucous membrane. Consult safety data sheet, available on request.
- Components provided in the kit are approved for use with the **Vitassay Yersinia enterocolitica O:3+O:9**. Do not use any other commercial kit component.
- Follow Good Laboratory Practices, wear protective clothing, use disposal gloves, goggles and mask. Do not eat, drink or smoke in the working area.

STORAGE AND STABILITY

Store as packaged in the sealed pouch either at refrigerated or room temperature (2-30°C/35.6-86°F).

The test is stable until the expiration date printed on the sealed pouch.

The test must remain in the sealed pouch until use.

Do not freeze.

MATERIALS

MATERIAL PROVIDED	MATERIAL REQUIRED BUT NOT PROVIDED
<ul style="list-style-type: none"> 25 tests/kit Vitassay Yersinia enterocolitica O:3+O:9 Instructions for use. 25 Vials with diluent for the sample dilution. 1 Vitassay Yersinia enterocolitica O:3 Positive Control swab. 1 Vitassay Yersinia enterocolitica O:9 Positive Control swab. 1 Vitassay Negative Control swab. 	<ul style="list-style-type: none"> Specimen collection container. Disposable gloves. Timer.

SPECIMEN COLLECTION

Collect sufficient quantity of feces: 1-2g or mL for liquid samples. Stool samples should be collected in clean and dry containers.

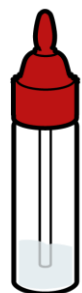
Samples can be stored in the refrigerator (2-8°C/35.6-46.4°F) for 1-2 days prior to testing. For longer storage, maximum 1 year, the specimen must be kept frozen at -20°C (-4°F). Samples must be brought to room temperature before testing.

SPECIMEN PREPARATION

1. Remove the cap of the vial with diluent for the sample dilution (figure 1).
2. Use the stick to collect sufficient sample quantity (approx. 125mg). For solid stool, insert the stick in 4 different areas of the stool sample taken approx. 125mg, (figure 2), and add it into the vial with diluent for the sample dilution. For liquid stool, take 125 µL of the sample using a micropipette and transfer it into the vial with diluent for the sample dilution.
3. Close the tube with the diluent and stool sample. Shake vigorously the vial in order to assure good sample dispersion (figure 3).



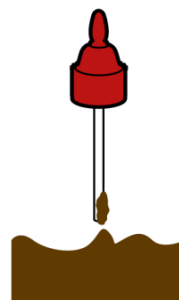
Vitassay



Vial for sample dilution.



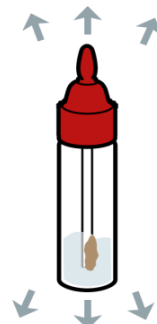
Vitassay



Insert the stick in 4 different areas of the stool.



Vitassay



Put the sample into the vial, close the cap and shake.

PROCEDURE

Allow the test, stool sample, controls and diluent to reach room temperature (15-30°C/59-86°F) prior to testing. Do not open pouches until the performance of the assay.

1. Shake the vial with the sample vigorously to obtain a good sample dilution.
2. Remove the **Vitassay Yersinia enterocolitica O:3+O:9** from its sealed bag just before using it.

3. Take the vial containing the diluted sample, cut the end of the cap (figure 4) and dispense 4 drops in the circular window marked with the letter A – Yersinia enterocolitica O:3 (figure 5) and 4 drops, using the same vial, in the circular window marked with the letter B – Yersinia enterocolitica O:9 (figure 6).

4. Read the results at **10 minutes**. Do not read the results later than 10 minutes.

If the test does not run due to solid particles, stir the sample added in the sample window with the stick. If it does not work, dispense a drop of diluent until seeing the liquid running through the reaction zone.



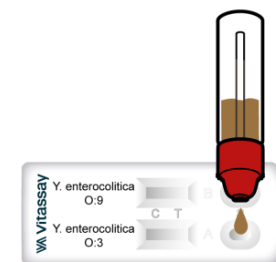
Vitassay



Cut the end of the cap.

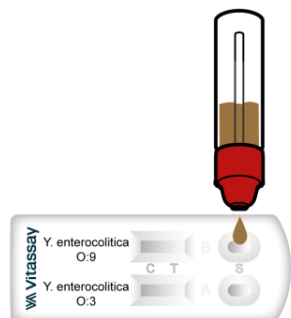


Vitassay



Dispense 4 drops in the circular window marked with the letter S to the strip A-Yersinia enterocolitica O:3.

6



Dispense 4 drops in the circular window marked with the letter S to the strip B-Yersinia enterocolitica O:9.

INTERPRETATION OF THE RESULTS

RESULTS	Strip A Yersinia enterocolitica O:3	Strip B Yersinia enterocolitica O:9	INTERPRETATION
	Negative	Negative	
	GREEN	GREEN	There is no Yersinia enterocolitica O:3 and/or Yersinia enterocolitica O:9 presence.
	Positive	Positive	
	GREEN-RED	GREEN-RED	There is Yersinia enterocolitica O:3 and Yersinia enterocolitica O:9 presence.
	Positive	Negative	
	GREEN-RED	GREEN	There is Yersinia enterocolitica O:3 presence.
	Negative	Positive	
	GREEN	GREEN-RED	There is Yersinia enterocolitica O:9 presence.

ANY OTHER RESULTS

Invalid result, we recommend repeating the assay using the sample with another test. **Note:** Wrong procedural techniques or deterioration of the reagents are the main reasons of control line failure. If the symptoms or situation still persist, discontinue using the test kit and contact your local distributor.

Notes: The intensity of the red colored test line in the result line zone (T) will vary depending on the concentration of antigens in the specimen.

QUALITY CONTROL

Internal procedural controls are included in **Vitassay Yersinia enterocolitica O:3+O:9**. Green lines appearing in the results window are internal controls, which confirm sufficient specimen volume and correct procedural technique.

LIMITATIONS

- **Vitassay Yersinia enterocolitica O:3+O:9** must be carried out within 2 hours of opening the sealed bag.
- An excess of stool sample could cause wrong results (brown bands appear). Dilute the sample with the diluent and repeat the test.
- The intensity of test line may vary depending on the concentration of antigens.
- The use of other samples different from human fecal samples has not been established.
- The quality of **Vitassay Yersinia enterocolitica O:3+O:9** depends on the quality of the sample; Proper fecal specimens must be obtained.
- Positive results determine the presence of Yersinia enterocolitica O:3 and/or Yersinia enterocolitica O:9 in human stool samples. A positive result should be followed up with additional laboratory techniques (biochemical and serological methods of by PCR) to confirm the results. A confirmed infection should only be made by a physician after the evaluation of all clinical and laboratory findings and must be based in the correlation of the results with further clinical observations.
- Negative results should not be considered as conclusive; it is possible that the concentration of antigen is lower than the detection limit value. If symptoms or situation still persist, a Yersinia enterocolitica O:3 and/or Yersinia enterocolitica O:9

determination should be carried out on a sample from an enrichment culture.

EXPECTED VALUES

Yersiniosis is a zoonotic bacterial disease with high public health relevance, especially in Europe because of its high incidence.

After *Campylobacter jejuni* and *Salmonella* spp., *Y. enterocolitica* is the third most common enteric pathogen associated with foodborne infections in Europe.

PERFORMANCE CHARACTERISTICS

Clinical sensitivity and specificity

An evaluation with stool samples was performed, comparing the results of **Vitassay Yersinia enterocolitica O:3+O:9** and another agglutination commercial test (Yersinia enterocolitica Agglutination Kit, Progen).

Results were as follows:

		Yersinia enterocolitica Agglutination Kit		
		Positive	Negative	Total
Vitassay Yersinia enterocolitica O:3+O:9	Positive	7	0	7
	Negative	0	56	56
	Total	7	56	63

		Yersinia enterocolitica Agglutination Kit		
		Positive	Negative	Total
Vitassay Yersinia enterocolitica O:3+O:9	Positive	2	0	2
	Negative	0	61	61
	Total	2	61	63

Vitassay Yersinia enterocolitica O:3+O:9 vs Yersinia enterocolitica Agglutination Kit			
Sensitivity	Specificity	PPV	NPV
>99%	>99%	>99%	>99%

Results showed that **Vitassay Yersinia enterocolitica O:3+O:9** has a high sensitivity and specificity to detect Yersinia enterocolitica O:3 and Yersinia enterocolitica O:9.

Cross reactivity










No cross reactivity was detected against other gastrointestinal pathogens that are occasionally present in feces:

<i>Campylobacter coli</i>	<i>Listeria monocytogenes</i>	<i>Shigella boydii</i>
<i>Campylobacter jejuni</i>	<i>Salmonella enteritidis</i>	<i>Shigella dysenteriae</i>
<i>Clostridium difficile</i>	<i>Salmonella paratyphi</i>	<i>Shigella flexneri</i>
<i>E. coli</i> O157: H7	<i>Salmonella typhi</i>	<i>Shigella sonnei</i>
<i>Helicobacter pylori</i>	<i>Salmonella typhimurium</i>	<i>Staphylococcus aureus</i>

REFERENCES

1. S. VIRTANEN; R. LAUKKANEN-NINIOS; P. ORTIZ MARTÍNEZ; A. SIITONEN; M. FREDRIKSSON-AHOMAA; H. KORKEALA. "Multiple-Locus Variable-Number Tandem-Repeat Analysis in Genotyping *Yersinia enterocolitica* Strains from Human and Porcine Origins". Journal of clinical Microbiology, July 2013, Vol 51, Number 7, pp. 2154-2159.
2. M. FREDRIKSSON-AHOMAS; N. CERNELA; H. HÄCHLER; R. STEPHAN. "*Yersinia enterocolitica* strains associated with human infections in Switzerland 2001-2010". European Journal of Clinical Microbiology and Infectious Diseases, July 2012, Volume 31, Issue 7, pp. 1543-1550.
3. KAROLINA SKOREK; ADRIANNA RACZKOWSKA; BARTŁOMIEJ DUDEK; KATARZYNA MIETKA; KATARZYNA GUZ-REGNER; ALEKSANDRA PAWLAK; ELZBIETA KLAUSA; GABRIELA BUGLA-PLOSKONSKA; KATARZYNA BRZOSTEK. "Regulatory Protein OmpR Influences the Serum Resistance of *Yersinia enterocolitica* O:3 by Modifying the Structure of the Outer Membrane". PLoS ONE, November 2013, Vol 8; Issue 11, e 79525.

SYMBOLS FOR IVD COMPONENTS AND REAGENTS

	in vitro diagnostic device		Keep dry
	Consult instructions for use		Temperature limitation
	Use by		Manufacturer
	Batch code		Contains sufficient for <n> test
DIL	Sample diluent		Catalogue number

