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 Ed01 August 2023

CE IVD EN

Vitassay

EN

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 enterolitica 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 enterolocolitica 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.
- Read the instructions for use carefully before using the test.
- Do not use the kit if the label sealing the outer carton is torn or if the bags are open or damaged on arrival.
- Do not use the tests if the desiccant material is missing or broken inside the aluminium pouch.
- Do not reuse. This is a single-use device.
- Specimens should be considered potentially hazardous and should be handled in the same manner as an infectious agent, following local/national regulations. A new test should be used for each sample to avoid contamination errors.
- Material exposed to samples should also be considered potentially hazardous and should be handled in the same way as an infectious agent, following local/national regulations.
- Used material and tests should be disposed of in an appropriate biohazard container after testing.

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- 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 nor reagents from other batches.
- Follow Good Laboratory Practices. These practices should include, but are not limited to, personal protective equipment (PPE), such as lab coat, surgical or appropriate mask or face shield, disposable gloves and eye protection. Take the necessary precautions during sample collection, transport, storage, handling and disposal. Each sample must be correctly and unequivocally identified to ensure proper traceability of samples.
- In case of spillage, clean thoroughly with a suitable disinfectant.
- Do not eat, drink or smoke in the workplace.
- The presence of yellow lines in the result window (control line area and test line area), before using the test, is completely normal and does not imply a failure in the functionality of the test.
- The visual interpretation of the results is done by coloured lines, the interpretation of the results should be done by a professional user without problems of visualisation and colour interpretation.
- A certificate of analysis can be provided upon request (not included).

STORAGE AND STABILITY

The storage temperature of the kits should be 2-30ºC.

Do not freeze.

Under these conditions, they can be used until the expiry date indicated on the kit label.

All kit components are for single use only and must remain in their primary packaging until use. The test must remain in the sealed pouch until use.

MATERIALS

MATERIAL PROVIDED	MATERIAL REQUIRED BUT NOT PROVIDED
▪ **25** tests/kit **Vitassay Yersinia enterocolitica O:3+O:9** ▪ Instructions for use. ▪ **25** Vials with diluent for the sample dilution.	▪ PPE, such as disposable gloves ▪ Specimen collection container ▪ Timer ▪ Micropipette (in case of liquid stool)

SPECIMEN COLLECTION

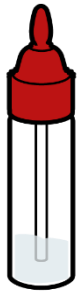
Collect sufficient quantity of feces: 1-2g or mL for liquid samples. Stool samples should be collected in clean and dry containers.

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

SPECIMEN PREPARATION

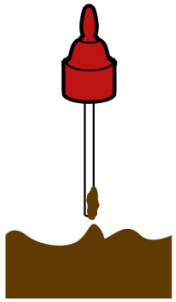
1. Remove the cap of the vial with diluent for the sample dilution (figure 1).
2. Use the stick to collect sufficient sample quantity (approx. 125mg). For solid stool, insert the stick in 4 different areas of the stool sample taken approx. 125mg, (figure 2), and add it into the vial with diluent for 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).

1



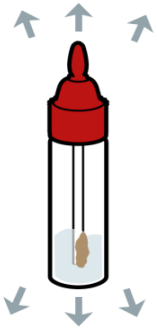
Vial for sample dilution.

2



Insert the stick in 4 different areas of the stool.

3



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

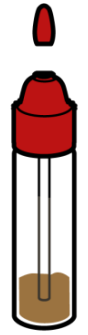
PROCEDURE

Allow the test, stool sample, controls and diluent to reach room temperature (15-30ºC) 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.

4



Cut the end of the cap.

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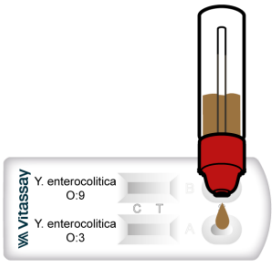
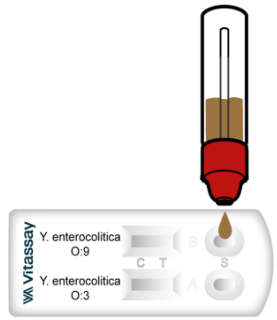
5



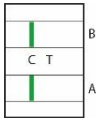
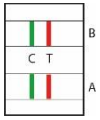
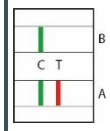
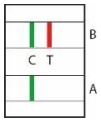
Dispense 4 drops in the circular window marked with the letter S to the strip A-*Yersinia enterocolitica O:3*.

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 enterolitica O:3*	Strip B *Yersinia enterocolitica O:9*	INTERPRETATION
	**Negative**	**Negative**	There is no *Yersinia enterocolitica O:3* and/or *Yersinia enterocolitica O:9* presence.
	GREEN	GREEN	
	**Positive**	**Positive**	There is *Yersinia enterocolitica O:3* and *Yersinia enterocolitica O:9* presence.
	GREEN-RED	GREEN-RED	
	**Positive**	**Negative**	There is *Yersinia enterocolitica O:3* presence.
	GREEN-RED	GREEN	
	**Negative**	**Positive**	There is *Yersinia enterocolitica O:9* presence.
	GREEN	GREEN-RED	
ANY OTHER RESULTS			Invalid result, we recommend repeating the assay using the sample with another test. **Note**: Wrong procedural techniques, insufficient sample volume or deterioration of the reagents are mostly the main reasons for control line failure. If the symptoms or situation 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 in the results window are internal controls, which confirm sufficient specimen volume and correct procedural technique.

LIMITATIONS

- 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 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.
- **Bloody** stool samples and/or **mucuos stool** samples can cause non-specific reactions in the test. Such positive samples should be followed up with other diagnostic techniques to confirm the result

EXPECTED VALUES

Yersioniosis 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
Y. enterocolitica O:3	**Total**	7	56	63

Table 1. Results of **Vitassay Yersinia enterocolitica O:3+O:9** compared to a commercial kit (Yersinia enterocolitica Agglutination Kit, Progen) for *Y. enterocolitica* O:3.

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

Table 2. Results of **Vitassay Yersinia enterocolitica O:3+O:9** compared to a commercial kit (Yersinia enterocolitica Agglutination Kit, Progen) for Y. enterocolitica O:9.

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

Table 3. Sensitivity, specificity, positive predictive values, and negative predictive values of the **Vitassay Yersinia enterocolitica O:3+O:9** compared to a commercial kit.

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.*

Analytical sensitivity

The Limit of detection (LoD) stablished for **Vitassay Yersinia enterocolitica O:3+O:9** was 3x105 UFC/mL for *Y. enterocolitica* O:3 and 2x107 UFC/mL for *Y. enterocolitica* O:9

Cross reactivity

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

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

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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 Procine Origins". Journal of clinical Microbiology, July 2013, Vol 51, Number 7, pp. 2154-2159.

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SYMBOLS FOR IVD COMPONENTS AND REAGENTS

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

Changes control		
Nº Version	**changes**	**Date**
IUE-7455032 Ed01 August 2023	Addition of analytical sensitivity section. Formatting has been updated. Limitations section has been updated. Grammatical and editorial changes have been made to Precautions, Limitations, Sample Collection, Storage and Stability, and Interpretation of Results. Material required, but not included updated with minor changes.	25/08/2023

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