VITASSAY

H. pylori + Transferrin

Rapid test for the qualitative detection of *H. pylori* and human transferrin in human stool samples.

IUE-7455021 Ed02 August2023

CE IVD EN

Vitassay

For professional *in vitro* diagnostic use only.

INTENDED USE

Vitassay H. pylori + Transferrin is a rapid immunochromatographic, one step assay for the simultaneous qualitative detection of *Helicobacter pylori* (*H. pylori*) and human transferrin in human stool samples.

Simple, non-invasive and highly sensitivity immunoassay for the detection of *H. pylori* that causes gastric and duodenal ulcers and the presence of gastrointestinal bleeding and moreover, to monitor patients' treatment (*H. pylori* eradication).

INTRODUCTION

Most cases of colorectal cancer arise from premalignant adenomatous polyps which release small amounts of blood into the stool.

H pylori infection is a common chronic infection that is associated with upper gastrointestinal diseases, including chronic gastritis, peptic ulcers, and gastric cancer.

Transferrin, which is mainly responsible for the transport of iron in the blood, has also been identified as a potential biomarker for colorectal cancer. Bleeding into the gastrointestinal tract releases both transferrin and hemoglobin into feces where it can be measured by immunoassay-based tests. Furthermore, it has been reported from in vitro studies, and implied from studies done on colorectal cancer screening populations, that hemoglobin is more sensitive to degradation by enterobacteria and digestive enzymes than transferrin.

When gastrointestinal bleeding occurs, serum transferrin enters the gastrointestinal tract and is excreted in feces. Previous proteomic studies identified transferrin as a potential protein marker for a number of epithelial cancers, including colorectal cancer.

PRINCIPLE

Vitassay H. pylori + Transferrin is a qualitative immunochromatographic assay for the detection of *H. pylori* and human transferrin in human stool samples.

Strip A: The test line zone of the nitrocellulose membrane is pre-coated with monoclonal antibodies against *H. pylori*.

Strip B: The test line zone of the nitrocellulose membrane is pre-coated with monoclonal antibodies against human transferrin.

During the process, the sample reacts with the antibodies against *H. pylori* (strip A) and/or Transferrin (strip B), forming conjugates. The mixture moves upward on the membrane by capillary action. If the sample is *H. pylori* 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 Transferrin 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.
- 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 the samples should also be considered potentially hazardous and should be handled in the same manner as an infectious agent, following local/national regulations.
- Do not reuse. This is a single-use device.
- Used material should be disposed of in an appropriate 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 H. pylori + Transferrin**. Do not use any other commercial kit component.
- 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.

Vitassay
Parque Tecnológico WALQA
Ctra. N.330, Km.566
22197-Cuarte (Huesca, SPAIN)
www.vitassay.com

- 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 on 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 H. pylori + Transferrin** ▪ Instructions for use. ▪ **25** H. pylori vials with diluent for the sample dilution. ▪ **25** Transferrin 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: 1-2 g or 1-2 mL for liquid samples. Stool should be collected in clean and dry containers.

Samples (for *H pylori* detection) 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 (for Transferrin detection) can be stored in the refrigerator (2-8ºC) for 7 days prior to testing. For longer storage, maximum 6 months, the specimen must be kept frozen at -20ºC.

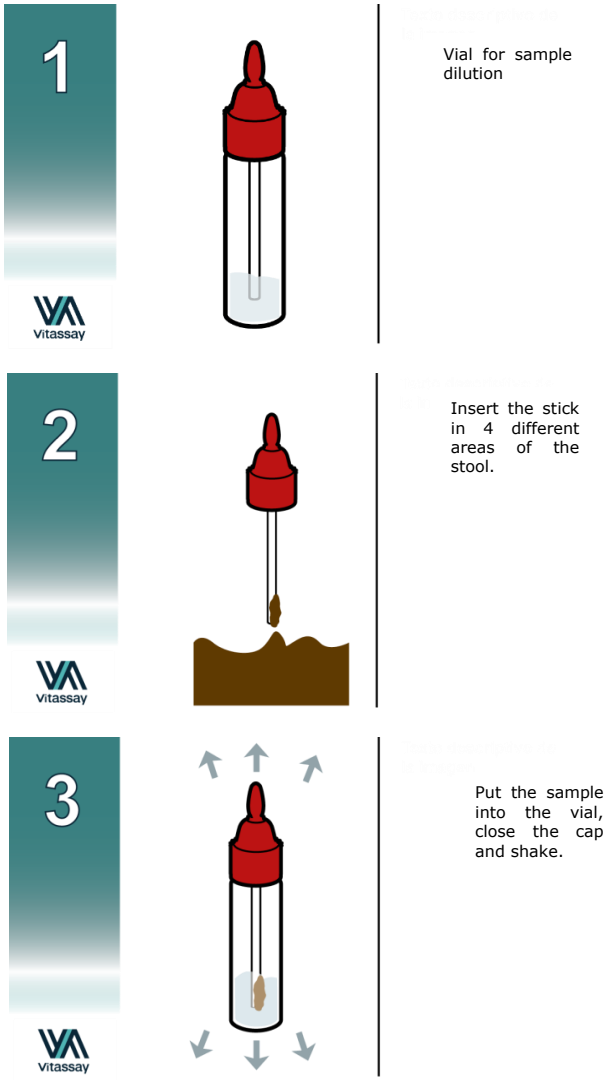
Samples must be brought to room temperature before testing.

SPECIMEN PREPARATION

Please take note that there are two different vials with diluent for sample dilution. One for H pylori strip and the other one for Transferrin strip.

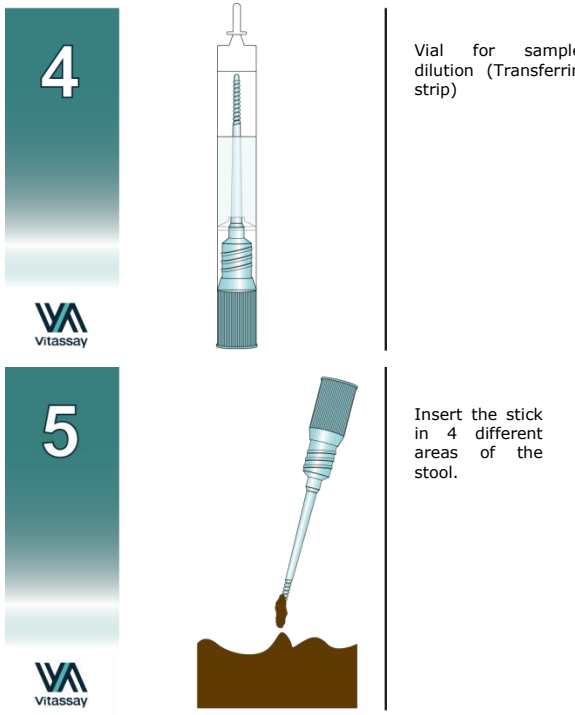
H. pylori procedure (specimen preparation)

1. Remove the cap of the vial with diluent for the sample dilution (figure 1).
2. Use the stick to collect sufficient sample quantity. For solid stool, insert the stick in 4 different areas of the stool sample, taken approx. 50mg, (figure 2), and add it into the vial with diluent for the sample dilution. Not to exceed the stick´s screw to avoid wrong results. For liquid stool, take 125µL of the sample using a micropipette and transfer it into the vial with diluent for the sample dilution.
3. Close the vial with the diluent and stool sample. Shake vigorously the vial in order to assure good sample dispersion (figure 3).



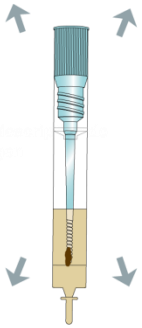
Transferrin procedure (specimen preparation)

1. Take out the cap of the vial with diluent for the sample dilution (figure 4).
2. Use the stick to collect sufficient sample quantity. For solid stool, insert the stick in 4 different areas of the stool sample (figure 5), and add it into the vial with diluent for the sample dilution. For liquid stool, take 20 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 dilution (figure 6). The stool collection vial with diluted sample can be stored at room temperature until 2 days or until 5 days in the refrigerator (2-8ºC) prior to testing.



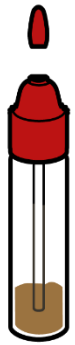
Vitassay
Parque Tecnológico WALQA
Ctra. N.330, Km.566
22197-Cuarte (Huesca, SPAIN)
www.vitassay.com

6



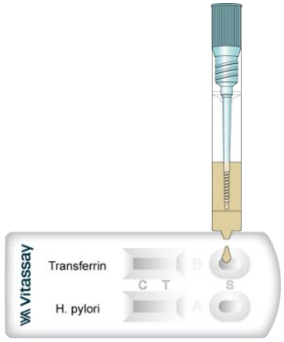
Put the sample into the vial, close the cap and shake.

7



Cut the end of the cap.

10



Dispense 3 drops in the circular window marked with the letter S for the strip B – Transferrin.

Precautions: Patients should not collect samples during their menstrual period, if they have bleeding hemorrhoids, blood in urine or if they have strained during bowel movement.

PROCEDURE

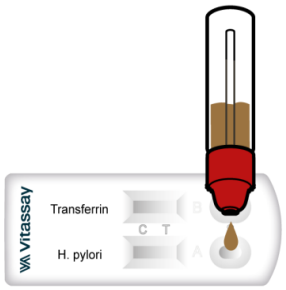
Allow the test, stool sample and controls to reach room temperature (15-30ºC) prior to testing.

Do not open pouches until the performance of the assay.

1. Shake the stool collection tube vigorously to obtain a good sample dilution.
2. Remove the **Vitassay H. pylori + Transferrin** from its sealed bag just before using it.
3. Take the *H. pylori* stool collection tube containing the diluted sample, cut the end of the cap (figure 7) and dispense 3 drops in the circular window marked with the letter S for the strip A–*H. pylori* (figure 8).
4. Take the Transferrin stool collection tube containing the diluted sample, cut the end of the cap (figure 9) and dispense 3 drops in the circular window marked with the letter S for the strip B–Transferrin (figure 10).
5. 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.

8

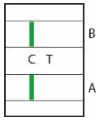
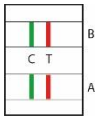
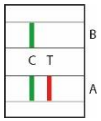
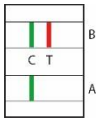


Dispense 3 drops in the circular window marked with the letter S for the strip A – *H. pylori*.

9

Cut the end of the cap.

INTERPRETATION OF THE RESULTS

RESULTS	Strip A H. pylori	Strip B Transferrin	INTERPRETATION
	**Negative**	**Negative**	No infection caused by *H. pylori* and no gastrointestinal bleeding problem.
	GREEN	GREEN	
	**Positive**	**Positive**	Posible infection caused by *H. pylori,* with gastrointestinal bleeding, which might mean gastrointestinal diseases (stomach and duodenum) like gastrtitis, peptic ulcer disease and gastric carcinona.
	GREEN-RED	GREEN-RED	
	**Positive**	**Negative**	Posible infection caused by *H. pylori*, without gastrointestinal bleeding.
	GREEN-RED	GREEN	
	**Negative**	**Positive**	No infection caused by *H. pylori* but presumptive gastrointestinal bleeding problem.
	GREEN	GREEN-RED	

Vitassay
Parque Tecnológico WALQA
Ctra. N.330, Km.566
22197-Cuarte (Huesca, SPAIN)
www.vitassay.com

Any other results	Invalid result, either A or B, it is recommended to repeat 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 control is included in **Vitassay H. pylori+Transferrin**. Green line appearing in the results window is an internal control, which confirms 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 antigen's concentration.
- The use of other samples different from human samples has not been established.
- The quality of **Vitassay H. pylori+Transferrin** depends on the quality of the sample; Proper fecal specimens must be obtained.
- Positive results determine the presence of H. pylori and/or human transferrin in stool samples. However, this may be due to various causes, such as haemorrhoids, blood in urine or stomach irritation. A positive result should be cross-checked with other invasive diagnostic techniques (endoscopy) to determine the source and cause of blood in the stool sample.
- Infection should be confirmed by a qualified specialist or physician, after evaluation of clinical tests and laboratory findings, taking into account the correlation that may exist with all clinical observations.
- A negative result should not be considered as conclusive, it may be that the concentration of H. pylori and/or human transferrin in the stool sample is below the limit of detection or cut-off value. If symptoms or the situation continues, the H. pylori test should be repeated with the sample previously subjected to enrichment or other techniques for H. pylori determination. Negative transferrin results do not exclude bleeding caused by some polyps or colorectal cancer, as they may cause bleeding intermittently or not at all during some stages of the disease. In addition, blood may not have been evenly distributed in the stool sample.
- To detect transferrin in strip B (to avoid false positive results) patients should not collect samples during their menstrual period, if they have bleeding hemorrhoids, blood in urine or if they have strained during bowel movement.
- **Bloody stool** samples and/or mucous stool samples can cause non-specific reactions in the test. Such positive samples should be followed up with other diagnostic techniques to confirm the results.
- To detect transferrin on the B-strip (avoiding false positives), samples should not be obtained from patients during their menstrual period, if they have bleeding haemorrhoids, blood in urine or constipation.
- Stool samples whose antigen concentration is close to the **Vitassay H. pylori+Transferrin** cut-off value (B:Transferrin strip) may be lost if the sample has been diluted and stored for up to 2 days at room temperature or up to 5 days at 2-8°C before testing. For stool samples close to the cut-off value of the test it is best to dilute the sample and test immediately.

EXPECTED VALUES

Colorectal cancer (CRC) is the second leading cause of cancer in Canadian men, and the fourth leading cause of cancer death in the world.

The prevalence of H. pylori is estimated at ≈30% in the adult US population, based on data from the National Health and Nutrition Examination Survey, with higher rates with increasing age. Indviduals infected with H. pylori have a 15% (vs 4% among noninfected individuals) risk of developing peptic ulcer disease, and are at tenfold-higher risk of gastric adenocarcinoma. A large majority (70%-90%) of patients with peptic ulcer disease are infected with H. pylori, and its eradication is an important component of treatment for preventing ulcer recurrence.

PERFORMANCE CHARACTERISTICS

Analytical sensitivity (detection limit)

Detection limit range is: 0.78 ng/mL-0.09 ng/mL of Helicobacter pylori recombinant outer membrane protein.

Cut-off value of **Vitassay H. pylori+Transferrin** (Transferrin strip) is 4 ng/mL (0.4µg hTf/g of faeces) for human transferrin.

Clinical sensitivity and specificity

An evaluation, with faecal samples, was performed comparing **Vitassay H. pylori + Transferrin**, and qPCR technique VIASURE Helicobacter pylori Real Time PCR Detection kit, CerTest), and another commercial immunochromatographic assay (Human Hexagon, OBTI). The samples were taken from patients with the same as *Helicobacter pylori* infection symptoms arrived in Gastroenterology area in a Spanish hospital.

Results were as follows:

		qPCR test: Viasure Helicobacter pylori Real Time detection kit		
		Positive	**Negative**	**Total**
Vitassay H. pylori + Transferrin	**Positive**	54	1	55
	Negative	1	60	61
H. pylori	**Total**	55	61	116

Table 1. Results of **Vitassay H. pylori+Transferrin** (H. pylori) compared to a commercial kit

Vitassay H. pylori+Transferrin vs VIASURE Helicobacter pylori Real Time PCR Detection Kit		
	Mean Value	**95% confidence interval**
Sensitivity	98.2%	90.3-100.0%
Specificity	98.4%	91.2-100.0%
PPV	98.2%	90.3-100.0%
NPV	98.4%	91.2-100.0%

Table 2. Sensitivity, specificity, positive predictive values, and negative predictive values of the **Vitassay H. pylori+ Transferrin** (H. pylori) compared to a commercial kit.

In addition, **Vitassay H. pylori+Transferrin** was compared with an ELISA test for cut-off values of 0.4 µg hTf/g **Vitassay H. pylori+Transferrin** vs ELISA test:

		Human Transferrin ELISA kit		
		Positive	**Negative**	**Total**
Vitassay H. pylori + Transferrin	**Positive**	38	3	41
	Negative	12	47	59
Transferrin	**Total**	50	50	100

Table 3. Results of **Vitassay H. pylori+Transferrin** (Transferrina) compared to a commercial kit

Vitassay H. pylori+Transferrin vs Human Transferrin ELISA kit		
	Mean Value	**95% confidence interval**
Sensitivity	76%	61.8-86.9%
Specificity	94%	83.5-98.7%
PPV	92.7%	80.1-98.5%
NPV	79.7%	67.2-98%

Table 4. Sensitivity, specificity, positive predictive values, and negative predictive values of the **Vitassay H. pylori+ Transferrin** (Transferrin) compared to a commercial kit.

Vitassay
Parque Tecnológico WALQA
Ctra. N.330, Km.566
22197-Cuarte (Huesca, SPAIN)
www.vitassay.com

The results showed that **Vitassay H. pylori + Transferrin** has a high sensitivity and specificity- to detect *H. pylori* and human transferrin.

Cross reactivity

No cross reactivity was detected against other fecal markers that are occasionally present in feces:

For the strip A: *H. pylori*

Astrovirus	*Entamoeba histolytica*	*Legionella pneumophila*	*Shigella boydii*
Adenovirus	*Escherichia coli O111*	*Listeria monocytogenes*	*Shigella dysenteriae*
Calprotectina humana	*Escherichia coli O26*	Norovirus GI	*Shigella flexneri*
Campylobacter coli	*Escherichia coli O157*	Norovirus GII	*Shigella sonnei*
Campylobacter jejuni	*Giardia*	Rotavirus	Streptococcus pyogenes
Clostridium difficile antigen GDH	Hemoglobina humana	*Salmonella enteritidis*	*Streptococcus pyogenes*
Clostridium difficile Toxin A	Hemoglobina bovina	*Salmonella paratyphi*	*Yersinia Enterocolitica O:3*
Clostridium difficile Toxin B	Hemoglobina de cerdo	*Salmonella typhi*	*Yersinia Enterocolitica O:9*
Clostridium perfringens	Lactoferrina humana	*Salmonella typhimurium*	*Transferrina humana*

Para la tira B: Transferrin

Astrovirus	*Entamoeba histolytica*	*Legionella pneumophila*	*Shigella boydii*
Adenovirus	*Escherichia coli O111*	*Listeria monocytogenes*	*Shigella dysenteriae*
Calprotectina humana	*Escherichia coli O26*	Norovirus GI	*Shigella flexneri*
Campylobacter coli	*Escherichia coli O157*	Norovirus GII	*Shigella sonnei*
Campylobacter jejuni	*Giardia*	Rotavirus	Streptococcus pyogenes
Clostridium difficile antigen GDH	Hemoglobina humana	*Salmonella enteritidis*	*Streptococcus pyogenes*
Clostridium difficile Toxin A	Hemoglobina bovina	*Salmonella paratyphi*	*Yersinia Enterocolitica O:3*
Clostridium difficile Toxin B	Hemoglobina de cerdo	*Salmonella typhi*	*Yersinia Enterocolitica O:9*
Clostridium perfringens	Lactoferrina humana	*Salmonella typhimurium*	*Transferrina humana*
Cryptosporidium	Hemoglobina humana	*Salmonella typhimurium*	

REFERENCES

1. WAEL L. L. DEMIAN; STACY COLLINS; CANDACE FOWLER; JERRY MCGRATH; SCOTT ANTLE; ZOE MOORES; DEBORAH HOLLOHAN; SUZANNE LACEY; JOSEPH BANOUD; EDWARD RANDELL. "Evaluation of the analytical performance of the novel NS-Prime system and examination of temperature stability of fecal transferrin compared with fecal hemoglobin as biomarkers in a colon cancer screening program". Practical Laboratory Medicine 2 (2015), pp. 29-36.

2. SUSAN H. BOKLAGE; ALLEN W. MANGEL; VARUM RAMAMOHAN; DEIRDRE MLADSI; TAO WANG. "Impact of patient adherence on the cost-effectiveness of noninvasive tests for the initial diagnosis of Helicobacter pylori infection in the United States". Patient Preference and Adherence 2016:10 45-55.

3. PENG JIN; ZI-TAO WU; MING-MING MENG; XIN WANG; XIAO-WEI WANG; LI-JUAN GONG; DONG-LIANG YU; HUI XIE, AI-QIN LI; SHI-RONG LI; LAWRENCE YEN; JIANYU RAO; JIAN-QIU SHENG. "Combined Fecal Transferrin Test and Immuno Fecal Occult Blood Test for Detetcting Colorectal Cancer and Advanced Adenoma in Asymptomatic and Symptomatic Populations". Cancer Science and Therapy, 2012, volume 4(8) 243-248.

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**	**Fecha**
IUE-7455021 Ed02 August 2023	Change of format. Addition of a new evaluation.Cross-reaction section has been updated. Limitations section has been updated. Transcription error in interpretation section has been corrected. Wording and grammatical changes have been implemented in Precautions, Limitations, Specimen collection, Storage and Stability. Material required but not included updated with minor changes.	29/08/2023

Vitassay

Parque Tecnológico WALQA
Ctra. N.330, Km.566
22197-Cuarte (Huesca, SPAIN)
www.vitassay.com