VITASSAY

Transferrin
-Turbidimetric Assay-

Rapid test for the quantitative detection of Transferrin in human stool samples.

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CE IVD EN

Vitassay

For professional *in vitro* diagnostic use only. Professional trained in Turbidimetric techniques.

INTENDED USE

Vitassay Transferrin –Turbidimetric Assay- is a rapid Turbidimetric assay for the quantitative detection of transferrin in human stool samples.

Simple, non-invasive and highly sensitivity assay for the detection of human transferrin in feces that helps in the search for gastrointestinal bleeding problems. This product has been optimized for several automated analyzer.

INTRODUCTION

Transferrin (Tf) is an iron-transporting protein (molecular weight=76.500 Da) that is synthesised mainly in the liver and is present at a concentration of 2.0-3.0 g/L in normal serum. The blood concentration of Transferrin is 1%-2% that haemoglobin (Hb), but Transferrin is highly stable and is considered to be a more sensitive indicator of bleeding than Hb, even when the intestinal retention time for faeces is long.

PRINCIPLE

Vitassay Transferrin –Turbidimetric Assay- is a quantitative turbidimetric assay for the detection of transferrin in human solid stool samples.

Transferrin Turbidimetric Assay is based on antigen-antibody agglutination reactions between the antigen contained in the sample and the antibodies anti-antigen coated on polystyrene latex particles.

Such agglutination is measured as an increase in absorbance proportional to the quantity of antigen contained in the sample.

The use of two external controls, Control 1 and Control 2, is used to verify that the test is working properly.

PRECAUTIONS

- For professional *in vitro* use only.
- Do not use after expiration date.
- A trained person in Turbidimetric technique and autoanalyzer use is required.
- Do not use the test if its primary containers are damaged.
- Specimens should be considered as potentially hazardous and handle in the same manner as an infectious agent. Avoid contamination errors, follow proper work procedure.
- The reagents after use 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 Transferrin -Turbidimetric Assay-**. Do not use any other commercial component.
- Follow Good Laboratory Practices, wear protective clothing, use disposal gloves, goggles and mask. Do not use any other commercial kit component.
- If measure range is exceeding, use the sample diluent to dilute the sample and repeat the assay again.
- Read and follow up the instructions for use provided in the kit.
- Prepare and adjust the analyzer before starting measurements.

STORAGE AND STABILITY

Store as packaged in the original primary container, the reagents should be preserved at refrigerated temperature (2-8ºC/35.6-46.4ºF), the sample diluent could be preserved refrigerated or at room temperature (2-30ºC/35.6-86ºF).

The product is stable until the expiration date printed on the label, if they have been preserved under the recommended conditions.

Do not freeze and keep away from the sunlight.

MATERIALS

MATERIAL PROVIDED	MATERIAL REQUIRED BUT NOT PROVIDED
▪ Reagent R1 (Enhancer buffer) ▪ Reagent R2 (Latex particles) ▪ Calibrator 0 ▪ Calibrator 1 ▪ Calibrator 2 ▪ Calibrator 3 ▪ Calibrator 4 ▪ Calibrator 5 ▪ Control 1 ▪ Control 2 ▪ Universal vials with diluent for the sample dilution. ▪ Instruction for use. ▪ Additional screw cap.	▪ Specimen collection container. ▪ Disposable gloves and laboratory equipment. ▪ Vortex. ▪ Automatic analyzer. ▪ Microcentrifugue (10000g).

SPECIMEN COLLECTION

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

Samples can be stored in the refrigerator (2-8ºC/36.5-46.4ºF) for 3 days prior to testing. For longer storage, maximum 6 months, the specimen must be kept frozen at -20ºC (-4ºF). Samples must be

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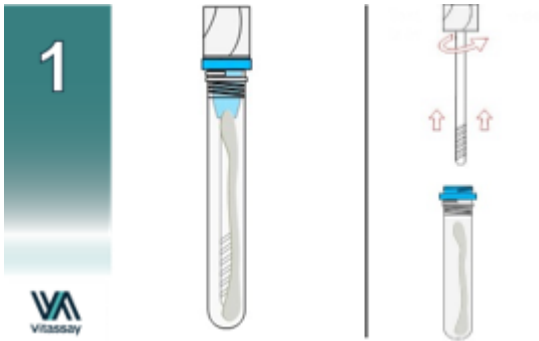
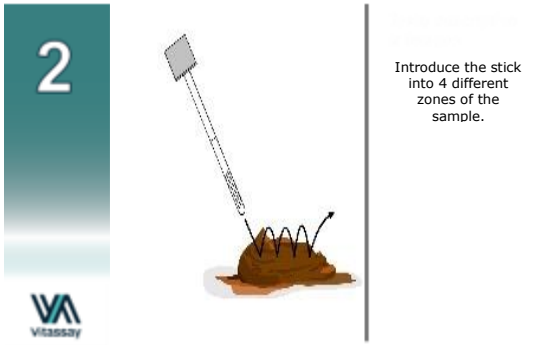
brought to room temperature before testing. Homogenize the sample as thoroughly as possible prior to preparation.

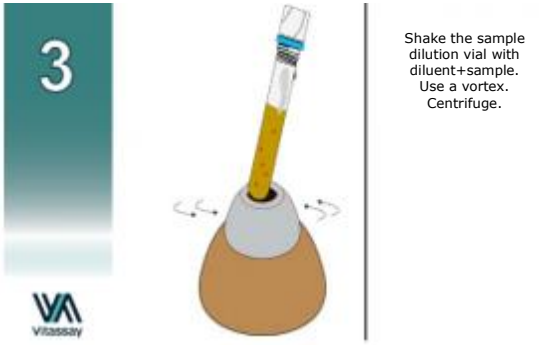
SPECIMEN PREPARATION

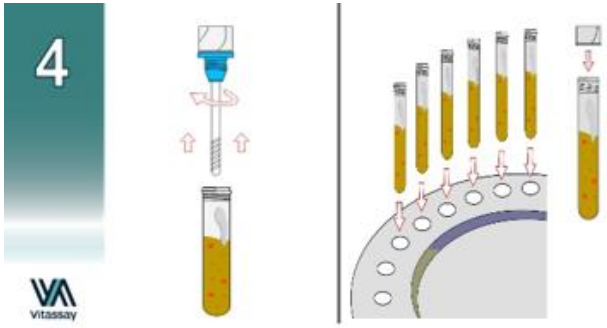
1. Label the vial with the patients´s identification.
2. Open the cap of the vial (anticlockwise) without removing the blue separator and use the stick to pick up sufficient quantity of sample (figure 1).
3. Use the stick to collect sufficient sample quantity. Introduce the stick into 4 different zones and rotate the stick in order to make the samples as homogenous as possible and make sure all grooves are filled with faecal material (figure 2).
4. Remove the stool excess by rotating the stick on the internal wall of the sterile container.
5. Insert the stick with the sample into the vial through the blue separator. Close the white cap by holding the blue separator to prevent it from opening.
6. Shake each vial (30-60 seconds) by Vortex in order to assure good sample dispersion (figure 3). Proceed to step 7 if the grooves are visually empty of any stool material, otherwise repeat the shaking up to 120 seconds and proceed to step 7 regardless some residual stool sample in the grooves. The sample dilution vial with diluted sample can be stored for 7 days in the refrigerator (2-8ºC) prior to testing and bring the samples to room temperature.
7. Centrifuge the vial for 10 minutes at 1000-3000 x g in order to remove the possible residuals of faecal material. Alternatively, let the vial sit upright for a minimum of 15 minutes.
8. Remove the white screw cap and the blue separator from vials by rotating the separator (clockwise). The faecal extract is now ready to be tested. Insert the opened device directly into the clinical chemistry analyser or transfer the supernatant to an adaptor cup (not provided) (figure 4).
9. Close the collection vial by the provided additional screw cap (figure 4).

Note: Do not use the sample vials directly in the analyzer.









PROCEDURE

Reagent R1 y Reagent R2 are ready to use.

Calibration curve

For calibration only use transferrin Calibrator: Cal0, Cal1, Cal2, Cal3, Cal4 and Cal5. Contain transferrin antigen at diferent concentrations indicated on the label of each of the vials.

	Reference	Calibrator 1	Calibrator 2	Calibrator 3	Calibrator 4	Calibrator 5
Con.	0 ng/mL	10 ng/mL	25 ng/mL	50 ng/mL	100 ng/mL	250 ng/mL
Vol.	1000 µL	1000 µL	1000 µL	1000 µL	1000 µL	1000 µL

Reagents are ready to use. The frequency in the realization of the calibration curve must be established by the end user in the function of the criteria established for the clinical laboratory.

Note: See section quality control.

Analytical procedure

Measure range: 5 – 250 ng HTf/mL.

Procedure	Steps	
R1 addition	200 µL	0 s
Sample addition	30 µL	10 s
R2 addition	55 µL	300 s
Blank measure	505 nm – 800 nm	310 s
Mainly measure	505 nm – 800 nm	610 s

*Data obtained by the analyzer Biolis i24 (Tokio Boeki)

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INTERPRETATION OF RESULTS

Positive results values: Higher or equal than the cut off fixed by the clinical lab.

Recommended: 10 ng of transferrin/mL (1 µg of transferrin/g of stool) for diagnostic procedures.

Positive results determine the abnormal presence of transferrin antigen in human stool samples.

Vitassay Transferrin -Turbidimetric Assay Cut-off value:

Transferrin concentration values lower than 10 ng/ of hTf/mL are considered normal values and that is not indicative of bleeding in the gastrointestinal tract.

Transferrin concentration values equal or higher than 10 ng hTf/mL are considered abnormal values and that is indicative of bleeding in the gastrointestinal tract.

QUALITY CONTROL

Transferrin C1 & C2 Controls are ready to use.

Transferrin Control 1: is liquid control at a certain concentration of transferrin (lower than Control 2). Concentration is indicated on the label of the vial.

Transferrin Control 2: is liquid control at a certain concentration of transferrin (higher than Control 1). Concentration is indicated on the label of the vial.

The use of controls at two different concentrations is recommended to verify test precision.

If the obtain results are out of the tolerance range, the equipment, the reagents or the technique must be reviewed. If the problems persists, stop using the reagents and contact your distribuitor.

LIMITATIONS

- **Vitassay Transferrin -Turbidimetric Assay** should be only used for the detection of transferrin in human stool samples.
- The quality of **Vitassay Transferrin -Turbidimetric Assay-** depends on the quality of the sample; Proper fecal specimens must be obtained.
- Positive results should be followed up with additional invasive techniques (endoscopy) to confirm the results.
- Negative results do not excluded bleeding, as some polyps and colorectal cancers may bleed intermittently or not during certain stages of the disease. Moreover, sample is heterogeneous in terms of the distribution of blood in it.
- Patients should not collect samples during their menstrual period, if they have bleeding hemorrhoids, blood in urine or if they have strained bowel movement.

EXPECTED VALUES

The incidence of Colorectal Cancer (CRC) has increased rapidly and continues to be a major public health threat around the world. CRC is the third most common cancer globally, causing approximately 1.4 million new causes and 700.000 deaths per year. There is ample evidence to support that screening and early detection reduce the mortality of colorectal cancer.

PERFORMANCE CHARACTERISTICS

Analytical sensitivity:

Detection limit: 5 ng hTf/mL.

Prozone:

Lower concentrations of 10 µg of transferrin/mL of stool do not show prozone effect and no false negative results have been observed.

Within-Run Precision:

	Low (15 µg/g)	Media (80 µg/g)	High (200 µg/g)
N	20	20	20
Media (µg/g)	16.1	80.6	197.2
DS (µg/g)	1.2	3.6	10.3
CV (%)	7.4	4.7	5.2

*Data obtained by the analyzer Biolis i24 (Tokio Boeki)

Cross reactivity:

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

Bovine Hemoglobin	*Human Hemoglobin*
Pig Hemoglobin	*Human Lactoferrin*
Bovine Transferrin	*Human Calprotectin*

REFERENCES

1. Takashima Y., Shimada T. and Yokozawa T. Clinical benefit of measuring both haemoglobin and transferrin concentrations in faeces: demostration during a large-scale colorectal cancer screening trial in Japan. Diagnosis 2015; 2(1): 53-59.
2. Jin P., Wu Z., Meng M., Wang X., Wang X., Gong L., Yu D., Xie H., Li A., Li S., Yen L., Rao J. and Sheng J. Journal of Cancer Sceince & Therapy 2012, 4.8. DOI: 10.4172/1948-5956. 1000149.

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
	Keep out of the sunlight		

ADAPTED EQUIPMENT

- Biolis 24i/Biolis 50i (Tokio Boeki)
- BS200E (Mindray)
- ChemwellT (Awareness)
- TC220 (Tecom)

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