1. NAME OF THE MEDICINAL PRODUCT

LacTEST 0.45 g powder for oral solution

2. QUALITATIVE AND QUANTITATIVE COMPOSITION

One sachet contains 0.45 g of gaxilose.

For the full list of excipients, see section 6.1.

3. PHARMACEUTICAL FORM

White or almost white powder for oral solution.

4. CLINICAL PARTICULARS

4.1. Therapeutic indications

This medicinal product is for diagnostic use only.

LacTEST is indicated for the diagnosis of hypolactasia in children and adolescents aged 6 years or over, adults and elderly patients presenting clinical symptoms of lactose intolerance.

4.2. Posology and method of administration

This medicinal product should be prescribed by paediatrician and physicians experienced in the management of hypolactasia and must only be administered by duly authorized healthcare professionals under suitable medical supervision.

Posology

In adults, elderly patients and adolescents aged 12 years or over and children 6-11 years old, 0.45 g of gaxilose by oral administration.

Paediatric population

No dose adjustment is needed.

The safety and efficacy of LacTEST in children aged 0 to 5 years have not been established. No data are available.

Patients with renal impairment

The safety and efficacy of LacTEST in patients with renal impairment have not been established (see section 4.4). In patients with serious kidney disease, the use of LacTEST is contraindicated (see section 4.3.).

Patients with hepatic impairment

The safety and efficacy of LacTEST in patients with hepatic impairment have not been established. In patients with portal hypertension (ascites, cirrhosis), the use of LacTEST is contraindicated (see section 4.3.).

Method of administration

Precautions to be taken before handling or administering LacTEST

Carefully dissolve the entire contents of the sachet in about 100 ml of water. A clear and colourless solution will be obtained. The patient must immediately drink this solution and the time of the ingestion must be recorded. The total urine from 0 to 5 hours will be collected.

The performance of the test will have the following sequence: the patient will empty his/her bladder two hours before the test, and again 15-30 minutes before the start thereof. The LacTEST product will be administered. From that moment, the patient should drink up to 500 ml of water to facilitate diuresis and will collect the urine in a suitable vessel for the 5 hours and will empty his/her bladder before the end of the test. This will allow determining the total amount of xylose in accumulated urine of 0 to 5h. If the patient vomits during the performance of the test, it is necessary to repeat it. If it is necessary to repeat the procedure, it must not be done until at least 3 days have passed.

For patient preparation, see section 4.4.

For instruction on reconstitution of the medicinal product before administration and instruction for use, see section 6.6.

4.3. Contraindications

- Hypersensitivity to the active substance or to any of the excipients listed in section 6.1
- Galactosemia
- Pentosuria
- Patients that have a serious kidney disease, portal hypertension (ascites, cirrhosis), myxedema (severe hypothyroidism) or have medical records of total gastrectomy and/or vagotomy.

4.4. Special warnings and precautions for use

Patients with renal impairment

The use of LacTEST is not recommended in adults and elderly with abnormal renal function (proven by previous measurement of glomerular filtration rate), since renal excretion of xylose may be reduced and the safety and efficacy of LacTEST have not been established in these populations.

Paediatric population

For information on the use in paediatric population, see section 4.2.

The use of LacTEST is not recommended in children 6-11 years old and adolescents aged 12 years or over with abnormal renal function since renal excretion of xylose may be reduced and the safety and efficacy of LacTEST have not been established in this population.

Patient preparation

During the performance of the test in urine, the patients will be asked to drink up to 500 ml of water to facilitate diuresis. The patients must fast from 10 hours before the test starts and during its performance. As both aspirin and indomethacin have been reported to diminish urinary excretion of xylose, subjects will be asked to avoid taken either of these drugs from at least 48 h before performance of the gaxilose test to until the test is completed.

Children from 6 to 11 years old must have a low-carbohydrate breakfast, without lactose, xylose and arabinose 3 hours before ingesting Gaxilose, containing all or some of these meals: white rice, fried egg, grilled chicken breast and lactose-free serrano/serrano ham.

Specific warnings

Since urinary elimination of xylose may be reduced in patients with impaired renal function, it is mandatory to evaluate patients to detect any potential renal dysfunction when this impairment is suspected.

4.5. Interaction with other medicinal products and other forms of interaction

No interaction studies have been performed.

Both aspirin and indomethacin have been reported to diminish urinary excretion of xylose; thus, subjects will be requested to avoid the intake of any of these drugs from at least 48 h before LacTEST 0.45g intake and until the completion of the test.

The possibility of interaction between LacTEST 0.45 g and the food containing arabinose cannot be completely discarded; therefore, the patients will be warned of avoiding the intake of foods containing arabinose at least 10 hours (maximum 24 hours) before LacTEST 0.45 g intake and until the completion of the test.

Paediatric population

No interaction studies have been performed.

4.6. Fertility, pregnancy and lactation

Pregnancy

No effect during pregnancy is anticipated since systemic exposure to gaxilose is negligible.

LacTEST can be used during pregnancy.

Breastfeeding

No effect during breastfeeding is anticipated since systemic exposure to gaxilose is negligible.

LacTEST can be used during breastfeeding.

4.7. Effects on ability to drive and use machines

LacTEST has no or negligible influence on the ability to drive and use machines.

4.8. Undesirable effects

The gaxilose clinical program consisted of 3 studies in healthy volunteers and the intended population in which 540 subjects received oral gaxilose (113 mg – 5.4 g) and 12 received placebo. No treatment related serious adverse, nor events resulting in withdrawal, were detected. Only 13 adverse events were considered as probably or possibly related to the product under investigation, and none of them led to withdrawal. Four of them were moderate in intensity (pruritus and urticaria) and 9 were mild (abdominal distension, abdominal pain, vomit, diarrhea, nauseas and migraine).

A phase IV clinical trial was conducted in the intended population. A total of 74 patients were exposed to 0.45 g of gaxilose, and 70 received a second similar dose of gaxilose with a wash-out period of 5+/- 2 days. In the first test, 37 adverse events related to Gaxilose (24 mild and 13 moderate) were suffered by 22 patients (29.73% of the patients who performed the first test). In the second test, 15 adverse events related to gaxilose (12 mild and 3 moderate) were reported by 8 patients (11.43% of the patients who performed the gaxilose retest). No serious or severe adverse events were reported.

The adverse events reported are listed in the table below according to frequencies and system organ class.

The frequencies of adverse events are ranked according to the following: Very common (≥1/10); Common (≥1/100, <1/10); Uncommon (≥1/1,000, <1/100); Rare (≥1/10,000, <1/1000); Very rare (<1/10,000); Not known (cannot be estimated from the available data).

System Organ Class and frequency	**Adverse Events**
Nervous system disorders	
Uncommon	Headache.
Frequency not known*	Dizziness, syncope.
Gastrointestinal disorders	
Uncommon	Abdominal distension, abdominal pain, nausea, diarrhea, vomiting.
Frequency not known*	Flatulence, abdominal discomfort, abdominal pain upper, gastrointestinal sounds.
Skin and subcutaneous tissue disorders:	
Uncommon	Pruritus, urticaria.
Frequency not known*	Rash.

*Note: The adverse events indicated with "Frequency not known" are those that emerged during the Phase IV clinical trial. Not enough data are available.

In the population of 6-11 years old in healthy volunteers, no adverse events observed during the study were related to Gaxilose.

Reporting of suspected adverse reactions

Reporting suspected adverse reactions after authorisation of the medicinal product is important. It allows continued monitoring of the benefit/risk balance of the medicinal product. Healthcare professionals are asked to report any suspected adverse reactions via Spanish Pharmacovigilance System of Human Medicines: www.notificaRAM.es

4.9. Overdose

Due to the fact that only 0.45 g of gaxilose is ingested, an overdose is not expected.

5. PHARMACOLOGICAL PROPERTIES

5.1. Pharmacodynamic properties

Pharmacotherapeutic group: Other diagnostic agents, ATC code: VO4CX.

No pharmacodynamic activity has been described for the amount of 0.45 g of gaxilose.

LacTEST is a non-invasive method which allows evaluating the overall activity of intestinal lactase *in vivo* by means of the colorimetric determination of xylose in urine or blood after the oral administration of 4-O-ß-D-galactopyranosyl-D-xylopyranose (gaxilose), a disaccharide produced by means of enzymatic synthesis which works as a structural analog of lactose, the physiological substrate of intestinal lactase. Alternatively, xylose in urine samples can also be measured by an enzymatic method. After its oral administration, gaxilose is hydrolyzed by the intestinal mucosa enzyme into galactose and xylose, and these physiological hydrolysis products pass into the blood. The appearance of xylose in urine is closely correlated with the levels of intestinal lactase activity determined post-mortem in experimental animals, therefore, the use of gaxilose allows evaluating *in vivo* the activity of this enzyme.

The critical point for classifying patients as having normolactasia or hypolactasia by means of this diagnostic test is the following: the patients with a total amount of xylose excreted in urine of 0-5 hours equal to or greater than 37.87 mg of xylose are considered as having normolactasia if xylose is determined by the phloroglucinol method. If xylose is quantified by the Xylossay enzymatic method or by Osaxyl electrochemical method, patients are considered as normolactasic when the amount of xylose in urine of 0-5 hours is equal to or greater than 19.18 mg. If the amount of xylose (according to the method used) is lower than the previous values, the patient will be considered as having hypolactasia.

5.2. Pharmacokinetic properties

After its oral administration oral, this compound is hydrolyzed by the intestinal mucosa enzyme into galactose and xylose, and these physiological reaction products pass into the blood.

Absorption

Gaxilose is not absorbed by the intestinal mucosa as it has been previously described. This compound is hydrolyzed by intestinal lactase into its monosaccharide constituents galactose and xylose, which are absorbed by the intestinal mucosa, carried to the blood and one of them, xylose, is eliminated in the urine.

Distribution

The product is hydrolyzed by intestinal lactase into galactose and xylose which, after being absorbed, pass into the bloodstream and undergo normal physiological pathways. Galactose is transformed into glucose in the liver and xylose is partially metabolized in an endogenous manner [about 50% of the ingested xylose regardless of the dose and age, demonstrated in experimental animals], and the rest appears in blood and is finally eliminated in the urine. The non-hydrolyzed disaccharide is eliminated through the intestine.

Metabolism

For the assessment of the metabolization rate of gaxilose and of the extent in which the xylose produced is metabolized, it was previously necessary to study the assimilation of xylose in rats at 15, 18 and 30 days of age evaluating the proportion in which this monosaccharide is recovered in urine. It was observed that approximately 48% of the xylose administered is eliminated in urine.

A clinical trial performed in 12 healthy volunteers (which received 113, 225, 450, 900, 2700 and 5400 mg of gaxilose and placebo) clearly showed that the ingestion of gaxilose was followed by the appearing of xylose in blood and urine, in such a way that it depended on the oral dose administrated. This evidences that the synthetic disaccharide is a substrate in vivo of the intestinal lactase activity in human beings and that it can be used for the non-invasive evaluation of this enzymatic activity. The minimum dose of gaxilose for a reliable detection of xylose in the urine was 450 mg, to guarantee that a sufficient amount of eliminated xylose is collected.

Elimination

Xylose is passively absorbed in the small intestine and although a portion of it is metabolized (as has been indicated above), the rest is eliminated in the urine.

Renal/Hepatic impairment

The pharmacokinetics in patients with renal or hepatic impairment has not been characterised.

5.3. Preclinical safety data

The assays at different doses in animals have not detected toxicity at large doses. The lethal dose 50 in rats and mice both by oral route and by intravenous route was greater than 4000 mg/kg.

6. PHARMACEUTICAL PARTICULARS

6.1. List of excipients

None.

6.2. Incompatibilities

This medicinal product must not be mixed with other medicinal products.

6.3. Shelf life

Unopened sachets: 4 years.

After reconstitution, the solution should be used immediately.

6.4. Special precautions for storage

Unopened sachets. This medicinal product does not require any special temperature storage conditions.

Keep the sachets in the outer carton.

For storage conditions after reconstitution of the medicinal product, see section 6.3.

6.5. Nature and contents of container

Pack size of one sachet for single use.

Sachets formed by a Coated paper/Aluminum/Polyethylene and Polyethylene/Polyester, containing 0.45g gaxilose

6.6. Special precautions for disposal and other handling

The measurement of xylose levels in the 0-5 hours collected urine samples may be performed using the enzymatic kit (Xylossay) with the automated analyzers, the electrochemical method (Osaxyl) with PoC or making use of the phloroglucinol manual technique described hereinafter. Patients with a total amount of xylose excreted in urine of 0-5 hours equal to or greater than 19.18 mg of xylose, using the enzymatic kit Xylossay or the electrochemical method Osaxyl, and equal to or greater than 37.87 mg of xylose, quantified making use of

the manual phloroglucinol method, are considered normolactasic. Should the amount of xylose is less than the previous values (according to the method used), the patient will be considered hypolactasic.

Any unused medicinal product or waste material should be disposed of in accordance with local requirements.

Analysis of samples and specification of the assay for laboratories

Xylose determination using the enzymatic kit (Xylossay) with automated analyzers

The enzymatic kit for xylose quantification includes the following reagents:

Vial 1. Phosphate buffer 50 mM, pH 8.0 (solution)
Vial 2. Lyophilized NAD^+
Vial 3. Lyophilized Xylose dehydrogenase enzyme
Vial 4. Calibrator: Standard xylose solution (3.75 mg/dl)

Preparation of Kit reagents

Important: reagents preparation must be carried out in the following position.

1. Add phosphate buffer (vial 1) in the vial 3, to dissolve the enzyme lyophilized at a final concentration of 0.24 mg/ml. Mix genteelly to avoid loss of activity in the suspended enzyme. Keep cold during use if possible. This will be called **REACTIVE 2**.
2. Add phosphate buffer (vial 1) in the vial 2 (lyophilized NAD^+) and mix until it is totally dissolved (vortex if necessary).
3. Add the complete dissolved NAD^+ (NAD^+ + buffer) to the vial 1 Thus, the vial 1 will contain phosphate buffer plus NAD^+ in the appropriated concentration for the assays (3.4 mg/ml). Keep cold during use if possible. This will be called **REACTIVE 1**.
4. The Standard xylose solution is ready for use.

Automated analyzer assay protocol:

Sample preparation: Every urine sample must be shaken after thawing and centrifuged in order to homogenize it and remove any precipitate that could affect the assay.

Instrument settings: Reaction kinetics with two reagents (R1 and R2), reaction endpoint, wavelength 340 nm. Reaction will be carried out at the standard device temperature (usually 37°C). The instrumental application for testing must be designed and incorporated by the technical manager of the team, according to the following specifications:

GENERAL ANALYZER ASSAY PROTOCOL:

In this protocol, the final volume of the reaction (V_R) is the addition of the volumes of reactive 1, reactive 2 and distilled water (for the blank), sample or xylose calibrator.

Step	Reactive	Blank	Calibrator	Sample
1	Distilled water	0.175 x V_R µl	-	-
	REACTIVE I (phosphate buffer +NAD^+)	0.725 x V_R µl	0.725 x V_Rµl	0.725 x V_R µl
	Sample (Urine)	-	-	0.175 x V_R µl
	Xylose Calibrator (3.75 mg/dl)	-	0.175 x V_R µl	-
Mix the reaction and incubate for 5 min. After this, read the absorbance (A1)				
2	REACTIVE 2 (Xylose dehydrogenase)	0.1 xV_Rµl	0.1 xV_R µl	0.1 xV_R µl
Mix the reaction, incubate for 5 min and read the final absorbance (A2)				
Note: Each time xylose is measured in urine samples, xylose controls must be performed after calibration. These controls, one with low concentration and the second with high concentration, must be measured as if they were samples.				

Example for V_R = 200 µl:

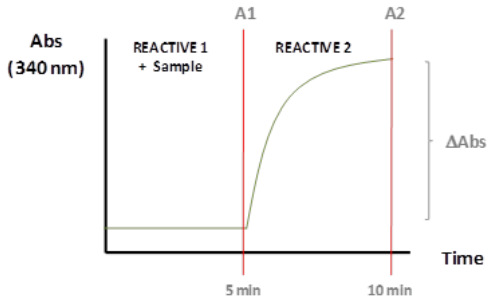
Step	Reactive	Blank	Calibrator	Sample
1	Distilled water	35 µl	-	-
	REACTIVE I (phosphate buffer +NAD^+)	145 µl	145 µl	145 µl
	Sample (Urine)	-	-	35 µl
	Xylose Calibrator (3.75 mg/dl)	-	35 µl	-
Mix the reaction and incubate for 5 min. After this, read the absorbance (A1)				
2	REACTIVE 2 (Xylose dehydrogenase)	20 µl	20 µl	20 µl
Mix the reaction, incubate for 5 min and read the final absorbance (A2)				
Note: Each time xylose is measured in urine samples, xylose controls must be performed after calibration. These controls, one with low concentration and the second with high concentration, must be measured as if they were samples.				

Observation: the shown protocol has been designed according to general specifications. However, final volumes and dilutions of reactives might be different among analyzers, as long as their final concentrations and ratios are maintained in the final assay mixture (2.46 mg/ml of NAD^+, 0.024 mg/ml of xylose dehydrogenase, phosphate buffer 50 mM).

Calculations:

Total amount of xylose in urine samples

Typical assay profile:



Two values of Absorbance (340 nm) will be recorded from each assay:

- A1 = initial Absorbance of the mix **REACTIVE 1** + Sample (incubated 5 min)
- A2 = final Absorbance after addition of **REACTIVE 2** (incubated 5 min)

Differences between the two Absorbance values are proportional to xylose concentration which could be calculated using the provided xylose standard solution as reference:

- ΔAbsorbance (340 nm) = ΔAbs = A2 - A1
- Concentration of xylose in Sample (Urine) = [*Sample*] (*mg/dl*)
- Concentration of xylose in Standard solution = *3.75 mg/dl*

$$[Sample]\ (mg/dl) = \frac{\Delta Abs\ (Sample\ assay)}{\Delta Abs\ (Calibrator)}\ x\ 3.75\ mg/dl$$

The total amount of xylose in the Sample (mg) will be calculated from its total volume:

$$Xylose\ (mg) = [Sample]\ (mg/dl)\ x\ Volume\ Sample\ (dl)$$

Example:

Determination of Total amount of xylose in an urine sample:

- Total volume of sample (urine) = 557 ml = 5.57 dl

1) Sample assay: A1 = 0.122
A2 = 0.146

$$\Delta Abs\ (Sample\ assay) = 0.146 - 0.122 = 0.024$$

2) Standard assay: A1 = 0.087
A2 = 0.166

$$\Delta Abs\ (Calibrator) = 0.166 - 0.087 = 0.079$$

$$[Sample](mg/dl) = \frac{0.024}{0.079}\ x\ 3.75\ mg/dl = 1.139\ mg/dl$$

$$Xylose\ (mg) = 1.139\ \frac{mg}{dl}\ x\ 5.57dl = \mathbf{6.34\ mg}$$

Normal values in children and adolescents over 6 years of age, adults and elderly patients

Test	Xylose in urine (mg)
LacTEST 0.45 g	≥ 19.18

Values below 19.18 mg are considered indicative of hypolactasia.

Note:
Considerations to be taken into account concerning the urine samples storage.

1- It is recommended to store the urine samples for the measurement of xylose, at 4°C if the determination is going to be carried out on the day; or otherwise, when the measurement is not being performed on the day, samples should be stored at -20°C or -70°C (samples will remain stable at least 2 months after freezing).
2- If measurements need to be repeated, samples can be frozen and thawed at least three times in a period of 2 months.

Validation of xylose quantification method in urine using the enzymatic kit (Xylossay)

Analytical parameters (*)	
Range of the calibration curve	From 0.25 to 15 mg/dl
Linearity	$r^2 \geq 0.9996$
Limits	Limit of Blank: 0.046 - 0.072 mg/dl Limit of Detection: 0.13 - 0.49 mg/dl Limit of Quantification: 0.42 - 1.64 mg/dl
Precision (coefficients of variation)	Within-run precision < 13.6% Between-run precision < 11.5%
Accuracy (percent of the nominal value)	Within-run accuracy ≤ 12% Between-run accuracy ≤ 5.9%
Carry-over	3.3 - 4.0%
Error	0.095 - 0.76%

(*) Range of data calculated from 3 different automatic analyzers.
Note: Before implementing the enzymatic test into a specific analyzer, analytical validation must be performed following the approved current Guidelines.

Xylose determination using the electrochemical method (Osaxyl)

The electrochemical detection includes the following:

- Osaxyl^{7000}. Reader for the quantitative detection of xylose in urine samples.
- Osaxyl test strips. Pre-calibrated strips for a single use in Osaxyl^{7000}.
- Dummy cell.
- Charger.
- Dilution buffer.
- Micropipette of 100 µL.
- Micropipette tips
- Empty vials for sample pre-treatment.
- Leaflet
- Quick guide of reference

Preparation of the procedure:

- Collect the urine sample following the indications described in the LacTEST 0.45g package insert.
- Take note of the total volume of urine in the container.
- Check the expiration date of the test strips and dilution buffer.
- Remove a test strip from its aluminum pouch with clean, dry hands.
- Allow 10 minutes to warm to room temperature (20-30°C), the strip, the urine sample, and the dilution buffer.
- Put a clean tip on the pipette.
- Add, using the pipette, 100 µL of the dilution buffer to an empty vial.
- Without removing the pipette tip, add the same volume of the urine sample to the vial to which you added the dilution buffer before.
- Close the vial and shake it to obtain a homogenous mixture.
- Start the analysis procedure on the reader following the indications of the Osaxyl^{7000} User Manual.
- When the reader indicates it, insert the test strip and add to the end of the same, the mixture previously prepared by using the pipette.
- Once the analysis is finished, the result appears on the display.
- Remove the used test strip from the reader and discard it together with the vial and the pipette tip.
- If the reader has requested you to repeat the analysis with a higher dilution factor, use a new test strip and prepare a new urine sample dilution on another vial by adding 3 volumes (300 µL) of the dilution buffer and one volume (100 µL) of the urine sample. Start the analysis procedure on the reader following the indications of the Osaxyl^{7000} User Manual for the analysis of samples with higher dilution factor.

Normal values in children and adolescents over 6 years of age, adults and elderly patients

Test	Xylose in urine (mg)
LacTEST 0.45 g	≥ 19.18

Values below 19.18 mg are considered indicative of hypolactasia.

Note:
Considerations to be taken into account concerning the urine sample storage and dilution.

1. It is recommended to measure the xylose sample when it arrives.
2. If the determination of xylose is going to be carried out the same day but later, the urine sample should be store at 4ºC.
3. When it is managed urine volumes lower than 150 ml and Xylose values higher than 10 mg/dL, it should be used a dilution factor of 1:4.

Validation of xylose quantification method in urine using the electrochemical method Osaxyl

	Osaxyl fresh urine	**Osaxyl Frozen urine**
Range of the calibration curve	From 0.75 to 10 mg/dl	From 0.75 to 10 mg/dl
Linearity	$r2 \geq 0.9986$	$r2 \geq 0.9952$
Limits	Limit of Blank: -0.05 mg/dl Limit of Detection: 0.40 mg/dl Limit of Quantification: 0.75 mg/dl	Limit of Blank: 0.05 mg/dl Limit of Detection: 0.75 mg/dl Limit of Quantification: 0.75 mg/dl
Precision (coefficient of variation)	Within-run precision < 4.4% Between-run precision <3.5%	Within-run precision < 4.2% Between-run precision < 6.7%
Accuracy (percentage of the nominal value)	Within-run accuracy ≤ 12.6% Between-run accuracy ≤ 13.2%	Within-run precision < 1.4% Between-run precision < 3.0%
Error	12.6-13.2%	1.4-3%

General recommendation:

When there is uncertainty about the diagnosis, a clinical follow-up will help to conclude a definitive diagnosis.

Xylose quantification using manual phloroglucinol method.

It consists of the spectrophotometric determination of a coloured compound formed between the reagent phloroglucinol (1,3,5-OH-benzene) and furfural, which is the product of the reaction of xylose in a strongly acidic medium.

A validated protocol for determination of xylose in urine samples of patients, who received LacTEST 0.45 g, including recommendations for treatment and storage of samples, is indicated below.

Xylose Determination Protocol

Prepare:

a) 6.66 mM solution of xylose (100 mg/dl) in Milli Q water. From this solution, a 0.66 mM (10 mg/dl) solution, which will be used in the calibration curves, is prepared.

b) Colour reagent with phloroglucinol.
- 0.5 g of phloroglucinol
- 100 ml of glacial acetic acid
- 10 ml of hydrochloric acid.

The resulting mixture is enough to process 50 samples (50 tubes) and it should be prepared fresh before its use. Once it has been prepared the reaction mix should not be used for more than 5 hours.

c) Prepare a xylose standard curve ranging from 0.0125 to 0.5 mg/dl (0.25 to 10 µg).

The standard curve is expressed both in mg/dl and in µg of xylose, since the value of xylose in urine is expressed as the total amount (mg) referred to the volume of urine collected for each subject (amount units are used because the diagnosis in urine is expressed as the total amount of xylose present in urine). The standard xylose solution should be prepared fresh on the day when the xylose measurements are performed. Once it has been prepared the standard solution should be used within 5 hours.

	Xylose Standard Curve								Samples			
Xylose, mg/dl	0	0.0125	0.025	0.05	0.1	0.2	0.4	0.5				
Xylose, 10 mg/dl (µl)	-	2.5	5	10	20	40	80	100	-	-	-	-
Urine (µl)	-	-	-	-	-	-	-	-	25	50	75	100
Milli Q water (µl)	100	97.5	95	90	80	60	20	-	75	50	25	0
Phlorogl. reagent (ml)	1.9 ml →											

- 10 ml Polypropylene tubes, acids and heat resistant, are used. The samples are added. A xylose standard curve is made, with the xylose amounts indicated above.
- The phloroglucinol reagent is added to each tube until completing a volume of 2 ml.
- Tubes are incubated in a 100ºC bath for 4 minutes exactly and then cooled in water.
- The absorbance at 554 nm is measured in a colorimeter or spectrophotometer immediately after cooling the reaction.
- The spectrophotometer is adjusted to zero absorbance, before reading the standard solutions and samples, with a reagent blank containing water and phloroglucinol reagent. This "reagent blank" is heated and cooled along with the other samples.
- Calculation: The concentration of xylose in urine is calculated extrapolating the values from the calibration curve. The total volume of urine collected during 5hours after intake of the product needs to be recorded, to calculate the total amount of xylose in the 5-hours urine.

Normal values in adults, elderly patients and adolescents aged 12 years or over

Test	Urine xylose (mg)
LacTEST 0.45 g	≥ 37.87

Values below 37.87 mg are considered indicative of hypolactasia.

Note:

Considerations to be taken into account concerning the urine samples storage:

1. It is recommended to store the urine samples for the measurement of xylose, at 4^{o}C if the determination is going to be carried out on the day; or otherwise, when the measurement is not being performed on the day, samples should be stored at -20^{o}C or -70^{o}C (samples will remain stable at least 2 months after freezing).
2. If measurements need to be repeated, samples can be frozen and thawed at least three times in a period of 2 months.

Regarding work solutions and processed samples of urine, the following aspects should be considered:

1. The colour reagent of phloroglucinol is only stable for 5 hours after its been mixed.
2. Once samples have been processed by heating in the presence of the phloroglucinol reagent, samples will only be stable during the first three hours, after processing.

Validation of the method of measurement of xylose in urine with the manual phloroglucinol method.

Calibration Curve Range:	0.5 – 20 mg/dl
Linearity:	$y = 1.2225x + 0.0095$ $r^2 > 0.9997$
Sensitivity:	Quantification limits for urine and serum samples is at 0.5mg/dl.
Reproducibility:	CVs between different samples must not be greater than 15%.
Accuracy:	CVs between different samples must not be greater than 15%.
Measurement error:	0.48 – 6.45%

Sensitivity and specificity values of the phloroglucinol, Xylossay and Osaxyl methods.

Method	Sensitivity	Specificity	Reference method
Phloroglucinol method	0.935	0.918	Biopsy
Enzymatic method	0.955	0.993	Phloroglucinol method
Electrochemical method	0.962	0.938	Enzymatic method

7. MARKETING AUTHORISATION HOLDER

Venter Pharma S.L.
c/ Almagro, 1, 1º dcha
28010 Madrid
Spain

8. MARKETING AUTHORISATION NUMBER(S)

9. DATE OF FIRST AUTHORISATION/RENEWAL OF THE AUTHORISATION

10. DATE OF REVISION OF THE TEXT

Detailed information on this medicinal product is available on the website of the Spanish Agency for Medicines and Medical Devices (AEMPS) http:// www.aemps.gob.es