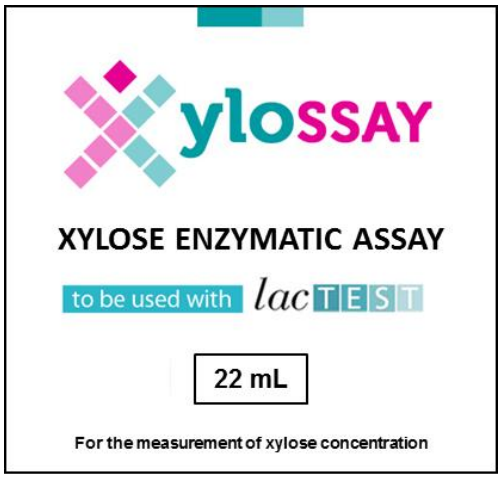
VERSION FOR SIEMENS DIMENSION VISTA 1500 AUTOMATED ANALYZER



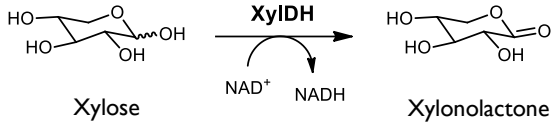
IVD CE

DESCRIPTION:

Xylossay® is a reliable and automatable method for the quantification of xylose present in urine. **Xylossay®** is the recommended method for xylose detection after the administration of **LacTEST 0.45 g.**

METHOD RATIONALE:

The enzyme xylose dehydrogenase (XylDH)[1] catalyzes the specific oxidation of xylose to yield xylonolactone[2]. As XylDH is a NAD^+ dependent enzyme, this reaction requires a concomitant reduction of this cofactor to NADH:



Xylose Xylonolactone

Production of NADH can be spectrophotometrically detected and quantified by the increase of the absorbance at 340 nm during the reaction. This way, absorbance increments will be directly proportional to the amount of xylose present in the sample.

COMPONENTS OF THE KIT:

Xylossay® is supplied in a format suitable for performing 110 xylose determinations. Each kit contains a vial of each one of the following components:

VIAL	CONTENT AND FORMAT	AMOUNT
Brown	Phosphate buffer, 50 mM, pH 8.0 (Solution)	22 mL
Orange	β-Nicotinamide Adenine Dinucleotide (NAD^+) (Lyophilized)	66.34 mg ± 12%
Purple	D-Xylose Dehydrogenase (Lyophilized)	0.6 mg ± 10%
Pink	Calibrator: D-(+)-Xylose (Solution)	2 mL; 3.75 mg/dL ± 5%.

1. Sánchez-Moreno, I. *et al. J. Biotechnol.* (2016) 234:50-57.
2. Stephens, C. *et al. J. Bacteriol.* (2007) 189:2181-2185.

NON SUPPLIED ADDITIONAL EQUIPMENT:

Preparation of the reagents requires the use of micropipettes or systems suitable for dispensing the specified volumes. Vortex agitators can be used when indicated. Xylose quantification controls.

PREPARATION OF REAGENTS:

IMPORTANT: the preparation of the reagents must be conducted in the following order:

1. Dissolve the content of the purple vial (Xylose dehydrogenase, lyophilized) in ***2.5 mL*** of buffer (Phosphate buffer 50 mM, pH 8.0; brown vial). Mix genteelly to avoid loss of activity in the suspended enzyme. Keep cold during use if possible. This solution will be called **REACTIVE 2**.

2. Add 2 mL of buffer (Phosphate buffer 50 mM, pH 8.0; brown vial) in the vial that contains the lyophilized NAD^+ (orange vial) and mix vigorously until its complete dissolution (vortex agitators can be used).

3. Add the totality of the 2 mL of dissolved NAD^+ (orange vial + buffer) in the buffer vial (brown vial). Thus, the brown vial will contain ***19.5 mL*** of phosphate buffer plus NAD^+ at the appropriated concentration. Keep cold if possible. This solution will be called **REACTIVE 1**.

4. The solution of xylose (Calibrator: standard xylose solution, pink vial) is ready for use.

CONSERVATION AND STABILITY:

Xylossay® must be stored at temperatures between 4° and 8°C until use, being stable under these conditions for at least 18 months. After being prepared, REACTIVES 1 and 2 are stable for at least 39 days when maintained at temperatures between 4 and 10°C (stability in automated equipments). Both REACTIVE 1 and REACTIVE 2 can be frozen at -20°C without any activity loss. Both reagents can be frozen and thawed at least 6 times without altering their function.

IMPORTANT: to use frozen reactives, these must be completely thawed (at room temperature). Once completely thawed, they must be agitated for total homogenization before use.

ASSAY PROTOCOL:

SAMPLE PREPARATION: Collection of urine samples must be performed strictly following the specifications indicated in **LacTEST 0.45 g** summary of product characteristics. Centrifugation of urine samples is recommended (according to the protocol established in each laboratory) to remove any possible precipitate that could affect the assay. Frozen samples must be agitated after thawing for their homogenization.

PARAMETERS: wavelength 340 nm, using the standard device temperature (usually 37°C). The instrumental application for testing must be designed according to the following specifications.

CALIBRATION AND CONTROL PROCEDURES: Calibration of xylose determination in the analyzers (**CAL**) must be carried out using the standard xylose solution supplied with the kit (Calibrator, pink vial) as concentration reference (3.75 mg/dL). For calibration and xylose quantification control it is recommended the use of at least two certified and validated levels of control: a low level, with xylose concentration between 0.8 and 3 mg/dL, and a high level,

with xylose concentration between 5 and 8 mg/dL. These controls are not supplied with the kit, and must be purchased independently.

STANDARD ASSAY PROTOCOL:

The shown volumes can be increased or reduced if the final proportion between them is maintained.

Reactive	Blank	Sample	CAL
Distilled water	**24 µL**	-	-
REACTIVE 1	**100 µL**	**100 µL**	**100 µL**
Sample	-	**24 µL**	-
Calibrator	-	-	**24 µL**
Mix the reaction and incubate for 5 min. (**A1**)			
REACTIVE 2	**14 µL**	**14 µL**	**14 µL**
Mix the reaction and incubate for 5 min. (**A2**)			

FINAL NUMBER OF MEASUREMENTS WITH EACH KIT:

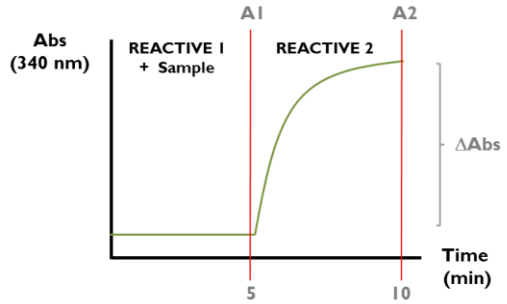
Due to the dead volume of some automated analyzers, the number of determinations can be reduced. The next formulas allow calculating the final number of assays in function of the dead volume:

$$\boldsymbol{REACTIVE\ 1} = \frac{(\mathbf{19.5\ mL} - \text{Dead volume in mL}) \text{ x } 1000}{\text{Volume of REACTIVE 1 in the assay (µL)}}$$

$$\boldsymbol{REACTIVE\ 2} = \frac{(\mathbf{2.5\ mL} - \text{Dead volume in mL}) \text{ x } 1000}{\text{Volume of REACTIVE 2 in the assay (µL)}}$$

CALCULATIONS:

Absorbance values (340 nm) per assay:



A1 = Initial absorbance of the mixture REACTIVE 1 + Sample (incubated for 5 min).

A2 = Final absorbance after adding REACTIVE 2 (incubated for 5 more minutes, total time of reaction: 10 min).

Differences between the two absorbance values will be proportional to xylose concentration, which can be calculated using the standard xylose solution provided with the kit (Xylose standard solution, pink vial):

ΔAbsorbance (340 nm) = ΔAbs = A2 - A1

Sample concentration = [*Sample*] (*mg/dL*)

Xylose concentration (Calibrator) = *3.75 mg/dL*

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

The total amount of xylose in the Sample (mg) can be calculated using the total volume of urine collected during the test.

$$Xylose\ (mg) = [Sample]\ x\ Volume\ of\ Sample\ (dL)$$

EXAMPLE:

Determination of the total amount of xylose in the urine sample of a patient:

Volume of Sample (urine) = **557 mL** = 5.57 dL

- **Sample assay**: A1 = 0.122

A2 = 0.146

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

- **Calibrator assay**: A1 = 0.0870

A2 = 0.166

$$\Delta Abs\ (Calibrator) = 0.166 - 0.0870 = 0.079$$

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

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

REFERENCE VALUES

Normal values in adults:

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

Values below 19.18 mg indicate hypolactasia.

ANALYTICAL PARAMETERS:

The analytical parameters of **Xylossay®** application were determined during the validation of the technique in three automated analyzers (Roche Cobas c502, Werfen ILab 600 and Siemens Dimension Vista 1500 analyzers).

Linearity: until at least 15 mg/dL in all the cases.

Limit of detection: between 0.13 and 0.49 mg/dL.

Reproducibility: The coefficients of variation between samples cannot be higher than 15%.

Accuracy: ≥ 88 %.

Carry-over: ≤ 4 %

ANALYTICAL PERFORMANCE:

The analytical parameters established for **Xylossay®** can slightly vary in function of the characteristics of the equipment used. Presence of some sugars as L-arabinose or glucose at high concentrations can affect xylose determination. For this reason, sample collection requires a fasting period of 10 hours. Under these conditions, no interference has been found in the collected urine.

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ANNEX – APPLICATION PARAMETERS

The following parameters were optimized in a Siemens Dimension Vista 1500 automated analyzer:

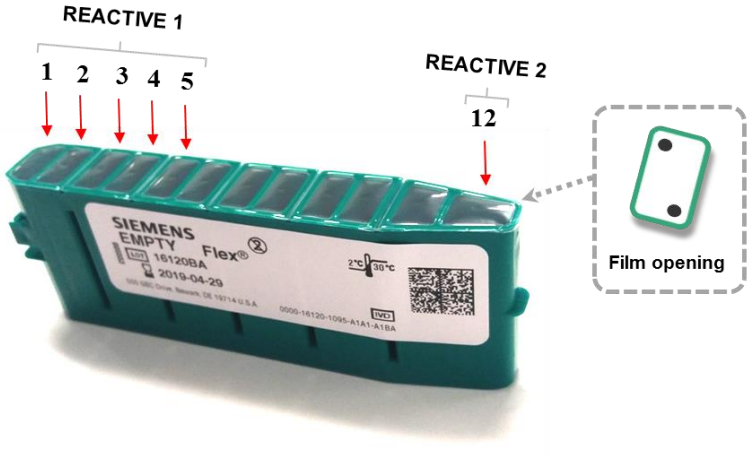
Advanced menu > **Configuration** > **User-Defined Methods** > **Create New**

Method name	XILO	ID	xxxx	Units	mg/dL	Mode	PHOTOMETRIC

Execution | Reagent | Calculation | Calibration | Sample

Delivery	Time	Component 1		Remix	Component 2		Remix	Chase		Total Volume	Mix
D1	-21	R1	100 µL	None	---	0 µL	None	0	µL	100 µL	None
S1	0	S	24.0 µL					0	µL	24 µL	Moderate
D2	392	R2	14 µL	None	---	0 µL	None	0	µL	14 µL	Moderate
D3	39	---	0 µL	None	---	0 µL	None	0	µL	0 µL	None
S2	662	S	0.0 µL					0	µL	0 µL	None

Total Volume 138 µL



Reagent cartridge for User-defined tests in Dimension Vista 1500 equipment from SIEMENS (SIEMENS EMPTY Flex®, SIEMENS reference: KS999). In the case of XYLOSSAY®, **Reagent 1** must be loaded in **positions 1-5**, and **Reagent 2** in **position 12**. To add the reagents, the film that covers the wells of each position must be perforated in two of its corners, as indicated in the figure insert.

Execution | **Reagent** | Calculation | Calibration | Sample

	Reagent	Tests	Life (hours)	Volume
1	R1	27	2160	2992
2	R1	27	2160	2992
3	R1	27	2160	2992
4	R1	27	2160	2992
5	R1	27	2160	2992
6	Empty	0	0	0
7	Empty	0	0	0
8	Empty	0	0	0
9	Empty	0	0	0
10	Empty	0	0	0
11	Empty	0	0	0
12	R2	131	2160	2186

Onboard Life (hours)
2160

Execution | Reagent | **Calculation** | Calibration | Sample

Format: Absorbance
Measuring: 340
Blanking: ---

	Time	Dilution		
☑ P1	310	1.00	IOD ---	0
☑ P2	620	1.00	FOD ---	0
☐ P3	-21	1.00	FOD ---	0
☐ P4	-21	1.00	FOD ---	0

Name	Formula
maurl	R93[340]
mau2	(r179[340])
CalculationNumber	mau2–maurl

☐ Errors

Check	Read 1		Read 2	Trigger	
---	-21	–	-21	---	0

Absorbance | Add

Execution | Reagent | Calculation | **Calibration** | Sample

Std. Curve: LINEAR Interval (days): 30

Calibrator Levels

2

Level	Weight	Replicates
1	1.00	3
2	1.00	3

Calibrator Dilutions

Create	Component	Diluent	Dilution Factor	Minimum Aspiration
☐ L1				200
☐ L2				200

Execution | Reagent | Calculation | Calibration | **Sample**

Urine

☑

	Low	High
Assay Range	0.2	15
Reference Range	0	15

Expiration (minutes): 120

Predilute

☐

Sample volume: 0
Dilute Factor: 0
Diluent: SDIL

Autodilution

☑ ☑ Above ☑ Below

	Above	Below
Sample volume	15	70
Dilute Factor	5	2
Diluent	SDIL	

Advanced menu > **Calibration** > **Calibrators** > **New**

Product Name	XILOCal
Open Channel	YES
Lot Number	XXXXX
Expiration Date	XXXX-XX-XX
OnBoard Stability	720
Open Vial Stability	720
Max Punctures	720
Fluid Type	Urine
Obsolete	NO

Bottle Values

	A	B	Unit
Volume	2000	2000	µL
XILO	0	3.75	mg/dL

Calibration Point Values

Level	1	2	Unit
XILO	0	3.75	mg/dL

☐ Calibrator prepared externally