Chuangxingwell

Lactose Intolerance Rapid Test Kit

For in-vitro diagnostic use only.

IVD

INTENDED USE

This kit is intended for the in vitro qualitative detection of galactose in human urine. It is clinically used solely as an adjunct to the diagnosis of lactose intolerance and is not intended for the diagnosis of hereditary galactosemia.

CLINICAL BACKGROUND

Lactose intolerance (hypolactasia) results in the inability to digest lactose, the predominant sugar of milk. This inability results from a decrease in the activity of lactase enzyme, which is produced in the small intestine. Lactase breaks down milk sugar (lactose) into monosaccharides (glucose and galactose), which can then be absorbed into the bloodstream.

Lactase activity begins to decrease after weaning. It is estimated that approximately 15-20% of Western and Northern Europeans, and 90% of Asians, Africans and Native Americans suffer from lactose intolerance. Often the symptoms remain undiagnosed, and, thus lactose intolerance may remain untreated for years. Lactose intolerance may cause the following gastrointestinal symptoms after the intake of lactose: nausea, flatulence, abdominal bloating, cramps and pain, as well as diarrhea.[1-3]

PRINCIPLE

Urine undergoes adsorption by activated carbon within the urine purification device, filtering out interfering substances such as bilirubin, glucose, and vitamin C. Galactose in the urine undergoes enzymatic conversion to form galactose-1,2-dihydroxy-1,3-dihydroxypropane-2,5-diol and hydrogen peroxide. The latter oxidizes 3,5-dichloro-2-hydroxybenzenesulfonic acid to produce a red color. The intensity of this coloration is positively correlated with the concentration of galactose in the urine.

WARNINGS AND PRECAUTIONS

Wear gloves during operation to avoid contact with urine samples. All samples should be treated as potential sources of biological contamination. If reagents come into contact with skin or eyes, immediately rinse thoroughly with copious amounts of water and seek prompt medical attention. Do not mix reagents from different batches. Expired reagents are prohibited. Once opened, reagents must be used within the specified timeframe. Discard testing devices, pipettes, and other consumables as medical waste. Dispose of remaining reagents and samples according to laboratory biosafety protocols. Read the entire instruction manual thoroughly before use. Improper handling may invalidate test results.

SPECIMEN COLLECTION AND HANDLING

Biopsy specimens are recommended to be taken with e.g. 5 mm forceps from the mucosa of the upper part of the small intestine at any site of the postbulbar duodenal wall during gastroscopy.

The bulbus should be avoided, because of the gastric acid related changes in the lactase activity in the bulbar mucosa in some patients. Total lactase content in the biopsy depends on the size of the biopsy specimen used. Estimation of the recommended biopsy size can be done visually by comparing the size of each biopsy to the picture below. Recommended size of biopsies: Diameter (ø) between 2 mm - 1.5 mm.

When smaller forceps are used (for e.g. small children), it is recommended to use two biopsies instead of one.

The performance of the Lactose Intolerance Rapid Test Kit was evaluated using biopsies in this size range. If the biopsy is smaller than recommended, there is a risk of a false positive (hypolactasia) result. If the biopsy specimen is too large, there is a risk of a false negative (normolactasia) result. Before performing the test, blood must be removed from the biopsy specimen by placing it briefly on the sterile gauze pad. It is recommended to perform the test immediately.

Do not contaminate the biopsy forceps or biopsy specimen with formalin or other tissue fixatives. It is recommended that the biopsies for test are collected first, before any biopsies are collected for histology. If the forceps become contaminated with formalin or other tissue fixatives rinse thoroughly in water before collecting the biopsies for test.

KIT CONTENTS AND REAGENT PREPARATION

Each Lactose Intolerance Rapid Test Kit contains reagents for 10 or 25 tests. Store the kit components refrigerated (2-8 °C).

1. Test Plates

Contents: 10 or 25 test plates in a bag, each containing a well for the biopsy specimen. Preparation: Ready for use.

2. Substrate Solution, Bottle 1, SUBS

Contents: 1 vial containing 2 ml or 4 ml of acetate buffer and lactose with 0.002% thiomersal as preservative. Preparation: Ready for use. Stability: Stable until expiration date. In-use stability 4 months.

3. Chromogen Solution, Bottle 2, CHRO

Contents: 1 vial containing 0.75 ml of chromogen solution in acetic acid with 0.002 % thiomersal as preservative.

Preparation: Ready for use. Stability: Stable until expiration date. In-use stability 4 months.

WARNING: Acetic acid iscorrosive and can cause irritation!

4. Signal Reaction Solution, Bottle 3, SIGN

Contents: 1 vial containing 2 ml or 4 ml of enzyme solution with 0.002 % thiomersal as preservative. Preparation: Ready for use. Stability: Stable until expiration date. In-use stability 4 months.

5. Instructions for Use

6. Interpretation color chart

MATERIALS REQUIRED, BUT NOT PROVIDED

Forceps, timer, sterile gauze pads, gloves. Performance of the kit may be tested with Lactase CONTROL+.

STORAGE AND STABILITY

Store the Lactose Intolerance Rapid Test Kit refrigerated (2-8 °C). In-use stability of the kit is 4 months. Do not use reagents after the expiration date printed on the label.

TEST PROCEDURE

Read the complete assay procedure before starting. Allow all the reagents to reach room temperature (20-25 °C) for at least 15 minutes before use. Mix all the reagents before using by turning the bottles upside-down for a few times. Then tap the bottles on the table to ensure the liquid returns to the bottle. When adding the drops into the wells, hold the bottles in vertical upside-down position.

STEP 1: LACTASE REACTION

1. Open the label covering the well on the plate. Place the biopsy specimen into the well. See figure 1.
2. Add 2 drops (80 µl) of the substrate solution (Bottle 1) into the well. Close the label.
3. Mix the plate by shaking 5-6 times vigorously sideways on the table.
4. Incubate for 15 minutes at room temperature (20-25 °C).

STEP 2: SIGNAL REACTION

1. Open the label again. Add 1 drop (10 µl) of the chromogen solution (Bottle 2) into the well. See figure 2.
2. Immediately add 2 drops (80 µl) of the signal reaction solution (Bottle 3). Close the label.
3. Mix the plate by shaking for 5-6 times vigorously sideways on the table.
4. Incubate for 5 minutes at room temperature (20-25 °C) and interpret the lactase content with the color chart included in the kit package.

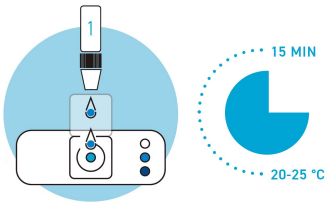

Figure1. Lactase Reaction
(lactose -> galactose + glucose)

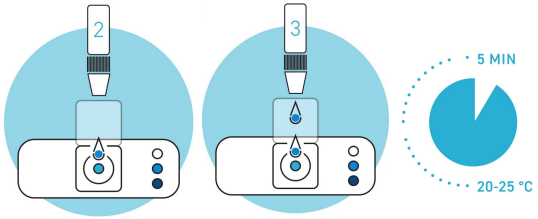

Figure2. Signal Reaction
(glucose + color reagents -> blue color)

RESULTS

Presence of functional lactase in the specimen can be interpreted with the color chart given on the label. See the example below.

- **POSITIVE** (hypolactasia)
- **MILD POSITIVE** (mild hypolactasia)
- **NEGATIVE** (normolactasia)

In case of a negative result hypolactasia can be excluded. For the more detailed interpretation of the results please refer to the interpretation chart included in the kit.

LIMITATIONS

The recommended diameter of biopsies is 1.5-2.0 mm in order to avoid imprecision in test results. As with any diagnostic procedure the Lactose Intolerance Rapid Test Kit results must be interpreted in the light of the patient's clinical presentation and any other information available to the physician.

PERFORMANCE CHARACTERISTICS

Correlation With Other Methods

The Lactose Intolerance Rapid Test Kit results were compared with the results of biopsy specimens subjected to the biochemical determination of lactase activity in which ≥10 U/g indicates normal lactase activity and <10 U/g mild or severe lactose intolerance.

Sensitivity: 95 %, Specificity: 100 %, Positive predictive value (PPV): 100 %, Negative predictive value (NPV): 98 %.

27.5% of the patients had severe hypolactasia. The validity of the lactase activity was confirmed by determining the lactase/saccharase ratio. The number of patients tested was 80. When Lactose Intolerance Rapid Test Kit was compared to the combination of H2 and CH4 breath test results, the following characteristics were shown:

Sensitivity 96%, specificity 96%, PPV 96%, NPV 96%, N=50.

Analytical sensitivity and precision tests were performed according to CLSI EP12-A2 guideline. Analytical sensitivity: C5-C95 interval between Positive - Mild positive was found to be 0.8-1.34 mU and between Mild positive - Negative 7-11 mU.

Precision: 98-100 %. Tested sample concentrations were near the C5-C95 intervals.

REFERENCES

1. Enattah NS, Sahi T, Savilahti E, Terwilliger JD, Peltonen L, Jarvela I. Identification of a variant associated with adult-type hypolactasia. Nat¬Genet 2002 Feb; 30(2): 233-237.

2. Kuokkanen M, Myllyniemi M, Vauhkonen M, Kääriäinen I, Karesvuori S, Linnala A, Härkönen M, Järvelä I, Sipponen P. A biopsy-based guick test in the diagnosis of duodenal hypolactasia in upper gastrointestinal endoscopy. Endoscopy 2006; 38(7):708-712.

3. Ojetti V, La Mura R, Zocco MA, Cesaro P, De Masi E, La Mazza A, Cammarota G, Gasbarrini G, Gasbarrini A. Quick test: a new test for the diagnosis of duodenal hypolactasia. Dig Dis Sci. 2008 Jun; 53(6): 1589-92.

SYMBOLS

	Caution	IVD	In Vitro Diagnostic Medical Device		Do Not Use if Package is Damaged
	Manufacturer	LOT	Batch Code		Contains Sufficient for <n> Tests
	CE Marking	REF	Catalogue Numbe	EC REP	Authorized representative in the European Community
	Keep Dry		Use-By Date		Keep Away From Sunlight
	Temperature Limit		Do Not Re-use		Date of Manufacture

Manufacturer
Jinan Chuangxingwell Biotech Co., Ltd.
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EC REP

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