A Genetic Lab-on-Phone Test for Point-of-Care Diagnostic of Lactose Intolerance near Patient and in less than 90 Minutes

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Background: The −13910 C/T single nucleotide polymorphism located within the *MCM6* gene, an enhancer region located upstream of the lactase-phlorizin hydrolase gene, is associated with lactase persistence/non-persistence traits among the Caucasian population. The performance of a new point-of-care CE-IVD (*In Vitro* Diagnostic) marked isothermal lab-on-phone lactose intolerance assay, using crude samples, was assessed in comparison with Sanger sequencing using purified DNA, as reference method.

Methods: The study was conducted following a non-probability sampling using direct buccal swab (n = 63) and capillary blood (n = 43) clinical samples from a total of 63 volunteers. A 3 × 3 confusion matrix/contingency table was used to evaluate the performance of the isothermal lab-on-phone lactose intolerance assay.

Results: The isothermal lab-on-phone lactose intolerance assay successfully detected the −13910 C/T variant with a limit of detection of 5 cells/assay and demonstrated an overall accuracy of 98.41% (95% CI, 91.47%–99.96%) for buccal swab samples and 100% (95% CI, 91.19%–100%) for capillary blood, taking just 90 min from sample to result, with only 2 min hands-on.

Conclusions: The lab-on-phone pocket-sized assay displayed good performance when using direct buccal swab and capillary blood samples, enabling a low-cost, real-time, and accurate genotyping of the −13910 C/T region for the rapid diagnosis of primary lactose intolerance at point-of-care, which enables a prompt implementation of appropriate diet habits and/or intolerance therapies. To our knowledge, this is the first point-of-care genetic test for lactose intolerance to be made available on the market.

INTRODUCTION

Lactose intolerance is a clinical condition characterized by the impaired ability to digest lactose, which can be caused by a reduced or absent activity or synthesis of the brush border enzyme lactase-phlorizin hydrolase (LPH) (1), commonly known as lactase, that hydrolyses lactose into its constituent monosaccharides (2). After the weaning phase, during the transition into adulthood, there is a genetically programmed down-regulation of LPH expression (3). This phenotype

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IMPACT STATEMENT

The present study will benefit individuals with suspected lactose intolerance, as the described lab-on-phone assay can rapidly diagnose primary lactose intolerance using direct buccal swab or capillary blood samples with high accuracy and low cost. The evidence presented demonstrates the good performance of the new assay compared to the reference method of Sanger sequencing. This manuscript contributes to the advancement of knowledge in the field by introducing the first point-of-care genetic test for lactose intolerance, which allows for timely implementation of appropriate diet habits and/or lactose intolerance therapies.

is known as lactase non-persistence (LNP) or adult-type hypolactasia, which is the ancestral state and the norm in most humans (4). Individuals with LNP have reduced ability to digest lactose and may experience symptoms of lactose intolerance, including diarrhoea, discomfort, bloating, flatulence, and intestinal cramps (5). Nevertheless, most lactase non-persistent individuals can tolerate small amounts of lactose and some can even consume large quantities without experiencing symptoms (6). The expression of the lactase gene (*LCT*) is regulated by an upstream transcriptional enhancer called *MCM6* (minichromosome maintenance complex component 6) (3). Changes in this region, known as single-nucleotide polymorphisms (SNPs), have been shown to lead to an alternative path for *LCT* expression, causing it not to be downregulated as the original pathway, allowing the digestion of lactose throughout life, and this phenotype is known as lactase persistence (LP) (5). The first SNP to be associated with this trait, −13910 C/T (rs4988235), was identified in 2002 by studying Finnish families (7), and it was later shown that this variant is a *cis*-acting enhancer of the *LCT* promoter (8, 9). In addition to this variant, a total of 23 known SNPs underlie the genetic aetiology of the LP phenotype (3).

The diagnosis of lactose intolerance is essential for the implementation of dietary adaptations or other appropriate therapies, and for this purpose different methods can be used (1). These include the hydrogen breath test (HBT), lactose tolerance test, direct testing of lactase enzymatic activity on duodenal biopsies, and genetic testing (1, 3, 10). HBT is considered the "gold standard" and its advantages include being non-invasive, inexpensive, highly sensitive and specific, and easy to perform (1). Nevertheless, in addition to having to be carried out by health professionals, this method does not allow the distinction between primary or secondary intolerance, is time-consuming (3 to 6 h) and can be influenced by exogenous factors (1). Since the identification of polymorphisms associated to LP, genetic testing has become a standard technique for the diagnosis of primary hypolactasia using a simple EDTA blood sample or buccal swab (11). Currently, there are several lactose intolerance PCR-based *in vitro* diagnostic tests available for the detection of the −13910 C/T variant (12, 13). Other methods being used for the detection of SNPs associated with LP include restriction fragment length polymorphism (RFLP), Sanger sequencing, microarrays, reverse-hybridization, or pyrosequencing (10, 14–16). Although some of these techniques already allow the simultaneous detection of several variants, they still require centralized laboratories, expensive equipment, and specialized personnel. Compared with the methods mentioned above, the loop-mediated isothermal amplification (LAMP) methodology offers more advantages achieving similar performance, having even been

considered one of the best candidates to replace the PCR method (17). In particular, LAMP offers a fast, reliable, isothermal amplification of DNA or RNA templates in only 5 to 50 min, without the need for a thermocycler, being more robust and tolerant to inhibitors that frequently affect PCR, which allows for the use of crude samples, such as direct buccal swabs or capillary blood (18). The presence of a predisposing SNP does not imply intolerance, nor does it allow prediction of, if and when an individual will develop lactose intolerance (1). Nevertheless, genetic testing can be crucial for a better diagnostics of lactose intolerance (11) as it allows the distinction between primary and secondary hypolactasia (1), and its clinical value improves with age (19). A shortcoming of this approach is that the different SNPs that can be present in the enhancer region of the lactase gene are strictly correlated with the ethnicity of individuals (11), and while the detection of the −13910 C/T SNP is indicative of LNP in Caucasians, the same is not true for patients with African or Asian heritage (10). For example, in some sub-Saharan African populations, the −13910CC genotype is common and not linked with lactose intolerance (20).

STAB VIDA has developed an isothermal lab-on-phone lactose intolerance CE-IVD (*In Vitro* Diagnostic) marked point-of-care diagnostic assay based on LAMP followed by mutation probe-based melting curve analysis that is automatically processed in real time in an inexpensive (i.e., costing 30× less than common laboratory quantitative PCR [qPCR] devices), portable, and reusable device that is controlled 100% via a user-friendly mobile app (Fig. 1). Previously, STAB VIDA developed a point-of-care test for the detection of SARS-CoV-2 using the same device, obtaining very good results and a geographically widespread commercial exploitation (21).

Herein, we assessed the performance of such a pocket-sized diagnostic assay to automatically and reliably detect the presence of the −13910 C/T polymorphism in direct buccal swabs and capillary blood samples within 90 min—from sample to result—with only 2 min hands-on time, and compared it with the reference method of Sanger sequencing.

MATERIALS AND METHODS

Data Collection and Ethical Considerations

The validation study was conducted following a non-probability sampling with a total of 63 volunteers from Portugal. Buccal swab (n = 63) and capillary blood samples (n = 43) were collected from volunteering individuals, after a signed informed consent was obtained—see Fig. 2 for more details on the flow of participants. All samples were anonymized after being collected, before testing.

Sanger Sequencing

As reference method, Sanger sequencing for the −13910 C/T polymorphism was performed blindly on blood samples collected in a QIAcard FTA™ Micro (Qiagen) or buccal swab samples collected in a QIAcard FTA Elute Indicating Micro (Qiagen) following STAB VIDA's lactose intolerance service laboratory routine. Briefly, one 2 mm punch was taken from each FTA card—including a control—with a micro-punch and washed with Whatman® FTA® purification reagent (Whatman) and nuclease-free water. After purification of FTA's captured nucleic acid, the *MCM6* region was amplified by PCR and, after purification, the amplicons were sequenced and analyzed following the certified internal service procedure.

Isothermal lab-on-phone lactose intolerance assay

In parallel to Sanger sequencing assays, the isothermal lab-on-phone lactose intolerance assays (STAB VIDA Lda) were performed blindly on direct buccal swab and capillary blood samples following manufacturer instructions. Briefly, buccal swab and capillary blood samples were collected in 200 µL of lysis buffer, and 10 µL of the final lysis

Fig. 1. Portable pocket-sized lab-on-phone device for isothermal amplification and melting curve analysis of the −13910 C/T region for the diagnosis of Lactose Intolerance.

buffer–sample mix were directly added to the reaction tube of the lactose intolerance assay, prior to running the assay for approximately 90 min, all hands-free after clicking "Start" on the companion mobile app (namely, "Dr Vida Pocket PCR" app, available for free at Google Play and Apple's App stores). Results were transferred and stored in real time to an application programming interface (API) server, via the companion mobile App, and automatically self-analyzed to deliver a final result that is presented in real time to the end-user via the mobile App and email.

Limit of Detection

For limit of detection (LOD) determination, A-549 cells from ZeptoMetrix, with a −13910TT genotype, were used at different concentrations (1 to 100 cells/reaction, i.e., 0.02 to 2 cells/µL, considering a final reaction volume of 50 µL), using lysis buffer as sample diluent.

Statistics

All data was analyzed using a 3×3 confusion matrix/contingency table, after exclusion of invalid results, and the overall and genotypes class statistics were calculated using the statistical computing software R (R foundation, version 4.2.2) and its "caret" library (version 6.0-94) (https://www.rdocumentation.org/packages/caret/versions/6.0-94/topics/confusionMatrix).

RESULTS

For analytic validation, the LOD was determined. A-549 cells at 6 levels of concentration ranging from 1 to 100 cells/reaction in triplicate were used to determine the LOD of the isothermal lab-on-phone lactose intolerance assay, without any DNA extraction or other pre-treatment. The lowest target level demonstrating a >95% detection rate of the −13910 C/T SNP was found for 5 cells per reaction, in a total reaction volume of 50 µL (i.e., 0.1 cells/µL)—see Table 1.

The results and statistics from validation for the isothermal lab-on-phone lactose intolerance assay using buccal swab and capillary blood direct crude samples are summarized in Table 2, in comparison to the reference method—Sanger

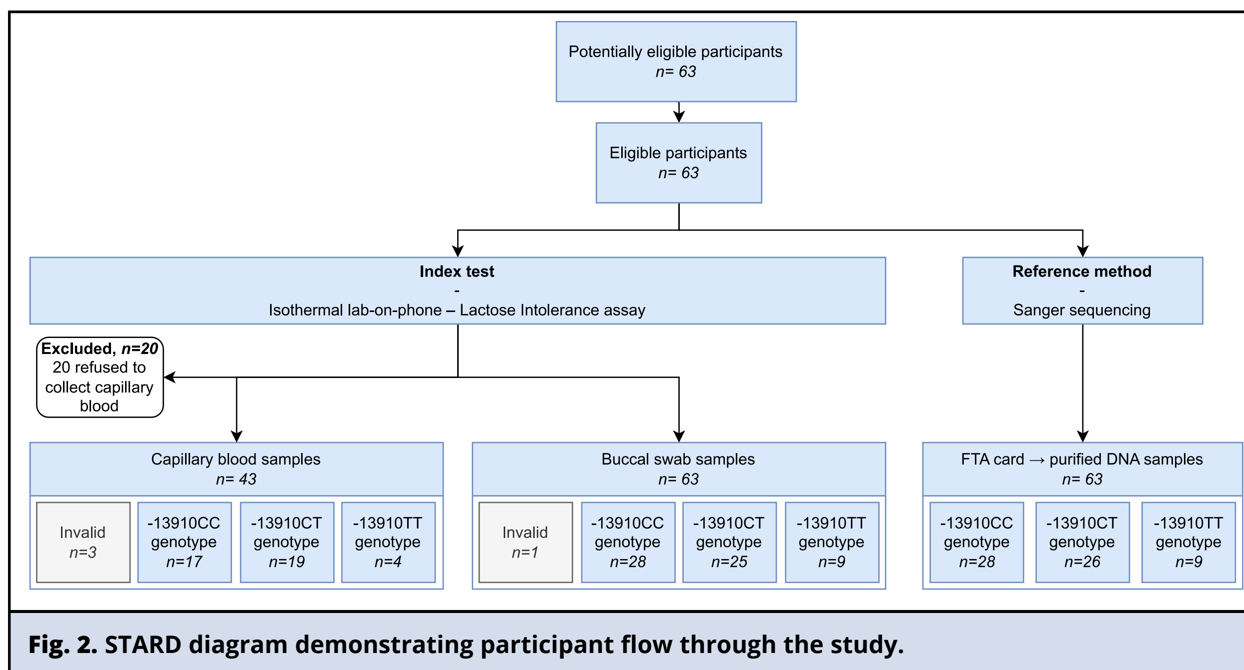

Fig. 2. STARD diagram demonstrating participant flow through the study.

Table 1. Isothermal lab-on-phone lactose intolerance assay LoD study for direct cell crude samples.

Target level (per reaction)	A-549 cells detection rate
1	33% (1/3)
5	100% (3/3)
10	100% (3/3)
50	100% (3/3)
75	100% (3/3)
100	100% (3/3)

sequencing using purified DNA from FTA cards. Examples of the melting curves obtained with the isothermal lab-on-phone lactose intolerance assay for each genotype (−13910CC, −13910CT, and −13910TT) and invalid results are shown in Fig. 3.

DISCUSSION

The pocket-sized isothermal lab-on-phone lactose intolerance assay has been demonstrated to detect as low as 5 cells per reaction (i.e., 0.1 cells/μL), which represents an equivalent LOD to gold standard molecular methods (i.e., PCR and Sanger sequencing). For direct buccal swab samples, the study results obtained with the isothermal lab-on-phone lactose intolerance assay showed an overall accuracy of 98.41% (95% CI, 91.47%–99.96%) when compared to Sanger sequencing using purified DNA samples. In a total of 63 crude samples, only one failed to be correctly genotyped and 1 was determined as invalid by the isothermal lab-on-phone lactose intolerance assay (online Supplemental Table 1). In comparison, when testing direct capillary blood samples, an overall accuracy of 100% (95% CI, 91.19%–100%) was achieved. In a total of 43 tested capillary blood crude samples, 3 were determined as invalid (Supplemental Table 1), perhaps due to a miscollection of blood or sample inhibition.

While blood has traditionally been the main source of genomic DNA, buccal swabs have also proven to produce sufficient yield and quality (22–24). Although our results were slightly superior for capillary blood, and even though buccal

Table 2. Performance of the isothermal lab-on-phone lactose intolerance assay using direct buccal swab and capillary blood crude samples in comparison to reference method Sanger sequencing (using purified DNA).

Buccal swab samples
Number of invalid assays: 1/63 (1.59%)

		Reference method—Sanger sequencing			
		−13910 CC	**−13910 CT**	**−13910 TT**	**Total**
Isothermal lab-on-phone lactose intolerance assay	−13910 CC	28	0	0	28
	−13910 CT	1	24	0	25
	−13910 TT	0	0	9	9
	Total	29	24	9	62
Overall statistics					
Accuracy: 98.41%					
95% CI: 91.47%–99.96%					
No information rate: 47.62%					
P-value [Acc > NIR]: < 2.2e-16					
Kappa: 0.9739					
Class statistics by genotype:		**−13910 CC**	**−13910 CT**	**−13910 TT**	
Sensitivity		96.67%	100%	100%	
Specificity		100%	97.44%	100%	
Pos pred value		100%	96.00%	100%	
Neg pred value		97.06%	100%	100%	
Prevalence		47.62	38.10%	14.29%	
Detection rate		46.03	38.10%	14.29%	
Detection prevalence		46.03	39.68%	14.29%	
Balanced accuracy		98.33%	98.72%	100%	

Capillary blood samples
Number of invalid assays: 3/43 (6.98%)

		Reference method—Sanger sequencing			
		−13910 CC	**−13910 CT**	**−13910 TT**	**Total**
Isothermal lab-on-phone lactose intolerance assay	−13910 CC	17	0	0	17
	−13910 CT	0	19	0	19
	−13910 TT	0	0	4	4
	Total	17	19	4	40
Overall statistics					
Accuracy: 100%					
95% CI: 91.19%–100%					
No information rate: 47.50%					
P-value [Acc > NIR]: 1.169e-13					
Kappa: 1					
Class statistics by genotype:		**−13910 CC**	**−13910 CT**	**−13910 TT**	
Sensitivity		100%	100%	100%	
Specificity		100%	100%	100%	
Pos pred value		100%	100%	100%	
Neg pred value		100%	100%	100%	
Prevalence		42.50%	47.50%	10.00%	
Detection rate		42.50%	47.50%	10.00%	
Detection prevalence		42.50%	47.50%	10.00%	
Balanced accuracy		100%	100%	100%	

Acc, accuracy; NIR, negative predictive value; Pos pred, positive predictive value; Neg pred, negative predictive value.

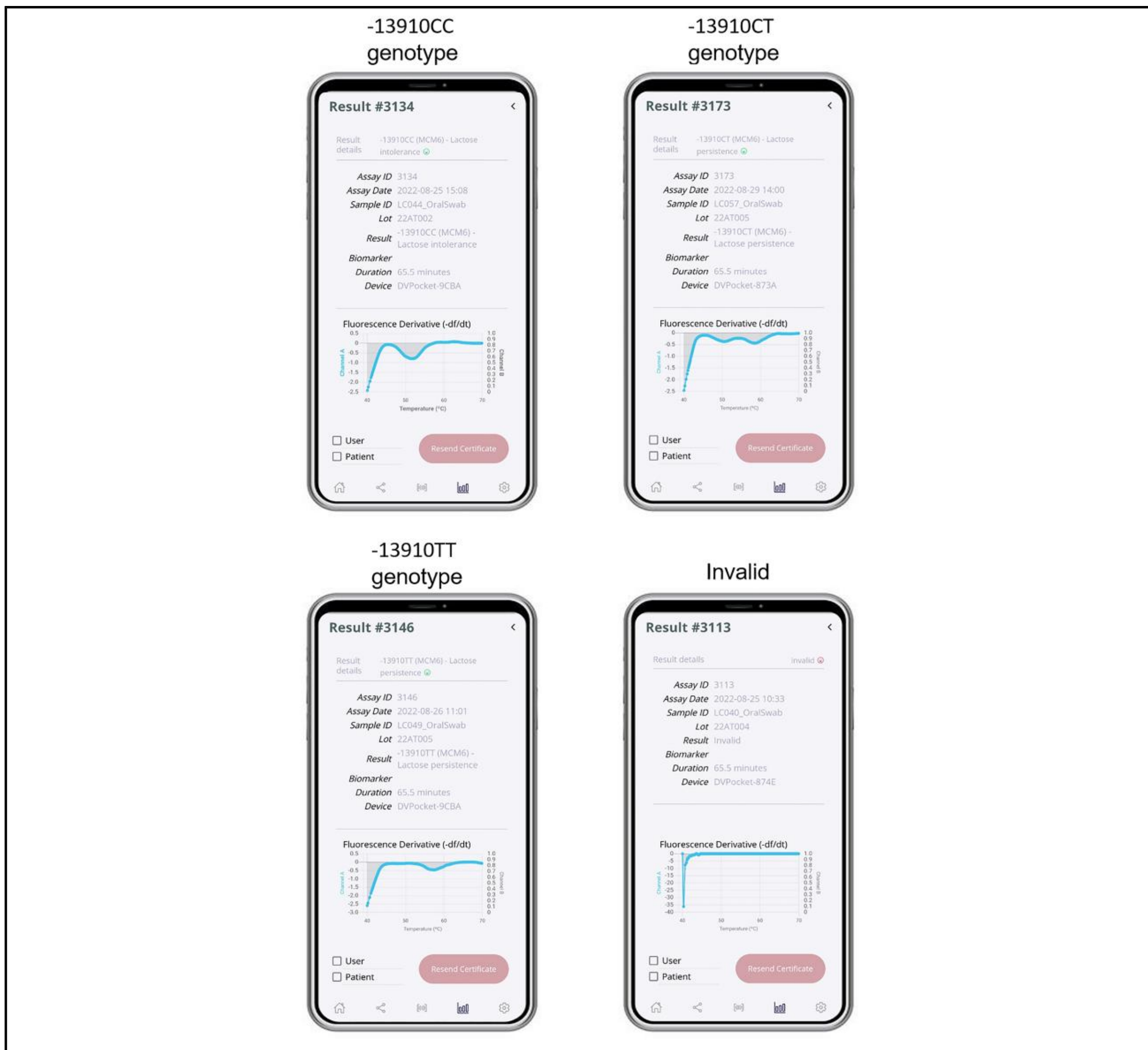

Fig. 3. Example of different possible results of the isothermal lab-on-phone lactose intolerance assay, as assessed through its mobile app.

swabs are often contaminated with DNA from bacteria, fungi, and food, the collection of this sample has simpler logistics and people are less reluctant to perform the test (25). Overall, the performance of the isothermal lab-on-phone lactose intolerance assay for direct buccal swab and capillary blood samples can be considered very good, especially given that it detects the −13910 C/T SNP using crude samples (i.e., without any pre-treatment required), whereas in the case of Sanger sequencing the tested sample is purified genomic DNA. Additionally, this assay offers the advantages of portability and affordability compared to currently available tests on the market.

Specifically, the device can cost 30 times less than common qPCR devices and the assay can be performed at a cost 5 times less than similar laboratory tests available on the market.

The isothermal lab-on-phone lactose intolerance assay helps to better manage the patient's lactose intolerance symptoms, given that it enables point-of-care testing for primary hypolactasia, through genotyping of the common −13910 C/T variant, without the need to send samples to a centralized laboratory and allows prompt implementation of new diets and/or appropriate therapies. Furthermore, since dairy restrictions may not be necessary in patients with secondary hypolactasia or other causes of lactose intolerance after diagnosis and therapy of their primary diseases, a prompt distinction from those with primary adult-type hypolactasia is beneficial (12). Nonetheless, although the described Lactose Intolerance assay possesses numerous strengths, it still can only detect one variant associated to the Caucasian population, but other similar assay(s) could be developed to diagnose other variants associated in other populations, at point-of-care.

CONCLUSION

In conclusion, the isothermal lab-on-phone lactose intolerance assay has been demonstrated to be a reliable pocket-sized portable system for point-of-care diagnostics that can be easily used in the field, over-the-counter, or at-home testing for primary lactose intolerance diagnostic. The assay provides rapid real-time results to identify the genotype of a patient with suspected lactose intolerance, which can be a very important factor for the implementation of appropriate therapy and diet adjustments. To our knowledge, this is the first point-of-care genetic test for lactose intolerance available on the market.

SUPPLEMENTAL MATERIAL

Supplemental material is available at *The Journal of Applied Laboratory Medicine* online.

Nonstandard Abbreviations: SNP, single-nucleotide polymorphism; LP, lactase persistence; LOD, limit of detection.

Human Genes: *MCM6*: minichromosome maintenance complex component 6; *LCT,* lactase gene.

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