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RESEARCH PAPER

A novel galactose electrochemical biosensor intended for point-of-care measurement of quantitative liver function using galactose single-point test

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Abstract

Liver disease has emerged as a healthcare burden because of high hospitalization rates attributed both to steatohepatitis and to severe hepatic toxicity associated with changes of drug exposure. Early detection of hepatic insufficiency is critical to preventing long-term liver damage. The galactose single-point test is recommended by the US FDA as a sensitive means to quantify liver function, yet the conventional method used for quantitation of circulating galactose still relies on the standard colorimetric method, requiring time-consuming and labor-intensive processes, and is confined to the medical laboratory, thus limiting prevalence. To facilitate time- and cost-effective disease management particularly during a pandemic, a pocket-sized rapid quantitative device consisting of a biosensor and electrochemical detection has been developed. An in vitro validation study demonstrated that the coefficient of variation was less than 15% and deviations were between −4 and 14% in the range of 100–1500 µg/mL. The device presented good linear fit (correlation coefficient, $r = 0.9750$) over the range of 150–1150 µg/mL. Moreover, the device was found to be free from interference of common endogenous and exogenous substances, and deviated hematocrit, enabling a direct measurement of galactose in the whole blood without sample pre-treatment steps. The clinical validation comprising 118 subjects showed high concordance ($r = 0.953$) between the device and the conventional colorimetric assay. Thus, this novel miniaturized device is reliable and robust for routine assessment of quantitative liver function intended for follow-up of hepatectomy, drug dose adjustment, and screening for galactosemia, allowing timely and cost-effective clinical management of patients.

Keywords Bioassay · Biological samples · Biosensors · Biotechnological product · Catalysts · Enzymes

Introduction

Chronic liver disease-related hospitalization has emerged as healthcare burdens because of an increasing number of alcohol-associated liver disease and non-alcoholic steatohepatitis patients with cirrhosis, or end-stage disease resulting from delayed diagnosis and treatment of liver dysfunction [1]. Besides complications of chronic liver diseases, 30% of cirrhotic liver patients suffer dose-dependent drug adverse toxicity; a study revealed that 20% of prescribed drugs were dosed incorrectly in cirrhotic patients who have impaired hepatic elimination of drugs leading to a change of drug

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exposure and consequential adverse drug reactions [2]. Quantifying liver function at bedside is therefore critical in clinical management of liver disease. Several approaches have been used to assess liver functions, aiming for monitoring progressive pathological status and tracking liver dysfunction due to liver diseases, drug-induced toxicity, or hepatectomy. The Child–Pugh score, which adopts ascites and encephalopathy as parts of the clinical measures, sometimes underestimates mild or moderate hepatic impairment. Imaging and biopsy visualize liver for histopathological status, yet are not recommended for repeated testing. Biochemistry tests on alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, albumin, total protein, and bilirubin provide static information regarding hepatic functional reserve. These traditional assessments though only reflect the severity of hepatocytes injured by chemicals or viruses, but not the functioning of hepatocytes. To fill the unmet medical need, we developed the galactose single-point (GSP) test [3] and have successfully transformed the US FDA-recommended test to a non-invasive oral GSP (OGSP) test by converting the intravenous route of delivering galactose to an oral administration (data to be published elsewhere).

Galactose is an essential nutrient, especially for neonatal infants; hereditary galactosemia can be fatal if elevated levels of galactose or its metabolites are not detected early. Furthermore, galactose is safe as a tracer to investigate hepatic blood flow-dependent galactose uptake; this has led to the development of the GSP test, which evaluates liver dysfunction by measuring the residual galactose level after administration of galactose [4]. The underlying principle of GSP test is based on the high extraction of galactose by the liver (hepatic extraction fraction = 0.91) according to the "well-stirred model" [5–7]. Once the metabolic capacity of the liver is within the saturation/linear pharmacokinetic phases, galactose elimination will be dependent on two factors—the overall metabolic capacity of the liver (manifested in the saturation phase) and effective hepatic blood flow (manifested in the linear pharmacokinetic phase) [8]. For a compound with very high hepatic extraction following linear pharmacokinetics (such as galactose), its hepatic clearance will be dictated by hepatic blood flow which can be impacted by liver diseases [9–12]. Additionally, when the galactose is saturated, its clearance reflects the enzyme activity [4]. Thus, the GSP test can be used as early diagnosis of silent liver dysfunction and failure, and can assist in drug dose adjustment on a case-by-case basis, owing to its capability to track rapid changes in liver function.

The conventional protocols used in the GSP test and screening for galactosemia require quantitation of the clinical galactose level in blood. Circulating galactose can be determined either by the standard colorimetric method on dried blood spots [13] or by the high-performance liquid chromatography coupled with refractive index detection [14] or mass spectrometry, both of which involve a large volume of blood sample with multiple processes to eliminate the effect of complex matrices. Additionally, the transportation of biological samples to an analytical lab and their storage are critical factors for maintaining sample integrity. Hence, in this work, we reported a miniaturized, rapid, accurate, and robust point-of-care (POC) biosensor for clinical quantitation of galactose in capillary blood from the fingertip, intended for routinely assessing liver dysfunction as well as other potential uses. In particular, the handheld device is applicable to whole blood taken from critically ill patients with escalated oxygen tension or deviated hematocrit level. The operation can be carried out by both medical professionals and lay users, requiring no advanced instruments or skilled operators in a centralized laboratory. Such a modality is suitable in various clinical settings and also for home use.

Materials and methods

Galactose rapid quantitative detection system

The galactose rapid quantitative detection system adopts enzyme electrochemical sensing technology in which the test strip is coated with galactose dehydrogenase to catalyze a chemical reaction, yielding signal to the meter. As illustrated in Fig. 1A, the system comprising a galactose meter (connector, calculation unit, A/D converter, and processor) and a test strip of biosensor (with galactose dehydrogenase coated on a screen-printed, carbon-pasted dry electrode) was manufactured by Apex Biotechnology Corp. (Hsinchu, Taiwan). Measurement is initiated by placing one drop of capillary blood on the reaction zone. The concentration-dependent current generated in the presence of galactose is mediated by the electron mediator and detected by the electrode. Then, the electric signal is quantified into digital form and displayed on the screen of device. The disposable dry electrode test strips were supplied in individually sealed aluminum foil packs and were stored in 2–8 °C before use.

Reagents

All chemicals were purchased from Sigma-Aldrich (St. Louis, MO). Galactose dehydrogenase, and galactose oxidase were acquired from Nipro Corporation (Osaka, Japan) and Sigma-Aldrich (St. Louis, MO) respectively. The galactose solution for the GSP test was prepared by dissolving 40 kg galactose (Sigma-Aldrich) in 100 L of distilled water; this was performed by a PIC/S cGMP manufacturer Nang-Kuang Pharmaceutical Company in Taiwan.

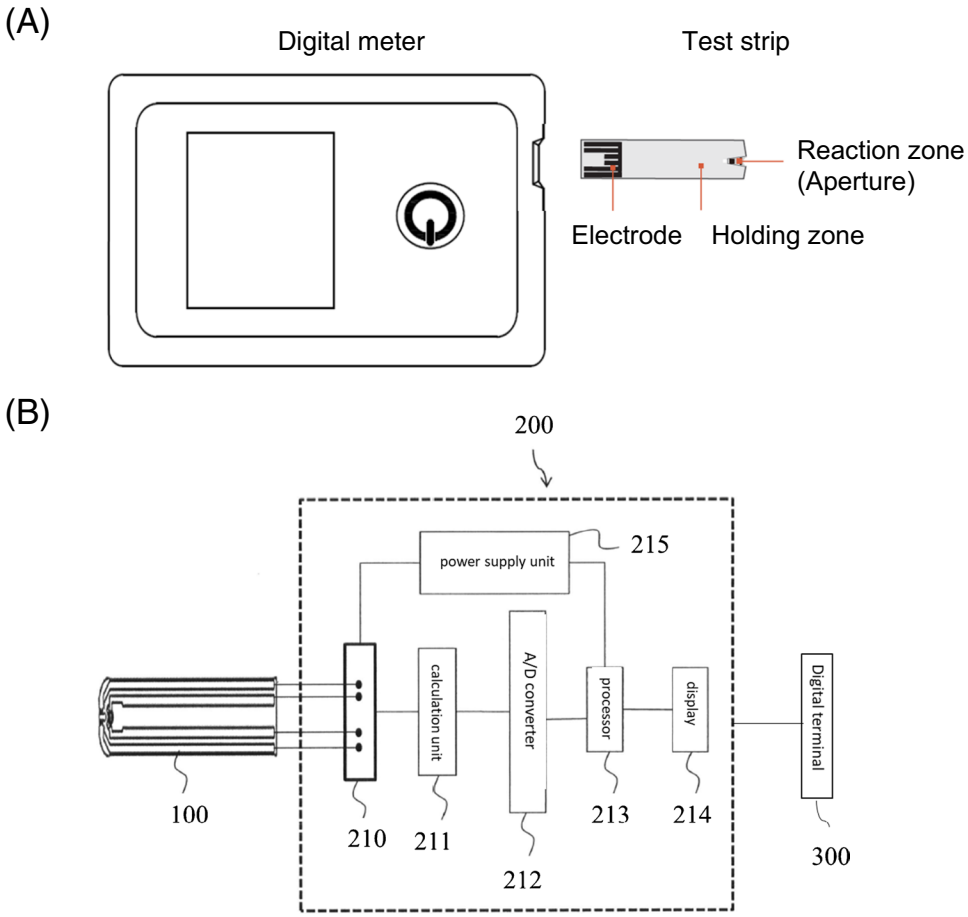

Fig. 1 Diagram of the galactose rapid quantitative detection system. **A** Frontal view of the system, comprising a digital meter and a test strip. **B** Block schematic view

Standard solution

A stock solution of galactose at 20 mg/mL was prepared with reverse osmosis water, and five standard solutions ranging from 0.8 to 20 mg/mL in water were made by serial dilution. Aliquots of these standard solutions were added to aliquots of blank blood. Unless otherwise specified, the final galactose concentrations for the assay validation of galactose in blood were 100, 200, 500, 900, 1200, and 1500 µg/mL.

Assay validation in vitro

Unless otherwise specified, the following performance test was conducted at room temperature (25 ± 5 °C) with a relative humidity of 20–60%. Blood galactose was measured using the galactose rapid quantitative detection system. The mean of galactose concentration (µg/mL), standard deviation (SD), and coefficient of variation (%CV) were calculated. The galactose concentrations of the spiked blood samples were verified by a YSI 2900 Biochemistry Analyzer (YSI Incorporated, USA).

Accuracy Accuracy was assessed with six levels of galactose (100, 200, 500, 900, 1200, and 1500 µg/mL). Precision evaluation consisted of within-run and between-run testing. The within-run precision test was performed in 1 day with five replicates. Additionally, a between-run test was performed to determine the galactose level in each sample in triplicate over 8 days (one measurement per day).

Repeatability The repeatability evaluation was conducted by determining the above six spiked concentrations of galactose using three individual galactose meters. Each sample was measured six times on one meter. All tests were performed on the same day.

Sensitivity and specificity Sensitivity and specificity were evaluated using three levels of galactose (200, 500, and 1,000 µg/mL) in the presence of six sugar mixtures (including sorbitol, mannitol, sucrose, glucose, fructose, and maltose; each at 1,000 µg/mL) and five galactose metabolite mixtures (including galactose-1-phosphate, glucose-1-phosphate, UDP-galactose, UDP-glucose, and glucose-6-phosphate; each at 1,000 µg/mL). In addition, an interference test was performed in the presence of common exogenous substances and metabolites, as recommended by the National Committee for Clinical Laboratory Standards (NCCLS EP7-A2).

Sample volume requirement The sample volume requirement was investigated using five spiked concentrations of galactose (190, 311, 552, 712, and 906 μg/mL) with a designated sample volume from 1.0 to 1.4 μL and test strips from three lots. Each detection was performed ten times. An underfill warning will appear on the screen of device if the volume is too low to detect galactose. A minimal sample volume was determined by meeting the criterion of successful ten readouts without a single underfill warning.

Linearity Linearity study was performed by measuring nine levels of spiked galactose concentration from 150 to 1,100 μg/mL in venous blood with ten galactose meters. Each detection was performed twice. System acceptability was evaluated by the regression.

Altitude effect An altitude test was performed to evaluate the impact of high altitude at 3,150 m (10,335 ft) on the robustness of the system in a mountain located in central Taiwan. Each concentration was determined three times using test trips from three lots.

Hematocrit effect The effect of hematocrit (Hct) was examined using five levels of galactose in oxygenated venous blood samples with hematocrit ranging from 20 to 60% using 43% as the reference control. Hematocrit was determined using the micro-hematocrit method. Each concentration was determined three times using test trips from three lots.

Stability The real-time stability of the galactose test strips was examined at 2 °C and 8 °C in relative humidity of 85%. The evaluation was performed with three levels of galactose (240, 500, and 850 μg/mL). Each concentration was determined by ten meters with a meter using test trips from three lots.

Clinical validation of the galactose rapid quantitative detection system

Blood samples were collected from participants undergoing GSP test performed in the Tri-Service General Hospital, Taipei, Taiwan (ClinicalTrials.gov: NCT03457311). The study protocol was approved by the Institutional Review Board of the Tri-Service General Hospital. Written informed consent was obtained from all participants, and the trial was conducted in accordance with the Declaration of Helsinki and under good clinical practice. One-hundred and eighteen participants were subjected to a 30-day screening period, followed by an intravenous GSP test after at least 6 h of fasting. Sixty minutes after intravenous administration of 1.25 mL/kg galactose solution (400 mg/mL of galactose), a drop of whole blood was taken from the participant's finger for galactose determination using both the galactose rapid quantitative detection system and the standard colorimetric method [13].

Statistical methods

Data were analyzed using the analysis of variance (ANOVA) followed by the multiple range test. The least significant difference was used when the result of the ANOVA was significant; otherwise, nonparametric multiple range tests were used. Linear regression and Pearson's correlation coefficient test were also applied where appropriate using SPSS Statistics for Windows (version 13.0, IBM, Armonk, NY, USA).

Results

Instrumentation of the galactose rapid quantitative detection system

The galactose rapid quantitative detection system adopts a disposable dry enzyme electrode technology. Figure 1B illustrates a schematic view of the galactose detection system. The system comprises a test strip 100 and a meter 200. The meter 200 includes a connector 210 connected to the external test strip 100, a calculation unit 211 for calculating the concentration, an A/D converter 212, a processor 213, and a display 214. When the power supply unit 215 applies a signal to the test strip via the connector 210, the galactose in the biological sample and the enzyme in the test strip react through an electrochemical reaction to produce electrochemical information. The signal reacting with the electrochemical information produces a corresponding response signal, which is then transmitted to the calculation unit 211 of the meter 200 via the connector 210. The calculation unit 211 calculates the corresponding response signal, which is output to the A/D converter 212 and is transformed into a digital reaction signal that is further processed by the processor 213. The measurement result is displayed on the display 214 in μg/mL. Furthermore, the digital reaction signal can also be transmitted to a digital terminal 300, by which the result can be sent to a cell phone or computer through Bluetooth or wireless connections.

A novel galactose biosensor with an enzyme-sensing mechanism

The electrochemistry-based biosensor is designed as a test strip of electrode containing a reaction zone where galactose in the blood sample is to react with galactose metabolic enzymes by which electrochemical signal is generated (Fig. 1A). Two galactose-catalyzing enzymes, galactose dehydrogenase (GD) and galactose oxidase (GO) capable of

generating electrochemical information, were selected for embedded immobilization and drying onto the electrode. GO-based amperometric test strip gave a linear galactose-dependent response (Fig. 2A). However, previous publication revealed that GO catalyzes the oxidation of primary alcohols and sugars (including D-galactose and lactose) in the presence of oxygen, thus impacting the accuracy of quantitation [15]. Aggravatingly, excessive oxygen competes with the electron mediator of the oxidase-based amperometric strip for electrons, thus resulting in underestimation of galactose measurement [16]. This is problematic when it comes with a point-of-care setting that employs fresh whole blood sample with a high oxygen content resulted from prolonged exposure to atmosphere, or taken from critically ill patients under oxygen support.

Galactose dehydrogenase, on the other hand, catalyzes D-galactose with high specificity without the involvement of oxygen [17]. The enzyme was therefore used to be immobilized and dried onto the biosensor. Unexpectedly, no substrate-dependent signal was observed, suggesting the activity of GD was collapsed after immobilization onto the surface of sensor and drying at 40 °C, whereas the response of enzyme in solution state as a control presented a linear response with galactose concentration (Fig. 2B). Similarly, the substrate-dependent electrochemical response was not observed after immobilization and drying at 0 °C to avoid heat inactivation of enzymatic activity (Fig. 2B). Since galactose dehydrogenase is unstable in neutral and alkaline pH, it is supplied in 3.2 M ammonium sulfate pH 6 solution to stabilize the enzyme. Under such a condition, irreversible protein denaturing might occur due to an abrupt escalation of ammonium sulfate concentration and/or pH change in the process of immobilization and drying that led to a loss of catalytic activity. The GD-based biosensor was therefore fabricated using desalted enzymes. The electrochemical data was again independent of galactose concentration (Fig. 2C), suggesting ammonium sulfate not the cause of inactivation.

Stabilization of labile proteins during drying requires protection of the protein structure against dehydration stresses. In biopharmaceutical industry, albumin, polymers, and

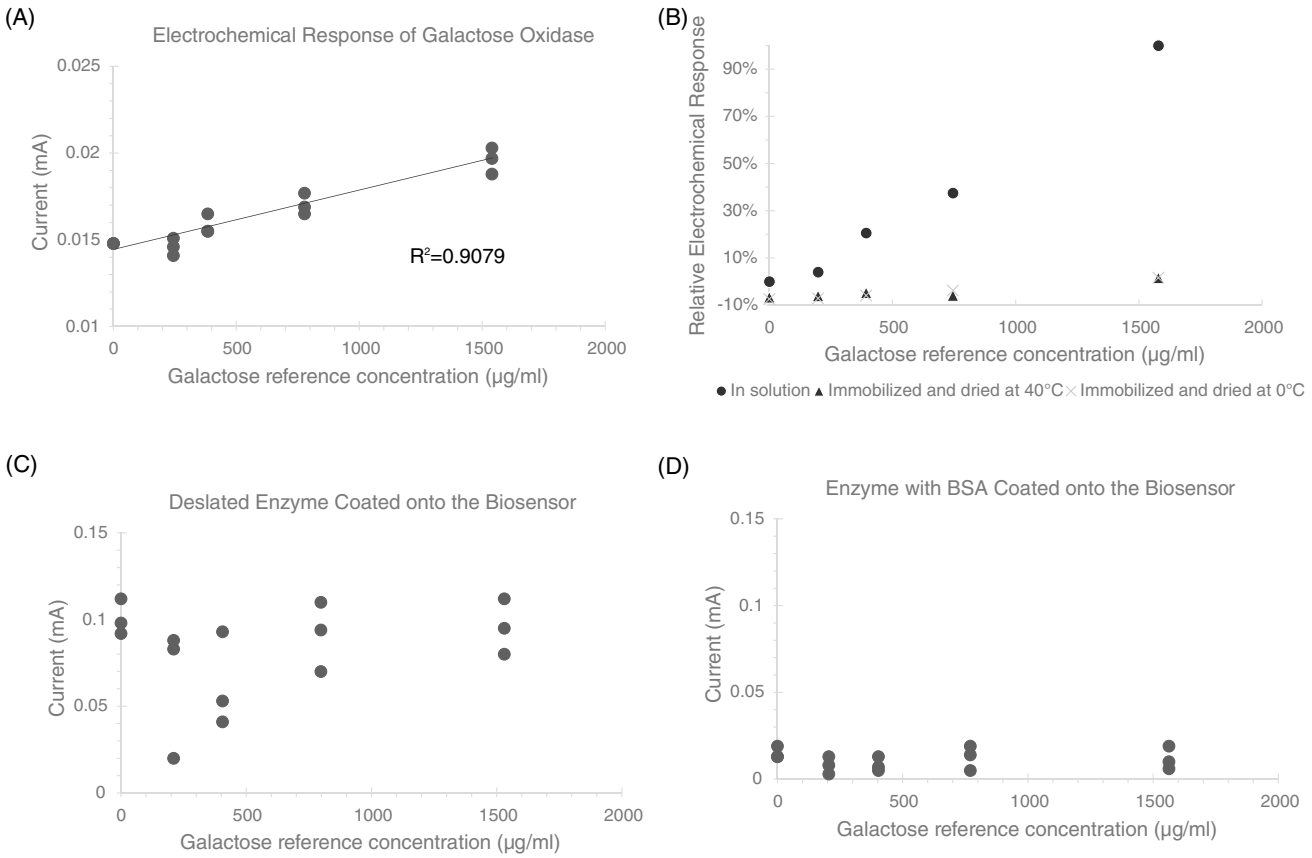

Fig. 2 Electrochemical responses after enzyme immobilization and drying onto the biosensor. **A** A plot of current (mA) induced by galactose oxidase (GO)-galactose reactions versus galactose concentration (µg/mL). **B** A plot of electrochemical responses of immobilized galactose dehydrogenase (GD) in solid state dried at 40 °C (▲) and 0 °C (✖), or in solution phase (●), relative to the response initiated by 1579 µg/mL galactose in solution, versus galactose concentration (µg/mL). **C** A plot of current (mA) induced by immobilized, dried, and desalted galactose dehydrogenase on biosensor versus galactose concentration (µg/mL). **D** A plot of current (mA) induced by galactose dehydrogenase immobilized and dried with BSA on the biosensor versus galactose concentration (µg/mL)

sugars are excipients added to stabilize biotherapeutics in the process of lyophilization. Likewise, bovine serum albumin (BSA) and D-trehalose were selected as protectants to examine their effects on the process of enzyme immobilization and drying. The result of using BSA as a stabilizer to fabricate the GD-based biosensor is shown in Fig. 2D, showing an absence of galactose-dependent electrochemical signal. However, using D-trehalose as a stabilizer to fabricate the GD-based biosensor successfully protected the activity of enzyme as proven by the linear correlation between galactose concentration and output readings reverted from electrochemical responses (Fig. 3A). Next, the performance of this galactose rapid quantitative detection system was validated with regard to specificity, interference, linearity, accuracy, precision, and stability as described below, for its use as a POC device utilizing unprocessed blood sample.

Accuracy, precision, and repeatability

The accuracy of the reading was evaluated by analyzing 24 replicates at each galactose level (100, 200, 500, 900, 1200, 1500 µg/mL) spiked in blood samples. The results tabulated in Table 1 show an accurate determination of galactose, with deviations between theoretical and experimental results (bias%) in the range of − 1 to 11%. The between-run precision expressed in coefficient of variation (%) was evaluated by analyzing three replicates at each concentration level over 8 days (one measurement/day); overall between-run precision is 5–14% (Table 1). Additionally, the within-run precision was evaluated by analyzing five replicates on the same day; within-run precision is 2–12% (Table 1). Repeatability was demonstrated by measuring six galactose concentrations on three meters, with each meter taking six measurements. The results in Table 1 indicate robust measurement, with %CV and bias% in the range of 7–15% and 0–18%, respectively. The analytical performance was found to be comparable to that of US FDA-approved colorimetric method used to determine the galactose level from dried blood spots for galactosemia screening, which has %CV and a mean bias of 3.0–28.7% and 12.8%, respectively [18]. Overall, the galactose rapid quantitative detection system could precisely measure galactose concentrations in blood samples.

Selectivity and specificity

Selectivity and specificity were evaluated with three galactose levels (200, 500, and 1000 µg/mL) in the presence of other sugars (sorbitol, mannitol, sucrose, glucose, fructose, and maltose) or galactose metabolites (galactose-1-phosphate, glucose-1-phosphate, UDP-galactose, UDP-glucose, and glucose-6-phosphate). Individual galactose result fell

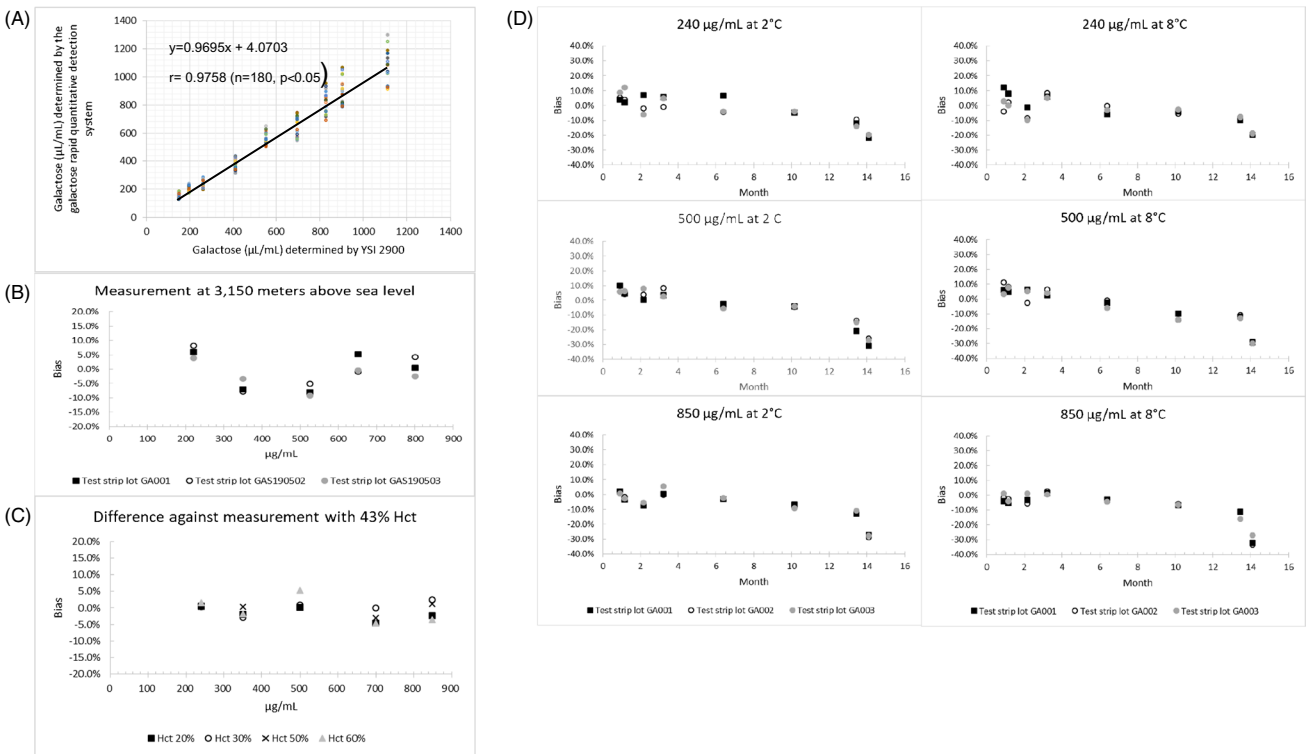

Fig. 3 In vitro validation of the galactose rapid quantitative detection system. **A** Linearity study; **B** altitude test of the system at 3,150 m; **C** measurement with hematocrit (Hct) ranging from 10 to 60% with 43% as the baseline; and (**D**) real-time stability of test strip in cold condition

Table 1 Accuracy, precision, and repeatability

Spiked conc. (μg/mL)	Estimated conc. (μg/mL) Mean ± SD	%CV	Bias%
Accuracy			
100 ($n=24$)	111 ± 10	9	11
200 ($n=24$)	209 ± 19	9	5
500 ($n=24$)	484 ± 29	6	−3
900 ($n=24$)	892 ± 65	7	−1
1200 ($n=24$)	1191 ± 60	5	−1
1500 ($n=24$)	1487 ± 92	6	−1
Between-run precision			
100 ($n=24$)	114 ± 14	12	14
200 ($n=24$)	223 ± 32	14	12
500 ($n=24$)	482 ± 26	5	−4
900 ($n=24$)	871 ± 67	8	−3
1200 ($n=24$)	1212 ± 85	7	1
1500 ($n=24$)	1507 ± 105	7	0
Within-run precision			
100 ($n=5$)	111 ± 13	12	11
200 ($n=5$)	211 ± 16	8	6
500 ($n=5$)	529 ± 10	2	6
900 ($n=5$)	893 ± 18	2	−1
1200 ($n=5$)	1213 ± 35	3	1
1500 ($n=5$)	1486 ± 25	2	−1
Repeatability			
100 ($n=18$)	118 ± 15	13	18
200 ($n=18$)	207 ± 30	15	4
500 ($n=18$)	499 ± 49	10	0
900 ($n=18$)	902 ± 70	8	0
1200 ($n=18$)	1214 ± 93	8	1
1500 ($n=18$)	1496 ± 107	7	0

within ± 13% of the reference galactose concentration at 200–1000 μg/mL, with a precision %CV ≤ 8% (Table 2).

Potentially interfering compounds in biological samples may also arise from endogenous and exogenous sources, including pathological metabolites, intervention drugs, and diet. Therefore, specificity was further demonstrated in biological samples containing common exogenous and endogenous substances in accordance with the National Committee for Clinical Laboratory Standards (NCCLS EP7-A2). The results tabulated in Table 3 show that only exceptionally elevated levels (beyond normal ranges) of ascorbic acid, triglyceride, and uric acid slightly affected the accuracy, yet a bias was within an acceptable range of ± 18.4%. All these results suggested the galactose rapid quantitative detection system free from many interference of endogenous and exogenous substances deriving from diet, therapeutic agents, and abnormal medical conditions. Unlike the conventional colorimetric assay that requires tedious sample pre-treatment to remove interfering blood matrix, this system is able to determine galactose level from whole blood directly in a minute or two.

Sample volume requirement

The minimal sample volume for the galactose rapid quantitative detection system was explored with a designated sample volume starting from 1.0 μL. While volumes of 1.0 and 1.1 μL led to 10 and 2 underfill warnings respectively out of ten measurements from each lot of test strip, a volume of 1.2 μL and more resulted in 10 successful readouts without a single warning. Therefore, the minimal requirement of volume was found to be 1.2 μL. Specificity and sample volume requirement together suggested a small drop of whole blood from fingertip sufficient for accurate determination of galactose.

Table 2 Selectivity and specificity testing for the galactose rapid detection system under various sugars and metabolites

Item	Galactose nominal concentration					
	200 μg/mL ($n=6$)		500 μg/mL ($n=6$)		1000 μg/mL ($n=6$)	
	Mean ± SD (%CV)[4]	Bias%	Mean ± SD (%CV)[4]	Bias%	Mean ± SD (%CV)[4]	Bias%
Galactose alone	220 ± 14 (6%)	10	485 ± 18 (4%)	−3	968 ± 33 (3%)	−3
Galactose plus D-lactose[1]	208 ± 17 (8%)	4	472 ± 28 (6%)	−6	925 ± 23 (3%)	−8
Galactose add 6 various sugar[2]	216 ± 7 (3%)	8	479 ± 14 (3%)	−4	951 ± 58 (6%)	−5
Galactose add 5 various metabolites[3]	225 ± 18 (8%)	13	474 ± 22 (5%)	−5	946 ± 30 (3%)	−5

[1] D-Lactose (1000 μg/mL)

[2] 6 various sugars (1000 μg/mL) included sorbitol, mannitol, sucrose, glucose, fructose, and maltose

[3] 5 various metabolites of galactose (1000 μg/mL) included galactose-1-phosphate, glucose-1-phosphate, UDP-galactose, UDP-glucose, and glucose-6-phosphate

[4] Acceptance criteria: %CV ≤ 15%

Table 3 Selectivity and specificity testing for the galactose rapid detection system under various exogenous and endogenous interferants. A normal range of each substance is shown in brackets

Spiked substances (normal range)	Lot of test strip					
	Lot No. GA001		Lot No. GA002		Lot No. GA003	
	Galactose concentration (μg/mL)					
	245	515	245	515	245	515
	Bias (%)					
L-Ascorbic acid (0.8–1.2 mg/dL)						
3 mg/dL	3.2	2.6	5.2	5.8	5.4	9.4
6 mg/dL	18.4	15.0	17.1	16.4	16.5	15.1
Cholesterol (114–201 mg/dL)						
300 mg/dL	6.2	−4.4	1.1	7.4	2.4	3.7
500 mg/dL	4.7	−1.2	2.7	2.9	3.3	3.6
Triglyceride (30–300 mg/dL)						
800 mg/dL	1.3	−2.8	4.1	5.0	7.5	5.1
1500 mg/dL	7.4	3.0	5.0	−3.2	6.4	5.1
Glucose (70–110 mg/dL)						
800 mg/dL	8.8	2.5	6.2	0.3	7.3	6.7
1000 mg/dL	4.0	−2.5	2.1	4.5	7.2	1.2
Mannose (N/A)						
2 mg/dL	8.5	2.5	2.5	0.8	−2.5	3.7
4 mg/dL	4.0	−2.1	4.1	1.2	3.9	8.7
Fructose (1–6 mg/dL)						
10 mg/dL	3.0	−9.0	8.8	−6.4	8.4	1.3
20 mg/dL	7.1	1.1	6.0	−7.0	−8.0	−2.3
Acetaminophen (1.0–3.0 mg/dL)						
10 mg/dL	2.0	1.4	−6.9	−5.1	0.3	−3.2
20 mg/dL	4.2	−4.7	−5.2	−9.5	−1.6	5.2
Fresh hemoglobin (100–200 mg/dL)						
50 mg/dL	−6.7	8.3	6.7	6.3	−0.7	1.9
200 mg/dL	9.5	−4.3	0.2	−2.3	−0.4	−5.7
Uric acid (2.5–8 mg/dL)						
10 mg/dL	8.7	9.6	8.2	8.6	8.7	8.2
20 mg/dL	17.6	16.3	16.1	−15.5	15.6	16.3
Bilirubin-unconjugated (0.3–1.2 mg/dL)						
10 mg/dL	−1.9	−1.9	−0.9	1.8	−2.8	0.6
20 mg/dL	−3.4	−7.0	−7.2	0.7	−3.1	−2.8
Bilirubin-conjugated (0–0.3 mg/dL)						
15 mg/dL	4.5	6.4	−2.5	−4.3	−6.3	−1.9
30 mg/dL	−7.8	−0.8	−3.9	−2.0	−4.4	0.6

Linearity study

The GSP test for liver dysfunction suggested a threshold of moderate liver impairment at 280 μg/mL [4]. Linearity of the system was therefore demonstrated with nine levels of galactose ranging from 150 to 1,150 μg/mL, determined by ten individual meters. Results are plotted in Fig. 3A, showing the coefficient of correlation $r = 0.9758$ and p value < 0.05 ($n = 180$). The high linearity confirmed a very good correlation with reference standards in the detection range and absence of saturation effects at high levels of analytes.

Impact of high altitude and abnormal hematocrit

The simplicity of the galactose rapid quantitative detection system allows it to be operated by non-technique-dependent users. Due to the increased prevalence of high-altitude activities, the impact of high altitude on device performance was evaluated to ensure reliable monitoring of liver function during travel. Five galactose levels were determined using test strips from three lots at an altitude of 3,150 m (10,335 ft). The results in Fig. 3B show a bias within ± 11%, suggesting an analytical performance

comparable to that performed at the sea level. The result supported routine liver function monitoring operated by lay users at home or during a travel without visiting hospital, thus significantly enhancing quality of life.

Hematocrit is the percentage of the blood that is composed of erythrocytes, and can be affected by many medical complications, such as anemia and edema. The baseline range of hematocrit for men and women is 42–50% and 37–47%, respectively. The test investigated the effect of hematocrit ranging from 20 to 60% using 43% as the reference. The results taken from five galactose levels with three lots of test strips are summarized in Fig. 3C, showing insignificant variations in the device performance due to abnormal hematocrit when compared to the reference result (43% hematocrit), with acceptable bias ranging from − 4.7 to 2.4%. Therefore, the galactose rapid quantitative detection system has been demonstrated to be free from interference of the above very common medical complications.

Stability of the galactose test strip

Galactose dehydrogenase is unstable in neutral and alkaline pH, and therefore, it is supplied in 3.2 M ammonium sulfate pH 6 solution. After optimization of the formulation by adding trehalose to stabilize enzymatic activity in the processes of immobilization and drying onto the electrode, the real-time stability of test strips from three lots was examined at 2 °C and 8 °C respectively. As summarized in Fig. 3D, both groups of test strips stored at 2 °C and 8 °C were found to be stable for up to 13.5 months, with bias within ± 20%.

Clinical validation of the galactose rapid quantitative detection system

The clinical viability of the POC device for determining blood galactose levels was explored using capillary blood samples taken from 118 participants, who were classified based on their liver function as normal, mild/moderate, or severe liver dysfunction. The GSP values determined by the POC meter were compared to those determined by the conventional colorimetric assay on dried blood spots (Fig. 4). The results showed that both modalities shared a close correlation ($r = 0.953$), confirming that the system gave comparable results to those determined by the current standard method ($p < 0.0001$, $n = 118$).

Discussion

Galactose in blood is a pivotal biomarker for the diagnosis of several clinical disorders, including liver dysfunctions and galactosemia. Assessment of potential liver damage is challenging because the liver is a silent organ and its damage can only be detected in late stages by tools such as serological testing and liver biopsy which is the current gold standard. The GSP test is a sensitive quantitative approach to detect liver dysfunction at the early stage and has been recommended by the US FDA as a means of quantitative assessment of liver function and has been quoted in popular textbooks [19, 20]; indeed, the elevated 60-min blood galactose concentration has been shown to correlate sensitively with hepatic dysfunction [3]. However, the intravenous administration of galactose limits its clinical prevalence. We have successfully improved the GSP test by changing

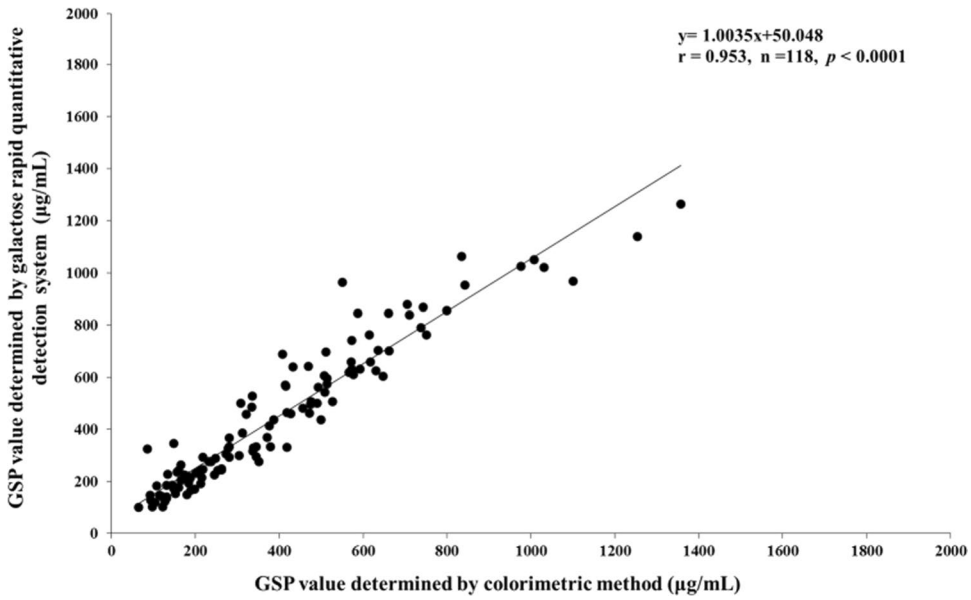

Fig. 4 Correlation of galactose levels in participants undertaking GSP test determined by the galactose rapid quantitative detection system versus the colorimetric method

the administration route from injection to oral consumption in a clinical trial. This trial found that the normal baseline GSP value was < 343 μg/mL and that GSP values increased with elevated degree of liver dysfunction (data to be published elsewhere).

Again, the lengthy and tedious quantitation process of colorimetric assay limits its clinical prevalence. Like conventional quantitation method for GSP test, many diagnostic tests are not compatible with whole blood samples and thus require sample pre-treatment step to eliminate interfering factors, including endogenous substances, drugs, blood composition, and blood viscosity, that are attributed to diet, medical interventions, or complications. The present galactose rapid quantitative detection system can accurately and precisely determine blood galactose concentration in the range of 100–1,500 μg/mL without the interference of galactose metabolites and other sugar complex matrices, environmental conditions, and certain medical complications. These features could facilitate usage of the present device as a POC platform to measure galactose in unprocessed blood sample. For example, patients receiving chemotherapy or hepatectomy may experience drug-induced anemia or high blood oxygen tension respectively. Liver function test is essential for evaluation of the eligibility of sequential cycles of chemotherapy or for post-operative management after hepatic resection. This device then assists in guiding optimal decision-making for practitioners at bedside.

Screening for galactosemia is another application of the present system. Galactosemia is a hereditary disease, which is attributed to a deficiency of galactose clastic enzyme, leading to the accumulation of galactose or its metabolites in the body, with symptoms including sleepiness, emesis, diarrhea, incapability of normal growth, and jaundice. This POC galactose meter is a suitable alternative to the conventional colorimetric method with dried blood spot as a means of quick screening. For example, by sampling from a newborn's toe tip, if the total galactose in capillary blood is greater than 150 μg/mL, it represents a high risk of neonatal galactosemia, and a follow-up biochemical test is needed to confirm the causative disease.

Coupling with the newly developed oral GSP test, this pocket-sized POC device for electrochemical determination of galactose concentration can be used in hospitals and at home, the value of which has become clear during a pandemic when access to hospitals becomes restricted. This quantitative liver function test can be completed in an hour, allowing routine clinical management of patients, including rapid routine screening, clinical assessment, follow-up, and adjustment of drug dosing in these patients, thus ultimately saving time and operational expenses. The features of simple, cost-effective operation and repetitive testing are particularly useful for patients receiving hepatectomy with a need for repeated monitoring of liver function, and for rapid screening purpose for the low-risk population of liver disease without the risky and costly gold standard liver biopsy. Taken together, this novel POC diagnostic device allows the screening and diagnostic process to be completed in due course and could relieve the financial burden of patients with chronic liver diseases, thereby improving healthcare accessibility and patient outcomes.

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Author contribution K-M Yu and O. Y-P. Hu conceived and designed the trial. T-Y. Huang was the principal investigator for the clinical trial. P. Yang and O. Y-P. Hu were responsible for laboratory studies, assay development, and sample collection. J. Y-N. Lau and O. Y-P. Hu provided guidance of the study. K-M. Yu, J. Y-N. Lau, and O. Y-P. Hu prepared the manuscript and contributed to data analysis and interpretation; T. Y-S. Shen, J. Y-N. Lau, and O. Y-P. Hu contributed to the device design and manufacturing. O. Y-P. Hu assumed final responsibilities for the manuscript.

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Declarations

Ethics approval and source of biological material Blood samples were collected from clinical participants undergoing GSP test performed in the Tri-Service General Hospital, Taipei, Taiwan (ClinicalTrials.gov: NCT03457311, registered on March 7, 2018). The protocol was approved by the Institutional Review Board of the Tri-Service General Hospital. Written informed consent was obtained from all participants, and the trial was conducted in accordance with the Declaration of Helsinki and under good clinical practice.

Conflict of interest Avalon HepaPOC Limited owns the intellectual property rights of this technology, and J. Y-N. Lau and O. Y-P. Hu are shareholders of this entity. No conflict of interest has been declared by the other coauthors.

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