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The LabTube – A novel microfluidic platform for assay automation in laboratory centrifuges

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Assay automation is the key for successful transformation of modern biotechnology into routine workflows. Yet, it requires considerable investment in processing devices and auxiliary infrastructure, which is not cost-efficient for laboratories with low or medium sample throughput or point-of-care testing. To close this gap, we present the LabTube platform, which is based on assay specific disposable cartridges for processing in laboratory centrifuges. LabTube cartridges comprise interfaces for sample loading and downstream applications and fluidic unit operations for release of prestored reagents, mixing, and solid phase extraction. Process control is achieved by a centrifugally-actuated ballpen mechanism. To demonstrate the workflow and functionality of the LabTube platform, we show two LabTube automated sample preparation assays from laboratory routine: DNA extractions from whole blood and purification of His-tagged proteins. Equal DNA and protein yield were observed compared to manual reference runs, while LabTube automation could significantly reduce the hands-on-time to one minute per extraction.

Introduction

Over the last years, numerous biochemical assays have been developed in modern biotechnology, thereby revolutionizing the analytical opportunities in clinical diagnostics [1], food analysis [2,3], biosafety [4,5] and forensics [6]. Manual assay processing is based on cumbersome and error-prone liquid handling of sample and reagents and therefore requires trained staff. Above a certain threshold of sample throughput, automation becomes cost-efficient and enables the successful implementation of complex analytical procedures into the daily routine of diagnostic and research labs, also when performed by less trained personnel [7,8]. The largest fraction of clinical and other laboratory tests is performed in centralized labs which process samples at high throughput for example by application of 96-well plate robotic systems which are costly in acquisition, operation and maintenance. For sample preparation at medium throughput systems like the QIAcube (Qiagen) or Maxwell 16 (Promega) are available for parallel processing of 12 and 16 samples, respectively, by combining pipetting robots with processing devices such as shakers, magnetic particle separators or centrifuges. Although highly developed, these systems are unattractive for small laboratories or for rarely demanded tests, as they cause high costs of acquisition and consume a large amount of laboratory space [7]. Moreover, these conventional automation systems have functional disadvantages: First, the user needs to load the instrument with buffer reservoirs and disposables before each run. Second, disposables are not fully closed during operation. Both issues bear the risk of cross-contamination.

Microfluidic integration offers an alternative concept to conventional, robotic systems which has successfully been demonstrated for automation of various assays and even sample-to-answer workflows [9–16]. Microfluidic systems, also named lab-on-a-chip systems, use enclosed, disposable chips with networks of fluidic channels for implementation of the required fluidic unit operations such as fluid transport, valving, mixing and separation. Since each sample is processed in an enclosed, disposable chip, the risk of cross-contamination is minimized. Despite very promising academic demonstrations for instance in the field of point-of-care testing [17–19], such microfluidic systems are not used in routine work today for two reasons: First, the implementation of reagent prestorage and reagent release at a defined point of time during the protocol is still an incompletely solved challenge [20]. Second, assay-specific processing devices are developed for each application to enable processing at ideal conditions. Therefore, microfluidic systems suffer from a high market entry barrier caused by costs for the processing device as well as a limited customer base per developed chip [21].

Here we introduce the LabTube as a flexible microfluidic platform for assay automation in standard laboratory

centrifuges enabling cost-efficient automation even at low sample-throughput. The platform is based on disposable LabTube cartridges, which include all reagents and functional components for automation of entire assay protocols controlled by changing centrifugal accelerations. A main feature is the decoupling of laboratory automation from the (high) investment for a specific processing device which presumably lowers the market entry barrier compared to other Lab-on-Chip platforms. Therefore, the LabTube platform represents a cost-efficient alternative for assay automation in laboratories with low to medium sample throughput, such as research or small hospital laboratories. As the LabTube features a completely closed cartridge, its application promises particular benefits for processing of infectious samples, even outside a biological safety laboratory. In this article, we present the basic principles of the LabTube platform and demonstrate its applicability to automation of sample preparation routines, which today is still considered a bottleneck in microfluidic process automation [22–24]. We discuss advantages, limitations and future prospects of the LabTube platform based on the demonstration of bind-wash-elute extractions of human genomic DNA from whole blood and purification of his-tagged proteins from cell lysates.

System design of the LabTube platform

Workflow overview

For LabTube automated processing, the user adds the sample into a dedicated cavity of the LabTube cartridge, closes the cartridge and places it into a centrifuge equipped with a swing-bucket rotor for 50 ml centrifuge tubes (see Figure 1). Controlled via a computer interphase such as RS232 or USB, the centrifuge executes an assay-specific centrifugation-time-protocol, which can be read in via a barcode label. This way, the centrifuge actuates unit operations implemented in the LabTube cartridge for reagent release, mixing, extraction and product-waste-separation. After completion of the automated protocol, the user removes the LabTube cartridge from the centrifuge and finds the eluate in a detachable collection tube.

Cartridge design and integrated unit operations

The disposable LabTube cartridge for automated DNA extraction is schematically shown in Figure 2. The cartridge is composed of a cartridge shell, a spring and three revolvers for liquid processing which are stacked on top of each other: Revolver I is designed for sample uptake as well as for prestorage and release of reagents. Revolver II performs sample processing including mixing and solid-phase extraction. Revolver III collects separate liquid fractions released from Revolver II.

The prestorage of reagents and their release at a defined stage during the assay protocol is a common challenge in the design of microfluidic systems [20]. This problem is solved in a very simple way by the LabTube platform. All reagents are stored in Revolver I which is sealed with an aluminum foil at bottom- and topside. One cavity is reserved for sample addition. Sample and reagent release from the cavities of Revolver I is realized by thorns at the topside of Revolver II which sequentially pierce the sealing foil of the prestorage cavities. The movement of the thorns is controlled by an integrated ballpen mechanism which rotates Revolver II horizontally in relation to the other components and vertically in relation to Revolver I. Comparable to clicking a ballpen, the centrifuge actuates the ballpen mechanism in the LabTube cartridge based on the interplay between two forces, spring force and centrifugal force, whereas the latter is controlled by a centrifugation-time-protocol as schematically illustrated in Figure 3a. The DNA extraction cartridge is designed to perform one switching process (state 1 to 1') when accelerated to a centrifugal acceleration $a_c > 300$ g (state 1 to 2) and subsequently decelerated to $a_c < 80$ g (state 2 to 1'). During deceleration, Revolver II is rotated and thus its thorns pierce the next cavities in state 1'. Piercing events only result in liquid release when piercing occurs in positions with fluidic interconnection to the prestorage cavity, as shown in Figure 3b. For DNA extractions, a ballpen mechanism is applied that enables eight switching processes and thus the sequential release of up to eight liquids in total.

Thorns at different radial positions are used to release reagents to different zones of Revolver II, enabling the generation of parallel fluidic paths. Figure 3c and 3d illustrate the thorn position and movement designed for DNA-extraction: Piercing by the two thorns marked in green guides liquids to the mixing system and subsequently from the mixing system onto the extraction column. A third thorn, marked in blue, releases liquids onto a path directly leading to the extraction column. In DNA extractions, the direct path to the column (blue path) is used for the elution buffer to avoid any contamination by potential sediments formed in the mixing pond.

Since the outlet of Revolver II is placed off-center (see Figure 2), the rotation induced by the ballpen mechanism enables fluidic routing to different cavities of Revolver III: During binding and washing steps of the DNA extraction protocol, the nozzle at the outlet of Revolver II is placed above of the waste chamber. Further switching rotates the nozzle to the collection tube and forms a separate fluidic path for collection of the eluate fraction. A 2D visualization of the relative movement of the revolvers in combination with fluidic routing is shown in Figure E2 and E3 in the electronic supplementary information.

Mixing is a crucial unit operation in many biochemical assays. Under centrifugation, mixing is a challenge, since centrifugation typically leads to separation instead of homogenization and special means are required to reach a sufficiently high distribution and homogeneity of sample and reagent. On centrifugal microfluidic systems, shake-mode mixing has been demonstrated as powerful mixing method for applications such as DNA extraction [25]. During shake mode mixing, high inertia forces cause swirling of the liquid layers, created by quickly changing rotational directions. Since standard laboratory centrifuges have a fixed direction of

rotation this method cannot be used in LabTube automation. As an alternative, a mixing system has been invented for the LabTube cartridge which utilizes the transfer of liquids between Revolver II and a spring-mounted "mixing pond" to invert fluidic layers, as shown in Figure 4. Liquid transfer to the mixing pond and retransfer to the Revolver II compartment is controlled by alternating centrifugal acceleration and two transfer passages P1 and P2. Passage P1 has a significantly smaller diameter and thus higher fluidic resistance compared to P1, leading to a preference of liquid transfer along P2. When the cartridge is accelerated to 2500 g the upper liquid layer in the Revolver II compartment flows into the mixing pond via P2. As the liquid mass in the mixing pond increases (state b) the spring gets more and more compressed and the lower liquid level from Revolver II empties through the narrow channel P1 (150 µm diameter) into the mixing pond. This leads to inversion of the previously upper and lower liquid layers. According to their densities, the previously lower liquid layers moves downwards through the previously upper liquid layer after being transferred. While crossing each other, the liquid layers get mixed. To pump the liquid back into the Revolver II compartment the centrifuge is decelerated to 200 g (state c). Also during retransfer, liquids are predominantly transferred via P2. Thus the upper layer is transferred first to the bottom of the Revolver II compartment and the lower layers are given on top, creating a second inversion of the liquid layers. After several alternations (typically 60 alternations for lysis-mixing in DNA extractions), the homogenized liquid is released by piercing an aluminum sealing of the mixing pond at centrifugal accelerations > 4000 g as depicted in state d.

Protocol implementation

For implementation of fully automated processing, centrifugation-time-protocols are used to connect the above discussed fluidic unit operations in the assay-specific sequence. Figure 5 illustrates the centrifugation-time-protocol for a DNA extraction, controlling reagent release, mixing, release of the mixture, solid phase extraction and liquid routing for separate collection of buffers and the eluate. In a first step, the cartridge is accelerated to a_c =600 g to activate switching process no.1 (see Figure 3a – state 2) and to centrifugally release the lysis buffer to the mixing system. Deceleration to a_c = 20 g rotates Revolver II by 45° and completes switching process no.1 (see Figure 3a – state 3→1'). At the same time the next cavity is pierced which contains Proteinase K and the sample. Both liquids are guided into the mixing system. Subsequently, alternations in centrifugal acceleration between a_c = 2500 g and 250 g are applied for 15 min of lysis-mixing to create a homogeneous lysate composed of lysis buffer, proteinase K and sample. In standard manual protocols, lysis is usually performed at 56 to 70 °C. Since standard laboratory centrifuges do not provide temperature control, lysis in LabTube protocols has to be performed at room temperature. After lysis-mixing, the centrifuge decelerates to 20 g for completion of switching process no.2 and for transferring the binding buffer into the mixing system. Binding buffer and lysate are mixed and subsequently the mixing pond is opened at 6000 g to release the mixture onto the extraction column (silica matrix). Passing the extraction column, the mixture is guided to the waste chamber of Revolver III at the maximum centrifugal acceleration which is 6000 g for the combination of centrifuge and swing-bucket rotor (Z 326 K, Hermle, Germany) used in our experiments. Following the binding of DNA to the extraction matrix, washing and elution buffers are released subsequently by switching processes no.4, no.5 and no.7. Prior to switching process no.7, the nozzle of Revolver II rotates from the waste collection area of Revolver III to the eluate collection area, resulting in a separate collection of the DNA eluate.

Experimental

Cartridge fabrication, assembly and operation

CAD designs of all cartridge components were constructed in compatibility with the constraints of injection molding to enable future mass fabrication. CAD-based illustration of the components and assembly is available in Figure E1 of the electronic supplementary information. At current, injection molding has only been applied to the fabrication of Revolver I (polypropylene) and the mixing pond (polypropylene). The other components were fabricated by rapid prototyping technologies in synthetic resin materials using LED scanning technology (FotoMed® LED.A, Innovation MediTech, Germany) for Revolver III and stereolithography (Watershed XC 11122, DSM Somos®, USA) for shell and Revolver II components.

Revolver I and the mixing pond were sealed at the bottom with a 20 nm heat seal coated aluminum foil (no. 112-2093, amcor, Germany). Sealing was performed at 156 °C for 4 s in a self-made heat press operated at 5 bar. After sealing Revolver I, parts were loaded with assay-specific buffers and got sealed at the topside with a pressure-activated sealing foil (no. 900320, HJ-Bioanalytik, Germany) to avoid spilling. In the experiments demonstrated here, the sample is added to Revolver I together with the buffers. Future users will add the sample to a prefilled Revolver I, only, through an integrated standard interface such as Luer or Monovette. Revolver II parts were equipped with the assay-specific extraction column which were pulled out of the corresponding commercial spin columns using a blunt object. E.g. for DNA extraction, such an extraction column consists out of a glass frit, several layers of silica membranes and a sealing ring. In three consecutive steps, the glass frit, silica membranes and sealing ring were inserted into the column cavity of Revolver II. To realize a tight sealing, the column cavity has exactly the same dimensions as in commercial spin columns and the column components were pressed into the column cavity using a blunt object having the same diameter as the sealing ring. For the ballpen mechanisms, the cartridges were equipped with a metal spring (length: 29 mm, diameter 8 mm diameter) with a spring force of 2.0 N mm^{-1} (D-143J, Gutekunst, Germany). For the mixing system, a smaller spring

of 3.2 mm diameter was used, which has a spring force of 2.8 N mm^{-1} (D-085F, Gutekunst, Germany). Assembled cartridges were transferred into a 50 ml centrifugation tube. The centrifugation tube was used to guarantee leak-tightness during system development and will not be part of the future cartridge design.

For processing, the assembled LabTube cartridges were placed into a swing-bucket rotor (220.72 V06) of an off-the-shelf refrigerated centrifuge (Z326K, Hermle Labortechnik, Germany) with adapters for 50 ml centrifugation tubes. Via an RS232 interface, this centrifuge was connected to a computer which transferred a list of centrifugation parameters including centrifugal acceleration, rates for acceleration changes, temperature and process times. During centrifugation, the cooling function was used to regulate the temperature to 22 °C for DNA extractions and 4 °C for protein purifications, respectively.

Extraction of genomic human DNA from whole blood

EDTA blood samples were freshly taken from internal volunteers at each day of the experiments. Table 1 shows the parameters used for manual and LabTube automated extractions based on the commercial DNA extraction kit "NucleoSpin Blood Mini" (Macherey-Nagel, Germany). Manual protocols were executed in SpinColumns following the original protocol as suggested by the supplier as well as in modified version adapted to the constraints of LabTube automation: maximum centrifugal acceleration of 6000 g and all operations at room temperature. Mixing in LabTube automated runs is performed by 60 mixing cycles which corresponds to a mixing+incubation time of 15 min, each. Moreover, an elongated time of 7 min instead of 1 min is used for drying of the column to counterbalance the lower maximum centrifugal acceleration.

For quantification of the extracted human genomic DNA, a real-time PCR was performed. Three DNase-free, HPLC-purified oligonucleotides were synthesized (Biomers.net GmbH) corresponding to a section of the human 18S gene locus. The sense 18S primer 5′-CGCCGCTAGAGGTGAAATTC-3′ and the antisense 18S primer 5′-GGCAAATGCTTTCGCTCTG-3′ were used in combination with the 18S probe 5′-TGGACCGGCGCAAGACGGA-3′. Stock solutions of 100 µM were prepared in RNase- and DNase-free water according to the manufacturer's protocol. The PCR reaction mix was conducted using the components of a QuantiFast Probe PCR kit (Qiagen, Germany) together with the 18S oligonucleotides, with RNase- and DNase-free water and with the extracted DNA. One PCR reaction mix was composed as follows: 2X QuantiFast mix buffer (5 µl), 10 µM each primer (0.3 µl), 10 µM probe (0.2 µl) and RNase- and DNase-free water (3.2 µl). A well-mixed bulk solution of this PCR reaction mix was prepared, performing triplicate reactions for each sample. For each PCR reaction, 1 µl of extracted DNA was added to 9 µl of the bulk solution. Real-time PCR experiments were performed on a Rotor-Gene 6000 instrument (Corbett Life Science) with the following thermocycling program: 95 °C for 10 min, followed by 45 cycles of 95 °C for 20 s, 50 °C for 30 s and 70 °C for 25 s. To determine the unknown human DNA concentration in the samples, a purified human genomic DNA standard (Roche) was measured in parallel. In a serial dilution, the human standard DNA (200 ng $µl^{-1}$) was diluted down to 0.02 ng $µl^{-1}$ in RNase- and DNase-free water. The quantification of the DNA was performed using the Rotor-Gene Q series software (Qiagen, Germany).

Lactose intolerance testing was performed with eluates generated in LabTube automated extractions to demonstrate the applicability of the LabTube extractions for genomic analysis. EDTA blood samples were taken from three volunteers which represented wild type, heterocygotic and homocygotic mutation in respect to the single nucleotide polymorphism C/T-13910, which is used for gene-based detection of lactose intolerance [26,27]. During this investigation, reference eluates were generated by a MagNA Pure LC automation system (Roche, Germany) in the routine workflow of MVZ Clotten. These reference extractions feature a sample volume of 200 µl and an elution volume of 100 µl, equal to LabTube automated runs. With the blood of each volunteer, three extraction runs were performed in parallel.

Real-time PCR melting curve analysis for C/T-13910 transition was performed according to a proprietary protocol for detection of single nucleotide polymorphisms at MVZ Clotten. In brief, a real-time PCR was performed using LightCycler (Roche, Germany) and fluorescently labeled nucleotide probes (hybridization probes, TIB Molbiol, Germany) complementary to wildtype sequence. A melting point of about 63°C corresponds to wildtype nucleotide sequence. The C/T-13910 transition is characterized by a melting point of 58°C.

Purification of His-tagged proteins

The baculovirus/ insect cell system was used to express the target protein – histidyl-tRNA synthease [28]. cDNA encoding the histidyl-tRNA synthetase was cloned into a transfer vector. Recombinant baculoviruses were generated by homologous recombination of the transfer vector with linearized baculovirus DNA in *Spodoptera frugiperda* (Sf9), identified and cloned by plaque assays. Isolated baculoviruses were propagated and used to infect Sf9 cells cultured in spinner flasks at 27 °C. After 68 h the infected cells were harvested by centrifugation. The pellets were washed in PBS and frozen for storage at -70 °C. Cell lysates were prepared by resuspending the frozen cell pellet in extraction buffer (20 mM HEPES, pH 8.0; 0.2 % Triton X-100, 1 M NaCl) and stirring for 30 min on ice. Cell debris was separated from soluble proteins by centrifugation (20 min; 40000 g). Finally the lysates were stored at -20 °C.

For protein purification, a different LabTube cartridge was used compared to DNA extractions, since the implemented assay (Ni-NTA spin columns, Qiagen, Germany) requires no mixing. Thus, Revolver II is designed without any insets and all thorns directly guide the released liquids onto the extraction matrix.

Since the protein content of every flow-through fraction of the assay contains relevant information, Revolver III includes cavities for the separate collection of six washing and elution fractions. In the current version of the protein purification cartridge, the user needs to disassemble the cartridge for accessing the collected fractions. This disassembly is simple for experienced users, but for future versions, the collection part will be realized as drawer, which can be detached from the cartridge without disassembly. To enable one equilibration, three consecutive lysate addition and six washing/elution steps, the ballpen mechanism is modified compared to the DNA cartridge to enable ten instead of eight switching processes.

Table 2 shows the parameters used for manual processing and the centrifugation-time protocols of LabTube automated runs. Manual centrifugation steps use 270 g and 890 g to centrifuge the reagents through the extraction matrix. For some steps and in dependence of the lysate composition, these forces are insufficient for a complete liquid transfer. For such cases, the user manual suggests to repeat the corresponding step until the transfer is completed. Since this cannot be proven during an automated run, the LabTube protocol uses centrifugal forces up to 2000 g in the end of all centrifugation steps. This way complete liquid removal is guaranteed.

To evaluate yield and purity in the purification experiments, the separately collected flow-through fractions of the lysate addition, washing and elution steps were analyzed by SDS-PAGE with subsequent Coomassie staining. 15 µl of a 1:1 mixture of sample and Laemmli sample buffer (161-0737, Bio-Rad, Germany) and 0.1 mM DTT were applied to precast gels (456-9033, Bio-Rad). Electrophoresis was carried out for approximately 80 minutes at 100 V in a Mini-PROTEAN Tetra Cell system (Bio-Rad). For Coomassie staining the Blue R Staining Kit (Serva, Germany) was used with three destaining steps of one hour to reduce the unspecific background.

Western blots were performed for specific identification of His-tagged proteins and confirmation of the molecular size of the target protein. For this purpose, additional gels from SDS-PAGE were blotted using the Trans-Blot Turbo system with corresponding membranes (170-4159, Bio-Rad). For His-tag specific detection PentaHis monoclonal was used as primary antibodies (34660, Qiagen), Rabbit anti-Mouse IgG-Fc-AP as secondary antibody (31332, Pierce Biotechnology, USA) and BioFX BCIP/NBT Purple AP (Sur Modics, USA) as membrane substrate.

For quantitative evaluation of the target protein yield, Coomassie stained SDS-PAGE gels and western blots were analyzed by densitometry using the image processing software Image J (http://rsbweb.nih.gov/ij/). Here line plots of the color intensity were generated and integrated in the region corresponding to the molecular weight of the target protein. The integrated line plot peak of the molecular weight marker at 50 kD (161-0374, Bio-Rad) was used as reference intensity for comparison of the target protein content between fractions processed on different gels or membranes, respectively. As further evaluation method, the BCA protein assay (71285 (Novagen), Merck Chemicals, UK) was used to determine the total protein content in the eluate fractions. Presuming high purity (confirmed by SDS-PAGE), the total protein content corresponds to the target protein content.

Experimental Results

Demonstration: Extraction of genomic human DNA

Following the protocol illustrated in Figure 5, genomic DNA was extracted from 200 µl human whole blood EDTA samples using LabTube cartridges which were loaded with reagents and silica membranes of a commercial DNA extraction kit (Nucleospin Blood Mini, Macherey-Nagel). LabTube automated DNA extractions exhibited a DNA yield of 114 ± 23 % compared to manually performed reference runs, as shown in Figure 6a for seven extraction runs, performed on two different days and from the blood of two different volunteers.

To test whether LabTube extractions also allow molecular downstream analysis, eluates of LabTube extractions were used for nucleic acid based testing of lactose intolerance. For this purpose, DNA extractions were performed from blood samples of three volunteers representing the three potential nucleotypes with respect to the single nucleotide polymorphism C/T-13910 located 13 910 base pairs (bp) upstream of the lactase gene at chromosome 2q21-22 [27]. In parallel to LabTube automated extractions, DNA extractions were also performed using the MagnaPure LC instrument (Roche, Germany) in an accredited, supra-regional diagnostic lab (MVZ Clotten, Freiburg, DIN EN ISO 15189, 17025). Following DNA extraction, the eluates of both extraction methods were analyzed in a real-time PCR followed by melting curve analysis using sequence-specific fluorescently labeled oligonucleotides as hybridization probes. Here, all eluates showed temperature peaks at equal positions and therefore lead to the same diagnosis, successfully demonstrating the applicability of LabTube automated extractions to such genetic tests (see Table E1 in the electronic supplementary information for data).

Demonstration: Purification of His-tagged proteins

The fast purification of high numbers of different recombinant proteins in microgram quantities represents a bottleneck in protein analysis for drug screening or quality control [24,29]. After cell cultivation, the cells are harvested and suspended in lysis buffer (> 50 ml). The resulting lysate is aliquoted and stored. For each lysate, suitable purification conditions need to be evaluated with respect to e.g. binding, washing and extraction parameters. For this purpose, some aliquots need to be purified at different conditions, causing a high routine work load. Consequently, there is a high interest in reduction of the hand-on-time required for this time-consuming routine screening work.

To demonstrate LabTube automated purification of His-tagged proteins, histidyl-tRNA synthetase expressed in the baculovirus/insect cell system was used as target protein [28] and dry Ni-NTA resin (Ni-NTA Spin Columns, Qiagen) as

extraction matrix. Variation of the reagents loaded in Revolver I can be used to answer different questions. One relevant question is to investigate which imidazole concentration is required for elution. For this purpose, LabTube cartridges were equipped with reagents for two washing and four elution steps with increasing imidazole concentrations within the elution buffer. Figure 6c shows the result of such an experiment analyzed by SDS-PAGE: For the target protein histidyl-tRNA synthetase (~ 50 kD) the expected bind-wash-elute pattern is observed, confirming specific binding to the resin. The target protein is visible in the flow-through fraction resulting from lysate addition (FT) and in the elution fractions (E_1-E_4) at imidazole concentration of $\geq$ 250 mM, but not in the washing fraction (W_1-W_2). Densitometric analysis of the SDS-PAGE results showed that only 3 % of the eluted protein mass was eluted to the fractions E_1 and E_2. The highest protein yield is obtained with 500 mM imidazole.

Figure 6d compares the SDS-PAGE results of manually performed and LabTube automated purifications obtained with a protocol using three washing and three elution steps (500 mM imidazole each). For both purifications, a similar distribution of the target protein is observed, indicating a comparable yield. This result is confirmed by three different quantification methods evaluated in Figure 6b. All methods show an in average equal yield of the target protein in the three elution fractions of the LabTube automated compared to the manual reference purifications. Furthermore, the purity of the eluted proteins is comparable between LabTube and manual runs (see Figure 6d).

Evaluation of hands-on-time

A major goal of laboratory automation is the reduction of hands-on-time. With LabTube automated processing, the user handling is limited to sample addition, starting of the centrifuge and removal of the product, which overall takes about 1 minute hands-on-time. Thus, the user is able to walk away from the instrument while LabTube processing takes place and several parallel laboratory tasks such as documentation or preparation of detection reactions can be performed. This is a significant difference to manual processing, as evaluated in Table 3: For instance when manually performing a DNA extraction from whole blood, the total processing time is approximately 26 minutes including preparation. Adding the hands-on-time for liquid handling, mixing and centrifuge operation, the user's hands-on-time is about 5 minutes. Nevertheless, during repeated steps of 1 minute centrifugation, no parallel work is possible. Thus, only the 15 minutes of incubation during lysis allows the user to walk away and perform parallel work. The overall process time for LabTube automated DNA extraction from whole blood is in the range of commercial automation systems.

When LabTube-automation is used in combination with self-made buffers, as done within the protein purification experiments shown in Figure 6c, manual filling of Revolver I is required. Nevertheless, the user profits from a reduced hands-on-time and a long walk-away-time since all pipetting steps can be performed at once in the beginning and all other manual handling becomes obsolete.

Discussion

System advantages

The application of standard laboratory centrifuges for automated processing is an outstanding advantage of the LabTube platform and thus faces a substantially lower market-entry barrier compared to other lab-on-a-chip approaches. LabTube automation can be performed with up to 20 samples in parallel (e.g. rotor S-4-104 for centrifuge 5810, Eppendorf, Germany) according to the commercially available swing-bucket rotors for standard benchtop centrifuges. For cost-effective mass fabrication of the disposable cartridges, all seven components of the cartridge assembly are designed suitable to injection molding. At high quantities, costs for fabrication and assembly per cartridge are expected to be less expensive than the reagents. Thus, a cost-efficient automation with overall hand-on-times of one minute can be realized even at low sample throughput. Therefore LabTube automation is in particular interesting for rarely demanded tests, applications in research or for diagnostics in smaller laboratories such as in hospitals or remote areas. Besides low invest, LabTube automation can be beneficial to standard automation or manual processing since each sample is processed in a closed and disposable cartridge. Thus, cross-contamination is virtually eliminated and the risk for personnel is minimized when working with infectious material. Moreover, LabTube processing requires no high hygienic laboratory standards due to the completely closed cartridge, which is of particular interest for applications in remote areas. Furthermore, internal design studies exploiting all available space in a 50 ml centrifugation tube showed that the functional components can be scaled up to allow for the use of sample volumes of up to 1 ml. Thus, the LabTube platform will increase the processable volume range compared to typical microfluidic platforms[9]. This is in attractive for diagnostics, since here the typical sample sizes range from 0.1 to 1 ml.

System limitations

For LabTube based assay automation, the use of standard laboratory centrifuges involves two limitations: First, the maximum centrifugal acceleration of standard laboratory centrifuges is between 4000 and 6000 g when using a swing-bucket rotor which is required for liquid handling in LabTube cartridges. In fixed-angle rotors, the non-horizontal filling levels in respect to the cartridge would impede a simple liquid transfer between the single components and thus exclude volume-efficient cartridge designs. The limitation to 4000 to 6000 g can in particular result in constrictions when complex or highly viscous liquids have to be centrifuged through solid phase extraction membranes. In case of significant performance

reduction, longer centrifugation times or modified extraction membranes with a larger cross-section or pore size compared to manual processing can be applied for compensation in LabTube runs. Alternatively, also bead-based extractions could be implemented to avoid the necessity for high centrifugal accelerations.

The second technical limitation is temperature control. Refrigerated laboratory centrifuges allow for cooling of the in-cabin gas volume, but they cannot be applied for temperature control inside the LabTube cartridges which is recommended in DNA extraction protocols for example during lysis incubation (typically 56 or 70 °C). As can be seen from the results shown in Figure 6a, temperature control is not essential for the demonstrated assays, although elevated temperatures were prescribed in the original protocols for lysis incubation and elution buffer, respectively. For assays that show a significant performance reduction when operated without temperature control, we propose two approaches: The first is integration of a battery based temperature control into the LabTube cartridge. The second approach is a passive temperature control via exothermal reactions balanced in temperature by phase-change-materials [30].

Instrumentation

Today, all laboratory centrifuges have a computer interface which is used for automated quality control tests prior to delivery. This interface is usually covered by the enclosure of the centrifuge after quality control, since until today there hasn't been a demand for computer connection. Thus, many standard centrifuges require a rework of the enclosure to enable accessibility to the computer interface. Once an interface is available, it can also be used in combination with a user-friendly bar-code reader system, as visible in Figure 1. Via a barcode label on the cartridge, the system reads the required centrifugation-time-protocol and controls whether all loaded cartridges require the same protocol. Furthermore, such a system enables direct connection to a laboratory information management system (LIMS) which registers all incoming samples, their processing and diagnostic results. This way, a very convenient implementation of LabTube automated assays can be offered for the use in diagnostic or clinical labs.

Conclusion

In conclusion, the novel LabTube platform was successfully demonstrated as a walk-away system for assay automation of routine sample preparation assays. An equal yield in DNA or target protein was obtained in demonstration of DNA extraction from whole blood (114 ± 23 %) and purification of His-tagged proteins from cell lysates (117 ± 23 % via BCA-assay), respectively. The hands-on-time is reduced to one minute: The user only has to load the sample, start the protocol and detach the collection tube when the run is completed. During the complete processing time, the user can walk away and perform parallel tasks. Based on closed disposable cartridges for operation in standard laboratory centrifuges, this innovative platform requires minimum standards of infrastructure because contamination risks are minimized without the need to purchase a specialized processing device. In contrast to automation in common microfluidic systems the LabTube is capable of processing sample volumes up to the milliliter range which is required in many diagnostic and analytical applications. Hence, LabTube automation is even cost-efficient at low sample throughput, features simple and safe handling, and requires no trained staff.

Future work will focus on the integration of sample purification from different sample matrices such as pulmonary or swab samples with different targets such as viral DNA and RNA and bacterial DNA. Instead of simply automating the protocols of commercial assays, the extraction chemistry could be adopted to the advantages and disadvantages of the LabTube technology. This way, we expect to enable reduced cartridge complexity and process times. Cartridge integrated downstream processing and detection, e.g. the prestorage of lyophilized reagents for DNA amplification in the detachable collection tube will be addressed as well. Additionally, the integration of completely different kinds of assays (e.g. immunoassays) is currently investigated. In summary, the LabTube platform could provide cost-efficient diagnostics at the point of care, in particular for areas with low infrastructure. Large laboratories could also profit from LabTube automated sample-to-answer tests. Here, processing highly hazardous samples or the analysis of rarely demanded tests could be automated in a cost-efficient way.

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Notes and references

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Electronic Supplementary Information (ESI) available: Figure E1 shows CAD-views of the cartridge, Figure E2 and E3 present a standard illustration approach for structured chronological presentation of a LabTube protocol and Table E1 presents the temperature peak data of the Real-time PCR melting curve analysis for lactose intolerance testing. Moreover, an animation movie is available for visualization of assembly and ballpen mechanism. See DOI: 10.1039/b000000x/

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Table 1. Protocol parameters used for DNA extraction from whole blood.

Process step	Manual protocol (fixed angle rotor)	LabTube protocol (swing-bucket rotor)
Lysis	Addition of 200 µL blood, 25 µL Proteinase K and 200 µL lysis buffer (B3) → Vortex mixing for 20 s → Incubation for 15 min at 70 °C)	Addition of 200 µL blood, 25 µL Proteinase K and 200 µL lysis buffer (B3) → 60 mixing cycles (15 min centrifugal acceleration is alternated between 200 and 2500 g, room temperature)
Binding	210 µl ethanol → vortex mixing for 20 s → 1 min @ 11000 g	210 µl ethanol → 60 mixing cycles → 2 min @ 6000 g
Washing 1	500 µl buffer (BW) → 1 min @ 11000 g	450 µl buffer (BW) → 1 min @ 6000 g
Washing 2	600 µl buffer (B5) → 2 min @ 11000 g	450 µl buffer (B5) → 7 min @ 6000 g
Elution	100 µl buffer (BE, preheated to 70 °C) → 1 min incubation → 1 min @ 11000 g	100 µl buffer (BE) → 1 min @ 20 g (incubation) → 2 min @ 6000 g

Table 2. Protocol parameters used for purification of histidyl-tRNA synthease from cell lysate. Precooled cartridges and the centrifuge's temperature control unit were used to maintain 4 °C during processing.

Process step	Manual protocol (fixed angle rotor)	LabTube protocol (swing-bucket rotor)
Equilibration	Addition of 350 µL, equilibration buffer [a] → 2 min @ 890 g	Addition of 350 µL equilibration buffer [a] → 2 min @ 890 g
Lysate addition	450 µl lysate → 15 min @ 270 g	3x 150 µl lysate → 5 min @ 100-2000 g [b]
Washing [c]	350 µl washing buffer [a] → 2 min @ 890 g	350 µl washing buffer [a] → 2 min @ 100-2000 g
Elution [c]	100 µl elution buffer [d] → 2 min @ 890 g	100 µl elution buffer [d] → 2 min @ 100-2000 g

[a] Equilibrium and washing buffer: 20 mM HEPES solution (pH = 8.0) with 5 mM imidazole
[b] During LabTube processing, centrifugal force is increased step by step within the listed limits.
[c] Numbers of washing and elution steps were varied as indicated in the results section and Figure 6, respectively.
[d] Elution buffer: 20 mM HEPES solution (pH = 8.0) with imidazole concentrations between 50 mM and 500 mM as indicated in Figure 6.

Table 3. Evaluation of hands-on-time and walk-away-time. Hands-on-time includes only the time required for handling steps for each sample. Centrifugation times are excluded.

Application	Method	Overall process time	Hands-on-time	Walk-away-time (percentage of overall process time)
DNA from whole blood	Manual	26 min	6 min	1x 15 min [a]
	LabTube	59 min [b]	1 min	58 min [b] (98 %)
	QIAcube [c]	~ 60 min [d]		
	Maxwell 16 [c]	30-40 min [d]		
	Magna Pure LC [c]	60-90 min [e]		
Purification of His-tag proteins	Manual	33 min	5 min	1x15 min (45 %)
	LabTube	33 min	1 min	32 min (97 %)
	QIAcube [c,e]	82 min [d]		
	Maxwell 16 [c,e]		60 min [d]	

[a] incubation time during lysis, [b] elongated process time for LabTube automated runs results from longer mixing times, [c] different kit and protocol compared to manual and LabTube automated runs, [d] manufacturer information (technical hotline of Qiagen, Promega user manuals for Maxwell 16), [e] protocols include resuspension of cell pellets or bacterial culture

Figure 1. LabTube workflow. LabTube cartridge and centrifuge with swing-bucket rotor. If desired, a barcode scanner can be used for transfer of the assay specific centrifugation-time-protocol. After protocol completion, the eluate (colored in pink) is obtained in a detachable collection tube.

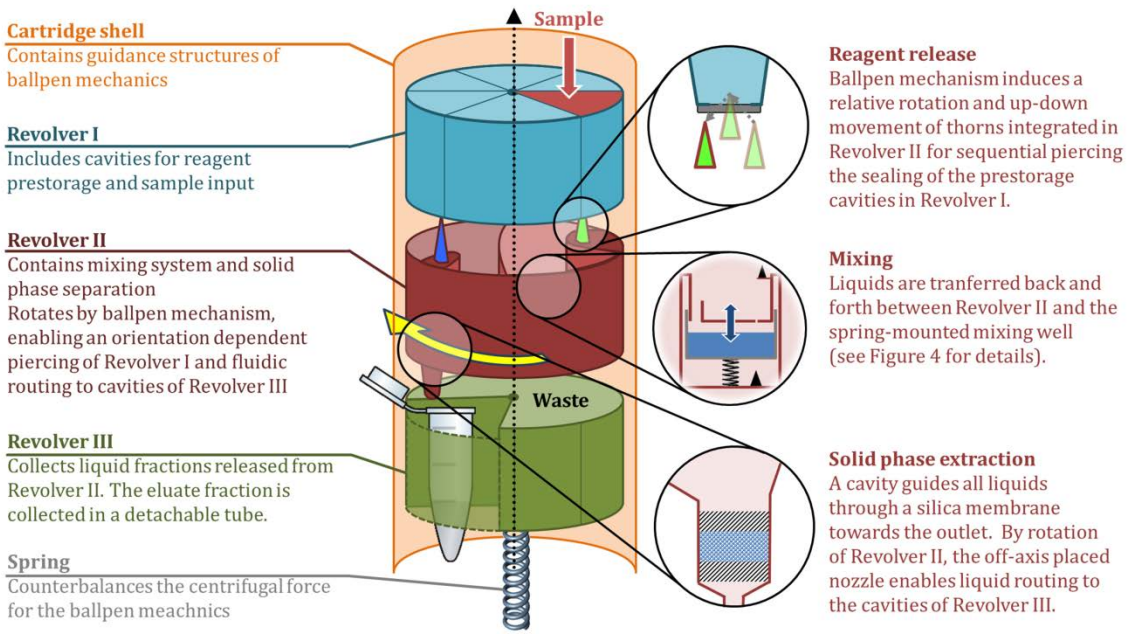

Figure 2. LabTube cartridge components and their functionality.

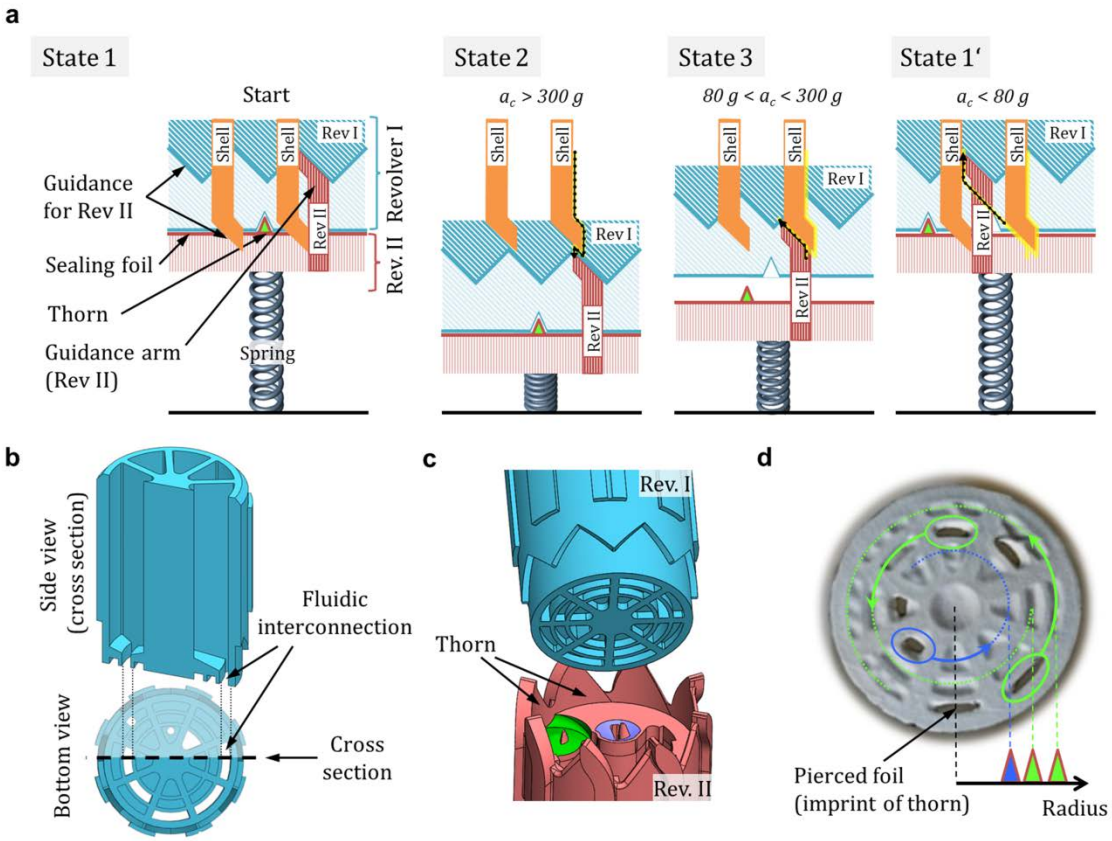

Figure 3. Integrated ballpen and piercing mechanism for reagent release and liquid routing. a) Centrifugally-controlled movement of Revolver I and Revolver II in relation to the cartridge shell during a single switching process. Yellow trace and dotted arrows illustrate the overall and current movement of the tip of the guidance arm of Revolver II (highlighted in dark red). b) Cross section and bottom view of Revolver I (CAD image). c) Bottom of Revolver I and top of Revolver II (CAD image). Rev. II contains three thorns, the green path guides liquids into the mixing system, the blue one guides liquids directly onto the column. d) Photograph of an aluminum foil sealed bottom of Revolver I in same orientation as shown in Figure 3b. After two switching processes, two holes were pierced into the foil by each thorn. Green and blue lines indicate the rotation of the corresponding thorns along the bottom.

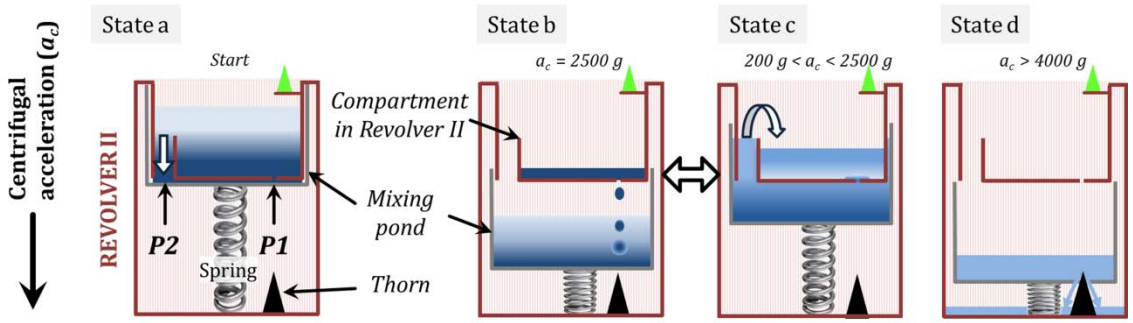

Figure 4. Mixing system integrated in Revolver II.
Liquids are transferred back and forth between Revolver II and the mixing pond (between state b and c) for continuous inversion of liquid layers. The green thorn is used for reagent release to the mixing system as shown in Figure 2. A density gradient is assumed after liquid addition (state a), which is illustrated as a color gradient. Two passages P1 and P2 are integrated for liquid transfer between the two compartments. Fluidic resistance of passage P1 is by orders of magnitude higher than P2 to create a preference of passage P2 and thus an inversion of the liquid layers. For release of the mixture, a thorn opens an aluminum sealing of the mixing pond at high centrifugation (state d).

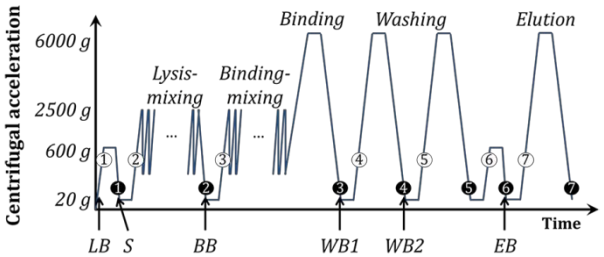

Figure 5. Schematic illustration of a centrifugation-time-protocol for fully automated DNA extraction. Activation and completion of switching processes by the integrated ballpen mechanism are indicated by numbers of white and black background, respectively. Letters mark the release of lysis buffer (LB), sample+Proteinase K (S), binding buffer (BB), washing buffer 1 (WB1), washing buffer 2 (WB2) and elution buffer (EB).

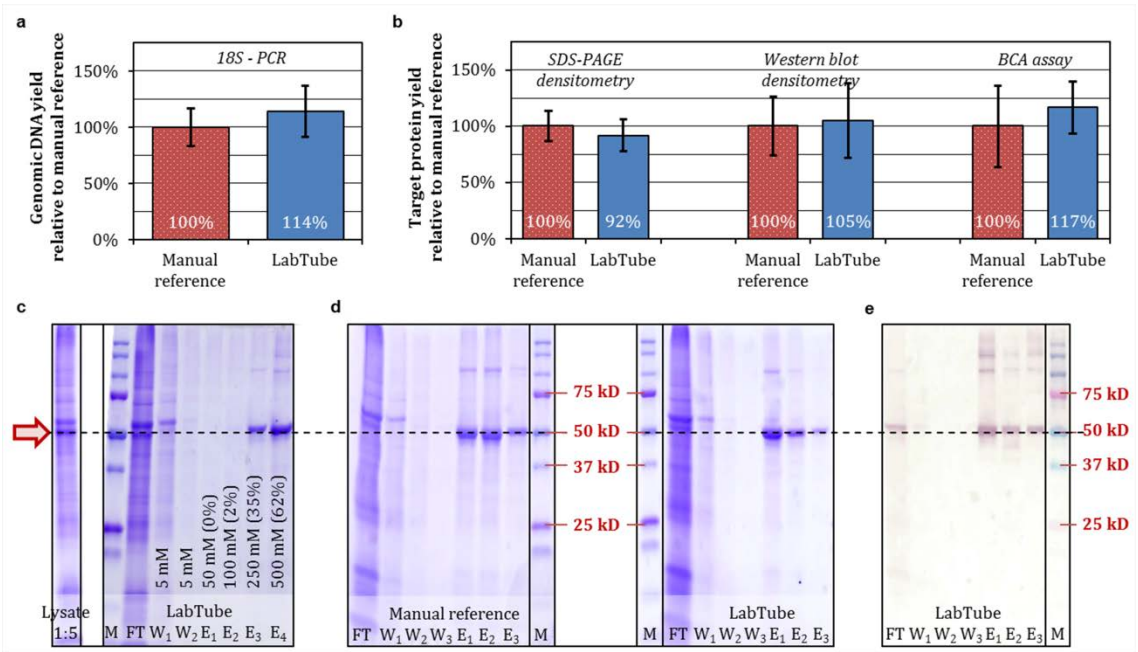

Figure 6. Yield comparison of LabTube automated to manually performed reference runs. a) Extraction of human genomic DNA from whole blood EDTA samples analyzed via 18S-PCR (n = 7; 2 series with 3 and 4 parallel LabTube runs). b) Protein purifications from histidyl-tRNA synthease analyzed via three different methods as indicated in the graph (4 parallel runs each). c) SDS-PAGE gel of a representative LabTube automated purification for the analysis of the required imidazole concentration in the elution buffer. Gel images show molecular weight marker (M) as well as the separately collected fractions of lysate flow-through (FT), 2x washing (W1-W2) and 4x elution (E1-E4). Imidazole concentrations and percentage of the overall eluted protein yield are indicated in parentheses within the figure. The arrow and the dashed line indicate the molecular weight of the target protein (histidyl-tRNA synthease). Additionally, the figure shows the unprocessed lysate (dilution 1:5) for comparison. d) Comparison of selected manual and LabTube automated purifications with lysate flow-through (FT), 3 washing (W1-W3, 5 mM imidazole each) and 3 elution steps (E1-E3, 500 mM imidazole). e) Western blot of the LabTube automated eluates shown in Figure 6d.

