Video Article

Filtration Isolation of Nucleic Acids: A Simple and Rapid DNA Extraction Method

Sally M. McFall[1], Mário F. Neto[1], Jennifer L. Reed[1], Robin L. Wagner[2]

[1]Center for Innovation in Global Health Technologies (CIGHT), Department of Biomedical Engineering, Northwestern University

[2]Pritzker School of Medicine, The University of Chicago

Correspondence to: Sally M. McFall at smmcfall@gmail.com

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Abstract

FINA, filtration isolation of nucleic acids, is a novel extraction method which utilizes vertical filtration via a separation membrane and absorbent pad to extract cellular DNA from whole blood in less than 2 min. The blood specimen is treated with detergent, mixed briefly and applied by pipet to the separation membrane. The lysate wicks into the blotting pad due to capillary action, capturing the genomic DNA on the surface of the separation membrane. The extracted DNA is retained on the membrane during a simple wash step wherein PCR inhibitors are wicked into the absorbent blotting pad. The membrane containing the entrapped DNA is then added to the PCR reaction without further purification. This simple method does not require laboratory equipment and can be easily implemented with inexpensive laboratory supplies. Here we describe a protocol for highly sensitive detection and quantitation of HIV-1 proviral DNA from 100 µl whole blood as a model for early infant diagnosis of HIV that could readily be adapted to other genetic targets.

Video Link

The video component of this article can be found at https://www.jove.com/video/54289/

Introduction

Several reports have discussed the development of paper- or membrane-based extraction methods for use in point-of-care (POC) devices [1-5] with the aim of making the exquisite sensitivity and specificity of molecular diagnostics available to all. The World Health Organization (WHO) Sexually Transmitted Diseases Diagnostics Initiative coined the term ASSURED (Affordable, Sensitive, Specific, User-friendly, Rapid and Robust, Equipment-free and Delivered to those who need it) to describe the ideal characteristics of a POC test [6]. Of these guidelines, the equipment-free characteristic is particularly challenging to achieve for molecular diagnostics. However, every innovation in the field will advance the goal of reaching those in most need, and there is hope for near-term improvements in test performance by adapting existing technology [7].

Here we describe a simple protocol for extracting DNA from whole blood that does not require complex chemistry or laboratory instrumentation. The FINA (filtration isolation of nucleic acids) sample preparation method was originally developed to extract leukocyte DNA from whole blood in order to detect the HIV-1 provirus as part of a sample-to-answer point-of-care (POC) quantitative PCR (qPCR) early infant diagnostic (EID) platform for use in limited resource settings [8-11]. FINA extraction differs from conventional purification methods which use silica membranes or silica-coated paramagnetic particles to reversibly bind DNA in the presence of chaotropic agents [12]. Instead, FINA uses vertical filtration via a separation membrane to extract cellular DNA from whole blood directly. The membrane containing the entrapped DNA can be placed directly in a PCR tube and either immediately used in a PCR reaction or air dried for later amplification [9]. No chaotropic agents, phenol, or alcohols are used in the sample extraction, eliminating the extensive washing steps needed to remove potent qPCR inhibitors derived from the extraction process [13,14].

The FINA membrane can capture either cells [9] or genomic DNA liberated by cell lysis [11] before the specimen is added to the membrane. For the cell capture, whole blood is added directly to the membrane. The cells are subsequently lysed in the membrane by adding 10 mM NaOH. The advantage to this method is that it involves only 3 steps: 1) sample addition; 2) cell lysis/wash and 3) filter disk placement in qPCR tube. The drawback to this method is that the membrane can only hold a defined number of cells directly proportional to the diameter of the membrane disk. To reach the limit of detection required for EID, 100 µl of whole blood is required for sample input which entails a filter that is too large to be placed in a qPCR tube. Lysing the blood cells with detergent before adding the sample to the collection membrane adds a processing step, but allows the use of a smaller filter for the same sample size. We were able to demonstrate high reproducibility, single copy detection, and quantification of as little as 10 copies of HIV-1 proviral DNA from 100 µl of blood using this test configuration [11].

In this report, we describe the FINA protocol as originally developed for laboratory use. The membrane/filter sandwich known as the FINA sample preparation module can be prepared in large batches and stockpiled for later use. When specimens are to be extracted this process takes 2 min and can be performed in varying size batches. The qPCR can be run immediately or the filters containing the embedded DNA can

be stored until it is convenient to perform qPCR. This method is very convenient for routine analysis of specimens in both low and high resource settings.

Protocol

Ethics statement: The whole blood specimens used in this study are not considered to be research involving human subjects. The specimens were obtained for clinical diagnostic purposes, which were satisfied, and the remaining portion of these specimens was provided for the FINA research assay. The specimens were coded such that the investigators were not able to readily ascertain the identity of individuals.

1. Preparation of FINA Sample Preparation Module

1. Prepare blotting pad by cutting a 35 x 35 mm^2 of a 2.6 mm thick 100% cotton pad. Cut one pad per specimen.
2. Prepare plastic paraffin film with a paper backing tape by a cutting piece of paraffin film (25 mm (h) by 50 mm (w)) and then punching a 7.14 mm diameter hole in the center of the tape using a hammer driven hole punch. Prepare one tape per specimen.
3. Prepare a bound glass blood separator membrane (capture membrane) disk by punching a 8.35 mm diameter circle using a hammer driven hole punch. Punch one disk per specimen. The disk has a particle retention capability of 2.3 µm.
4. Assemble the FINA sample preparation module by placing the capture disk in the center of the blotting pad using forceps. Place the paraffin film tape over the capture disk so that the hole of the paraffin tape leaves the center of the capture disk exposed.
5. Ensure maximum contact between the disk and the blotting pad by stretching the paraffin tape slightly to cover the blotting pad and pressing the paraffin tape firmly to stick it to the blotting pad around all edges of the disk.
6. Make as many modules as needed for the experiment or stockpile for future use.

2. Preparation of qPCR Tube

1. Prepare a 5.1 mm tape disk that will anchor the cell capture membrane to the side of the qPCR tube to prevent the membrane from blocking the fluorescence detection by punching a circle of double-coated polyester diagnostic tape with a hammer driven punch from a sheet of the tape. Prepare one tape disk per specimen.
2. Remove one side of the tape's sticker liner. Place the tape into a 200 µl PCR tube, sticking it onto one side and toward the bottom of the tube. Remove the second sticker liner. The tube is now ready to receive prepared disk.
3. Produce as many tubes as needed for the experiment or stockpile for future use.

3. Contrive Blood Specimen

Note: If a genuine test specimen is being prepared, proceed to step 4.

1. Thaw 8e5-LAV cells [15] harboring a single copy of the HIV-1 provirus obtained from the Virology Quality Assurance Laboratory (VQA; Rush Presbyterian/St. Luke's Medical Center, Chicago, IL).
   Note: They are stored as frozen cell pellets at a concentration of 4,000 cells/µl. Cell count was verified as described previously [9].
2. Prepare serial dilution in freezing medium (90% fetal bovine serum, 10% dimethyl sulfoxide) to concentrations ranging from 1 to 400 cells/µl.
3. Pipet 10 µl cells from each of the serial dilutions into a microcentrifuge tube. Add 100 µl of fresh HIV-1 negative EDTA treated whole blood sample to each tube to create a standard curve of 8e5-LAV copy numbers 10-4,000. Mix by gently flicking the bottom of the tube 5 times.

4. Perform FINA Extraction

1. Lyse blood cells by adding Triton-X100) to a final concentration of 1% (11 µl 10% Triton-X100 to 110 µl whole blood and cells from step 3.3).
2. Mix by gently flicking the bottom of the tube 5 times. The blood should turn translucent red.
3. Pipet all of the blood specimen onto the capture disk.
   Note: A water tight seal between the paraffin tape and the capture membrane ensures that the blood flows through the membrane, and good contact between the capture membrane and blotting pad assembly ensures rapid sample wicking.
4. Allow sample to soak through the filter.
   Note: The blood lysate wicks into the blotting pad due to capillary action, capturing the genomic DNA on the surface of the capture disk. The lysate has soaked into the disk when it appears matte.
5. Add 600-1,000 µl 10 mM NaOH drop-wise onto the capture disk.
   Note: The wash buffer can also be added as a bulk addition of 600 µl. The buffer will form a droplet on the hydrophobic paraffin tape and wick through the hole to the disk in approximately 20 sec. The filter will change from red to white indicating clearance of hemoglobin.
6. Separate the disk from the blotting pad with forceps and apply the disk to the tape in the prepared PCR tube. If there is any red color left on the filter add 50-100 µl of wash buffer to clear the filter before placing in the tube.
   Note: Infrequently, excess pressure applied during preparation of the FINA modules results in partitioning of the membrane layers during separation from the blotting pad. Discard these disks and prepare a fresh sample.
7. After the filter is placed in the PCR tube, analyze the samples right away. Alternatively, dry overnight in a box containing calcium sulfate desiccant, then cap and place in a foil pouch with silica desiccant and store until ready for analysis.

5. qPCR Reaction

1. Prepare 100 µl qPCR master mix per reaction using HIV specific primers and probes as described previously [9,11]. Include an internal amplification control to monitor for the presence of amplification inhibitors and to verify that a negative sample is a true negative. Be sure that filter is completely covered with master mix and that the filter stays fixed to side of tube throughout the reaction.
2. Perform amplification using a real-time PCR device as previously described [9].

Representative Results

The workflow for extracting proviral DNA from whole blood spiked with 8e5-LAV cells is shown in **Figure 1**. **Figure 2** shows the FINA sample module and prepared qPCR tube. This method allows for efficient amplification of HIV-1 provirus from 8e5-LAV cells at different copy numbers, as shown in the standard curve of contrived specimens (**Figure 3**). PCR had an efficiency of 103%, as calculated from Efficiency = $-1+10^{(-1/\text{slope})}$. Equation of the line was y= -3.25x + 27.95, with a correlation of $R^2 = 0.996$. The HIV proviral DNA standard curve replicates also have highly reproducible amplification, as evidenced in **Table 1**. Additionally, an internal control of 500 copies Hydroxypyruvate Reductase is present to show that there is no PCR inhibition resulting from extracting blood with FINA, independent of the number of copies of HIV-1 provirus present (**Figure 4**). IC amplification was efficiently obtained in all 18 samples tested, with an average Cq of 21.92 ± 0.25 and a CV of 1%.

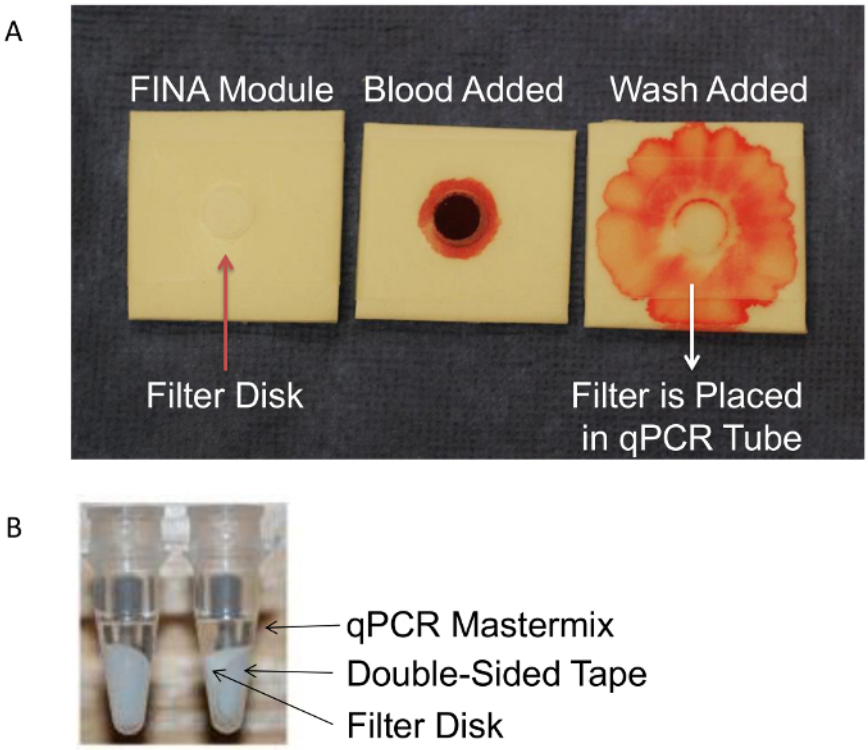

Figure 1: Workflow for detecting proviral DNA from whole blood spiked with 8e5-LAV cells to qPCR. (**A**) From left to right, the prepared FINA module, after blood is added to the capture membrane and after wash buffer was added. (**B**) qPCR tubes with capture disks stuck to double-coated tape with qPCR master mix added. Please click here to view a larger version of this figure.

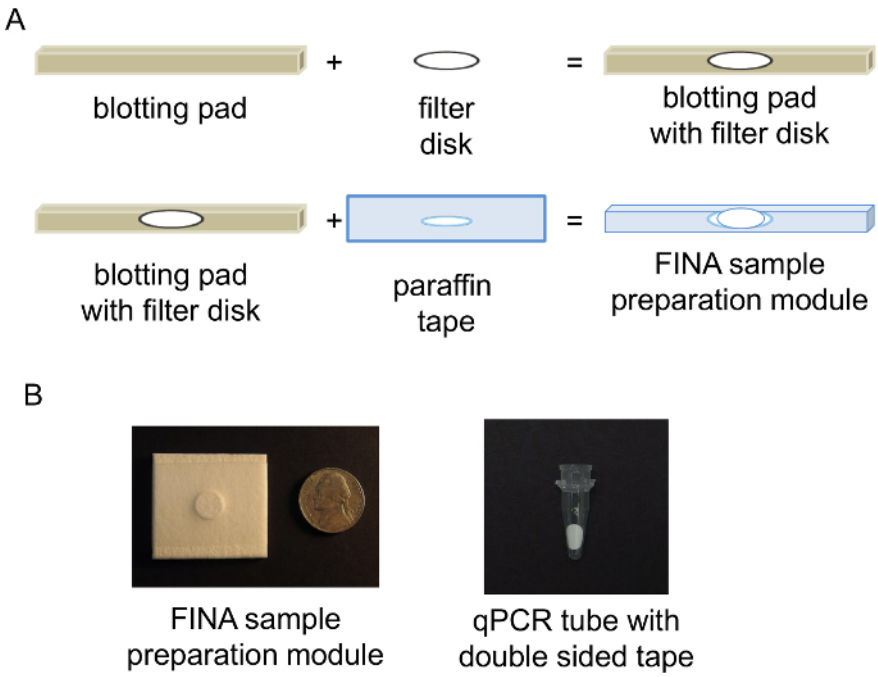

Figure 2: FINA in-house module and qPCR tube preparation. (**A**) An 8.35-mm diameter capture membrane disk was sandwiched between a square 707 blotting pad and a thin sheet of paraffin tape containing a 7.14-mm-diameter hole in the center such that the hole of the paraffin tape was centered on the capture disk for the direct application of lysed blood by pipet. (**B**) A 5.1 mm double-coated polyester diagnostic tape is stuck on side of 200 µl qPCR tube. The second liner of tape is removed to expose sticky surface for applying capture disk when ready. Please click here to view a larger version of this figure.

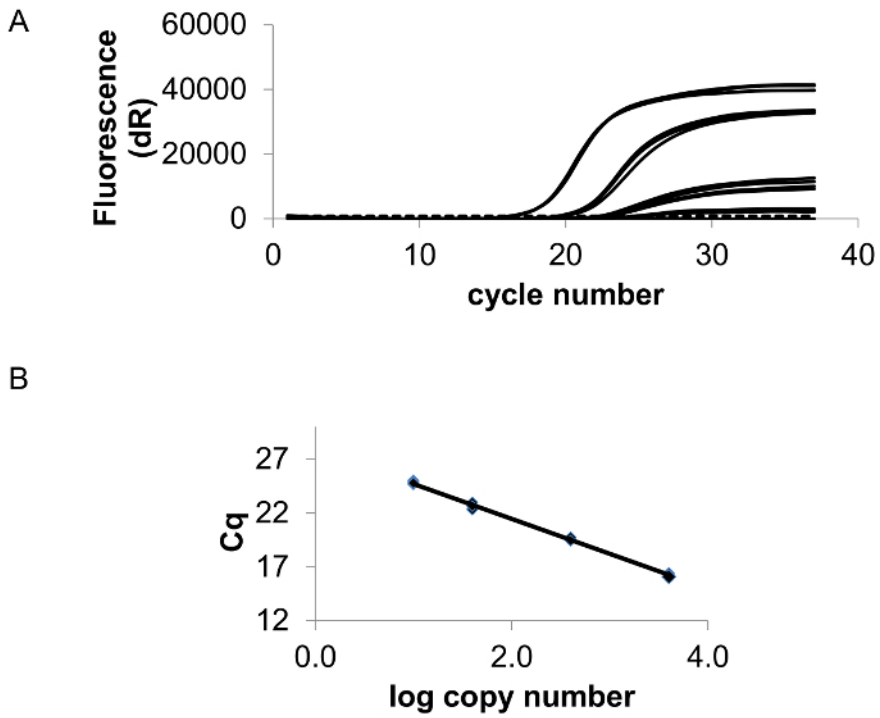

Figure 3: Standard curve of HIV-1 proviral DNA contrived specimens. (**A**) Amplification plots obtained from 100 µl of 4 replicates HIV-1 negative whole blood spiked with 4,000, 400, 40 and 10 8e5-LAV cells with 500 copies/reaction of the internal control Solid lines = amplification plots; Dotted line = threshold. Y-axis is fluorescence units and X-axis is qPCR cycle number. (**B**) Standard curves of Cq values calculated from amplification plot versus log copy number of 8e5-LAV cells per 100 µl whole blood. Equation of the line: y = -3.25x + 27.95; R^2 = 0.996; PCR efficiency = 103.23% calculated via Efficiency = $-1+10^{(-1/slope)}$ Please click here to view a larger version of this figure.

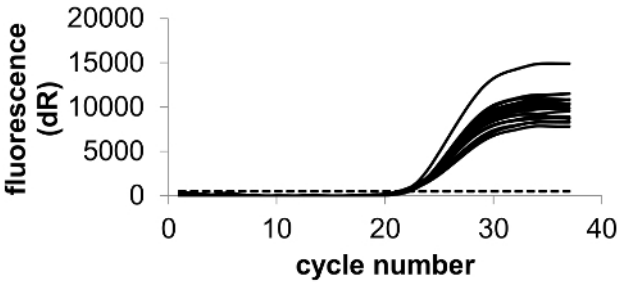

Figure 4: Internal control indicates no qPCR inhibition. 500 copies Hydroxypyruvate Reductase Amplification control plasmid added per reaction. Solid lines = amplification plots; Dotted line = threshold. Average Cq of IC = 21.92 ± 0.25. Cqs ranged from 21.21 to 22.32. Coefficient of variation (CV %) = 1%; N = 18. Please click here to view a larger version of this figure.

8e5-LAV cells/			
100 µl WB	**Ave. Cq**	**SD**	**CV (%)**
4,000	16.27	0.14	1
400	19.54	0.15	1
40	22.56	0.3	1
10	24.83	0.15	1

Table 1: Average Cq, standard deviation (SD) and coefficient of variation (CV %) of 4,000, 400, 40 and 10 copies of 8e5-LAV standard curve with FINA extraction and HIV-1 specific primers and probes. N = 4. WB = whole blood.

Discussion

EID linked to rapid treatment access has been demonstrated to reduce infant mortality due to HIV infection [16]. Because of the persistence of maternal antibodies in an infant's blood, rapid HIV antibody tests have limited utility in determining the status of HIV-exposed infants. The WHO recommends that all infants born to HIV-1 positive mothers should be tested at 4-6 weeks of age, using a virological test [17]. We have reported the development of an assay for the detection and quantitation of HIV-1 proviral DNA in whole blood specimens that is capable of single copy detection with demonstrated 100% sensitivity and specificity in a small field evaluation of 61 South African infants [11]. One hundred microliters or more of whole blood can be collected via finger or heel stick and processed via the FINA modules [18,19]. These blood samples can then be stored for at least one month before amplification making this method ideal for testing infants that are far from laboratories.

In the study presented in **Figure 3**, a standard curve of contrived specimens from HIV-1 negative whole blood spiked with thawed cells harboring HIV-1 proviral DNA is depicted. A caveat to this study is that some of the cells added to the sample could have already lysed due to the freeze/thaw, which could have potentially improved the detection level of the target. A more stringent experimental design would have been to have contrived the specimen using freshly cultured cells which would have more accurately mimicked whole blood.

The FINA sample prep method was designed for incorporation into an EID POC qPCR device (under development) for use in limited resource settings [8]; however, the manual format described here can also be a useful addition to a laboratory's sample prep portfolio. FINA does not use the bind, wash and elute strategy used in dried blood spots and other paper-based extraction systems that dilutes the extracted nucleic acids, possibly leading to sub-optimal detection sensitivity [5]. The FINA system is highly flexible and can be readily adapted for other genetic tests besides proviral HIV DNA detection. Lower volumes of blood or other biological specimens can be processed for abundant targets and higher volumes for less abundant ones. In our original embodiment of the FINA system, we capture the cells from whole blood using a blood separation membrane and then lyse them by the addition of 10 mM NaOH [9]. This version has the advantage of one fewer user steps, but the cell number/blood volume that can be processed is smaller. In addition to the bound glass membrane used in this report, other blood separation or blood collection filter papers have been used successfully with this method, and several different quantitative PCR instruments have also been shown to amplify template embedded in the filter disk [9]. The only stipulation is that the filter does not block the fluorescence reading of the real-time PCR instrument.

A significant limitation of FINA extraction is that there is a size constraint on the diameter of the capture membrane. A disk with a membrane larger than 9 mm cannot be placed in a 200 µl qPCR tube without the membrane overlapping in the tube which limits the surface area exposed to the reaction mix. Additionally, the larger the disk diameter, the more qPCR reaction volume is required to cover the filter which increases the cost and turn-around-time of the assay. If the filter is too small to efficiently capture the DNA in the specimen, it may clog preventing efficient washing.

Using the bound glass capture membrane, we were able to capture only HIV-1 proviral DNA and not viral RNA from whole blood samples (data not shown). We do not know if alternative membranes or buffers could be used to promote isolation of RNA instead of or in addition to DNA. If the reader would like to only isolate DNA from the specimen this protocol does not require the enzymatic removal of RNA required with some protocols. Detecting both HIV-1 RNA and DNA in clinical samples would improve the sensitivity of EID and this lack of RNA detection is a limitation of our diagnostic test.

The most critical step in adapting the FINA process to a new assay is to validate the size of the filter to accommodate the amount of DNA, *i.e.,* the number of cells, in the specimen to be tested. In general FINA extraction works best with specimens with smaller numbers of cells

so that smaller capture disks can be used. To determine the binding capacity of the filter, simply add a second filter disk to the FINA module stacked below the collection membrane. Add the sample and wash the membranes as in steps 4.1-4.5. Place each of the DNA capture disks into separate qPCR tubes and amplify. Using a standard curve, determine the percent of input DNA captured on each filter. The size of the filter can be optimized to the needs of the assay. For the FINA proviral DNA assay described here, more than 99% of human genomic DNA is captured when cell lysate is added to the filter (unpublished observations) enabling superior detection capabilities.

In addition to EID, future applications of FINA could include the detection and quantitation of proviral DNA as a biomarker for HIV-1 disease monitoring with patients who have achieved viral suppression due to antiretroviral therapy. Treatment monitoring aimed at HIV eradication could be performed by screening other inputs besides peripheral blood for reservoirs of HIV-1 infected cells. HIV proviral testing could also be used to screen vaccine trial subjects for true infection. The blood samples are stable during storage on FINA modules and can be collected under field conditions for later shipment to central laboratories for PCR analysis making this method ideal for epidemiological studies. In addition to HIV-1 detection, this highly flexible method could also be used to amplify precision medicine targets found in biological specimens such as pharmacogenetics screening or other SNP detection or for rapid genotyping of animal models. Isothermal amplification methods such as helicase dependent amplification (HDA) or loop-mediated isothermal amplification (LAMP) could also be used with FINA extracted template or the DNA-embedded on the filter could be used as template for conventional PCR amplification.

Disclosures

The authors have nothing to disclose.

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References

1. Byrnes, S. A. *et al.* One-step purification and concentration of DNA in porous membranes for point-of-care applications. *Lab Chip.* **15** (12), 2647-2659 (2015).
2. Connelly, J. T., Rolland, J. P., & Whitesides, G. M. "Paper Machine" for Molecular Diagnostics. *Anal Chem.* **87** (15), 7595-7601 (2015).
3. Govindarajan, A. V., Ramachandran, S., Vigil, G. D., Yager, P., & Bohringer, K. F. A low cost point-of-care viscous sample preparation device for molecular diagnosis in the developing world; an example of microfluidic origami. *Lab Chip.* **12** (1), 174-181 (2012).
4. Linnes, J. C. *et al.* Paper-based molecular diagnostic for. *RSC Adv.* **4** (80), 42245-42251 (2014).
5. Rodriguez, N. M. *et al.* Paper-Based RNA Extraction, in Situ Isothermal Amplification, and Lateral Flow Detection for Low-Cost, Rapid Diagnosis of Influenza A (H1N1) from Clinical Specimens. *Anal Chem.* **87** (15), 7872-7879 (2015).
6. Peeling, R. W., Holmes, K. K., Mabey, D., & Ronald, A. Rapid tests for sexually transmitted infections (STIs): the way forward. *Sex Transm Infect.* **82 Suppl 5** v1-6 (2006).
7. Mabey, D., Peeling, R. W., Ustianowski, A., & Perkins, M. D. Diagnostics for the developing world. *Nat Rev Microbiol.* **2** (3), 231-240 (2004).
8. Jangam, S. R., Agarwal, A. K., Sur, K., & Kelso, D. M. A point-of-care PCR test for HIV-1 detection in resource-limited settings. *Biosens Bioelectron.* **42** 69-75 (2013).
9. Jangam, S. R., Yamada, D. H., McFall, S. M., & Kelso, D. M. Rapid, point-of-care extraction of human immunodeficiency virus type 1 proviral DNA from whole blood for detection by real-time PCR. *J Clin Microbiol.* **47** (8), 2363-2368 (2009).
10. Kelso, D. M., Jangam, S., Yamada, D., & McFall, S. *Google Patents.* (2012).
11. McFall, S. M. *et al.* A Simple and Rapid DNA Extraction Method from Whole Blood for Highly Sensitive Detection and Quantitation of HIV-1 Proviral DNA by Real-Time PCR. *Journal of Virological Methods.* **214**, 37-42. (2015).
12. Boom, R. *et al.* Rapid and simple method for purification of nucleic acids. *J Clin Microbiol.* **28** (3), 495-503 (1990).
13. Radstrom, P., Knutsson, R., Wolffs, P., Lovenklev, M., & Lofstrom, C. Pre-PCR processing: strategies to generate PCR-compatible samples. *Mol Biotechnol.* **26** (2), 133-146 (2004).
14. Schrader, C., Schielke, A., Ellerbroek, L., & Johne, R. PCR inhibitors - occurrence, properties and removal. *J Appl Microbiol.* **113** (5), 1014-1026 (2012).
15. Folks, T. M., Powell, D. M., & Martin, M. A. *Google Patents.* (1991).
16. Violari, A. *et al.* Early Antiretroviral Therapy and Mortality among HIV-Infected Infants. *N Engl J Med.* **359** (21), 2233-2244 (2008).
17. WHO. (ed World Health Organization) *World Health Organization.* Geneva, Switzerland (2010).
18. Gruner, N., Stambouli, O., & Ross, R. S. Dried blood spots--preparing and processing for use in immunoassays and in molecular techniques. *J Vis Exp.* (97) (2015).
19. Maiers, T. J. *et al.* An investigation of fingerstick blood collection for point-of-care HIV-1 viral load monitoring in South Africa. *S Afr Med J.* **105** (3), 228-231 (2015).

