Lab on a Chip

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Storing self-contained gel capillary cassettes for POC medical diagnostics

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For effective clinical uptake of the lab on a chip/point of care technology (LOC-POC), in addition to cost advantages LOC-POC devices should offer multiple patient screening panels for related diseases as well as cold-chain transportation and storage abilities. We recently described a device that performs polymerase chain reaction (PCR) to simultaneously screen raw clinical samples from up to 16 patients for multiple infectious agents (Manage *et al.*, *Lab Chip*, 2013, **13**, 2576–2584). This cassette contains glass capillaries with desiccated semi-solid acrylamide gels that include all the reagents except for the sample, with integrated quality control. Here we report the development of protocols to store assembled PCR cassettes at room temperature, 4 °C or −20 °C as well as at +40 °C. We show that our cassettes are stable, with no loss of activity for at least 3 months at RT and at least 7 months at 4 °C and −20 °C. However, the activity of desiccated cassettes degrades when stored for more than 2 weeks at 40 °C, insufficient time for post-manufacture delivery and use of cassette PCR. To address this, we have evaluated two stage storage protocols. PCR cassettes can initially be stored at 4 °C and −20 °C for prolonged periods of time and removed for shorter term storage at RT, retaining activity for at least a month, which would facilitate transport to remote areas for testing. Effective use of cassette PCR in high temperature regions of the world, for experimental purposes defined here as 40 °C, appears to be feasible only after a first stage storage in the cold, followed by no more than 1 week at 40 °C. This should allow sufficient time for delivery by the manufacturer to a central area well served by power and refrigeration, for later ambient temperature transport and use in under-resourced areas that lack refrigeration.

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1. Introduction

Point of care or near point of care (POC) testing holds potential to significantly influence clinical care at the bedside. POC testing will allow confirmation of a diagnosis in order to provide optimal treatment. Currently, the health care industry relies on time consuming and expensive central laboratory testing. A successful POC device ideally should be self-contained, be user friendly with a simple means of sample introduction, should have no or minimal sample preparation steps, a rapid turnaround time, reasonable accuracy and must be affordable. Ideally a POC device should accept raw clinical samples and screen for multiple targets per patient. For applications in clinics with large patient volumes, it will be essential to implement simultaneous screening of multiple specimens in parallel. Previously we have introduced a flexible platform for molecular diagnostics.[1] We have developed a self-contained single-use and fully integrated multi-parameter cassette that contains all the reagents required for DNA amplification (PCR), including positive and negative controls. For the end user, cassette PCR requires only that unprocessed samples be introduced to the cassette. Sample delivery occurs through capillary force with no need for application of positive or negative pressure. This cassette consists of glass capillaries holding desiccated gels that include all PCR reagents. Each capillary reaction unit holds a different set of primers enabling detection of multiple disease targets on the same cassette. Capillaries are embedded in wax and the assembled cassette can be stored for later use. After use, the cassette can be safely discarded, with all the reaction units encased in solid wax. We have demonstrated that at least 16 different patients can be simultaneously screened on the same cassette for multiple sexually transmitted infections,[1] with integrated quality control, but with no need for pumps and valves to control fluid flow. The cassette is constructed from off the shelf components, avoiding expensive fabrication or clean room facilities.

Advances in POC technology has been summarized in several review papers.[2–4] Commercially available POC testing was reviewed by Niemz *et al.*[5] Some devices are capable of

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performing sample preparation steps and some devices are self-contained. Lounsbury *et al.* showed a three layer PMMA/PDMS chip that could be used in a POC setting,[6] with on-chip sample purification and a 45 min PCR. The device needs fresh reagents, a single actuation step for fluid handling, a centrifugation step for handling blood to analyze samples one at a time and does not include quality controls. Oblath *et al.* demonstrated a chip made from PDMS/glass with a monolithic aluminum oxide membrane (AOM) containing 7 separate wells to test up to seven primer sets with one sample.[7] Sample was loaded to each well separately and the vacuum was applied to the waste well to draw the sample through the AOM. Each well was then loaded with fresh reaction mixes containing different primers and mineral oil was placed as the vapor barrier before performing thermal cycling where real time PCR data was collected.

In order to develop devices that can perform faster diagnostic tests and can overcome implementation difficulties, especially in resource deprived areas, self-contained devices with dried PCR reagents are crucial. Several reports describe self-contained devices or conventional assays that can be stored. Sun *et al.* demonstrated a self-contained device made of cyclic olefin copolymer (COC) that contained gelified reagents in the PCR chamber.[8] The chip contains sample preparation steps, requires pumps for fluid handling, can be shipped at room temperature (RT) and be stored at 4 °C for extended periods. The chip was bonded after the reagents were gelified in the PCR chamber. Multiplex PCR was carried out to detect two bacterial strains. Once the sample is loaded to the chip, the gelified reagents were readily hydrated for PCR amplification. Chips were stored at RT or at 4 °C for up to 3 months. At RT, the half-life of gelified reagents was at least 3 months.[8] Qu *et al.* used a TaqMan probe-based assay for real time PCR to detect a set of pathogens, using vacuum dried reagents in 200 μL microcentrifuge tubes; all experiments were performed in a Roche LightCycler.[9] For this system, the reagents are stable for at least 49 days at 37 °C. Chen *et al.* demonstrated a single sample polycarbonate fluidic chip with dried reagents encapsulated by a paraffin film inside the PCR chamber and stored at RT for up to 5 months.[10]

In our previous work, we focused on validating our capillary cassette technology by analysing multiple patient samples and multiple sample types on the same cassette; we also briefly indicated that cassettes could be stored in vacuum sealed bags for up to 3 months at RT.[1] Since cassettes include DNA polymerase which is normally stored at −20 °C, methods are needed to ensure activity is preserved for prolonged periods of time. Two such methods of preserving reagents are lyophilization and desiccation. For both methods, reagents are dried in the presence of sugars to stabilize enzyme activity. Sugars such as trehalose and sucrose have been used as preservatives.[9,11–16] The research on preservation of reagents with different stabilizers has been reviewed.[14] For a Taq man probe-based assay to detect species of *Yersinia*, Qu *et al.* used 40% trehalose and 20% dextran as enzyme stabilizers during the vacuum drying.[9] For long term preservation of proteins, non-reducing sugars are required.[17] Trehalose is one of the most chemically unreactive and stable sugars and is a non-reducing disaccharide.[11] It is also an effective DNA preserving agent for long term storage,[18] an important concern for preservation of positive control templates. The amount of water retention after the desiccation is critical for maintenance of enzyme activity during reagent storage.

In this work, we focused on storage of the cassettes for longer periods of time and under different conditions, including a strategy to enable use of the cassettes in low resource locations with high ambient temperatures. Here, we show a self-contained cassette that can be stored at least 7 months at 4 °C, or at −20 °C, and at RT for at least 3 months without losing functionality. The cassette with desiccated reagents remains stable for at least a month at RT after prolonged first stage storage at low temperature. To assess the stability of the cassette under different storage conditions, as our model system we evaluated cassette stability for the detection of herpes simplex virus 1 and 2 (HSV-1 and HSV-2) from raw genital swab samples.[1]

2. Experiment

2.1 Samples

HSV-1 positive, HSV-2 positive, and negative controls were raw genital swabs in universal transport media (UTM; Copan Diagnostics Inc., Murrieta, CA, USA) as previously described.[1] They were frozen at −20 °C until use. Samples were applied directly to cassettes without any form of sample purification. Presence of the virus from each sample was identified in the clinical laboratory using tissue culture. In order to reduce the variability, the same set of HSV-1 and HSV-2 raw genital swab samples were used through the whole study.

2.2 Reagents

Separate reaction mixes were prepared with (positive controls) or without template for HSV-1 and HSV-2, and used to fill capillaries. The primer sets for detecting HSV-1, HSV-2 were published previously.[1] Each 100 μL reaction mix consisted of 20 μL of 5X PCR buffer (333 mM tris-sulfate, pH 8.6, 83 mM $(NH_4)2SO_4$ (Sigma, St. Louis, MO.); and 40% sucrose (Sigma)), 30 μL of 40% trehalose (Cargills Inc. Canada), 4 μL of 50 mM $MgCl_2$ (Fluka, Buchs), 2 μL of 10 mM [dNTP] (Sigma), 2 μL of 1%BSA (Sigma), 4 μL of 10 μM primer solution (Integrated DNA technologies, San Diego, CA) for each of the two primers, 10 μL of 10X LC Green Plus (Idaho Technology Inc., Salt Lake City, Utah) and 4 μL of Taq polymerase (20 units per μL), 10 μL of a 40% acrylamide (Sigma) + 4% bis-acrylamide aqueous solution (*N*,*N*-methylene bisacrylamide, BioRad, Hercules, CA), 2 μL of 3% azobis (Wako, Richmond), 1 μL of 10% TEMED (*N*,*N*,*N*′,*N*′-tetramethylethylenediamine, Sigma) and water. For the positive controls, 4 μL of raw sample was added, replacing water. The mixes were vortexed, centrifuged, and loaded into the capillaries as described below.

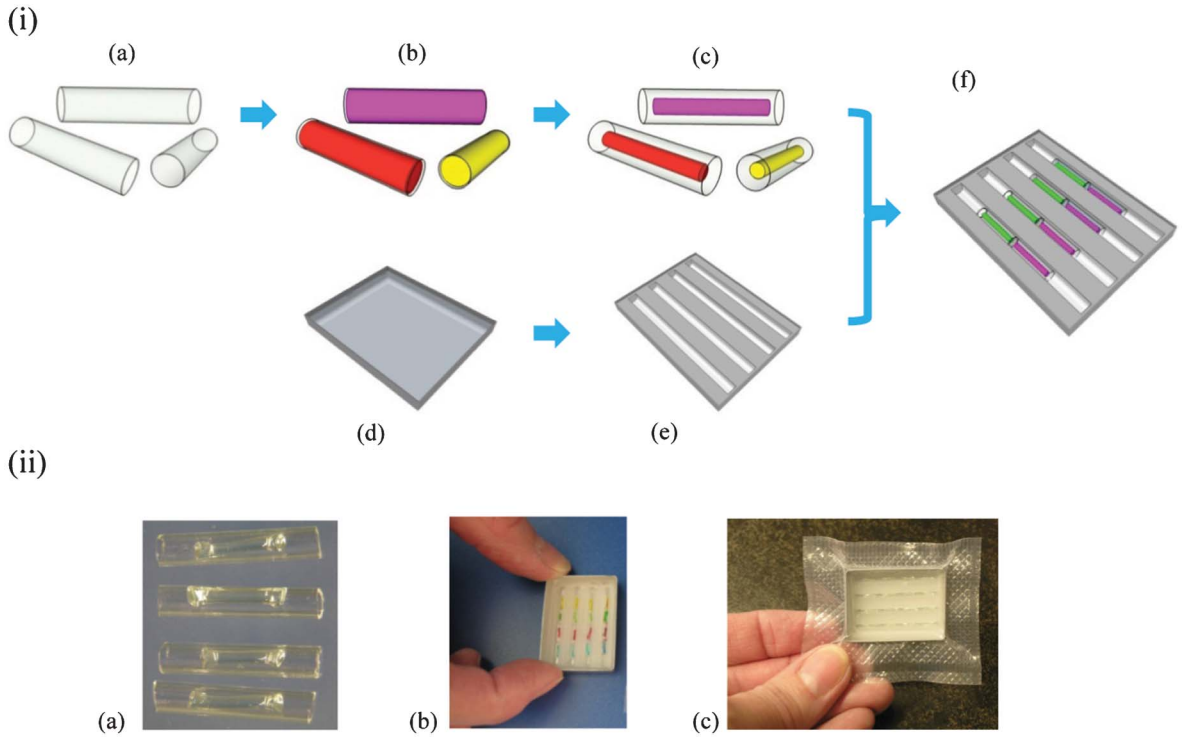

Fig. 1 (i) Procedure for making cassettes with capillaries: steps for making capillaries: (a) capillaries are cut to 6 or 7 mm in length, (b) filled with PCR/gel reaction mix and exposed to the UV light for photo-polymerization, and (c) desiccated to create a dried gel ''noodle'' inside the capillaries. Steps for making pans: (d) empty aluminum pan, (e) wax imprints to create trenches with different arrangements for placing capillaries, and (f) format for assembling HSV1 and HSV-2 capillaries and positive controls. Green and purple represent HSV-1 and HSV-2 capillaries respectively. The last row contains positive controls. Each capillary holds from 6–7 microliters of mixture. (ii) Photographs of capillaries/cassettes (a) dried gel inside capillary reaction units, (b) photograph of a cassette with capillaries arranged in 6 trenches. Capillaries are colored to represent different primers sets, and (c) photograph of a vacuum packed cassette.

2.3 Preparation of glass capillaries with desiccated gels

Steps for making glass capillaries with desiccated gels and arranging them in wax pans are shown in Fig. 1(i). Capillaries and details of preparation for PCR were as previously described.[1] Briefly, capillaries that served as positive controls were filled with the reaction mix containing the specified primer set and a known positive sample while all the others were filled by capillary action with reaction mix and the specified primer set but no template, and exposed to 370 nm UV light ($\sim$1 mW cm^{-2} on the capillaries) for 30 min to photo-polymerize the gel/reaction mix.

The capillaries with polymerised gel were desiccated in a vacuum oven (Isotemp Vacuum Oven 281A, Fisher Scientific) for 1 h, 2 h, and 24 h at 24 in of Hg in order to establish the residual moisture level that is required for reagent stability during long term storage. Desiccation of the gel inside the capillary creates a noodle shape (Fig. 1(ii)-a) of the dried gel which is important for the sample delivery where the sample flows through the space between the dried gel and the glass capillary walls by capillary force and rehydrates the gel. Several batches of capillaries were made, each for different time course series. These were stored in the cassettes as described below.

2.4 Preparation of the cassettes

Preparation of the pans for arranging capillaries holding reaction mix with different primers was described earlier.[1] Briefly, the wax was filtered to remove fluorescent particulates, and about 1.3 mL of molten wax was poured into an aluminum pan with dimensions (23.5 mm $\times$ 32 mm). A PDMS stamp was used for imprinting trenches in the wax to make a template for laying capillaries in the pan (Fig. 1(i)-e). The shape of the bottom of the trenches matches the shape of the capillary so that the capillary lies snugly in the wax trench. Capillaries for the long term study were laid in wax trenches as shown in Fig. 1(i)-f. Capillaries in the 4th trench have DNA polymerised inside the gel and serve as positive controls. A photograph of a cassette is shown in Fig. 1(ii)-b. Different sets of pans were made with capillaries desiccated for 1 h, 2 h and 24 h.

2.5 Cassette storage

Cassettes with wax/desiccated gel capillaries containing HSV-1 and HSV-2 primers were stored in vacuum-sealed bags as shown in Fig. 1(ii)-c. A commercially available food vacuum sealer was used to seal the packages. Different storage conditions were chosen in order to study reagent stability. Three different sets of sealed cassettes were stored at RT ($\sim$22–30 °C), in a refrigerator (4 °C), or in a chest freezer (−20 °C) for up to 8 months. RT fluctuated between 22–30 °C during different seasons. Cassettes were tested at different time

points from 1 week to 8 months. A set of cassettes were taken from 4 °C and −20 °C after storing at those temperatures for 2 months (first stage) and were then held at RT for the indicated periods of time (second stage). These cassettes were tested at the second stage for 1 or 4 weeks after 4 °C first stage storage and at 1, 4, and 8 weeks after −20 °C first stage storage. In order to test the performance of the cassettes at 40 °C, chosen to represent a warmer climate, a set of desiccated cassettes, either freshly made or after first stage storage at RT, 4 °C or −20 °C for 3 months, were stored in an incubator (Thermo Electron Corporation, Hepa class 100) at 40 °C. They were tested weekly for up to three weeks.

2.6 Moisture content after the desiccation

In order to calculate the residual moisture content in the desiccated gel capillaries, 5 sets with 5 empty glass capillaries in each set were weighed. The capillaries were then filled with PCR/gel reaction mix and the polymerized capillary sets were weighed immediately after polymerization. Five capillary sets with each set containing 5 capillaries were desiccated and then weighed after 1 h, 2 h, and 24 h. To find the anhydrous weight of the gels, the capillaries were baked at 100 °C for 24 h to remove any moisture in the gel. Methods were originally established by Shankar.[16]

2.7 DNA amplification in gel capillary cassettes

2.7.1 Sample delivery to the cassette to hydrate the desiccated capillary reaction units. The sample is delivered to a line of two or more capillaries that are placed adjacent to each other in the trench (Fig. 1(f)) by dispensing the sample at one end of the trench such that the sample flows through by capillary forces and rehydrates the gel in all capillaries within a given trench. The 1st and 2nd trenches received HSV-1 and HSV-2 samples respectively. The third and 4th trenches received water to hydrate the gels. The third trench contains negative controls while 4th trench contains positive controls. The raw genital swab samples were 10x diluted in water and were used directly to hydrate the capillaries. The gel takes about 7–8 min to rehydrate. The PCR reactions for each cassette are run independently, as the Gelcycler holds only one cassette at a time. Cassettes stored at different time points under different conditions were run separately, as described below.

2.7.2 PCR and MCA. A detailed description of the instrument (GelCycler) used for PCR and MCA was published previously.[1] Briefly, it contains a Peltier element for heating and cooling and a laser for fluorescence excitation, and a CCD camera for the image acquisition. After the sample was introduced into the cassette, the rehydrated cassette was then placed on the Peltier element of the GelCycler for thermal cycling. After a pre-denaturation step of 3 min at 94 °C, 35 cycles of DNA amplification for 3-step PCRs were carried out at 94 °C for 20 s, 60 °C for 30 s, and 72 °C for 30 s, followed by a final extension step of 120 s at 72 °C. For the 2-step PCRs, thermal cycling was carried out at 94 °C for 20 s, 62 °C for 20 s for 35 cycles while initial denaturation and final extension steps are similar to 3-step PCRs. MCA was performed from 65–90 °C and CCD images were taken at every 0.2 °C. The CCD images were analyzed as previously described,[1] but with slightly different image collection parameters for the CCD camera.

3. Results and discussion

Deterioration of the enzymes can occur over time. Thermal denaturation of an enzyme, resulting in a loss of its native shape, is one of the main reasons for loss of activity. Thermal stability of the enzyme is greatly influenced by excess water. Removing much of that water, improves enzyme stability by reducing the freedom of movement hence inhibiting the possibility for conformational changes of the enzyme.[19] When drying in the presence of trehalose or other sugars, the protein (enzyme) molecules are protected from denaturation by formation of hydrogen bonds between enzymes and sugars during the process of removing bonded water molecules with sugar molecules.[20] This drying mechanism is beneficial for long term storage of the enzyme and, is essential for long term storage of PCR cassettes consisting of glass capillaries filled with polymerized and then desiccated PCR/gel reagents. Among the sugars used for maintaining the enzyme activity during the storage, trehalose, a non-reducing sugar, was shown to confer excellent stability.[21]

In our cassette, glass capillaries serve as capsules or vessels for holding reagents during the polymerization and desiccation of the gel as well as during the rehydration process when the sample is introduced by capillary forces during POC testing. Melted wax during the PCR acts as a vapour barrier that stops any possibility of mixing of primers or amplicons during the PCR and MCA.[1] Previously, we have shown that the primers for both HSVs are specific and amplify only the appropriate samples.[1] We have sequenced the amplicons to confirm that the correct product was formed. No product was formed in the capillaries with mismatching primers. We have previously confirmed that the primers and amplicons do not cross-contaminate adjacent capillaries during sample delivery, rehydration, or PCR.[1]

3.1 Residual moisture content

Residual moisture content of the gel capillaries after the desiccation can be calculated by the ratio of (desiccated gel weight − anhydrous gel weight) to (wet gel weight − anhydrous weight). The final moisture content was calculated by averaging values from five capillary sets each containing five individual capillaries. The moisture content of the gels inside the capillaries that were desiccated for 1 h, 2 h, and 24 h are 14.18 ± 0.98%, 10.92 ± 0.87% and 7.59 ± 0.96% respectively.

3.2 DNA amplification with stored cassettes under different conditions

In order to study the best desiccation conditions for prolonged storage of cassettes, sealed cassettes with capillaries desiccated for 1 h, 2 h, and 24 h were tested at different time points from 1 week to 8 months. Three sets of storage conditions: RT,

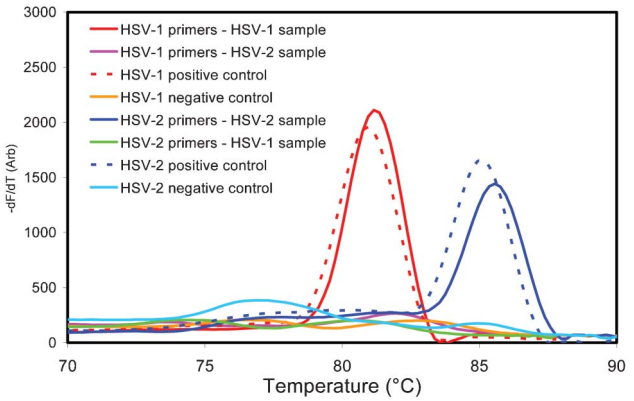

Fig. 2 Melt curve analysis (MCA) data for a cassette with capillaries stored at 4 °C for 2 week after 24 h desiccation. Each primer set amplified the DNA from the correct sample and produced melt peaks. Primers mismatched to the sample or the negative controls did not produce melt peaks. Positive controls that were hydrated with water produced appropriate melt peaks.

4 °C, and −20 °C were tested at each time point. Results from different time points were compared to results from control cassettes that were stored for 1–2 weeks. An example of MCA data obtained from a cassette containing capillaries desiccated for 24 h, stored at 4 °C and tested 2 weeks later is shown in Fig. 2.

The colour coding and the scale of the Y-axis are consistent throughout the manuscript. In all the experiments shown below, capillaries with mismatched primers and samples were run and gave the expected results, but are not shown. As demonstrated in Fig. 2, HSV-1 primers do not amplify HSV-2 DNA and HSV-2 primers do not amplify the HSV-1 DNA. We have previously confirmed the specificity of the HSV-1 and HSV-2 primers.[1]

The fluorescent dye, LC Green binds to any double stranded DNA, which means that the fluorescent signal read by the CCD camera is a combination of intensities from the amplified PCR product as well as the primer dimers, making Q-PCR curves a flawed measure of proper amplification. MCA is required to verify the correct product. PCR was performed similarly in all the stored cassettes using a 3-step PCR, except for the cassettes containing the capillaries that were desiccated for 2 h where PCR was done with 2-steps.

3.2.1 Capillaries desiccated for 24 h at different time points. MCA data obtained with the sealed cassettes containing the capillaries desiccated for 24 h and stored at RT, 4 °C or −20 °C and then tested at a range of time points are shown in Fig. 3. The MCA profiles of the control cassettes with freshly desiccated capillaries are consistent over multiple desiccation runs.

The performance of the cassettes containing capillaries that were desiccated for 24 h are stable only up to 3 months of storage at RT, showing loss of activity by 7 months, but similar cassettes stored at 4 °C and −20 °C did not change over 7 m of storage. This can clearly be seen in Fig. 3(b) where the intensities of the melt peaks are plotted at different time points. For storage at RT, the performance of the cassettes degraded by about 50% at the 7 m time point.

3.2.2 Capillaries desiccated for 1 h and 2 h at different time points. Melt peak intensities obtained with the sealed cassettes containing the capillaries desiccated for 1 h or 2 h and stored at RT, 4 °C or −20 °C, tested at different time points, are shown in Fig. 4(a) and 4(b) respectively. The DNA amplification in the cassettes containing capillaries desiccated for 2 h was performed with 2-step PCR.

Compared to MCA data from the 24 h desiccation protocol, shown in Fig. 3(b), the performance of the cassettes with capillaries desiccated for 1 h are considerably weaker at RT or 4 °C storage. With 1 h or 2 h desiccation and RT storage, capillaries lost activity as early as 1.5 months. Capillaries desiccated for 1 h or 2 h and stored at 4 °C degraded but not as extensively as those stored at RT. The MCA results after the 1 h or 2 h desiccation cannot be directly compared as the PCR reactions after the 2 h desiccation protocol were performed in 2-steps compared to the 3-step PCRs performed with a 1 h desiccation protocol, but the trend towards degraded activity is clear for both protocols stored at RT or 4 °C. However, with all three desiccation times, 1 h, 2 h and 24 h, the performance of capillaries stored at −20 °C appear to be independent of the desiccation time.

Comparison of Fig. 3(b) to Fig. 4 shows that when stored at RT or 4 °C, the capillaries desiccated for 1 h and 2 h have lost activity more quickly than those desiccated for 24 h. Capillaries desiccated for 1 h and 2 h have higher residual moisture levels than those desiccated for 24 h. Published work suggests that the higher moisture content causes degradation/aggregation of dried enzymes.[22,23] The reduced activity after 1 h or 2 h desiccation protocols may therefore reflect degradation of the enzyme caused by a higher moisture content in the gels.

These results suggest that the desiccation should be performed for ∼24 h in order to preserve capillaries for long term storage, especially if they are to be stored at RT or 4 °C.

3.2.3 Capillaries stored at RT after a first stage storage at 4 °C or at −20 °C for 2 months. In hot climates, the work above suggests that PCR activity will be quickly lost on storage. However it is possible to store cassettes at 4 °C or −20 °C for a prolonged period and then transfer them to RT storage for a shorter time during which they could be stored on the shelf in clinics despite high ambient temperatures. In order to check whether our cassettes could remain active when transitioned from cold storage to RT, a set of cassettes were stored at 4 °C and −20 °C for 2 months and then removed from the cold and stored at RT for a month. If they can survive at RT for few weeks, they could be transported to remote areas that may lack refrigeration. The MCA profiles for the two stage storage protocol are shown in Fig. 5.

The performance of the cassettes stored at RT for 4 weeks after first stage storage at 4 °C or −20 °C for 2 months can directly be compared with the performance of the cassettes continuously stored at 4 °C or −20 °C for 3 months in Fig. 5. These comparisons are shown in Fig. 5(b). The capillaries may have lost some activity after storing at RT for 4 weeks-3 months post-transfer from the first stage storage at 4 °C or −20 °C, but the majority of their activity still remains. More detailed future

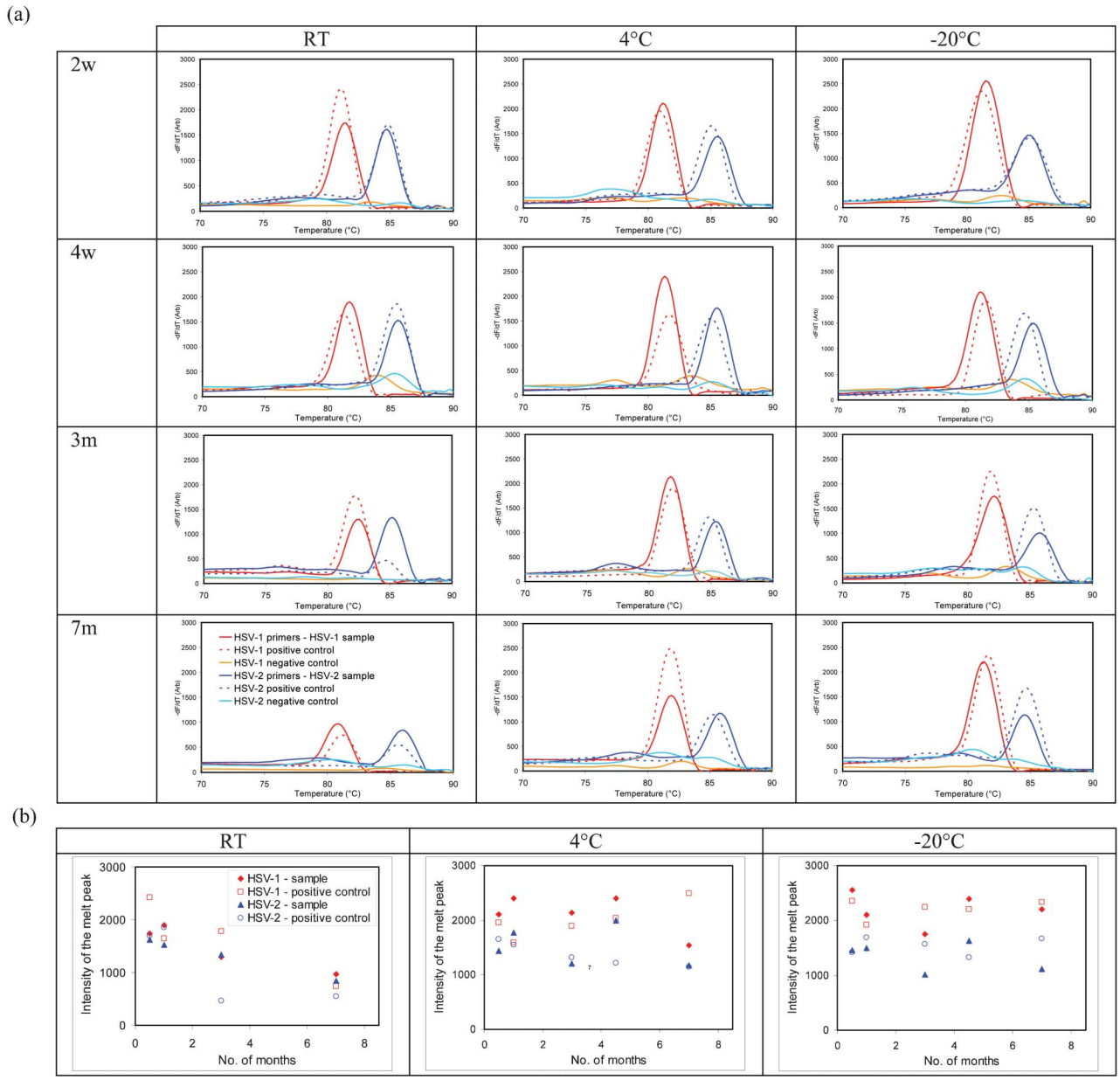

Fig. 3 Performance of the cassettes containing the capillaries desiccated for 24 h and stored at RT, 4 °C and −20 °C for up to 7 months. (a) MCA profiles (b) melt peak intensities of HSV-1 sample (solid red diamonds), HSV-1- positive control (red squares), HSV-2 sample (solid blue triangle) and HSV-2- positive control (blue circles) at different time points during the storage. The lengths of the capillaries that are cut by hand always vary in length from each other. This causes slight variations in the fluorescent intensities as slightly longer capillary has a greater gel volume and accepts higher sample volume. The consistency of the capillaries is being significantly improved by precise commercial cutting of capillaries.

studies will confirm whether or not this strategy is likely to be useful in the field.

3.2.4 Capillaries stored at 40 °C after storing at RT, 4 °C, or −20 °C for 3 months. In order to test whether our cassettes are stable at high temperature, *e.g.* at 40 °C, after desiccation and assembly, two sets of cassettes were stored in a 40 °C incubator. One set was placed at 40 °C after first stage storage at RT, 4 °C or −20 °C for 3 months and was tested after 1, 2, and 3 weeks at 40 °C. The other, a set of freshly made cassettes, was directly stored at 40 °C up to one month, with testing at 1–4 week time points.

Cassettes stored at 40 °C were stable for 1–2 weeks but lost considerable activity by week 4 (Fig. 6, far left panel). Since it is unlikely that cassettes could be delivered and used within a 2 week period this protocol does not seem feasible for clinical use. Hence we thus evaluated two stage storage protocols where storage at 40 °C was preceded by 3 months at RT, 4 °C or −20 °C. After 3 months at RT before a second stage at 40 °C, the cassettes lost most of their activity by week 1 (Fig. 6, 2nd panel from the left). Cassettes placed at 40 °C after 3 months at 4 °C retained activity for one week but lost activity thereafter (Fig. 6, 3rd panel from the left). Storage at 40 °C after 3 months

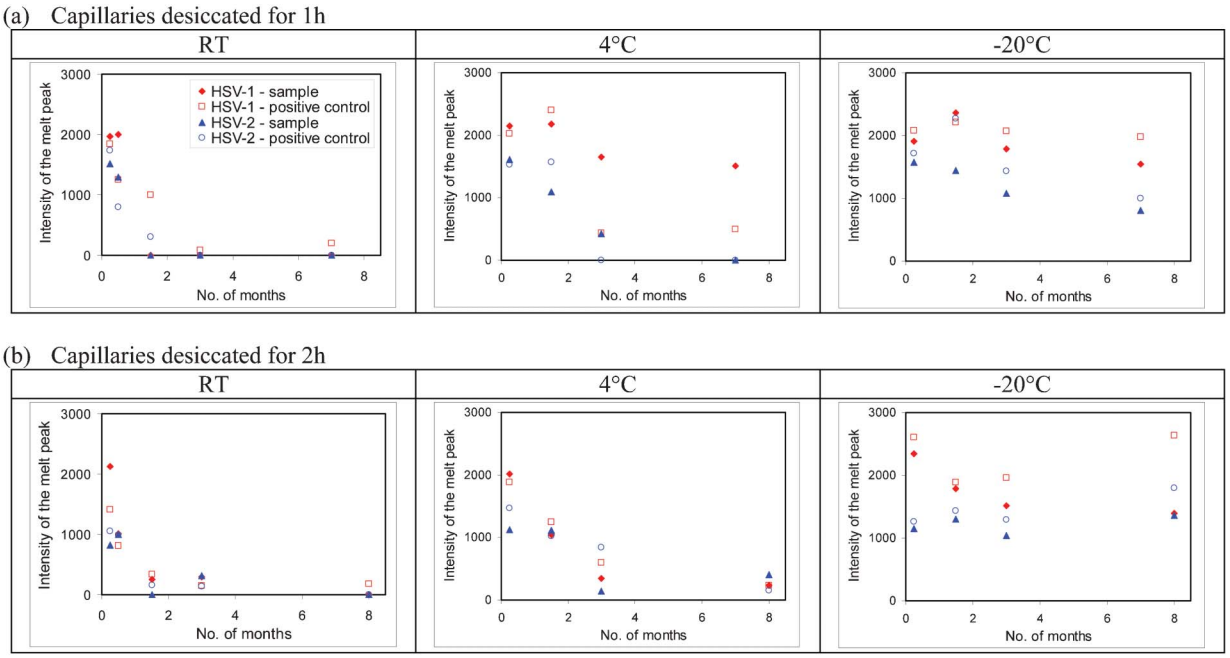

Fig. 4 Performance of the cassettes containing the capillaries desiccated for 1 h or 2 h, and stored at RT, 4 °C or −20 °C for up to 8 months. Melt peak intensities of HSV-1 sample (solid red diamonds), HSV-1- positive control (red squares), HSV-2 sample (solid blue triangle) and HSV-2- positive control (blue circles) at different time points during the storage of (a) desiccated for 1 h or (b) desiccated for 2 h.

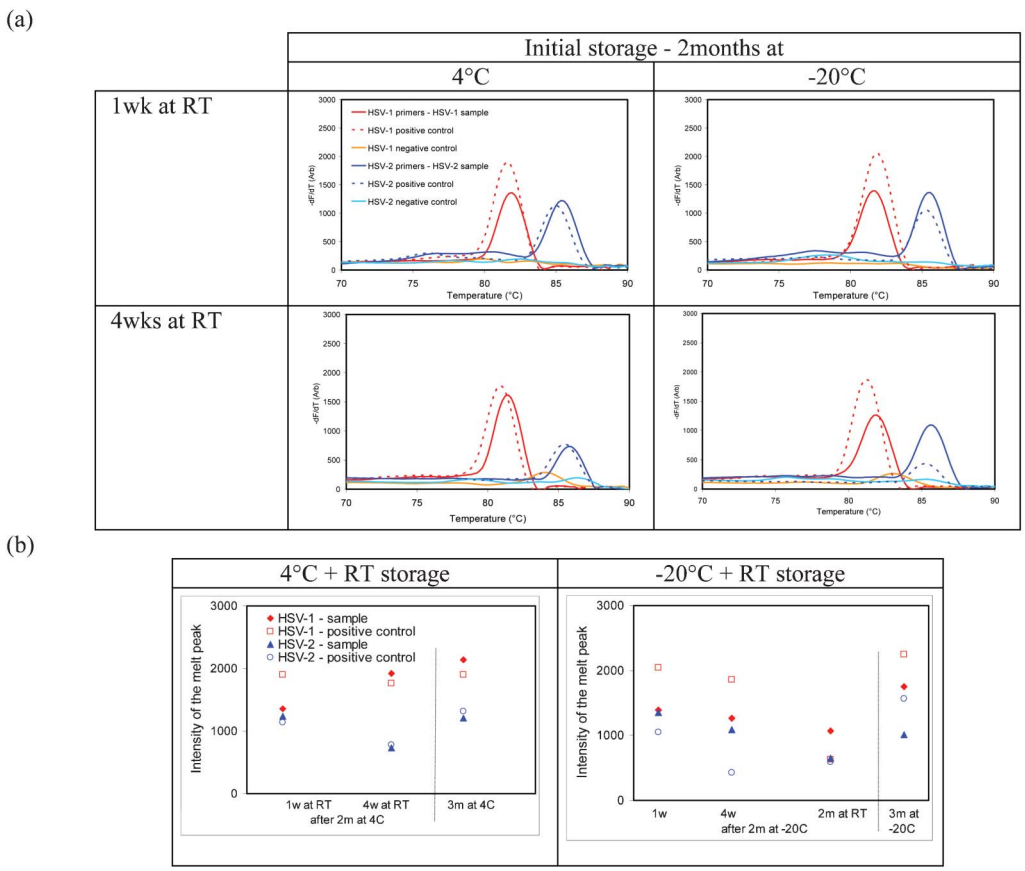

Fig. 5 Performance of the cassettes that were initially stored at 4 °C and −20 °C for 2 months and then stored at RT for 1–4 wks. (a) Melt curves of individual capillaries at 1 and 4 weeks and (b) comparison of intensities of melt peaks of capillaries stored at 2 m at 4 °C or −20 °C (first stage) and then 1–4 wk at RT (second stage), compared to capillaries stored continuously at 4 °C or −20 °C.

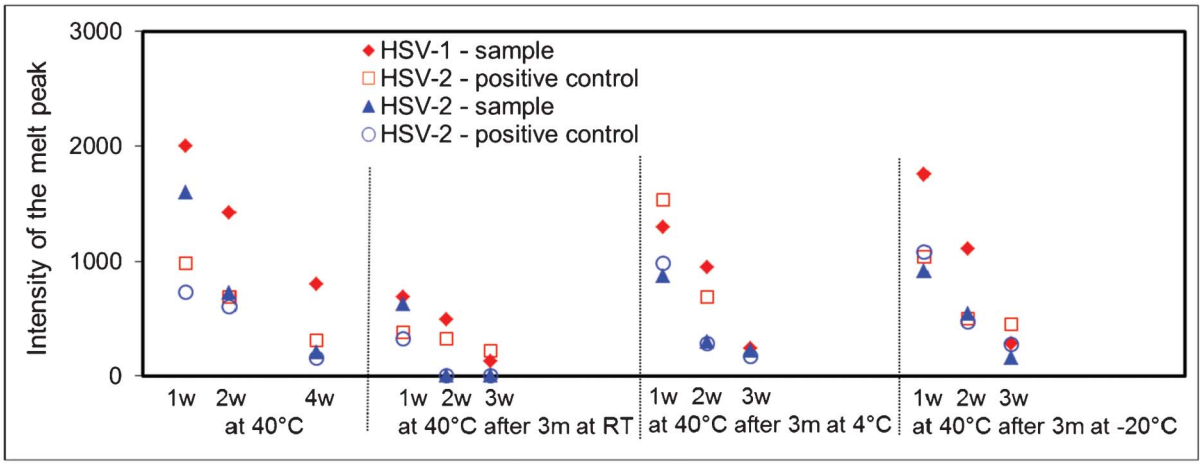

Fig. 6 Performance of the cassettes stored at 40 °C. One set was initially stored at RT, 4 °C, and −20 °C for 3 months and then moved to 40 °C for 1–3 wk. The first data set in the plot is from the cassettes that were directly placed in 40 °C.

at −20 °C preserved activity for 2 weeks but lost activity by 3 weeks (Fig. 6 right). These results suggest that the most feasible protocol for storing cassettes for use in high temperature regions of the world is to store them at −20 °C and use them no more than 1–2 weeks after the transition to ambient temperature.

4. Conclusions

The cassette used for this study is made from off the shelf components and is entirely self-contained. Sample delivery is achieved by capillary force after using a transfer pipette to introduce raw sample. This cassette can be stored at moderate room temperature (22–30 °C) for at least 3 months without loss of activity. A desiccated cassette retains full activity for at least 7 months when stored cold at 4 °C or −20 °C. If the cassette is removed from 4 °C and −20 °C storage and transitioned to moderate RT, PCR activity is maintained for at least a month. However storage of a cassette at high temperature, here defined as 40 °C, results in rapid loss of activity within 2 weeks. Two stage protocols that involve a transition from cold storage to high temperature are feasible, but our results suggest that cassettes should be stored at high temperature no longer than 1–2 weeks before use. Despite the short storage time at high temperature, this two stage protocol may make cassette PCR feasible in areas where refrigeration is limited or unavailable, providing time for transport from cold storage in urban laboratories to under-resourced rural clinics in tropical areas of the world. With the ability to perform multiple tests on one patient, to test multiple patients or to perform multiple tests on multiple patients, this cassette can be stored on the shelf for rapid use in clinics with large or small patient volumes, facilitating laboratory diagnosis and ultimately accurate treatment decisions before the patient leaves the clinic.

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