Dry-reagent storage for disposable lab-on-card diagnosis of enteric pathogens

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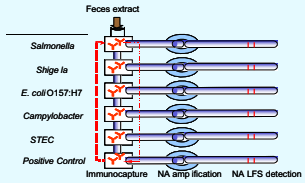
Introduction

❖ There is a need for non-centralized, point-of-care solutions which is rapid, low maintenance, easy-to-use, and sensitive and accurate for detection of enteric pathogens

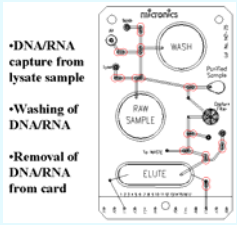
❖ The bacterial agent will be identified via selective antibodies from stool samples, nucleic acid extracted after lysis, and the virulence genes amplified by polymerase chain reaction (PCR)

Schematic of DEC approach



❖ A multiplex disposable enteric card (DEC) is being developed: It will be automated, rapid, point-of-care platform to simultaneously detect multiple enteric pathogens. This method is based on laminated microfluidic platform

Schematic diagram of a laminated lab card



❖ A prototype thermal electric peltier cooler (TEC) for extremely rapid temperature ramps is used to thermocycle the amplification chamber of the disposable microfluidic card

Microfluidics-enabled rapid PCR card and breadboard instruments
Prototype thermal electric cooler

Dry-Reagent Storage

❖ Present microfluidic devices requires additional bulky support equipment outside the system- a drawback for single use devices in point-of-care applications

❖ Dry reagents incorporated into the device can be resuspended by liquid through automated microfluidic circuitry

❖ On-card dry reagent preservation allows long-term storage of required biomolecules, reduces reagent waste, simplifies instrument operation and makes them portable

❖ Dry-reagent storage is critical in point-of-care applications in diverse environmental conditions such as high temperatures in tropical countries.

Methods

Carbohydrate matrix

❖ Trehalose, a non-reducing disaccharide, forms a protein-stabilizing glass in presence of dextran

❖ Protein is protected against degradation due to low molecular mobility in glassy state

❖ Native state of protein maintained largely due to substitution of its waters of hydration by the sugar during drying process

Reagents for dry-storage in DEC

❖ Tosyl-activated 1 µm magnetic beads (Dynal Biotech) covalently linked with anti-E coli antibody and suspended in PBS containing varying concentration of trehalose or trehalose-dextran (5-20 % w/v)

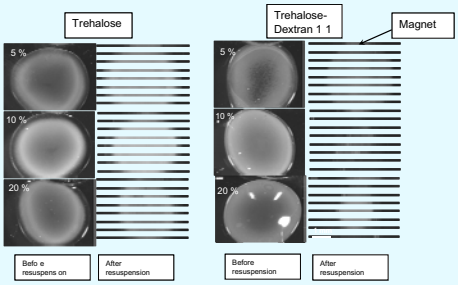
❖ Lysis buffer containing 4 5 M guanidium thiocyanate, 50 mM MES, pH 5 5, 20 mM EDTA, 1 % N lauroyl sarcosine, and 5 % Triton X-100 mixed with varying amounts of trehalose (0-40 %)

❖ PCR master-mix (Thermoscript ™ Plus platinum Taq mix from Invitrogen) containing dNTPs, polymerase enzyme, salmonella primer mix (Operon) and buffer in varying concentrations of trehalose and trehalose-dextran (0-20 %)

❖ All reagents were dried at 37° C, 15-18 % relative humidity and resuspended in water and tested for functionality

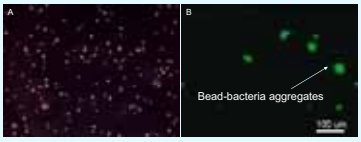
Results

Bead resuspension and functionality



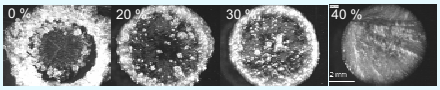
❖ End-point images at 5 min after resuspension of dehydrated antibody coated magnetic beads are shown above

❖ Trehalose alone results in beads sticking to the surface. Dextran aids in complete resuspension of beads



❖ Antibody-coated magnetic beads retained their ability to capture *E. coli* after rehydration as is shown in the above image **B** where SYTO 9 stained bacteria was used. The image **A** shows resuspended magnetic beads

Lysis buffer preservation



❖ Above image shows dry-down of lysis buffer at varying concentration of trehalose

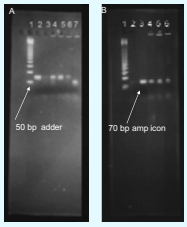
❖ Lysis buffer undergoes coarse crystallization in absence of trehalose which is undesirable for controlled rehydration in a microfluidic device

❖ Addition of trehalose forms cohesive matrix that readily goes back into solution during rehydration

❖ Resuspended buffer retained the function of lysing the bacteria

PCR master-mix preservation

Ethidium bromide stained DNA in Agarose gel



A. PCR after 24 h dry-storage. Lane 2 positive control. Lanes 4-6, master-mix preserved in 10, 15, 20 % trehalose respectively. Lane 7 has no trehalose. Lane 3 is negative control.

B. PCR after 28 days dry-storage. Lane 2 is negative control, Lane 3 positive control. Lanes 4-6, master-mix in 10, 15 and 20 % trehalose respectively.

❖ The PCR master-mix retained its activity after dry-storage in trehalose matrix for at least 28 days

❖ Absence of trehalose resulted in loss of enzyme activity within 24 h

❖ Addition of dextran to the storage mixture had a detrimental effect on PCR

Conclusions

❖ Reagents needed for immunocapture, lysis and PCR for pathogen capture can be stored in trehalose or trehalose–dextran matrix in dry form and still retain its activity

❖ A portable PCR microfluidic card with dry reagents has many advantages: it integrates the isolation, sample preparation and amplification necessary for PCR into a small, disposable, plastic device

❖ Such a device will minimize contamination, reduce sample/reagent amounts, diminish assay duration, and enable portability including point-of-care applications

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