LAB-ON-A-CARD ASSAY FOR ENTERIC PATHOGENS

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ABSTRACT

We describe the functional elements of a diagnostic instrument and disposable enteric card (DEC) system under development that rapidly identifies and differentiates Shigella dysenteriae serotype 1, Escherichia coli O157:H7, Campylobacter jejuni, and Salmonella in stool samples. These elements, currently realized as microfluidic subcircuits on individual cards, are: (1) pathogen capture and lysing, (2) nucleic acid capture, (3) on-chip rapid PCR, and (4) lateral flow detection of amplicons. The system utilizes a low-cost disposable lab-on-a-card platform designed to identify enteric bacterial pathogens in patients with acute diarrhea. Special emphasis is placed on the utility of the device to diagnose both intentionally released enteric biothreat agents as well as to provide a platform to identify infections that are common worldwide. All reagents are stored in dry form on the card.

In this paper we report on the design and validation of individual subcircuits, identification and validation of capture antibodies and strategy for organism immunocapture, and identification and validation of specific PCR primer sequences for over 200 clinical isolates of enteric pathogens as well as spiked and pathogenic stool samples.

THEORY

Figure 1. Culture plates representing a thorough clinical workup including Aeromonas, Campylobacte, E. coli O157:H, non-O157:H7 STEC, Plesiomonas, Salmonellae, Shigellae, Vibrios, and Yersiniae at a cost of ~ US$199.

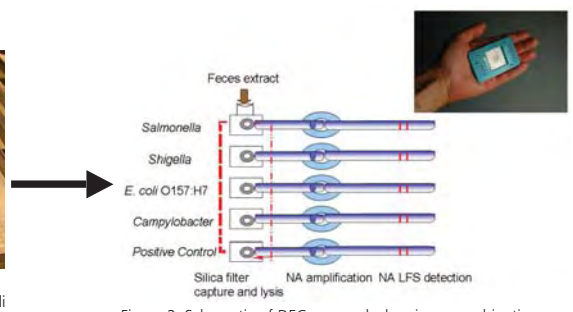

Figure 2. Schematic of DEC approach showing a combination of whole pathogen immunocapture (immunocapture agents for Salmonella, Shigella, and Campylobacter are grouped together), nucleic acid extraction, PCR, and lateral flow detection of amplicons at a projected assay cost of US$1 to $5.

EXPERIMENTAL DETAILS

Presently, we have implemented each of the four elements of the DEC as microfluidic subcircuits on individual cards before embarking on full integration of the disposable device. Fluid transfer between the subcircuits currently occurs by pipette. The subcircuits as described below are: (1) pathogen capture and lysing, (2) nucleic acid extraction, (3) on-card rapid PCR, and (4) lateral flow detection of amplicons. In addition, we have demonstrated the feasibility of drying and resuspending all reagents needed for the DEC, thus allowing the possibility of storage at ambient temperature.

Pathogen capture and lysing

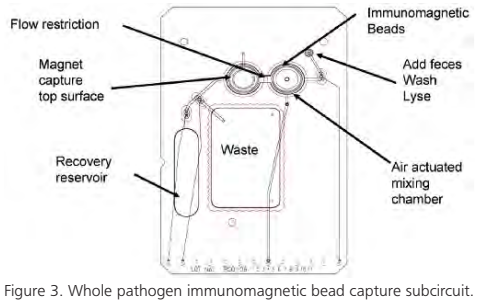

Figure 3. Whole pathogen immunomagnetic bead capture subcircuit.

Nucleic acid extraction on microfluidic card

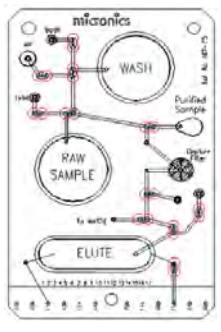

Figure 4. Schematic of nucleic acid extraction subcircuit. The operational steps currently are:
(1) sample injected
(2) nucleic acids captured on silica membrane
(3) wash
(4) dry membrane
(5) elute
(6) eluent taken for analysis
The purification of nucleic acid on the card is completed in < 5 minutes and is under complete software control.

PCR subcircuit

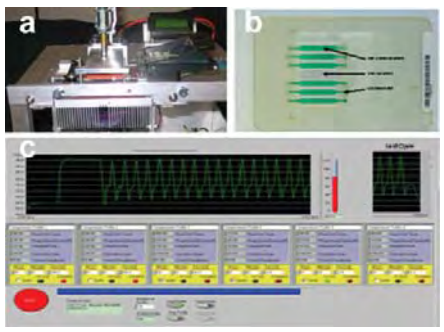

Figure 5. (A) Micronics-developed TCU. (B) PCR amplification lab card capable of performing six amplifications simultaneously, and (C) software interface with thermal couple trace showing 60 second 60°C reverse transcription followed by 16-second PCR cycles.

Figure 6. DEC instrument and disposable interface–curent prototype (shown without thermocycler attachment).

EXPERIMENTAL DETAILS (cont.)

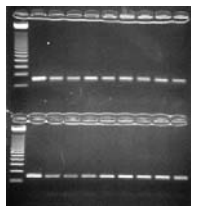

Figure 7. Ethidium bromide-stained DNA in agarose gel of salmonella amplified with the DEC PCR subcircuit. The left lane for the upper and lower gel is a MW marker; lane 1 for each gel is the result from a standard thermal cycler; the other 16 lanes are results for the DEC PCR subcircuit run for 15 minutes and 34 cycles.

Lateral flow detection of amplicons

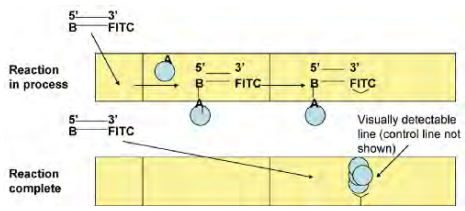

Figure 8. Schematic of amplicon detection using lateral flow strips. The strips operate by simple wicking (no incubations or washes are needed) and will be integrated on the DEC.

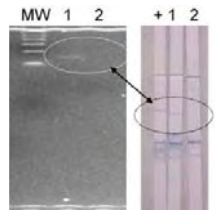

Figure 9. Gel and strip detection of Salmonella amplicon (MW = Molecular weight control; 1 = amplified on card template, 2 = on card no template control, + = thermocycler template control).

Dry reagent storage

On-card dry reagent storage will enable the use of the DEC system without refrigeration for reagents and disposables. In addition, it will remove the need for external, bulky, liquid reagents (except for stable aqueous resuspension and driving-fluid resevoirs). To determine the feasibility of the reagent drydown and resuspension process, three different reagent classes have been studied: (1) antibody-coated magnetic beads for pathogen capture, (2) lysis buffer for extraction of nucleic acids from the pathogens, and (3) amplification of DNA by polymerase chain reaction (PCR).

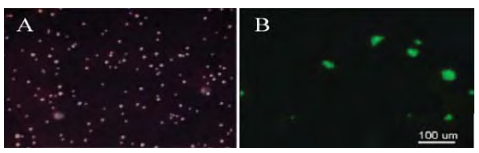

Figure 10. (A) Magnetic beads after resuspension from dehydrated state. (B) SYTO 9-stained E. coli captured by the magnetic beads.

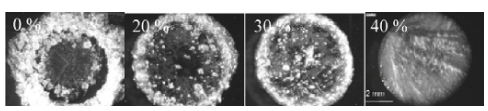

Figure 11. Stereomicroscope images of dehydrated spots of lysis buffer in varying concentration of trehalose (w/v).

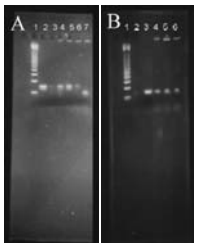

Figure 12. Ethidium bromide-stained DNA in agarose gel after PCR of trehalose preserved master-mix. (A) A 70 bp amplicon in lane 2 (positive control) and corresponding amplicon in lanes 4 and 5 of samples preserved in 10, 15, and 20% trehalose respectively for 24h. Lane 7: Sample without trehalose during dehydration and shows primer-dimer formation. Lane 1: 50 bp DNA ladder, and lane 3 is negative control (no DNA template). (B) Seventy bp amplified product after 28 days of dry preservation (lanes 4 through 6 corresponding to 10, 15, and 20% trehalose respectively). Lane 3 is positive control.

CONCLUSIONS

We have demonstrated the functionality of each subcomponent of the DEC system. We have demonstrated selective antibody capture for all target organisms directly from stool. We have shown pathogen lysis and nucleic acid extraction and capture on microfluidic cards. We have demonstrated PCR at the required sensitivity on microfluidic cards rapidly (8 minutes). We have shown that reagents needed for immunocapture, lysis, and DNA amplification by PCR for pathogen detection can be stored in trehalose matrix in dry form and retain their activity upon reconstitution. In the future, all of these components will be incorporated into a microfluidic platform, an on-chip device, for point-of-care diagnostic systems. Finally, we have shown detection of amplification products on lateral flow strips. Current work is directed towards integration and field validation.

REFERENCES

[1] Guerrant RA, Van Gilder T, Steiner TS, et al. Infectious Disease Society of America Guidelines: Practice guidelines for the management of infectious diarrhea. *Clinical Infectious Diseases*. 2001;32(3):331–351.

[2] NIAID Category A, B & C Priority Pathogens. Available at: www.niaid.nih.gov/biodefense/bandc_priority.htm. Accessed October 2003.

[3] Hatch A, Kamholz AE, Hawkins KR, Munson MS, Schilling EA, Weigl BH, Yager P. A rapid diffusion immunoassay in a T-sensor (invited review article). *Nature Biotechnology*. 2001;19:461.

[4] Weigl BH, Yager P. Microfluidic diffusion-based separation and detection. *Science*. 1999;15:346–347.

[5] Weigl BH, Morris CJ, Kesler N, Battrell CF, Bardell RL. Standard and high-throughput microfluidic disposables based on laminar fluid diffusion interfaces. In: Bornhop DJ, Dunn DA, Mariella DP Jr., et al., eds. *Biomedical Nanotechnology Architectures and Applications, [Proceedings of SPIE v 4626]*. 2002;421–428.

[6] Weigl BH, Bardell RL, Schulte TH, Battrell CF, Hayenga J. Design and rapid prototyping of thin-film laminate-based microfluidic devices. *Biomedical Microdevices*. 2001;3(4):267–274.

[7] Weigl BH, Bardell RL, Cabrera CR. Lab-on-a-chip for drug development [invited review article]. *Advanced Drug Delivery Reviews*. 2003;55(3):349–377.

[8] Gerdes JC. Self-contained cartridge integrating nucleic acid extraction, amplification and lateral flow detection. *Journal of Clinical Ligand Assay*. 1997;20:70–72.

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