Rapid sample preparation, DNA amplification and detection of Herpes Simplex Virus suitable for point of care applications

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Introduction

Overall goal:

- Develop rapid, simple, and inexpensive nucleic acid based diagnostic assays and related devices for identification of human pathogens in a user-friendly and closed system suitable for point-of-care (POC) settings

Targeted Pathogen:

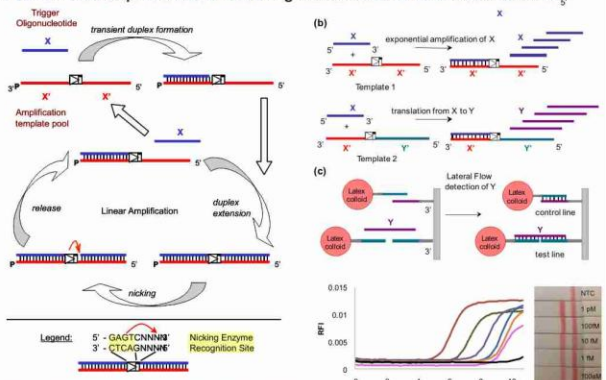
- Herpes simplex virus (HSV): POC detection of HSV infections important for HIV positive or immuno-compromised individuals, pregnant women, and newborns

Methods:

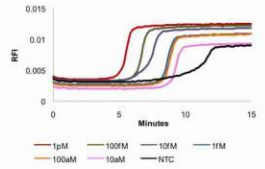
- DNA Amplification through the Exponential Amplification Reaction (EXPAR):
  - Very rapid: 10^6 – 10^9 fold amplification in under 10 minutes
  - Isothermal: simpler instrumentation for POC applications
- Sample Preparation: PureLyse™ System (Claremont BioSolutions)
  - Sample processing (from swab), DNA capture and elution in minutes
- Visual detection using nucleic acid lateral flow (NALF): simple, rapid, and inexpensive detection, suitable for POC settings

Isothermal Amplification of Oligonucleotides via EXPAR

- 10^6-10^9 fold amplification of short oligonucleotides at 55 °C within minutes



PNAS, 2003, *100*: 4504-4509; *Anal. Chem.*, 2005, *77*: 7984-7992



- Sensitivity limited by nonspecific amplification of negative control (NTC)
  - Suppressed or eliminated through mastermix optimization or implementation in hot-start format

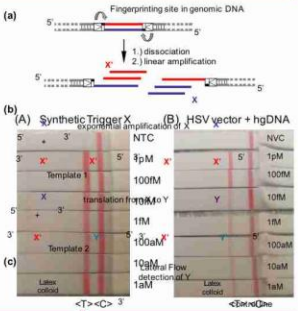
  Biochemistry, 2008, 47: 9987-9999

- Two-stage EXPAR : amplification of trigger X and conversion to a reporter oligonucleotide Y in a homogeneous reaction format
- For NALF detection: add lateral flow running buffer to amplified mastermix, incubate, and apply to a nitrocellulose test strip with immobilized DNA probes:
  - Line at test <t> and control <c> area = positive result
  - Line at control <c> area only = negative result

Fingerprinting: Trigger Generation From Genomic DNA

- Based on adjacent nicking enzyme recognition sites in genomic DNA
- Occurs under the same reaction conditions and mastermix as EXPAR

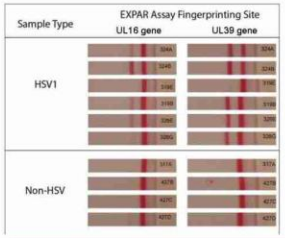
  Clinical Chemistry, 2007, 53: 2017-2020
- Trigger generation from 2 different sites of HSV1 genome:
  - 31mer sequence in gene UL16
  - 21mer sequence in gene UL39
- Proof of principle established with:
  - vector model system (pUC19 vector with HSV1 derived insert)

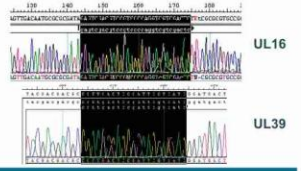


Clinical Samples

- HSV1 positive and negative samples from swab collections purified using standard sample preparation methods
- Amplification and detection via Fingerprinting / EXPAR / Lateral Flow
- Sequencing of clinical isolates reveals that fingerprinting sites are conserved

Sample ID	Type	PCR Ct	UL 16	UL39
324A	HSV1	20	Positive	Positive
324B	HSV1	20	Positive	Positive
319E	HSV1	35	Negative	Negative
319B	HSV1	25	Positive	Positive
326E	HSV1	20	Positive	Positive
326G	HSV1	45	Negative	Positive
317A	Non HSV	0	Negative	Negative
427B	Non HSV	0	Negative	Negative
427C	Non HSV	0	Negative	Negative
427C	Non HSV	0	Negative	Negative





Pouch-based system for EXPAR

- Small heater unit enables rapid and controlled heating of EXPAR master mix to 55°C inside a thin plastic pouch
- Temperature control: integrated circuit controls power applied to heater according to the temperature measured by an embedded thermocouple
- Two-stage EXPAR coupled with lateral flow demonstrated within this pouch based system

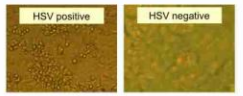
- Mastermix containing 1 pM trigger produces a definitive positive after 11 minute heating, with negative readout from the no trigger negative control (NTC)

Sample Preparation via PureLyse™ System

- Claremont BioSolutions PureLyse™ System: single-use, disposable, battery operated miniaturized bead blender
- Efficient mechanical lysis and solid phase DNA extraction in < 10 minutes from viruses, bacterial spores, and vegetative bacterial cells

Surrogate for Herpetic Lesion: HSV1 infected Shell Vials

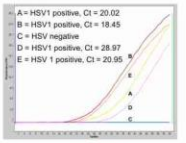
- Confluent monolayer of human lung carcinoma cell line (A549) on microscope cover slips
- Inoculated with residual swab samples (HSV1 positive or negative), incubated for 20 hours



PureLyse Purification of HSV DNA with PCR amplification

- Swab shell vial cover slips (HSV positive and negative), place swab into lysis buffer
- Perform DNA extraction with PureLyse™ blender (binding and elution)
- Amplify eluate using LC Green HSV1 real time PCR

  PCR protocol reported in Clinical Chemistry, 2007, 53: 1847-54



- Results: HSV viral DNA extracted via the PureLyse system is readily PCR amplified, with ct values indicative of $10e^7$ to $10e^5$ copies of HSV DNA

PureLyse purified plasmid containing HSV insert, with EXPAR / NALF

- Grow *E.coli* containing a pUC19 plasmid with HSV1-derived insert of the fingerprinting sites
- Perform DNA extraction with PureLyse™ or Qiagen kit
- Amplify using Fingerprinting / EXPAR, visualize with NALF

- Results: pUC19 plasmid with HSV1-derived insert extracted via the PureLyse system is readily amplified / detected using Fingerprinting / EXPAR / NALF

Conclusions and Future Directions

- Rapid, isothermal EXPAR amplification coupled with lateral flow detection offers an appealing approach for pathogen detection in POC settings
- EXPAR / NALF can be performed in a pouch-based cartridge, and can be coupled with nucleic acid extraction using the PureLyse™ blender

Future Efforts

- Determine clinical sensitivity and specificity of EXPAR / NALF compared to PCR (clinical study using DNA isolates from residual swab samples)
- Combine DNA extraction via PureLyse™ blender, pouch based EXPAR amplification and NALF detection into a fully integrated closed unit cartridge coupled to an inexpensive, battery operated handheld device
- Expand to other pathogens, addressing the need for simple, rapid, nucleic acid based infectious disease diagnosis at the POC in low resource settings

Acknowledgements

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