Abstract

Battery Powered DNA Testing System for POCT and Resource Limited Settings

POC diagnostics are not only intended to be used in a laboratory or clinic by a trained user, they also need to be operated by minimally trained community health workers in limited infrastructure settings. The platform we propose will have the ability to assess multiple pathogens and provide a broad test menu, is self contained and user friendly for the minimally skilled operator, and will permit patient intervention immediately after testing. The "X-bar I, All-in-One" is a fully automated, portable battery operated biological agent detection system. It consist of a rotary thermal cycler processing station for sample preparation and amplification, a reading station for DNA detection; A CPU for controlling system functions, solar panel battery recharging system for recharging the battery, an LCD digital monitor, a USB interconnection for printer and/or remote laboratory computer information management system, and keyboard for operator interface. All this in an easy to carry laptop size carrying case (similar to a laptop computer). This novel instrument is designed to enable convenient PCR testing in rugged environments with a thru-put of 108 test per hour.

Other features of this interesting device include:

- Positive sample identification
- 5-20 minutes to reaction endpoint using fluorescent tag chemistry
- Controls to prevent false positives and false negatives
- Potential for DNA amplification with detection monitored each cycle
- Broad test menu (more than 200 on-board test)

Benefits:

- Prompt results on the scene
- Very affordable instrument and test cost
- Closed tube to prevent cross contamination
- Can be operated in any country without power conversion devices

Point-of-care (POC) Testing with PCR

Step 1: Sample is placed in the funnel which contains buffers and PGEM extraction enzyme

Step 2: The capillary tubes are rotated to the 75˚C heater block and held for 15 minutes to extract DNA

Step 3: The capillary tubes are then rotated to the 95˚C heater block where the enzyme is activated. At the same time the wax plug is melted releasing the the polymerase and initiating the reaction, (Hot start Polymerase).

Step 4: The Total solution flows into the lower capillary tube where it is cycled thru all three temps (55˚C, 75˚C, 95˚C) 20 cycles to amplify the DNA.

Step 5: After the last cycle each capillary is rotated to the read station located in the center section of processing station and each capillary tube is read by the flouremeter individually in sequence.

Aim: Develop a fully automated, rapid, inexpensive, accurate, user friendly DNA testing system for use in POC settings.

Validation: Mouse tail. Results comparing X-bar vs electrophoresis results

Method: Sample preparation and analysis are integrated in a self-contained capillary tube using "Hot start Polymerase" The capillary tube is manufactured with probes and reagents specific to each assay.

Conclusion: Specific and sensitive analysis using nucleic acid amplification protocols can be prepared and performed using completely self contained packaging which controls contamination and allows high throughput in a variety of environments.