ASSISTING GOEIC IN ESTABLISHING A PCR LABORATORY

Technical Report

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Assisting GOEIC to establish a PCR Laboratory

(Technical Report)

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INTRODUCTION

Polymerase chain reaction (PCR) technology has been successfully used in testing food for pathogens (i.e., disease-causing organism). Compared to conventional microbiological analysis, PCR-based methods save time and resources, and produce fairly unambiguous results. These new methods are gaining the acceptance of organizations involved in setting standards, and are increasingly adopted in food testing laboratories. The PCR-based testing detects unique DNA sequences in the targeted organisms. These sequences are amplified instrumentally and a large number of DNA copies are produced and detected by various means. Advanced in PCR technology made it possible to automate the analysis and to get the results quickly. Some of these automated PCR equipment allows the analyst to observe the increase in DNA copies in real-time; this analytical format is referred to as 'real-time PCR.' Establishing a PCR laboratory for GOEIC should expose the Egyptian analysts to these modern analytical technologies and would facilitate the implementation of future standards. Setting up the PCR laboratory in Dekheila should be a high-priority, and details on how this can be accomplished are provided in this report.

EQUIPMENT AND FACILITY

Facility consideration

One of the major considerations in a PCR facility is applying a strictly linear work-flow to prevent contamination of the work area with the amplified DNA. If this type of contamination happens, food samples that are free from pathogens may test positive (i.e., false-positive results). Contaminated lab should be thoroughly cleaned before resuming sample analysis. Figures 1 and 2 show the layout and the instrumentation in the proposed PCR laboratory.

PCR equipment

Analyzing for pathogens in food by PCR-based methods involves these steps.

1. Enrichment of the food sample in the targeted pathogen (if present). This step takes 24-48 hours, depending on the food and the pathogen in question.
2. Automated PCR analysis (4 hours). If the sample does not contain the pathogen, the analysis is complete soon after the PCR analysis terminates. If the sample is positive by the PCR analysis, additional confirmation of these results is needed. It may take several days before the sample is conformed positive.

Automated PCR equipment from three US vendors have been investigated carefully during the preparation of this report. These are:

a. iCycler Real-Time PCR Detection System (BioRad Laboratories, http://www.bio-rad.com).

b. GeneXpert Technology (Cepheid, http://www.cepheid.com)

c. Bax PCR System (DuPont Qualicon, http://www.qualicon.com)

I recommend the automated system from Qualicon for these reasons:

1. **Methods approval.** Methods based on this system have been approved by the Association of Official Analytical Chemists (AOAC), the US Food Safety Inspection Service (FSIS), Health Canada, Association française de normalization (AFNOR), Ministry of Agriculture in Brazil, and NordVal of Denmark.

2. **Rapid and efficient testing.** The equipment can analyze from one to 96 samples simultaneously. Total time of analysis is approximately 4 hours, and most of analysis occurs without the intervention of the analyst.

3. **Equipment cost.** The list price for the complete Bax PCR system is approximately $37,000. However, the company provides incentives for buyers who plan to analyze large number of samples, or those who order multiple units. Included in the price are (i) a site-visit by company's representatives, (ii) equipment set-up, and (iii) 2-3 days of training at the customer's facility. In spite of several communications with Qualicon representatives, it was not clear whether this last statement applies to Egypt.

4. **Commercially available supplies.** PCR reagent kits are commercially available for the most-common pathogen: *Salmonella*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Enterobacter sakazakii*. Reagents sufficient to run a single analysis cost $8.5. A single analysis means one food sample is analyzed once for a single pathogen.

Additional laboratory equipment.

Before the PCR analysis, the food sample is prepared, homogenized, and enriched as done in the main microbiology laboratory (see part-1 of the report). Therefore, an incubator and a refrigerator are needed in the PCR lab. Instruments related directly to the PCR step of the analysis are:

- Two waterbaths (ambient to 95°C)
- Refrigerated micro-centrifuge
- Four vortexers
- Four sets of automatic pipetters (each set includes these sizes: 1000 µl, 100 µl, and 20 µl).

TRAINING

Training Egyptian analysts in PCR technology is urgently needed. Unfortunately, it was not possible during my visit to determine if credible PCR training is currently available in Egypt. In my judgment, analysts need a thorough understanding of the traditional PCR methods before

they move to the automated PCR technology. The training laboratory, as recommended in part-1 of my report, may serve this educational goal. It is not very costly to outfit this training laboratory with traditional PCR equipment (i.e., thermocycler, gel-electrophoresis system, waterbath, micro-centrifuge, etc.); this traditional PCR set costs less than $10,000.

RECOMMENDATIONS

1. I propose that GOEIC establishes a laboratory for advanced instrumentation, featuring automated PCR equipment. The proposed lab will help GOEIC keep track of emerging analytical technologies with great prospects for implementation as standard methods in the future.
2. Analysts should be trained intensively before using these instruments on routine basis.
3. In addition to the detection of pathogen, the PCR equipment should be suitable for detecting genetically-engineered ingredients (i.e., from GMO) in food.

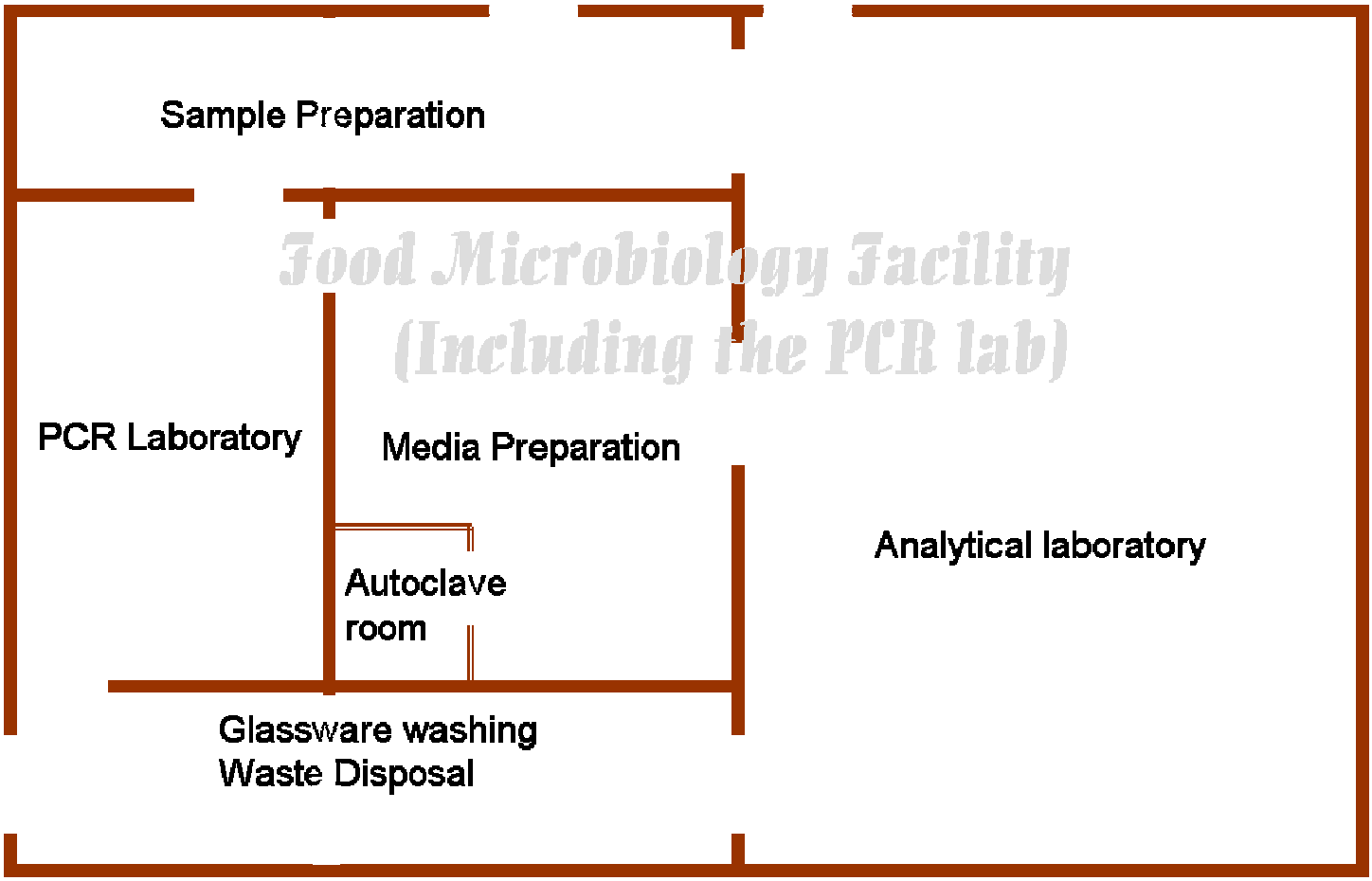

Fig. 1. A proposed layout of the food microbiology facility, including the PCR lab

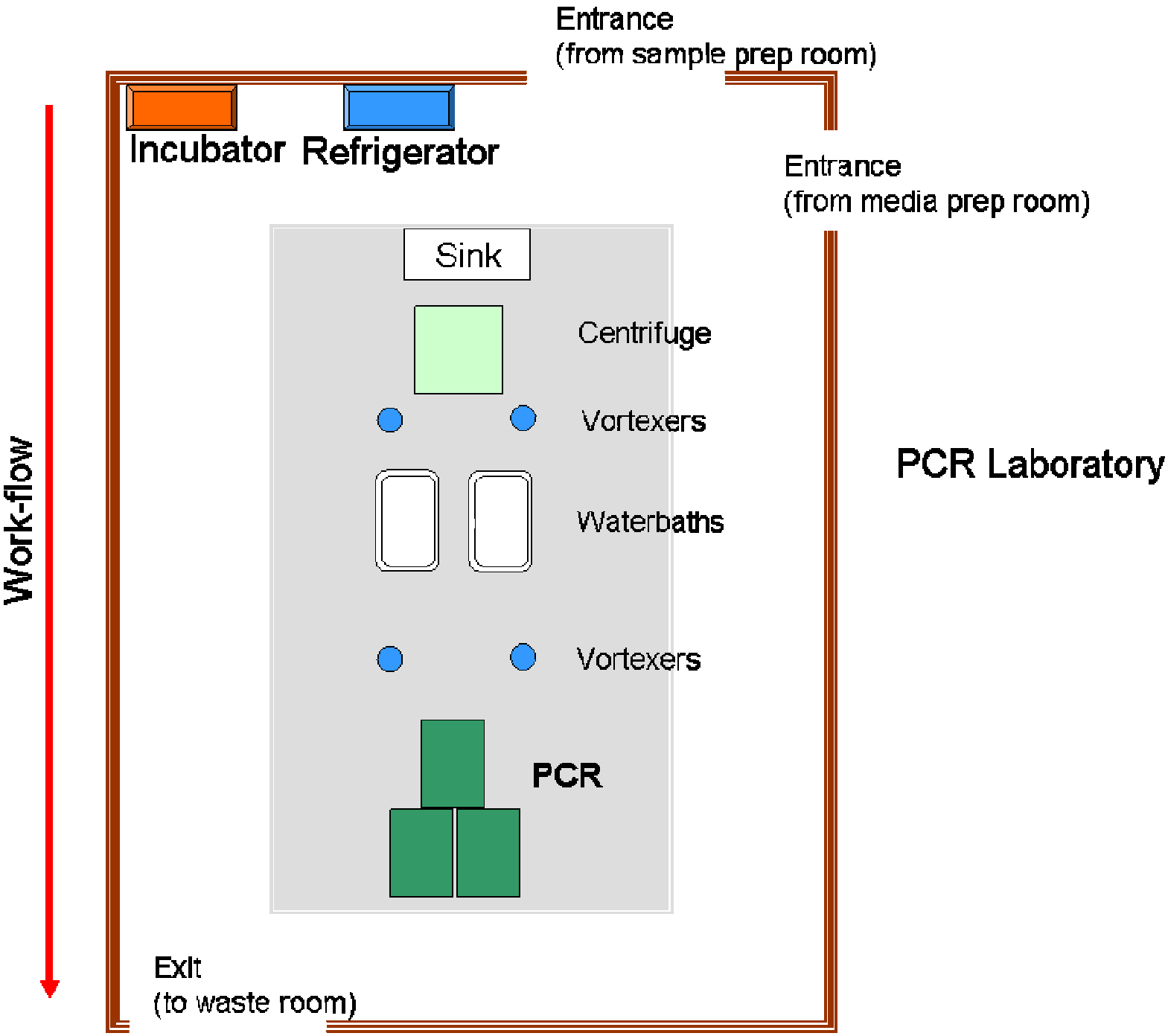

Fig. 2. Detailed PCR laboratory