## **RESEARCH PAPER**

## A DNA methylation assay for detection of ovarian cancer cells using a *HpaII/MspI* digestion-based PCR assay in an integrated microfluidic system

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**Abstract** Early and accurate diagnosis of cancer plays a very important role in favorable clinical outcomes. DNA methylation of tumor suppressor genes has been recognized as a diagnostic biomarker for early carcinogenesis. The presence of 5-methylcytosine in the CpG islands in the promoter region of a tumor suppressor gene is an important indicator of DNA methylation. However, the standard detection assay utilizing a bisulfite treatment and HpaII/ MspI endonuclease digestion is a tedious and lengthy process and requires a relatively large amount of DNA for testing. In this study, the methylated DNAs of various tumor suppressor genes, HAAO, HOXA9 and SFRP5, were chosen as candidates for detection of ovarian cancer cells. The entire experimental process for the DNA methylation assay, including target DNA isolation, HpaII/MspI endonuclease digestion, and nucleic acid amplification has been realized in an integrated microfluidic system. The limit of detection using this developed system has been experimentally determined to be  $10^2$  cells/reaction. The entire process from sample loading to analysis of the results only took 3 h which is much faster than the existing protocols. Different sources of biosamples, such as cells, ascites and serums, could be detected with the methylated DNA,

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Department of Obstetrics and Gynecology, National Cheng Kung University, Tainan 701, Taiwan indicating that this developed microfluidic system could be adapted for clinical use. Thus, this developed microsystem may be a promising platform for the rapid and early diagnosis of cancers.

**Keywords** DNA methylation · Microfluidics · MEMS · PCR · Enzyme digestion

## Abbreviations

CA-125	Cancer antigen 125
ddH <sub>2</sub> O	Double distilled water
DMEM	Dulbecco's modified Eagle medium
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide triphosphate
DTT	Dithiothreitol
EDTA	Ethylenediamine tetraacetic acid
EMV	Electromagnetic valve
FBS	Fetal bovine serum
HAAO	3-Hydroxyanthranilate-3,4-dioxygenase
HOXA9	Homeobox A9
LOD	Limit of detection
MEMS	Micro-electro-mechanical system
PBS	Phosphate-buffered saline
PCR	Polymerase chain reaction
PDMS	Polydimethyl siloxane
SFRP5	Secreted frizzled-related protein 5

## **1** Introduction

In most developed countries, such as the USA, 25 % of the total causes of death annually are cancer related, while about 0.5 % of the total population is diagnosed with cancer each year (Jemal et al. 2009). The incidence of

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