Title: Pre-analytic handling of body fluid specimen when BD Slideprep is used for liquid-based cytology.

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INTRODUCTION
While liquid-based cytology has replaced conventional Pap smear in most cytology laboratories in Hong Kong, liquid-based preparation (LBP) for non-liquid cytology is used by only a few hospitals. Gleneagles Hong Kong Hospital is a newly opened private hospital. Before cytology examination was applied on clinical specimens, we validated BD Slideprep LBP protocol with experiments by using four cancer cell lines from hematopoietic, esophageal, breast and nasopharyngeal cancers. The experiments were designed with the following objectives:

1) Identify tumour detection rate on diluted samples;
2) Compare cytology with ambient and 4 degree storage condition plus or without pre-Slideprep fixation;
3) Study cytology with different pre-Slideprep fixation times;
4) Investigate cytology with two different fixatives, Cytolytich Red (CRR) vs. 50% Alcohol.

MATERIAL AND METHOD
Material: four cancer cell lines were used in the experiments. Tumour cells were harvested from culture medium, washed with Trypsin and suspended in 15 ml Cytolytich Red.

EXPERIMENT
Each cell line varied in cell density. The breast cancer cell line (CUB) had the lowest density of cancer cells. Serial dilution at 4, 8 and 16 times reduced cancer cell density on the LBC smears. However, cancer cells can still be easily detected despite the reduced density. Urine epithelial cells and cancer cells were evenly distributed and well represented in BD Slideprep smears.

RESULTS

1) Each cell line varied in cell density. The breast cancer cell line (CUB) had the lowest density of cancer cells. Serial dilution at 4, 8 and 16 times reduced cancer cell density on the LBC smears. However, cancer cells can still be easily detected despite the reduced density. Urine epithelial cells and cancer cells were evenly distributed and well represented in BD Slideprep smears.

2) Specimens showed bacterial overgrowth and significant degeneration when placed in room temperature (RT) overnight compared with specimens stored in 4C (cold room). Delayed fixation with CRR could not restore degenerative changes, but it could remove some bacteria.

3) There were no identifiable differences in cytology when different fixation times of 30 minutes, 2 hours, 4 hours and overnight were applied before Slideprep processing. After centrifugation, fixation of specimens with 5-10 ml CRR for 30 minutes as recommended by the manufacturer was considered acceptable.

4) CUB and 50% Ethol had different effects on LBC smears.

DISCUSSION
Malignant effusion is an indicator of a bad prognosis and is reflective of late stage cancer. Accurate diagnosis could guide proper treatment. Clinicians and nurses need a precise guideline for effusion specimen collection. Our study with serial dilutions showed that cancer cell detection using BD Slideprep is a sensitive and reliable method for preparing good quality cytology specimens. As laboratories use 50 ml Falcon tube for centrifugation, a collection of effusion of 25-50 ml is considered adequate. Other studies on this issue also support our finding [1,2]. For the purpose of detecting cancer cells in effusion, the practice of collecting a large volume over a long period of time at room temperature is strongly discouraged. The proper preservation of effusion specimens before Slideprep processing is also important. Both pre-processing fixation and cold room storage can prevent bacterial overgrowth and autolysis-induced cell-degeneration. Fixation requires 1.5 volume of fixative to specimen in volume, but using proprietary Cytolytich Red may increase the overall cost. When Cytolytich Red is used as pre-processing fixative, the fixation times do not seem to improve cytology. However, we found that using different fixatives, such as Cytolytich Red and 50% alcohol, may cause variations in cell arrangement and nuclear details.

CONCLUSIONS
BD Slideprep liquid-based preparation is a robust method for cancer cell detection in effusion. A freshly collected 25-50 ml fluid refrigerated at 4 degrees before specimen processing is our recommendation to clinicians and nurses. Fixation with 1.5 volume of fixative to effusion fluid is unnecessary if specimens can be stored at 4 degrees or can be processed within 2-4 hours after being collected. If different fixatives are used in cytopreparation, the morphological characteristics of cancer cells may deviate across different preparations. A consistent cytopreparation protocol is preferred.

REFERENCES