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Evaluation of quality control procedures in an oesophageal cancer cohort study in Anyang, China

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SUMMARY

We wished to evaluate the efficiency of internal quality control procedures of a cohort study investigating human papilloma virus (HPV) infection as a key exposure factor in oesophageal cancer in Anyang, China. This was done by testing 2395 environmental/equipment swab and mock quality control samples from 14 loci on sample collection equipment for human β-globin and HPV DNA. Human β-globin was present in 3.88% of these samples but no HPV DNA was detected. There was no evidence of HPV DNA contamination in the sample collection or processing under the rigorous quality control in our ongoing cohort study. The study results indicated that use of disposable appliances, rigorous environmental cleaning and a high standard of sterilisation of reusable instruments are important in contamination prevention.

Introduction

Oesophageal cancer is the sixth commonest cancer among men and ninth among women and contributes significantly to the disease burden of cancer worldwide.[1] The rural area of Anyang, China has one of the highest incidences worldwide, although the principal risk factors there remain unclear.[1–3] In 1982, Syrjanen *et al.* found cells characteristic of human papilloma virus (HPV) lesions in oesophageal squamous cell carcinoma.[4] Our previous studies have also demonstrated HPV DNA in oesophageal biopsies which were microscopically normal, as well as in biopsies which showed dysplasia and carcinoma.

In 2006, a population-based cohort study of oesophageal cancer aetiology was initiated in rural Anyang, China to evaluate the role of HPV in the development of oesophageal cancer. A series of specimens was collected from individual participants to identify HPV infection, including oesophageal mucosal tissue and exfoliated cells of the palmar skin, oral mucosa, cervix and penis.

HPV infects squamous epithelial cells and these infected cells are regularly shed from the skin and the mucous membranes. HPV is relatively stable in the environment. For example, it may remain infectious within cells for up to seven days, even after desiccation.[5] Strauss *et al.* and Zhang *et al.* reported iatrogenic contamination by HPV via medical equipment and other objects such as environmental surfaces and surgeons' gloves.[6,7] Their results suggest that HPV cross-contamination is likely to occur in the collection or processing of specimens, and may result in false positive results. Therefore, rigorous quality control guidelines were established in our cohort study to minimise HPV cross-contamination in specimen collection and processing.

The primary goals of this study were to evaluate the validity of our quality control guidelines by testing for human β-globin and HPV DNA in mock and environment/equipment (E/E) samples, and to identify specimen collection and processing procedures associated with risk of HPV contamination. Our results may provide a basis for quality control management in other epidemiology studies involving biological sample collection.

Methods

Quality control procedure

Exfoliated cells and biopsies were collected by trained medical staff to evaluate for HPV infection, and quality control guidelines were established for these procedures to minimise the possibility of HPV cross-contamination. In brief, disposable devices were used for all sampling of exfoliated cells. The gastroscope was washed and

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decontaminated using a standardised protocol from the Chinese Ministry of Health, which consists of five washing steps including rinsing with fresh water, multi-enzyme solution (10 min), fresh water, 2% glutaraldehyde solution (10 min) and rinsing again with fresh water.[8] The procedure rooms and equipment used for sample collection and processing were decontaminated daily with bleach before and after sample collection. A standard operating procedure (SOP) was formulated and uniformly applied for sample collection and processing.

Collection of quality control samples

To evaluate the efficiency of these quality control guidelines, two types of quality control samples including mock and E/E swab samples were tested for human β-globin and HPV L1 DNA. Mock samples were used as a control to evaluate sample processing. Six mouse livers were placed randomly in a 72-well container which also contained exfoliated human cell samples, and both real and mock samples were processed identically. Swab samples consisted of material swabbed from selected loci in the working environment, such as the surfaces of procedure tables and centrifuges, and specific sites on the gastroscope and the sterilising equipment (Figures 1 and 2). Swab samples were obtained on the mornings of working days using cotton-tipped swabs soaked in normal saline (0.9% NaCl solution, pH 7.2) in order to collect cells and/or DNA.

β-Globin and HPV DNA detection

Samples were boiled in Tris–HCl buffer and tested for β-globin DNA using polymerase chain reaction (PCR) with GH20/PC04 primers prior to testing for HPV DNA. β-Globin DNA determinations were compared with the positive control. β-Globin-positive samples were subsequently evaluated for HPV DNA using PCR with SPF1/GP6+ primers. In a 96-well PCR reaction plate, three human blood DNA samples were used as negative controls, and serial dilutions of HPV-16-L1 plasmid (1, 10, 100, 1000 copies) and HPV-positive exfoliated oral cells were used as positive controls.

Statistical analysis

Group differences in detection rates were evaluated using the χ^2-test and Fisher's exact test. All statistical analyses were conducted using SPSS version 13.0 for Windows. All P values were two-sided and $P < 0.05$ was considered statistically significant.

Results

A total of 2395 mock and E/E swab samples were collected on 31 consecutive working days between October 2008 and January 2009, and during this time, a total of 16 318 human test specimens were collected. The average ratio was 1 quality control sample (swab and mock sample) per 6.8 human specimens. Table I shows the quality control sample distribution and detection rates of β-globin and HPV DNA. Overall, 93 (3.88%) quality control samples tested positive for β-globin DNA and statistically significant differences were found within different subgroups of sampling sites ($P < 0.001$). HPV DNA was not detected in any of these β-globin-positive samples.

Of the 987 mock samples, only 1 (0.10%) was positive for β-globin DNA which occurred in the processing of male genital test specimens. Differences within the four sampled body sites, including exfoliated cells of the oral cavity, palmar skin, male external genital and cervix, were not statistically significant ($P = 0.120$).

Eleven (6.88%) of 160 swab samples collected from the surfaces of procedure tables and centrifuges were positive for β-globin DNA. The detection rates varied from 0.00% to 10.00%, but the difference was not significant ($P = 0.575$).

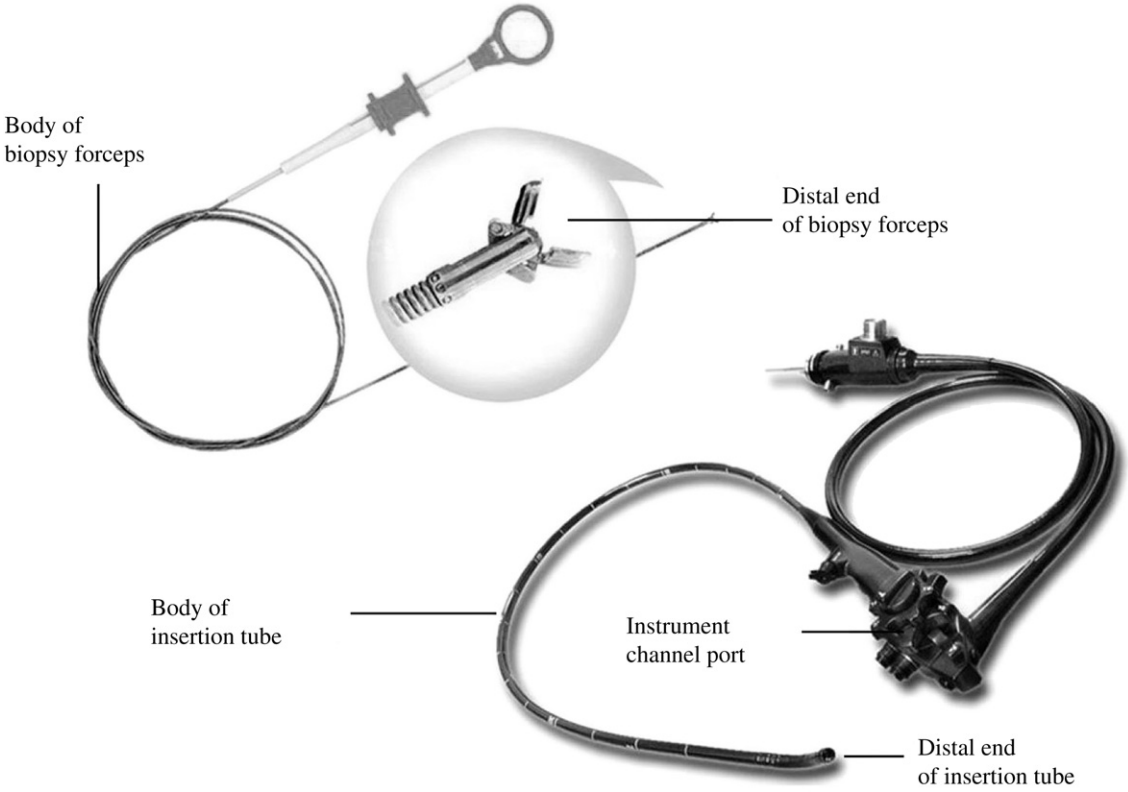

Figure 1. Sampling sites on the gastroscope (biopsy group).