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Letter to the Editor

eSwab flocked swabs unfit for viral culture

For many years, our laboratory has used the traditional tube culture method[1] for the detection of enterovirus in human samples. This technique relies on the appearance of cytopathogenic effect (CPE) in different cell lines, i.e. human hepatoma (PLC/PRF/5), rhabdomyosarcoma (RD) and HeLa cell lines, caused by viral infection.

We recently introduced new specimen collection devices for bacteriological (eSwab,[2,3] cat. no. 480CE, Copan Brescia, Italy) and virological (UTM[4,5] cat. no. 355CW, Copan, Brescia, Italy) analyses in our institution. Copan's patented flocked swabs comprise of a solid molded plastic applicator shaft with a tip that is coated with short nylon fibers that are arranged in a perpendicular fashion. This creates a highly absorbent thin layer with an open structure that allows complete elution of the specimen into the transport medium after sample collection. A patient collection pack is comprised of modified liquid Amies medium (eSwab) or Copan universal transport medium (UTM) and an identical size nylon flocked swab.

Following this introduction, we started to see an increased rate of cytopathogenic effect on our viral cell cultures used for the detection of enterovirus. This CPE was present to a variable degree in all three inoculated cell lines and was not present during our initial validation study that was performed before the introduction of the Copan UTM collection device. However, the evaluation study was conducted with cotton swabs instead of flocked swabs.

Further careful examination revealed the exchange between eSwab flocked swabs and those included with the UTM (FLOQswabs™) by nurses during the process of sample collection. Following these observations, we set up a study to confirm or deny the toxicity of eSwab flocked swabs on our viral cell cultures.

In a first experiment, 2 eSwab flocked swabs, 2 UTM flocked swabs (FLOQswabs™) and 2 cotton wood swabs each were inserted in a separate vial containing 3 ml of home-made viral transport medium (VTM). After 5 min of incubation, the swabs were removed, the vials were vortexed and the medium was filtered through a sterile 0.45 μm Minisart filter (Sartorius Stedim, Aubagne, France), before inoculating 0.2 mL filtrate onto human embryonic diploid skin/muscle-derived fibroblasts (E_1SM, normally used for the detection of herpesvirus), PLC/PRF/5, RD and HeLa cells, cultured in glass tubes. Cell cultures were incubated in stationary slanted racks at 37 °C and assessed daily for cytopathogenic effect for 4 consecutive days. A negative control consisting of VTM that had not come into contact with any swab type was included.

A second experiment was set up with 2 modifications: the medium now used was universal transport medium (UTM, Copan) and the swabs were inserted into the medium for 2 h prior to mixing and filtration.

The results of both experiments were similar and presented in Table 1. All duplicates had identical results. E_1SM cells were not affected. The PLC/PRF/5, RD and HeLa cell cultures all showed signs of toxicity after 24 h or 48 h of incubation when inoculated with VTM or UTM that had come into contact with eSwab flocked swabs. This was not the case when traditional cotton wood swabs or flocked swabs from UTM packages were used.

This toxic/cytopathogenic effect on PLC/PRF/5, RD and HeLa cells was characterized by a loss of the native shape of the cells to a globular shape. The confluent cell layer started showing tears, that gradually increased in size. By the 4th day, almost no cells remained. In contrast, E_1SM cells and cells that came into contact with FlOQswabs™ and cotton wood swabs were comparable to their respective negative controls.

Extensive communication with the manufacturer (Copan) determined that the difference between eSwab flocked swabs and the FLOQswabs™ included with UTM is the pretreatment of the former with a vegetal protein to sustain gonococci and anaerobic bacteria.[6] Since this is the only difference between eSwab and UTM flocked swabs, we hypothesize that this protein elutes from the swab and has a toxic effect on cell cultures.

The importance of these findings is obvious. During medical examination, multiple swabs are frequently collected simultaneously. Because both types of swabs look identical, except for a small red line at the breaking point of the eSwabs, they can easily be exchanged accidentally from eSwab to UTM collection tubes, leading to a CPE on several cell lines and possibly false positive results.

Following this investigation, an information campaign has been set up in our hospital to inform the clinical staff of the importance of putting the correct swab in the correct medium.

Table 1
Toxicity (CPE) seen in various cell lines with different nylon flocked swab types eluted in home-made viral transport medium/universal transport medium (Copan).

T (h)	E_1SM				PLC/PRF/5				RD				HeLa			
	24	48	72	96	24	48	72	96	24	48	72	96	24	48	72	96
E	N/N	N/N	N/N	N/N	ST/N	T/T	T/T	T/T	T/ST	T/T	T/T	T/T	T/ST	T/T	T/T	T/T
U	N/N	N/N	N/N	N/N	N/N	N/N	N/N	N/N	N/N	N/N	N/N	N/N	N/N	N/N	N/N	N/N
C	N/N	N/N	N/N	N/N	N/N	N/N	N/N	N/N	N/N	N/N	N/N	N/N	N/N	N/N	N/N	N/N
NC	N/N	N/N	N/N	N/N	N/N	N/N	N/N	N/N	N/N	N/N	N/N	N/N	N/N	N/N	N/N	N/N

E: eSwab flocked swab; U: UTM FLOQswab™; C: cotton wood swab; NC: negative control = VTM/UTM without contact with any swab type; N: no toxicity; ST: slight toxicity; T: toxic; E_1SM: human embryonic diploid skin/muscle-derived fibroblasts; PLC/PRF/5: human hepatoma cell line; RD: human rhabdomyosarcoma cell line; HeLa: HeLa cell line.

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To our knowledge, this is the first report of toxicity on viral cell cultures resulting from the inadvertent use of eSwab flocked swabs.

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Competing interests

None.

Ethical approval

Not required.

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