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Cellular DNA quantification in respiratory samples for the normalization of viral load: a real need?

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ABSTRACT

Background: Respiratory tract infections have an enormous social economic impact, with high incidence of hospitalization and high costs. Adequate specimen collection is the first crucial step for the correct diagnosis of viral respiratory infections.

Objectives: The present retrospective study aimed: i) to verify the cell yield obtained from sampling the nasal respiratory tract using mid-turbinate flocked swabs; ii) to evaluate the normalization of viral load, based on cell number; and iii) to compare the kinetics of viral infection obtained with normalized *vs* non-normalized viral load.

Study design: The number of cells were quantified by real-time PCR in residual extract of nasal swabs tested for respiratory viruses detection and stored at −80 °C in a universal transport medium (UTM™).

Results: A total of 513 virus-positive and 226 virus-negative samples were analyzed. Overall, a median of 4.42 $\log_{10}$ β2-microgolubin DNA copy number/ml of UTM™ (range 1.17–7.26) was detected. A significantly higher number of cells was observed in virus-positive as compared to virus-negative samples (4.75 *vs* 3.76; $p < 0.001$). Viral loads expressed as $\log_{10}$ RNA copies/ml of UTM™ and $\log_{10}$ RNA copies/median number of cells were compared in virus-positive samples and a strict correlation ($r = 0.89$, $p < 0.001$) and agreement ($R2 = 0.82$) were observed. In addition, infection kinetics were compared using the two methods with a follow-up series of eight episodes of viral infection and the mean difference was -0.57 $\log_{10}$ (range −1.99 to 0.40).

Conclusions: The normalization of viral load using cellular load compliments the validation of real-time PCR results in the diagnosis of respiratory viruses but is not strictly needed.

1. Background

Respiratory tract infections (RTI) have an enormous social and economic impact, with a high incidence of hospitalization and high public health care costs [1]. Because of similar clinical symptoms and simultaneous circulation of several different viruses, their etiology is often difficult to determine. Adequate specimen collection is the first crucial step for the correct diagnosis of influenza and other respiratory infections. Dilution correction in nasopharyngeal aspirates might improve the detection of respiratory infections [2]. Many studies have confirmed that mid-turbinate flocked swabs are less invasive than other specimen collection types (nasopharyngeal swabs, aspirates and washes), have good sensitivity in the detection of respiratory viruses and are therefore a good alternative for specimen collection [3–6]. Moreover, these mid-turbinate flocked nasal swabs are suitable for self-collection at home (either in adult patients or in children by their parents). Fast- and high-throughput molecular workflows require sample matrices that are suitable for automation. Respiratory swab specimens are better suited for this purpose compared to the more viscous nasopharyngeal aspirates. Universal Transport Medium (UTM™) is a room temperature stable viral transport medium for the collection, transport, maintenance and long term frozen storage of viruses and other pathogens such as Chlamydia, Mycoplasma and Ureaplasma.

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2. Objectives

The present retrospective study aimed: i) to verify the cell yield obtained from sampling the nasal respiratory tract using mid-turbinate flocked swabs, subsequently stored in UTM™; ii) to evaluate the normalization of viral load, based on cell number; and iii) to compare the kinetics of respiratory viral infection obtained with normalized *vs* non-normalized viral load.

3. Study design

3.1. Study design and samples

For the present study, a total of 739 residual UTM™ extracts stored at −80 °C and collected from December 2013 through April 2014 at the Molecular Virology Unit of the Fondazione IRCCS Policlinico San Matteo were included. A series of single or follow-up samples were also analyzed.

As part of standard diagnostic procedures, UTM™ extracts were tested with a panel of laboratory-developed real-time RT-PCR or real-time PCR to detect and quantify the following viruses: influenza virus A, human rhinoviruses (HRV), respiratory syncytial virus (RSV) types A and B, and human coronaviruses (hCoV) − OC43, -229E, -NL63, and -HKU1, as previously described [7,8].

Viral DNA/RNA was extracted from 500 µl (1:2 to ml) of mid-turbinate flocked nasal swabs (FLOQSwabs®, Copan Italia SpA, Brescia, Italy) stored in UTM™ (Copan Italia SpA, Brescia, Italy) on the automated extraction system NucliSENS® easyMAG™ (BioMerieux, Lyon, France). Elution volume was 55 µl, 5 µl (1:11) of which were used for respiratory viruses detection described above [7,8]. Viral RNA load was expressed per ml of UTM™ (copies or $\log_{10}$ copies/ml of UTM™) according to the extraction procedure dilution factor and calculated as follows: [(RNA copies number per reaction) × 22].

3.2. Cells quantification

In order to assess the sample adequacy, the number of respiratory epithelial cells was counted by quantifying the DNA of the housekeeping gene β2-microglobulin by real-time PCR, as previously described [9]. The number of cells was reported as β2-microgloubin DNA copy number/ml of UTM™ [10].

3.3. Viral load normalization

The normalized viral RNA load value was expressed as the number of viral RNA copies (copies or $\log_{10}$ copies) per median number of cells recovered in positive samples (4.65 $\log_{10}$ β2-microgolubin DNA copy number/ml of UTM™), and calculated as follows:

$$\text{normalized viral RNA load (ml of UTM™)} = \frac{[\text{RNA copies reaction} * 45282]}{\beta 2\,\text{microgl. DNA copies reaction} / 2} * 22\ (\text{dilution factor to ml})$$

3.4. Statistical analyses

All viral RNA load statistics were performed using $\log_{10}$ transformed viral load values. Quantitative variables were described as the mean and standard deviation, and/or median. Correlations between two quantitative variables were measured by the Spearman correlation test. Descriptive statistics and linear regression lines were performed using Graph Pad Prism software (version 5.00.288). Agreement between the viral load results reported as $\log_{10}$ RNA copies/ml of UTM™ and $\log_{10}$ RNA copies/median number of cells was assessed using the Bland and Altman analysis.

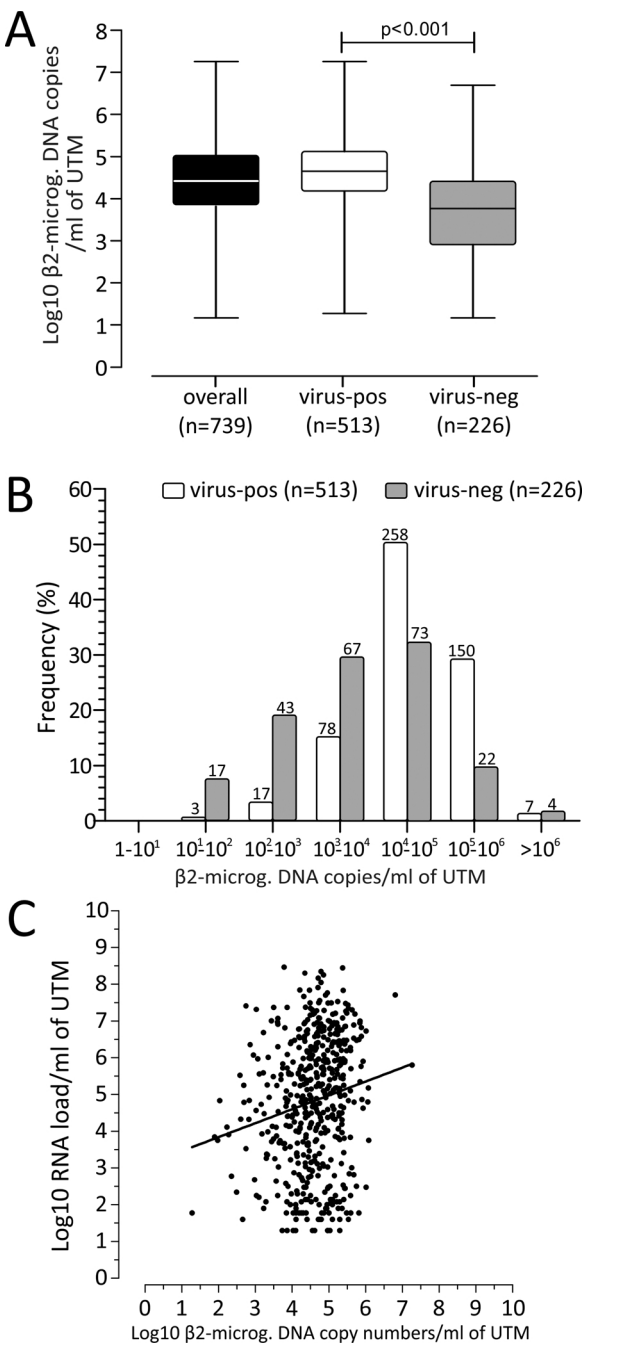

Fig. 1. Comparison of the number of cells measured in mid-turbinate flocked nasal swabs in the overall, virus-positive and virus-negative samples (A). Frequency distribution of the number of cells measured in respiratory virus-positive (white bars) *versus* virus-negative (grey bars) samples (B). Log-Log linear regression plots comparing the viral load and number of cells expressed as $\log_{10}$ β2-microgolubin DNA copy numbers/ml of UTM™ (C).

4. Results

A total of 739 samples were analyzed in this study. In 513 (69.4%) of these, at least one respiratory virus was detected, while 226 (30.6%) were negative. A total of 439/513 (85.6%) virus-positive samples were single samples collected from 439 patients, while 74/513 (14.4%) were follow-up samples collected from 29 patients. Among virus-positive samples, 190/513 (37.0%) were positive for HRV (median 4.85 $\log_{10}$ RNA copies/ml of UTM™, range 1.30 to 8.30 $\log_{10}$), 120/513 (23.4%) for influenza A (5.20 $\log_{10}$ RNA copies/ml of UTM™, range 1.30 to 8.47