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Quantitative real-time PCR detection of adenovirus in clinical blood specimens: A comparison of plasma, whole blood and peripheral blood mononuclear cells

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Abstract

Background: Detection and quantification of adenovirus (ADV) in peripheral blood specimens has become an increasingly important tool in the management of immunosuppressed patients. Investigators have described the use of whole blood (WB), peripheral blood mononuclear cells (PBMC), serum and plasma but no studies have compared the utility of these different sample types for use in a clinical diagnostic assay.
Objectives: To determine the optimal blood compartment for quantitative real-time measurement of adenovirus in peripheral blood specimens.
Study design: WB, PBMC, and plasma representing 338 samples from 148 patients were tested for ADV by quantitative real-time PCR (qrt-PCR) and the results compared for concordance of both qualitative sensitivity and viral load among positive specimens.
Results: There was no significant difference in qualitative sensitivity among the three tested specimen types. Quantitative values of WB and plasma were similar and tended to be greater than those found in PBMC samples. Comparison of consecutive positive samples within individual patients showed that viral loads tracked similarly over time, irrespective of the sample type tested.
Conclusion: While WB and plasma do not offer a significant increase in sensitivity over PBMC, they may offer benefits in terms of reduced processing costs and laboratory turn around time.

Keywords: PCR; Adenovirus; Transplant; Specimen

1. Introduction

Adenovirus infection has emerged as a serious challenge in the immunocompromised patient population. In healthy children, self-limiting adenovirus (ADV) infection primarily affects the respiratory tract, gastrointestinal system, genitourinary system, and conjunctiva (Horowitz, 2001; Ruuskanen et al., 2002). Infection is far more severe in the immunocompromised population with a case mortality rate as high as 80% in hematopoietic stem cell (HSCT) recipients (Hierholzer, 1992; Ison, 2006; Suparno et al., 2004). Cases in HSCT patients range from asymptomatic infection to disseminated disease with multi-organ failure. Distinct clinical syndromes include gastrointestinal processes such as diarrhea, hemorrhagic colitis and hepatic failure; fatal pneumonia; and urinary tract infections with associated hemorrhagic cystitis and renal failure (Hierholzer, 1992; Ison, 2006; Suparno et al., 2004; Walls et al., 2003). The incidence of ADV infection in this population is on the rise and some centers have begun

Abbreviations: ADV, adenovirus; CMV, cytomegalovirus; HSCT, hematopoietic stem cell transplant; PBMC, peripheral blood mononuclear cell; qrt-PCR, quantitative real-time PCR; WB, whole blood.

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routine testing for ADV in HSCT patients (Yusuf et al., 2006).

Diagnosis of ADV has traditionally been performed using culture-based techniques. While effective at detecting end organ infection in symptomatic patients, culture lacks the sensitivity for detecting low levels of circulating virus and may require weeks for definitive results (Heim et al., 2003; Horowitz, 2001; Koneman et al., 1997). Molecular diagnostic assays offer advantages in terms of speed, sensitivity, and the ability to quantify virus (Ebner et al., 2005; Flomenberg et al., 1997; Gu et al., 2003; Heim et al., 2003). They have been particularly useful for the detection of adenovirus in the peripheral blood, a finding that has been correlated with the risk of progression to disseminated disease in HSCT patients infected with ADV (Echavarria et al., 2001; Schilham et al., 2002; Teramura et al., 2004). In addition, the ability to monitor viral load offers potential in monitoring responsiveness to antiviral therapy which is becoming more practical (Hoffman et al., 2001; Legrand et al., 2001; Ljungman et al., 2003; Muller et al., 2005; Yusuf et al., 2006).

Several PCR-based assays have been described. Since investigators have targeted a variety of blood compartments, including WB, PBMC, serum and plasma, it has been difficult to directly compare methods (Ebner et al., 2005; Echavarria et al., 2001; Flomenberg et al., 1997; Gu et al., 2003; Heim et al., 2003; Lankester et al., 2002). While some in vitro work has suggested that lymphocytes may serve as a reservoir for the virus, no studies have compared the clinical utility of these different sample types in a clinical diagnostic assay. The use of different sample types in qrt-PCR may result in different performance characteristics, including sensitivity, quantitative accuracy, and precision. This study compares the use of WB, plasma, and PBMC for the detection of adenovirus in peripheral blood specimens from a pediatric HSCT recipient population.

2. Methods

2.1. Clinical samples and experimental design

Clinical samples were collected for routine diagnostic purposes from February 2003 to September 2005. The study was conducted following Institutional Review Board approval. Samples included 338 specimens from 148 patients. qrt-PCR was performed in a blinded fashion on each specimen in duplicate; the mean viral loads from replicate wells were used for all subsequent analyses. Replicate testing showed a high degree of concordance (p-values greater than 0.6875 for all three sample types tested). Dynamic trending graphs were produced from patients with three or more samples over time. Pair-wise statistical comparisons of the methods' detection probabilities and correlation of quantitative values for positive results were performed using a data set obtained by randomly selecting one sample per patient. Statistical comparisons were performed on this data set to avoid biases due to correlation of multiple samples per patient.

2.2. Viral amplicon standards and calibration curve generation

An amplicon quantitative standard was generated as previously described (Gu et al., 2003), using ADV type 5 primers. Each run included at least one no-DNA template control (sterile H_2O). PCR cleanup was performed with the QIAquick PCR Purification Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. Amplicon concentration and purity was assessed on a Biomate 5 Spectrophotometer (Thermo Electron Corporation, Waltham, MA). Copy number was calculated based on the theoretical molecular weight of each amplicon.

Ten-fold serial dilutions, from 10 to 10^6 copies/μl, of ADV amplicon standard in 10% Acetate EDTA Buffer were used to generate calibration curves for qrt-PCR. Each dilution was run in triplicate. A linear regression calibration curve was produced by Sequence Detection System (version 2.0) software, plotting mean cycle threshold versus concentration of viral DNA. Run acceptability was defined by R^2 values of $\geq$0.98.

2.3. Preparation and extraction of clinical samples

PBMC were prepared by centrifugation of 4 ml whole blood in Vacutainer™ Cell Preparation Tubes (CPT™, Becton Dickinson, Franklin Lakes, NJ). Samples were centrifuged at 1500–1800 RCF (2884–3159 rpm) for 30 min after which mononuclear cells and platelets formed a layer under the plasma. After removing the plasma, this layer was transferred to a 15 ml conical tube. Cells were washed with 15 ml of PBS, and then centrifuged at 300 RCF (1290 rpm) for 15 min. Supernatant was removed and the cell pellet resuspended in 200 μl of 10% AE Buffer. Whole blood, PBMC, and plasma were separately extracted using the BioRobot® M48 Workstation and the MagAttract DNA Tissue M96 Kit (QIAGEN Inc., Valencia, CA) according to the manufacturer's instructions. All extracts were frozen at −70 °C until use.

2.4. Quantitative real-time PCR (qrt-PCR)

qrt-PCR was performed as previously described (Gu et al., 2003), using an ABI PRISM 7900HT Sequence Detection system (Applied Biosystems, Foster City, CA). Briefly, a multiplexed PCR reaction targeted a 123 bp region of the highly conserved hexon gene. Each assay reaction was performed in a total volume of 50 μl and included 5 μl nucleic acid extracted from sample and a primer set containing eight degenerate sites and four Taqman® probes. Quantitative results, based on the linear regression curve described above, were expressed in copies/ml for all sample types. This assay was demonstrated to have a lower limit of detection of 100 copies/ml in WB and plasma and 17.5 copies/ml in