

A two-reaction real-time PCR assay covering the entire spectrum of human adenoviruses for early identification of patients at high risk of disseminated disease

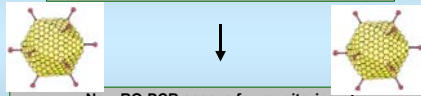
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Two-reaction RQ-PCR assay for all AdV-serotypes

Due to the high mortality of AdV-infections in immunosuppressed patients a rapid and sensitive AdV-diagnostics is needed.

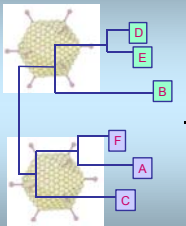


New RQ-PCR assays for monitoring or adenoviral infections provide an important improvement

-) by facilitating sensitive detection of all known serotype
-) at substantially reduced costs

Relationship among AdV species A-F

- based on hexon gene DNA-sequence

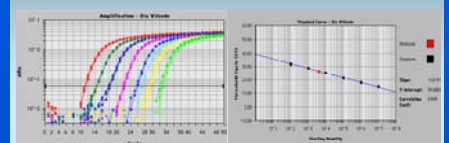


Design of primers/probes for the species

-) A, C and F
-) B, D, and E

all primers & probes within the hexon and fiber genes

Two-reaction real-Time PCR



Summary

Adenovirus (AdV) infection in the course of allogeneic stem cell transplantation (SCT) is associated with high transplant related morbidity and mortality. Disseminated AdV disease is lethal in most instances. Early detection of AdV infection and identification of patients carrying a high risk of disseminated disease therefore remain a major challenge. Yet, many of the commonly used diagnostic approaches based on PCR analysis do not effectively cover all potentially relevant AdV species. In a recently published paper we presented a five-reaction RQ-PCR assay for reliable diagnosis of invasive AdV infection prior to clinical signs of virus disease (Lion et al. *Blood* 102(3):1114-1120; 2003). Using this approach, we observed in a number of instances that AdV detection in stool preceded the onset of AdV viremia, thus raising the possibility that intestinal infections represent a potential source of virus dissemination in a subset of patients. To address this question, we have monitored 80 consecutive pediatric patients transplanted at our center for the presence and the load of AdV in stool and in PB. In order to substantially reduce time and costs for AdV screening we have established a two-reaction RQ-PCR assay permitting sensitive detection and quantification of all 51 currently known human AdV serotypes. The specificity and sensitivity of the novel two-reaction assay is comparable to the five-reaction RQ-PCR assay. Twenty two (28%) patients tested positive in serial stool samples, revealing adenoviruses of the subgenus A, B, C, D and F, with strong predominance of subgenus C. Fourteen patients revealed only low levels of AdV positivity in stool, not exceeding 10E5 copies/g. None of these patients have shown viremia during the posttransplant course. Eight patients had peak AdV levels ranging from 10E5-10E11 copies/g of stool. In six of these eight patients we were able to detect rapid virus proliferation in the intestinal tract to an extremely high AdV load. In four cases these kinetics preceded dissemination of the virus by several weeks. Our observations may therefore indicate a particularly high risk of progression of intestinal to invasive AdV infection in patients with rapid virus proliferation in the intestinal tract. In these patients the monitoring of AdV in stool may therefore permit timely onset of appropriate antiviral treatment, in attempts to prevent disseminated disease

AdV-RQ PCR Study in Children after allo-SCT

80 patients

Serial analysis: Stool ↔ PB

Intervals of sample analysis post SCT:

- until day + 28 → 7 days
- day +28 to + 100 → 1-2 weeks
- after day + 100 → clinical suspicion

Problems with the primer design

Species ACF (9 Serotypes) A single primer & probe system → not possible due to sequence heterogeneity

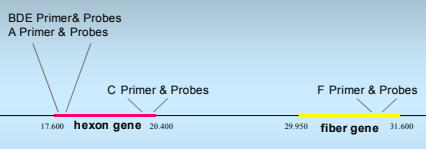
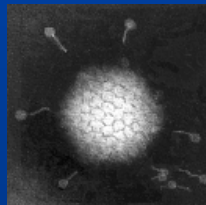
Primer & probe-mix within the hexon gene → not possible due to cross-reactions

Primer & probe-mix for each species within the hexon and fiber gene

Species BDE (42 serotypes) More sequence similarity than species ACF → single Primer&Probe system possible

Problem: a single serotype (Ad20) not detectable

New probe for Ad20 → 1 primer pair + 2 probes



Intestinal AdV Infections Followed by Dissemination

