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Cartas científicas

Unexpected results in BK virus detection by real-time PCR due to polymorphisms in the T-antigen gene



Resultados inesperados en la detección del virus BK mediante PCR en tiempo real debido a polimorfismos en el gen del antígeno T

Dear Sir,

BK virus (BKV) is recognized as a cause of allograft failure in renal transplant recipients and may also be associated with renal dysfunction in other immunosuppressed patients. In particular,

in patients who undergo hematopoietic stem cell transplantation, reactivation of BKV infection is associated with hemorrhagic cystitis.¹ In addition, there is no proven therapy, so, on-time detection of BKV replication and fast reduction of immunosuppressive therapy are recommended to prevent the development of BKV-associated nephropathy.² Quantitative real-time PCR assays have been developed, however, the significant variability in regions of the viral genome that are targeted for PCR-based diagnostic assays could result in either false negative or underquantitated viral load measurements. The TIB MOLBIOL LightMix[®] PCR assay is based on the amplification of a 175 bp fragment of the small T-antigen with specific melting point of 64°C. We report three cases of immunosuppressed patients with hematologic

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4201 ATGTGGAGTT AAAAAGAATA TAATATTATG CCCAGCACAC ATGTGTCTAC TAATAAAAGT
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4261 TACAGAATAT TTTTCATAA GTTTTTTATA CAGAAATTGA GCTTTTCTT TAGTAGTATA
.....

4321 CACAGCAAAG CAGGCAAGGG TTCTATTACT AAATACAGCT TGAATAAGAA ACTGGTGTAG
.....

4381 ATCAGAGGGA AAGTCTTTAG GGTCTTCTAC CTTTCTTTT TTTTGGGTG GTGTTGAGTG
..... .A..... .C..... .C..... .G.....

4441 TTGAGAATCT GCTGTTGCTT CTTTCATCACT GGCAACATA TCTTCATGGC AAAATAAATC
.....A.....

4501 TTCATCCCAT TTTTCATTAA AGGA ACTCCA CCAGGACTCC CACTCTTCTG TTCCATAGGT
.....G.....

4561 TGGCACCTAT AAAAAAATA ATTACTTAGG GCCTTTTAAT ATTTTATTAT TTATCTAAAT
.....A..... .C.....

4621 ATAAGTTAGT TACCTTAAAG CTTTAGATCT CTGAAGGGAG TTTCTCCAAT TATTGGACC
.....

4681 CACCATTGCA GAGTTTCTTC AGTTAGGTCT AAGCCAAACC ACTGTGTGAA GCAGTCAATG
.....

4741 CAGTAGCAAT CTATCCAAAC CAAGGGCTCT TTTCTAAAA ATTTTCTATT TAAATGCCTT
.....

4801 AATCTAAGCT GACATAGCAT GCAAGGGCAG TGCACAGAAG GCTTTTGGGA ACAAATAGGC
.....

4861 CATTCCTTGC AGTACAGGGT ATCTGGGCAA AGAGGAAAA CAGCACAAAC CTCTGAGCTA
...A.....

4921 CTCCAGGTTC CAAAATCAGG CTGATGAGCT ACCTTTACAT CCTGCTCCAT TTTTATATAC
.....T

4981 AAAGTATCA TTCTCTTCAT TTTATCCTCG TCGCCCCCTT
.....

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Fig. 1. Polymorphisms of the virus from the patients in relation to Dunlop reference strain.

malignancies that were monitored for BK virus in urine in which the real-time PCR assay rendered PCR products with melting temperature of 57 °C that was significantly different from those described by the manufacturer. Sequencing of the PCR products obtained from the amplification of the T-antigen region using the following primers: BK1fw (5'-TGCCAGCACACATGTGTCTA-3') and ATSrev (5'-TTTATCCTCGTCGCCCT-3') was done and showed polymorphisms in the T antigen gene (Fig. 1) one of which (G4471A) has never been described previously (Gene bank accession number KJ476629). Manna et al. have described clinical samples which yielded atypical amplification profiles, but the frequency with which this occurs is not clear from their data.³ Large and small T-antigen and anagoprotein sequences are sometimes regarded as attractive sites for assay development since it seems to have the lowest rate of genome variability. However, it should be kept in mind that, at this time a few number sequences of these genes have been known; so, it is also likely that additional gene polymorphisms will be discovered in these areas.⁴

The sequence obtained for VP1 region following the primers and conditions proposed by Li Jin et al.⁵ corresponded, in all cases, to the subtype VI (or Ib1 subgroup) that, according to epidemiological studies, has low rates in Europe showing the highest prevalence in the Southeast Asia, although it is widespread throughout the world.⁶ A recent study conducted by J. Ledesma in Spain reported that the subtype I was the predominant subtype detected in urine (61.2%) and plasma (38.2%) samples followed by subtype II and that the subtype found in urine can be different from that found in plasma.⁷

The abnormality of the RT-PCR results and, later, the polymorphisms found could indicate a common source of infection for the patients, likely from transfused blood products, since all of them have compatible blood groups and were transfused in the same period of time.

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A comparative evaluation of the sensitivity of one automated and one manual nucleic acid extraction methods for the performance of the Speed-oligo™ Direct Mycobacterium Tuberculosis assay



Evaluación comparativa de la sensibilidad de un método automatizado y uno manual para la extracción de ácidos nucleicos con el ensayo Speed-oligo™ Direct Mycobacterium Tuberculosis

Dear Editor,

Nucleic acid amplification techniques are becoming widely used for mycobacterial detection as they combine high sensitivity, high specificity, and the rapid turnaround of results. Among the different steps that participate in ensuring a reliable molecular detection method, DNA extraction is crucial.

The novel oligochromatographic assay, Speed-oligo™ Direct Mycobacterium Tuberculosis (SO-DMT) (Viracell S.L., Santa Fe, Granada, Spain), is based on PCR technology combined with a dipstick to detect the presence of *Mycobacterium* and specifically identify *Mycobacterium tuberculosis* complex (MTC) in clinical respiratory specimens.

In our previous study, we demonstrated a high sensitivity of SO-DMT in detection of *Mycobacterium* spp. in acid fast bacilli (AFB) smear-positive clinical respiratory specimens and a simultaneous differentiation from MTC species.¹

In this study, we compared on performance of the SO-DMT assay, related to mycobacterial culture, by using two different nucleic acid extraction methods: the one manual included in the SO-DMT assay (Viracell-NXM) and another automated performed with the GXT DNA/RNA nucleic acid extraction kit (Hain Lifescience, Nehren, Germany) (Hain-ANXM).

The purpose of comparison was, in case of a high correlation of results, to only perform the automated Hain-ANXM extraction for the SO-DMT assay with the aim of a single nucleic acid extraction with less hands-on-time which could also be used for the GenoType® MTBDRplus test in case of MTC detection.

SO-DMT and culture were used to prospectively assay 81 fresh respiratory specimens from 81 patients with suspicion of mycobacterial disease from August 2012 till June 2013. However, 23 could only be extracted by one method (21 by Viracell-NXM and 2 by Hain-ANXM). For this reason, they were excluded from the study as both extraction methods could not be compared. Also, 27 frozen respiratory specimens (culture results: MTC [12], and Nontuberculous mycobacteria (NTM) [15]) were retrospectively evaluated with the SO-DMT. Thus, a total of 85 specimens were included in the study. Respiratory specimens were decontaminated, examined by auramine staining according with a method previously published² and cultured following standard protocols.^{3,4} Generally, *Mycobacterium* were identified by GenoType™ *Mycobacterium* CM/AS assay (Hain Lifescience, Nehren, Germany) or BD MGIT™ TBc ID (Beckton Dickinson, USA). However, one NTM isolate included was finally identified as *Mycobacterium terrae* by sequence-based methods (Gregorio Marañón Hospital, Madrid (Spain)).