“Target-Plexing“ in PCR design increases safety in blood donation screening

I. Schupp[1], H. Fey[1], R. Himmelreich[1]

[1] Gesellschaft zur Forschung, Entwicklung und Distribution von Diagnostika im Blutspendewesen mbH, Frankfurt am Main, Germany

P244

Introduction & Aim

In 2009 the first transmission of HIV-1 by a cellular blood product after mandatory NAT screening in Germany was reported (1). Between 2007-2010 in total 17 HIV transmission cases were reported and submitted to root cause analyses (2). The analyses showed that low viral loads and mismatches in the primer/probe region led to the detection failures of the NAT tests.

As a reaction to the transmission cases the Paul Ehrlich Institute mandated the use of dual target HIV-1 NAT tests for donor screening in Germany (3). As a result, the next generation of HIV NAT tests cover at least two genome regions. We call this approach “Target-Plexing”.

We investigated whether ‘Target-Plexing’ may also be used to increase the sensitivity of an assay and not only compensate for target mutations.

Therefore, a new PCR assay to amplify and detect three genomic regions (‘amplicons’) of the DNA of CMV was designed. The probes of the multiple amplicons use the same fluorescent dye. Parallel amplification of the individual amplicons shall lead to increased net fluorescence signals compared to single-plex PCRs and lower Ct values in real-time PCRs. These two effects combined can lead to more sensitive test.

To support these hypotheses, the new triplex CMV assay was tested in comparison with single-plex and duplex PCRs.

The new triple target assay was then subjected to a system comparison test with two CE-marked IVD test systems for CMV DNA.

Methods

After multiple sequence alignments, a number of primers and probes were designed with the aid of bioinformatics software. The best performing triplex assay was developed to the standards of a commercial IVD product (Fig. 1).

Precursor single-plex and duplex assays were used as comparators. In its validation phase the new triplex assay (‘PoET CMV’) was evaluated in a system comparison test with two different quantitative, CE-marked CMV diagnostic tests.

PoET CMV was performed on GFE's PoET instrument (Fig. 2), and the other two tests on the respective automated accompanying systems. The performance comparison was conducted with a panel of 96 samples, which included 86 potentially CMV reactive and 10 CMV negative samples. The samples were split into three aliquots, one for each system. The sample panel consisted of ring trial material, seroconversion panels and the CMV WHO International Standard. The panel encompassed low CMV concentration samples and serial dilutions.

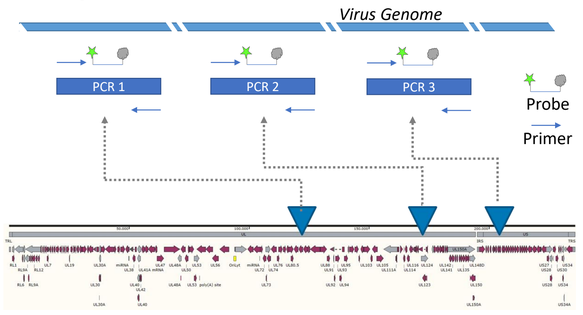

Figure 1. CMV genome map and the localization of the three PCR target genes and the Target-Plexing with the primer & probe design approach illustrated

Results

In the system comparison with the panel of 86 potentially CMV reactive samples, System 1 yielded 23 reactive samples (27 %), System 2 13 reactive samples (15 %) and PoET CMV 41 reactive samples (48 %). 35 samples were reactive with all three systems. Of those, no samples were exclusively reactive with System 1, 1 sample was exclusively reactive with System 2 and 16 samples were exclusively reactive with PoET CMV (Tab. 1, Fig. 4).

Figure 2. PoET Instrument by GFE

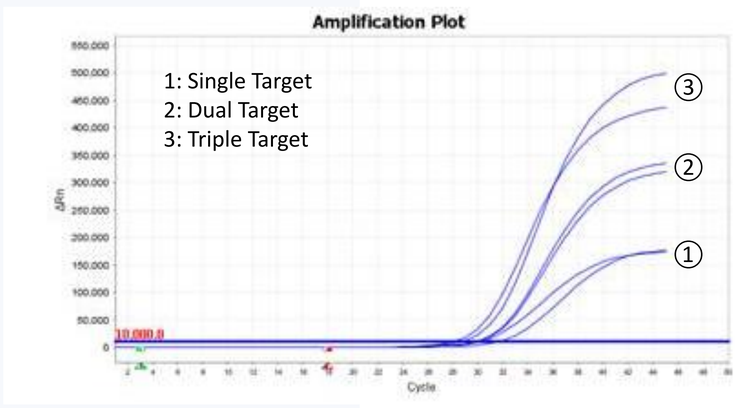

Figure 3. Comparison of the CMV PCR Design in single, dual and triple target format

Test systems	System 1	System 2	PoET CMV*
LoD 95	20,6 IU/ml	215 IU/ml	12,5 IU/ml

Table 1. Published LoD95 of the two comparators vs. PoET CMV

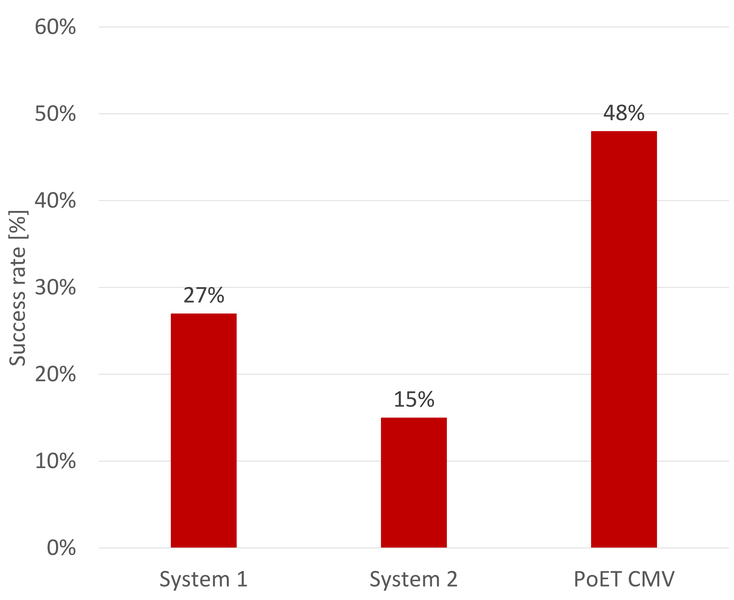

Figure 4. System comparison: Display of the success rate (percentage reactive results out of 86 potential reactive samples

Conclusion

Assays with Target-Plexing are more complex than singleplex assays and therefore need to be designed meticulously and are costlier to manufacture.

However, Target-Plexing has several advantages. It compensates for mutations in the genomes of pathogens and also increases the sensitivity of an assay. In blood screening, the intended use of PoET CMV, sensitivity of an assay if often of particular interest. Highly sensitive assays provide an extra margin of safety in the process of preventing transfusion transmitted diseases.

References

1. **Schmidt M et al.** First transmission of human immunodeficiency virus Type 1 by a cellular blood product after mandatory nucleic acid screening in Germany. Transfusion. 2009 Sep;49(9):1836-44. doi: 10.1111/j.1537-2995.2009.02203.x. Epub 2009 May 11..
2. **Chudy M, et. al**.. Risk Minimization Measures for Blood Screening HIV-1 Nucleic Acid Amplification Technique Assays in Germany. Transfus Med Hemother. Februar 2014;41(1):45–51.
3. **Paul Ehrlich Institute**; Instruction to implement measures for risk minimisation in using HIV-1 NAT test systems, June 2012

Contact information

Dr. Ralf Himmelreich

Gesellschaft zur Forschung, Entwicklung und Distribution von Diagnostika im Blutspendewesen mbH, Frankfurt am Main, Germany

Tel: +49 69 400 5513 15

ralf.himmelreich@gfeblut.de

www.gfeblut.de

GFE

Visit us at booth no. 1407