Testing of cadaveric blood samples for viruses by NAT

Recommendations for specific validation studies

Background

Cadaveric specimens are to be considered different when compared to routine blood donor specimens. A major problem is the potential inhibition of nucleic acid amplification technology (NAT) based assays by specimen components, e.g. haemoglobin, leading to false negative results. Predilution of the cadaveric specimens may remove or decrease inhibition. Internal controls should be used to monitor the extraction and amplification steps.

Validation studies including cadaveric specimens should be performed. Minimal study requirements for cadaveric indication should comprise the assay features sensitivity (limit of detection (LOD)), specificity, and reproducibility.

The German requirements for NAT sensitivity defined on basis of the individual blood donation have to be fulfilled for HIV-1- and HCV-NAT assays (HCV RNA 5,000 IU/mL; HIV-1 RNA 10,000 IU/mL).

In principal, both qualitative (screening) NAT assays and sensitive quantitative (diagnostic) NAT assays may be suitable for testing cadaveric specimens, after validation with this specimen type has shown its suitability.

Pre-analytics

Note: Whenever possible, serum or plasma specimens collected from donors prior to death may be tested instead of cadaveric blood specimens.

- Cadaveric blood specimens can be collected in clot or EDTA anticoagulant tubes. Follow sample tube manufacturer's instructions.
- Cadaveric specimens include serum and plasma specimens.
- For collection of specimens from cadaveric donors, follow general standards and/or regulations. Specimen stability is affected by elevated temperature.
- Whole blood (EDTA collection tube) or plasma may be stored for up to 72 hours at 2 to 8°C. Temperatures not exceeding 25°C are acceptable for up to 24 hours. Specimens may be stored an additional 5 days at 2 to 8°C following centrifugation. Plasma separated from the cells may be stored for up to 14 days at ≤ -70°C before testing. Do not freeze whole blood.
- Whole blood (clot tube) or serum may be stored for up to 72 hours at 2 to 8°C. Temperatures not exceeding 25°C are acceptable for up to 24 hours. Specimens may be stored for an additional two days at 2 to 8°C following centrifugation. Serum removed from the clot may be stored for up to 14 days at ≤ -70°C before testing. Do not freeze whole blood.

- Cadaveric plasma and serum samples should be subjected to not more than three freeze-thaw cycles.
- Specimens with visible precipitates or fibrinous material should be clarified by centrifugation for 10 minutes at 1000 to 3000 x g prior to testing. Do not test specimens without sufficient sample volume above the gel separator or red cell interface.
- Cadaveric blood specimens may be diluted to overcome potential sample inhibitory substances or specimen shortage. Plasma and/or serum may be diluted 1:5 in saline (0.9% sodium chloride) or in negative human plasma.

Performance characteristics

Whenever possible matched pairs of pre- and post-mortem specimens should be used for the spiking experiments.

If these matched samples are not available cadaveric serum/plasma specimens and control serum/plasma specimens should be used for the studies. Cadaveric specimens tested NAT- and seronegative may be used additionally for the spiking experiments (sensitivity/ reproducibility).

Specificity

- 20 NAT negative and seronegative cadaveric serum/plasma specimens and 20 control plasma specimens should be tested.

Sensitivity and reproducibility

- 20 cadaveric serum/plasma specimens and 20 control serum/plasma specimens should be spiked with the respective virus at 3x LOD (of the NAT validated for the normal serum/plasma samples) and tested. If samples are prediluted (1:5) before NAT testing, the respective dilution factor should be considered for the virus-spiked concentration (15x LOD).

Sensitivity performance with cadaveric specimens of diagnostic (quantitative) or screening (qualitative) NAT assays may also be indicated by monitoring the performance of the internal control or internal quantitation standard (e.g. Ct-values) provided that this control (co-extracted and co-amplified with the patient specimen) is present at lower concentration range.

There are several commercial NAT tests available that are already validated by the manufacturer for the use of cadaveric samples.