Proposal for the Validation of Anti-HIV-1/2 or HIV Ag/Ab Combination Assays, Anti-HCV-Assays, HBsAg and Anti-HBc Assays for Use with Cadaveric Samples

Introduction

According to the „Verordnung über die Anforderungen an Qualität und Sicherheit der Entnahme von Geweben und deren Übertragung nach dem Transplantationsgesetz", Annex 3, dated as of March 26, 2008 the following serological investigations are required for tissue donors:

- Anti-HIV-1/2
- HBsAg
- Anti-HBc
- Anti-HCV
- Syphilis

Further laboratory tests (HTLV-I, CMV, EBV, T. gondii, T. cruzi, Malaria, RhD, HLA) may be required depending on the history of the donor and on the tissue properties.

All assay systems used in the European Union have to comply with the requirements of the Directive 98/79/EC of the European Parliament and of the Council of 27 October 1998 on in vitro diagnostic medical devices. In addition assays for the detection of infections with HIV, HCV and HBV have to meet the Common Technical Specifications (CTS) [2009/886/EC]. The CTS require investigations on the sensitivity and specificity of the assays. These investigations are to be carried out using serum and plasma of patients and blood donors. By contrast, current CE-marked assays are usually not validated for the use of cadaveric specimens. After death rapid changes due to autolysis, haemolysis, bacterial growths etc. occur in the blood specimens. Hence the properties of blood are rapidly modified after death which might give false-negative or false-positive results in testing. In addition, the pre-mortal administration of blood and blood products and blood volume substitution may lead to significant dilution effects and the changed blood distribution may also contribute to sampling errors.

Therefore if the intended use of kits does not include testing of cadaveric serum samples, validation of assays is required if the results are used for the release of tissues for transplantation. The Paul-Ehrlich-Institut recommends the following validation scheme for serological assays using cadaveric samples.

Proposal for a Validation Study:

The following aspects should be covered with a validation study:

1) Accuracy

It is recommended to test a minimum of 20 post-mortem samples per analyte. Each sample should be divided into at least three aliquots. One aliquot should be investigated without any treatment (unspiked). Two aliquots should be spiked with the respective analyte in two different concentrations to check sensitivity, (1) low and (2) medium to high positive. The low positive aliquot should be near the cut-off (about 2 to 4 fold s/co ratio) and the medium to high positive aliquot should be within the linear measuring range of the assay. The same procedure should be performed with normal human serum or plasma from pre-mortem donors in parallel as reference to compare with the post-mortem sample results. The spiking volumes should not exceed a tenth of the sample volume in order not to change the composition of the sample too much. Whenever pre-mortem samples from the same post-mortem individual are available, matched pairs should be included in the study for comparison.

To ensure comparability of results, the following PEI or WHO reference materials and standards may be used for the validation studies to spike the cadaveric samples:

– Anti-HIV-1: PEI Anti-HIV-1 reference material or NIBSC HIV (antibody), 1st International Reference Panel, NIBSC code 02/210
– HIV-1 p24 Antigen, NIBSC code 90/636 (for HIV Ag/Ab combination assays)
– HBsAg: PEI HBsAg ad 1000 Standard or the 2nd International WHO HBsAg Standard, NIBSC code 00/588
– WHO Anti-HBc, NIBSC code 95/522
– PEI Anti-HCV reference material
– Anti-syphilitic plasma IgG and IgM, human, lyophilized, NIBSC code 05/132 or Anti-syphilitic plasma IgG, human, lyophilized, 2nd International Standard, 2007 NIBSC code 05/122

If HIV Ag/Ab or HCV Ag/Ab combination assays are used, it is reasonable to also validate the HIV-1 p24 antigen or HCV core antigen detection. For HCV core antigen a HCV-RNA high positive and anti-HCV negative specimen may be used for the spiking experiments. For HIV p24 antigen the above mentioned standard 90/636 is available.

PEI also accepts that own suitable (in-house) samples are used for the spiking experiments. If the concentration of the analyte in the in-house sample is unknown it may be examined beforehand to determine the spiking concentrations needed.

For some markers empirical values for the concentration ranges (final concentration in the cadaveric sample) can be given when using the above mentioned reference materials as shown in Table 1 below.

Table 1. Empirical concentration ranges for accuracy measurement

Reference Material	Catalogue No.	Concentration (low positive)	Concentration (medium to high positive)
HIV-1 p24 Antigen 1st WHO Standard	NIBSC code 90/636	2.0 – 5.0 IU/mL	10 - 20 IU/mL
PEI HBsAg ad 1000 Standard or	n./a.	0.05 – 0.2 PEI-U/mL	0.5 - 1 PEI-U/mL
2nd International WHO HBsAg Standard	NIBSC code 00/588	0.1 – 0.4 IU/mL	1 - 2 IU/mL
Anti-HBc 1st International WHO Standard	NIBSC code 95/522	1.0 – 3.0 IU/mL	5 - 10 IU/mL
PEI Anti-HCV Reference Material	n./a.	Diluted 1:50 to 1:100	≤ 1:20

The above mentioned concentration ranges are derived from the testing experience at the PEI and are only to be considered as a suggestion for the validation experiments. It should be noted that the analytical sensitivities may differ among the various test kits. In addition, with individual anti-HCV or anti-HBc assays the cut-off near dilutions may differ considerably and needs to be determined beforehand using a dilution series with the serum and plasma specimens.

With respect to HIV antibody detection by Anti-HIV-1/2 and HIV Ag/Ab combination assays, the analytical sensitivities mostly differ considerably from assay to assay. Therefore, a suggestion for adequate dilution ranges cannot be given and the sensitivities of the test systems used need to be investigated beforehand. If the assay systems are known to PEI, experience on the dilution range of the PEI-Anti-HIV-1 reference material can be shared upon request.

1a) Expected Results
The results are compared between pre-mortem donor samples ("normal") and the post-mortem samples by calculating the ratio between the spiked cadaveric and the respective spiked normal serum or plasma. The expectation is that results between pre-mortem and post-mortem dilutions are comparable within the range of precision but do not differ by more than 25% for the positive spiked samples.

1b) Confirmatory tests
In the case that the unspiked cadaveric or normal samples already yield reactive or discrepant results, further testing is necessary. This should be done as far as possible including a confirmatory or supplemental assay, NAT methods or by investigating further markers of infection (e.g. for HBV).

2) Precision
Precision studies should be performed ideally using one sample with reactivity close to the cut-off (e.g. s/co 2 to 4). This sample should be tested in several replicates. The resulting coefficient of variation (CV) should not exceed 15%.

3) Centrifugation procedures

When testing cadaveric specimens, centrifugation procedures probably need to be applied which are harsher than those defined by the instructions for use of the assay. If this is the case, centrifugation conditions need to be validated.

4) Time of blood sampling and testing

Validation studies should reflect the time point of blood collection for the post-mortem samples required and also consider the time it takes until testing.

5) Documentation

Presentation of the data of the validation study and submission to PEI should include at least:

1) Identification of tests kit(s), methods and samples used:
   – Full test name
   – Identification /catalogue no. of the test kit
   – Instrument(s) applied
   – Manufacturer
   – Lot no.
   – Cut-off
   – Expiry date of the test kit
   – Test dates
   – Description of samples and spiking materials
   – No. of replicates per sample

2) Measuring values should be identified as S/Co, COI, Index, OD (Abs.), IU etc. to allow an assessment of the data.

In addition, a submission of the data to the Paul-Ehrlich-Institut should contain a detailed description of the validation experiments to determine accuracy and precision including sample pre-treatment conditions, and a summary of the results and conclusions drawn from the validation studies.

The Table below can serve as an example for data presentation.

Table 2. Proposal for a template for data presentation

Analyte	Sample		Test result	Recovery (%)	Time points	
Anti-HIV 1/2, HBsAg, anti-HBc, anti-HCV, etc.			Mean value (S/Co, IU, etc.)	post-mortem / pre-mortem	Sampling post-mortem	Test date
1 Neat	Pre-mortem	unspiked				
	Post mortem	unspiked				
2 Low positive	Pre-mortem	spiked				
	Post mortem	spiked				
3 Medium to high positive	Pre-mortem	spiked				
	Post mortem	spiked				

6) Further aspects for undertaking a validation

For test systems where a validation (with post-mortem samples) has already been performed (and is known and acceptable to the Paul-Ehrlich-Institut), it is not necessary to repeat a complete study. Verification of suitability of using post-mortem samples can be demonstrated using two spiked samples per marker. Information on validated assay systems can be provided by the Paul-Ehrlich-Institut upon request (contact address pei-ivd@pei.de).

Test kits where the kit instructions for use admit applicability of post mortem specimens do not require validation.

7) Literature

The following literature may be useful:

- U.S. Department of Health and Human Services, Food and Drug Administration, Center for Biologics Evaluation and Research, November 2004. Recommendations for Obtaining a Labeling Claim for Communicable Disease Donor Screening Tests Using Cadaveric Blood Specimens from Donors of Human Cells, Tissues, and Cellular and Tissue-Based Products
- Kalus U, Wilkemeyer I, Caspari G, Schroeter J, Pruss A. (2011). Validation of the Serological Testing for Anti-HIV-1/2, Anti-HCV, HBsAg, and Anti-HBc from Post-mortem Blood on the Siemens-BEP-III Automatic System. Transfus Med Hemother 38: 365–372.
- Edler C, Wulff B, Schröder AS, Wilkemeyer I, Polywka S, Meyer T, Kalus U, Pruss A (2011). A prospective time-course study on serological testing for human immunodeficiency virus, hepatitis B virus and hepatitis C virus with blood samples taken up to 48 h after death Journal of Medical Microbiology 60: 920 - 926.
- Baleriola C, Johal H, Robertson P, Jacka B, Whybin R, Taylor P, Rawlinson WD (2012). Infectious disease screening of blood specimens collected post-mortem provides comparable results to pre-mortem specimens. Cell Tissue Bank. 13: 251 - 258.