Superior
Health Council

PREANALYTICAL VARIABLES INFLUENCING THE INTERPRETATION AND REPORTING OF BIOLOGICAL TESTS ON SAMPLES OF DONORS FOR HUMAN BODY MATERIAL

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Superior Health Council

ADVISORY REPORT OF THE SUPERIOR HEALTH COUNCIL no. 9525

Preanalytical variables influencing the interpretation and reporting of biological tests on samples of donors for HBM[1]

In this scientific advisory report, the Superior Health Council of Belgium aims at providing stakeholders in material of human origin with advice on a number of critical pre-analytical aspects of serological testing as well as NAT testing, to improve virological safety in the field of cell- and tissue donation

This version was validated by the Board on
May 2022

I INTRODUCTION

Screening of donors with biological tests remains a crucial assessment on which many decisions are made when releasing organ-, cell- and tissue donors for therapeutic use. The collection (collected “pre-mortem” or “post-mortem”) and storage conditions of the blood-, serum- and plasma samples used, on the one hand, and the specificity, sensitivity, accuracy, reproducibility of the tests used, on the other hand, define whether the testing results will accurately reflect the biological safety status of the donor.

While procurement from deceased donors increases the availability of organs and tissues, it also impacts the risk of transmission of donor-derived infections to recipients of organ- , cell- and tissue transplants.

Assays for standard virological screening are mainly optimised for use of sera collected from living donors. In addition, the overall quality, quantity and condition of samples obtained from deceased donors are often not ideal. This may lead to problems in achieving accurate and reliable results. Methods used for screening serum or plasma from cadaveric donors need to be validated for this purpose and the samples tested need to be collected and pre-conditioned correctly, in order to make accurate testing possible.

Even when using validated tests, performed on correctly collected and stored samples, falsely negative test results can be obtained due to massive blood loss of the donor and hemodilution of the donor’s blood by rapid infusion of blood, colloid and/of crystalloid solutions.

Pooling of samples before testing can also be seen as a form of hemodilution. Pooling of samples from several donors is common practice in blood establishments, but cannot be used in the field of cell- and tissue banking. If cell- and tissue banks outsource their serological testing to blood establishments, they should stipulate in their agreement with the blood

[1] HBM: Human Body Material

establishment that the serological as well as NAT[2] testing have to be performed separately in each individual blood sample.

But also many other aspects (e.g. time and route of blood sampling, type of collection tubes in relation to the testing requirements, storage of samples etc.) will define the accuracy of the testing result and must be taken into account.

When organ donors also become cell- and tissue donors, screening with biological tests can get very complicated. The organ donation legal framework and the cell- and tissue donation legal framework are different. The (serological and NAT testing) acceptance criteria of these different types of donors are discordant at some points.

The working party “biological pre-analysis” of domain “Cells, tissues and organs” of the Superior Health Council (SHC) studied these issues and formulates, through this document, advice on a number of critical pre-analytical aspects of serological testing as well as NAT testing, focused on virological safety, in the field of cell- and tissue donation (material of human origin).

[2] NAT: Nucleic Acid Tests

II CONCLUSION AND RECOMMENDATIONS

The Royal Decree (RD) of 28 September 2009 requires mandatory biological tests (serological tests and NAT[3] testing) to be performed on samples from tissue donors, to prevent transmission of infectious diseases from donor to acceptor.

As the R.D. does not provide guidelines concerning the storage and maintenance conditions of the blood samples, nor on the interpretation and reporting of the testing, the Superior Health Council (SHC) already published an advice with guidelines on the reporting and interpretation results of biological tests performed on samples from donors of Human body material (HBM) (SHC 9314, 2016).

The current advice of the SHC deals with the following preanalytical variables influencing the interpretation and reporting of biological tests on samples of donors for HBM:

1. **Sampling related variables**
   - 1.1. Type of samples
   - 1.2. Condition of the donor
   - 1.3. Collection of the samples
   - 1.4. Volume of the sample
   - 1.5. Choice of specific tubes
   - 1.6. Pre-mortem sampling
   - 1.7. Post mortem sampling
   - 1.8. Labelling of tubes (avoid mixing up of labels)

2. **Variables related to transport, storage and processing of blood samples**
   - 2.1. Transport
   - 2.2. Centrifugation and haemolysis
   - 2.3. Storage before and after centrifugation
   - 2.4. Serum versus plasma
   - 2.5. Serological tests versus NAT testing

3. **Variables related to dilution**
   - 3.1. Haemodilution
   - 3.2. Pooling of samples

4. **Available tests and validation**
   - 4.1. Available tests
   - 4.2. Validation

Considering the possible impact of the abovementioned preanalytical variables on the interpretation and reporting of the tests in donors of HBM, it is very important to discuss them in detail with the lab concerned, and to include them in the agreement between the establishment for HBM and the laboratory that performs the serological and NAT testing. A list is provided with the elements that should minimally be included in this agreement.

[3] NAT: Nucleic Acid Tests

Non-negative (grey area and reactive) screening results for anti-HIV[4] -1 and -2 must be confirmed by a recognised AIDS[5] Reference Laboratory (ARLs).

It seems appropriate to use the existing NIHDI[6] nomenclatures for similar tests in patients to set the cost in donors and to explicitly mention these costs in the agreement between the tissue establishment and the laboratory.

Keywords and MeSH descriptor terms[7]

MeSH terms*
"human" "cells" "tissues"

Keywords	***Sleutelwoorden***	***Mots clés***	***Schlüsselwörter***
Human body material	*Menselijk lichaamsmateriaal*	*Matériel corporel humain*	*Menschliches Körpermaterial*
Biological test	*Biologische test*	*Test biologique*	*Biologischer Test*
Pre-analysis	*Pre-analyse*	*Pré-analyse*	
Pre-analytical aspects	*Pre-analytische aspecten*	*Aspects pré-analytiques*	
Virological safety	*Virologische veiligheid*	*Sécurité virale*	
Donation	*Donatie*	*Don*	

MeSH (Medical Subject Headings) is the NLM (National Library of Medicine) controlled vocabulary thesaurus used for indexing articles for PubMed: http://www.ncbi.nlm.nih.gov/mesh.

III METHODOLOGY

After analysing the request, the Board of the Superior Health Council and the Chair of the working group "Cells, tissues and organs of human origin" identified the necessary fields of expertise. An *ad hoc* working group was then set up which included experts in tissue banking, virology and quality management. The experts of this working group provided a general and an *ad hoc* declaration of interests and the Committee on Deontology assessed the potential risk of conflicts of interest.

This advisory report is based on a review of the scientific literature published in both scientific journals and reports from national and international organisations competent in this field (peer-reviewed), as well as on the opinion of the experts. Moreover, companies in the field of diagnostic tests have been heard.

After the advisory report had been endorsed by the members of the *ad hoc* working group and by the standing working group "Cells, tissues and organs of human origin", it was ultimately validated by the Board of the SCH.

[4] HIV: Human immunodeficiency virus
[5] AIDS: Acquired immunodeficiency syndrome
[6] NIHDI: National Institute for Health and Disability Insurance – Belgium
[7] The Council wishes to clarify that the MeSH terms and keywords are used for referencing purposes as well as to provide an easy definition of the scope of the advisory report. For more information, see the section entitled "methodology".

IV ELABORATION AND ARGUMENTATION

List of abbreviations used

AIDS	Acquired Immunodeficiency Syndrome
Ag	Antigen
anti-HBc	Antibodies against Hepatitis B core antigen
anti-HBs	Antibodies against Hepatitis B surface antigen
anti-HCV	Antibodies against Hepatitis C Virus
anti-HIV 1,2	Antibodies against Human Immunodeficiency Virus 1,2
ARL	AIDS Reference Laboratories - Belgium
CLSI	Clinical & Laboratory Standards Institute: CLSI Guidelines
CMV	Cytomegalovirus
COVID-19	Coronavirus Disease 2019
DNA	Deoxyribonucleic Acid
EDQM	European Directorate for the Quality of Medicines
EDTA	Ethylene Diaminetetraacetic Acid
GDPR	General Data Protection Regulation
HBM	Human Body Material
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
HBsAg	Hepatitis B Surface Antigen
IVD	In Vitro Diagnostic
LOD	Limit Of Detection
NAT	Nucleic Acid Tests
NIHDI	National Institute for Health and Disability Insurance – Belgium
PCR	Polymerase Chain Reaction
RBC	Red Blood Cells
RD	Royal Decree
RNA	Ribonucleic Acid
RT	Room Temperature
SHC	Superior Health Council
TBV	Total Blood Volume
TPV	Total Plasma Volume

1 General considerations

On 28 September 2009, the Royal Decree (R.D.), establishing the quality and safety standards for the donation, removal, acquisition, testing, processing, storage and distribution of human substances that must be met by the banks for human body material, the intermediary structures for human substances and the production establishments, was implemented.
This R.D. of 28 September 2009 requires mandatory biological tests to be performed on samples from tissue donors. The tests are performed on donor serum or plasma. The tests are divided in serological tests and nucleic acid tests (NAT).
The goal of the mandatory tests is to prevent transmission of infectious diseases from donor to acceptor. Depending on the results, human body material (HBM) is rejected or released. (Theodoropoulos et al, 2018).
The R.D. states that biological tests are mandatory to detect the following antibodies and antigens: anti-human immunodeficiency virus 1,2 (anti-HIV 1,2), Hepatitis B surface antigen (HBsAg), antibodies against Hepatitis B core antigen (anti-HBc), if relevant antibodies against Hepatitis B surface antigen (anti-HBs) and antibodies against Hepatitis C virus (anti-HCV). A

screening test for syphilis is also mandatory, as well as HIV[8]1, HCV[9] and HBV[10] NAT. The R.D. stipulates that other tests are recommended under specific circumstances.
However, no guidelines are provided by the R.D. concerning the storage and maintenance conditions of the blood samples, nor on the interpretation and reporting of viral serology and NAT testing (R.D. 28 September 2009).

In 2016, the SHC published an advice *"reporting and interpreting biological tests performed on samples from human body material donors"*, (SHC 9314, 2016) with guidelines on the reporting and interpretation results of biological tests performed on samples from donors of HBM.

The current advice can be considered as a follow-up advice to this earlier advice SHC 9314.

Indeed, in order to reduce rejection of samples (and hence potential donors) due to steps prior to diagnostic tests and their interpretation, the SHC considered it appropriate to supplement its advice SHC 9314 with recommendations concerning the pre-analytical phase. Pre-analytical errors relate to multiple variables (Cudachar, 2013; Guder, 2012).

Plebani et al (2014) listed a number of pre-analytical errors linked to identification on the one hand and linked to the sample on the other hand:

- Preanalytical errors linked to identification:
    - unlabeled samples,
    - mislabeled samples,
    - insufficiently labeled samples,
    - samples suspected of being from the wrong patient ("wrong blood in tube"),
    - irregularities in transfusion labeling requirements (e.g. signature of phlebotomist),
- Preanalytical errors linked to the sample:
    - hemolyzed,
    - clotted,
    - icteric/lipemic,
    - incorrect filling level/inadequate quantity,
    - lost/not received,
    - damaged during transportation,
    - improperly stored.

A recent paper of Sareen et al (2017) confirms that the pre-analytical phase is an important component of total laboratory quality. Studies show that the preanalytical phase accounts for 46 % – 68.2 % of errors observed (Plebani et al, 2014).

The current advice is primarily related to the sampling step, in particular the choice of sample tubes, the choice between serum and plasma, the donor's condition (deceased or living), the timing of the sample in case of hemodilution (pre-transfusion or post-transfusion) and in case of deceased donors (pre-mortem or post-mortem).

[8] HIV: Human immunodeficiency virus
[9] HCV: Hepatitis C virus
[10] HBV: Hepatitis B virus

Secondly, storage and transport of the samples to the analysis laboratory, the centrifugation of the blood samples, and the practice of sample pooling are discussed.

Finally, we will examine the importance of standardisation of working methods and the choice of tests, taking into account the differences in diagnostic sensitivity and specificity between tests and the availability of validated tests on the market.

All these various steps are essential. For optimally reliable results, it is important to limit variability and to take into account all interfering factors.

The requirements for tissues and cells may differ from those for organs, as different regulations apply. However, the purpose of these recommendations is to propose test conditions that offer quality and safety guarantees for any HBM (tissues, cells and organs) and to provide sufficient information to understand possible risks as well. Given that donors may be multi-HBM-donors (including organs), it is appropriate to extend the routine test requirements for organ donation, to those for cells and tissue donation. Moreover, the national test requirements should be compatible with international requirements, in case the HBM is applied in another country.

The tests discussed in this advice are not limited to serological tests, but relate to biological tests in general (e.g. also includes NAT testing). Conversely, bacteriological tests are excluded from the recommendations in this advice.

The interpretation of, and decisions taken based on the obtained test results will not be discussed in this advice. For these aspects, the SHC refers to its earlier advice SHC 9314 (2016).

Finally, it always remains the responsibility of the transplant/implant physician to make a risk/benefit analysis based decision on rejection or acceptance of the HBM.

2 Sampling

2.1 Type of samples and tests to be carried out

Even though biological tests can be carried out on other body fluids and/or HBM, screening tests are in general performed on donor blood (mostly serum or plasma). The standard blood collection method is a venous puncture. For HBV, HCV and HIV screening of allogeneic donors, both serological testing and NAT of donor blood samples are needed. For syphilis, only serological tests need to be performed. In the case of autologous donors, NAT tests are not necessary (Kitchen et al, 2013).

2.2 Condition of the donor

Blood collection of allogeneic tissue donors is described in the R.D. or 28 September 2009. On the one hand, blood samples can be taken from living donors. In that case of non-reproductive HBM, the sample should be collected at the time of donation or within 7 days after the donation. In the case of living donors, there is a possibility to collect an additional sample at a later point in time if needed. On the other hand, in the case of deceased donors,

blood samples need to be taken within 48 hours pre-mortem, or within 24 hours post-mortem. In case of deceased donors, the possibility to collect an additional sample does not exist (R.D. 28 September 2009; Greenwald et al, 2018).
In the case of an autograft procedure, blood of the autologous donor needs to be collected within a month before transplantation or within 7 days after the donation (R.D. 28 September 2009).

2.3 Collection of the samples

Whole blood (to be processed into serum or plasma depending on the type of tube used) is procured in the donor. Collection of the samples should follow general standards and regulations (e.g., CLSI[11]. "Procedures for the Handling and Processing of Blood Specimens for Common Laboratory Tests: Approved Guideline", 4th Edition, CLSI document GP44-A4, 2010).

2.4 Volume of the sample

It is important to have a sufficient amount of blood in order to perform the necessary tests. In case of a deceased donor, there is no possibility to obtain a new sample, e.g. if a technical problem has arisen in the lab.
In the case of a post-mortem multi-tissue donor, the various stakeholders (e.g. different procurement organisations, tissue establishments, etc.) need results of biological screening. However, sharing of results, especially in the context of interactions with other banks and with organisations such as Eurotransplant (in case of multi-tissue donors) can help to reduce the amount of blood samples needed.
Therefore, it is not only important to define the necessary volume on beforehand in mutual agreement with the lab as well as the priority of the tests to be performed, but also to mention the added value of sharing lab results.

2.5 Choice of tubes

Depending on the need for serum or plasma for the tests, tubes with/without additives will be chosen.
biological tests for the detection of HIV, HBV, HCV and syphilis can be performed on serum or plasma. In most cases, serum is used for biological testing. A dry tube (without anticoagulant) or a tube with coagulation activator is a tube in which the blood coagulates and solidifies. After centrifugation, the supernatant liquid obtained is the serum.
PCR[12] or NAT testing can be executed on ethylenediaminetetraacetic acid (EDTA) plasma or serum. EDTA plasma is most often used for PCR testing. EDTA is an anticoagulant that inhibits blood and/or plasma from clotting, ensuring that the DNA[13] or RNA[14] to be detected is non-significantly changed prior to the analytical process. Anticoagulation occurs by binding calcium ions (Guder, 2012).

[11] CLSI: Clinical & Laboratory Standards Institute: CLSI Guidelines
[12] PCR: Polymerase Chain Reaction
[13] DNA: Deoxyribonucleic Acid
[14] RNA: Ribonucleic Acid

2.6 Pre-mortem sampling

If the sample is taken from a living, heart-beating donor, the blood quality, and more specifically with regard to the performance of the biological tests, is considered to be comparable with routine patient samples.

2.7 Post mortem sampling

However, if the sample is retrieved from cadaveric, post-mortem, non-heart-beating donors, the blood quality, and more specifically with regard to the performance of the biological tests, cannot be considered comparable with routine patient samples, due to potential blood changes after death (e.g., haemolysis, autolysis, or blood contamination).

The EDQM[15] guidelines state that in the case of a deceased donor, blood samples must have been obtained just before death or, if this was not possible, the time of sampling must be as soon as possible after death, and in any case within 24 h after death.

However, a number of studies have validated longer post-mortem times.

To verify the validity of NATs performed on blood specimens collected later than 24 h post-mortem, Meyer et al monitored viral nucleic acid concentrations in blood samples of deceased patients who were infected with HIV (n = 7), HBV (n = 5), and HCV (n = 17). Samples were taken upon admission and at 12 h, 24 h, 36 h, and 48 h post-mortem. HIV and HCV RNA were quantified using a Cobas TaqMan device (Roche), HBV DNA was quantified using an in-house PCR. A more than 10-fold decrease of viral load was observed in samples taken 36 h or 48 h post-mortem in one HIV-infected patient only. For all other tested patients, the decrease of viral load in 36 h or 48 h post-mortem samples was less pronounced. Specimens of 3 HIV- and 2 HBV-infected patients taken 24 h post-mortem, or later, were even found to have increased concentrations (> 10-fold), possibly due to post-mortem liberation of virus and/or its nucleic acid from particular cells or tissues. They concluded that their preliminary data indicate that the time point of blood collection for HIV, HBV and HCV testing using PCR may be extended to 36 h or even 48 h post-mortem, and thus improve availability of tissue donations (Meyer et al, 2012).

A more recent study was carried out by Schmack et al (2020). Twenty paired ante- and post-mortem blood samples from cornea donors were obtained and subsequently analyzed for hepatitis B surface antigen (HBsAg), hepatitis B antibody (anti-HBc), anti-HCV, HCV RNA, anti-HIV-1/2, and HIV p24 Ag using Abbott test systems. The sera were also spiked with reference materials in concentrations giving low and high positivity for HBV, HCV, and HIV markers. The spiked ante- and post-mortem sera from related donors showed similar results for HBsAg, anti-HBc, anti-HCV, HCV RNA, anti-HIV, and HIV p24 Ag, indicating a high stability of viral markers in cadaveric specimens. Three cornea donors had a medical history of HBV infection and revealed anti-HBc at similar levels in the ante- and post-mortem sera. In addition, there was a single post-mortem sample demonstrating a weak signal of antiHIV-1 and HIV-1 p24 Ag. False-positive or false-negative results were not detected. The results obtained with the Abbott ARCHITECT analyzer and Abbott RealTime HCV PCR showed no significant differences. The auteurs concluded that the analysed screening assays are suitable for the

[15] EDQM: European Directorate for the Quality of Medicines

detection of infectious markers of HBV, HCV, and HIV at similar levels in spiked ante- and post-mortem sera from cornea donors (Schmack et al, 2020).

2.8 Labelling of tubes

This is a very crucial aspect. On the one hand, the labelling of the primary tube must correspond with the right donor. But there might be a second source of mistakes if there is a step of aliquoting into a secondary tube before transport to the laboratory. This risk can be avoided by using tubes with gel. Tubes must be identified while respecting the rules of the GDPR[16]. In the case of allogeneic donors, a donor code is used, in order to preserve the necessary anonymity.

3 Variables related to transport, storage and processing of blood samples

The legislation does not provide clear guidelines concerning the storage and maintenance conditions of blood samples, although these variables may be of real importance in the context of donation of HBM, especially in the case of procurement at a different site than the laboratory site. In those cases, blood samples are obtained in a different hospital and might only be transported to the processing tissue establishment and/or the laboratory when the often elaborate and long-lasting HBM procurement procedures are finished.

The blood samples will be centrifuged to separate the different components in the blood. It is important to make a distinction between the storage conditions before and after centrifugation (McCaughey et al, 2017).
Red blood cells (RBC) are one of the major blood components. Haemolysis indicates the disruption of the RBC membrane, which causes the release of haemoglobin. This forms a problem because (major) haemolysis can lead to false-positive test results. The temperature of the blood during storage before centrifugation is an important factor in the process of haemolysis. A temperature above 40 °C and below 1 °C causes haemolysis.
After centrifugation, the plasma or serum (depending on the type of tube) does not contain RBC anymore. Therefore the sample can be stored at lower temperature than 1 °C. Other factors that affect haemolysis are sampling technique (e.g., intravenous catheter versus venous puncture, sample transport (by pneumatic tube versus by hand), sample tube volume and filling (McCaughey et al, 2017). As there are lab-specific differences, it is important that ranges of the storage conditions are mentioned explicitly in the convention between the tissue establishment and the lab.

Biological tests for the detection of HIV, HBV, HCV and syphilis can be performed on serum or plasma.

In most cases, serum is used for serological testing.
Serological tests can be divided in two types, the first one detects antigens while the second one detects antibodies (Greenwald et al, 2018). An antigen is any substance capable, under appropriate conditions, of inducing a specific immune response and/ or reacting with the products of that response. An antibody can be defined as an immunoglobulin molecule that has a specific amino acid sequence by virtue of which it interacts only with the antigen that

[16] GDPR: General Data Protection Regulation

induced its synthesis in cells of the lymphoid series, or with antigens closely related to it (Padalko et al, 2018). One does not detect a causal agent but the immunological reaction to an infection.

In addition to these two types of serological tests, another category of tests, NAT tests, can be executed. NATs are more appropriate to detect the infection at an early time point, as these tests are based on a molecular amplification technique to detect the DNA or RNA of the virus. NAT can be executed on EDTA plasma or serum but preferably on plasma.

Transport and storage conditions prior to testing are routinely carried out in accordance with the applicable rules for the safe transport of (risk-bearing) biological material.

Companies provide a diagnostic test package insert with instructions concerning storage timing and temperature that must be followed. These inserts invariably state that the reliability of assay results cannot be guaranteed if there are any deviations of the instructions in the package insert.

This means that different timeframes between blood sampling and centrifugation, different timeframes between centrifugation and initial storage (= transport conditions) and different timeframes between initial and final storage can possibly influence the biological test results and the reliability of the test. Variation in storage temperature before fractionation of the blood could also have an impact on the biological test results and the reliability and validity of the test.
Moreover, the recommendations of the companies in these inserts may differ in function of the use of plasma or serum, especially in the case of post mortem samples.

However, there are hardly any data concerning the impact of storage condition prior to centrifugation. In annex 1, some preliminary data gathered in the context of a master thesis are mentioned.

In the case of PCR screening for HBV, HCV and HIV, centrifugation should be executed within a certain time after blood collection according to the package inserts. However, this is not always possible. Especially in the case of HBM procurement from a multi-tissue donor in a different hospital than the site of the tissue bank, it is sometimes not feasible to comply with the timeframes mentioned in the package inserts.
Moreover, there are no data in the scientific literature on whether different conditions before centrifugation could influence the biological test results and the reliability of the test, especially for these three NAT tests.

Storage conditions for samples after centrifugation seem to be less critical. Although many authors have reported that the storage conditions could affect the RNA stability and, hence, HCV RNA detection, a number of studies have shown that storage after centrifugation does not have a major impact on test results. In 2003, José et al studied HCV RNA stability in plasma samples after storage at different temperatures (-70, -20, 5 and 25 °C). Samples containing different HCV titers were stored and analysed by qualitative or quantitative NAT techniques at defined time points. Samples containing high HCV RNA titers were stored at -20 °C and were followed-up during approximately 2.6 - 2.7 years, samples with intermediate concentrations during approximately 1 year and samples with 100 International Units/milliliter

(IU/ml) during 2.5 years. Independently of the HCV RNA concentration, the results show absence of decay in HCV RNA detectability. Samples stored at 25 °C maintained their HCV RNA titer during 14 days and samples at 5 °C were stable for at least 3 months. José et al, 2003. Similar results were obtained for other viruses (José et al, 2005; Baleriola C et al, 2011).

In 2017, Berger et al measured the influence of storage time on EDTA plasma samples stored at 4 °C. The viral load was measured, using Roche HCV Quantitative Test vs. 2.0, in 43 samples obtained from HCV-positive individuals. The mean reduction of the viral load after 4 °C storage for 6 - 8 days was 0.46 log10 IU/ml (range +0.17 to -1.66 log10 IU/ml). After 1 - 3 days a mean loss of 0.19 log10 IU/ml (range +0.30 to -1.41 log10 IU/ml) and after 3 - 5 days of 0.32 log10 IU/ml (range +0.36 to -1.81 log10 IU/ml) was observed. In 23.3 % of samples, a viral load reduction ≥ 1 log10 IU/ml (1.0 - 1.81 log10 IU/ml) was found after prolonged storage (5 - 8 days). In none of the samples did the HCV load fall below the detection limit. They concluded that plasma storage for up to 8 days can quantitatively reduce the HCV RNA load but has no influence on the reliability of a qualitative HCV RNA detection to determine the HCV status of serologically negative cornea donors (Berger et al, 2017)

In his review paper on the preanalytical practices in molecular diagnostic testing, Sotoudeh Anvari summarized data from the scientific literature on different types of samples. A part of the table, relevant for blood, plasma and serum samples, is copied underneath (Sotoudeh Anvari et al, 2021).

Table 1. Preanalytical recommendations for molecular analysis (Sotoudeh Anvari et al, 2021).

Specimen type	Target	Temperature	Time interval until the analysis
Whole blood	DNA	RT[17]	Up to 24 h
		2 – 8 °C	Up to 72 h optimal, but possible up to 6 days
Whole blood	DNA (HBV)	RT	4 – 6 h
	RNA (HIV, HCV)	4 °C	72 h
Serum	DNA	RT	24 h
	DNA (CMV[18])	RT	Less than one day
	DNA (CMV)	4 °C	2 days
	DNA (HBV)	RT	24 h
	DNA (HBV)	4 °C	7 days
Plasma	DNA	RT	24 h
		2 – 8 °C	5 days
		-20 °C	Longer than 5 days
		-80 °C	9 to 41 months
Plasma	RNA	4 °C	Up to 24 h
Plasma	DNA (HBV)	RT	24 h, 28 days
		4 °C	7 days, 28 days
Plasma	RNA (HIV, HCV)	4 – 8 °C	1 week
	HIV	RT	30 h
	HIV	RT	7 days

[17] RT: Room Temperature
[18] CMV: Cytomegalovirus

	HIV	5 °C	14 days
	HCV	RT	72 h
	HCV	25 °C	14 days
	HCV	RT	3 months

Another problem is the freezing of samples before analysis. Some companies have validated their test for freezing, but in most cases only for a certain time and for certain ranges of temperature.
But in the majority of cases, they do not indicate when after thawing the tests should be carried out or at what temperature the samples should be stored between the thawing procedure and the lab analysis.
It is also important to validate if freeze / thaw cycles on samples can be allowed.
All these items should be discussed between the tissue establishment and the lab and be specified in the convention document between the parties concerned.

4 Variables related to dilution: haemodilution and pooling

4.1 Haemodilution

Case report: Real-life practical example in Belgium
The Belgian Competent Authority inspected (2019) a Belgian Eurotransplant organ donor file. The inspector detected in the donor-file that the donor-blood had been (significantly) haemodiluted. He observed at that moment that also tissues had been harvested in this donor. As a result of this observation, and about one year after the organ and tissue donation, the transplant coordinator of the involved hospital informed the receiving tissue banks about the dilution issue. Unfortunately, all tissues harvested in this donor had already been grafted. The haemodilution information, which was inserted into the Eurotransplant file at the moment of organ donation, had not been shared with the tissue establishment at the moment of donation, due to the differences in legal frameworks and the lack of obligation to inform.

Uniforming both legal frameworks could also increase the number of available cell- and tissue donors. Today, organ donors are announced through the Eurotransplant network. Cell- and tissue banks are not always informed by this network of possible procurement opportunities. Ideally, the transplant coordination centre should forward the available donor information to relevant tissue establishment.

If transfusion or infusion of fluids (blood products, colloids and/or crystalloid) was performed shortly before donation, haemodilution may lead to a decreased detectability of the antibodies or antigens in the donor blood and possibly to false-negative results. An algorithm is applied to evaluate the degree of haemodilution (Kitchen et al, 2013). The EDQM states that it is current practice in a number of countries to consider 50 % calculated haemodilution to be the maximum allowable to minimize the risk of a false-negative test result due to serum dilution.

Examples of when a haemodilution calculation may need to be carried out include:

- *ante-mortem* blood sample collection: if blood components and/or colloids were administered in the 48 h preceding blood sampling, or if crystalloids were infused in the hour preceding blood sampling;

- *post-mortem* blood sample collection: if blood components and/or colloids were administered in the 48 h preceding death (circulatory arrest), or if crystalloids were infused in the hour preceding death (circulatory arrest).

We refer to Appendix 18 of EDQM 4th Edition 2019 for an example of a commonly used formula to assess the donor's potential haemodilution, or plasma dilution, that can be applied when the donor received any fluids that may lead to haemodilution. Adaptations of the algorithms may be needed for body sizes outside the normal adult range. Allowances may need to be made for a very large or a very small adult donor, or a paediatric donor. In brief, a donor's total plasma volume (TPV) and total blood volume (TBV) are estimated by calculations based on the donor's body weight, then direct comparisons are made to amounts of recent transfusions and/or infusions that were administered before circulatory arrest or before collection of the blood sample, whichever occurs first:

a. estimate TPV of donor (weight in kg × 40 mL/kg; or, weight in kg ÷ 0.025);
b. estimate TBV of donor (weight in kg × 70 mL/kg; or, weight in kg ÷ 0.015);
c. calculate total blood (mL) received in the last 48 h (**A**);
d. calculate colloids (mL) received in last 48 h (**B**);
e. calculate crystalloids (mL) received in the last 1 h (**C**);
f. add **B** + **C** and compare to TPV (fluid volumes are compared);
g. add **A** + **B** + **C** and compare to TBV (mass/fluid volumes are compared);
H. does either comparison show > 50 % dilution? if not, the blood sample qualifies and can be used for testing for infectious diseases.

Exceptionally, a tissue establishment may accept tissues and cells from a donor with plasma dilution of > 50 %, but only if each required test has been validated appropriately for use with a diluted test specimen. In such cases, to help reduce risk, additional testing should also be performed using molecular tests (i.e. NAT) for HIV, HBC and HCV, and possibly for other viruses, depending on the donor's travel history, underlying disease or other factors.

The blood collected can also be diluted if the sample is drawn in close proximity to an infusion or transfusion intravenous line, even if the donor is not actually haemodiluted or plasma-diluted. Samples should be drawn from the opposite side of the body in relation to the site of any infusion/transfusion.

Furthermore, in theory, a transfusion shortly before the donation can result in transmission of infectious agents to the donor.

4.2 Pooling samples

Pooling of samples is common practice for NAT testing in blood establishments. Chapter 9.5.2 of the EDQM guide (9.5.2. Nucleic Acid Amplification Techniques (NAT)) provides guidelines for the detection limits against which the NAT tests must be validated and describes the aspects that are important when performing pooling.
The blood establishments work according to the instructions of the manufacturer, rely mainly on the package leaflet of the manufacturer of their NAT equipment and diagnostic reagents.

They take care to achieve the minimum LOD[19] stipulated in the guideline, and make sure that it is achieved even if the samples are diluted eg. 1/6.

Pooling of samples cannot be used in the field of cell- and tissue banking. Also more in general, clinical diagnstic labs have to work on individual samples and that is also the case for molecular tests.

Pooling is only minimally[20] discussed in EDQM for tissues and cells, but the following statement is made for pooling of samples of the same donor: specimens of blood, serum or plasma from the same donor must not be mixed together for testing, whether collected at the same time or at a different time.

If cell- and tissue banks outsource their biological testing to blood establishments, they should stipulate in their agreement with the blood establishment that the serological as well as the NAT testing has to be performed on strictly individual, not pooled, blood samples.

5 Available tests

Many biological tests, including serological and molecular tests, used in the clinical laboratories are not specifically validated for the use on cadaveric blood samples.
The companies providing this type of tests have been heard by the working group of the SHC at two different occasions, once in the initial phase and once just before finalisation of the advice. There was hardly any evolution in the availability between the start and the finalization of the advice (over 3 - 4 years - delayed due to COVID-19)[21]. This illustrates, the limited interest, to be expected from the companies, with regard to post-mortem tests.

When a clinical laboratory is performing tests on cadaveric blood samples, the lab should check if the particular assay is validated for use on cadaveric blood by the company that markets this assay. The validation of the test should be per parameter (e.g. HIV, HBV, HCV in case of molecular testing and anti-HIV 1,2; HBsAg, anti-HBs, anti-HBc, anti-HCV and syphilis serology separately) and per specimen type (e.g. EDTA plasma, serum) used.
Testing on other specimen types than blood-derived is in this particular setting not applicable.
In case the test is validated for use on cadaveric blood samples for the sample type by the manufacturer of the assay, the verification procedure in the clinical laboratory should be in accordance with its quality system.
In case the test is not validated for use on cadaveric blood samples for the sample type by the manufacturer of the assay, the clinical laboratory should perform a validation specifically for cadaveric blood samples.

[19] LOD: Limit Of Detection
[20] EDQM: Molecular assays from deceased donors should be performed in individual samples (see current legislation of each country), not in pooled samples. Some of these NAT assays are combination tests that can detect HIV, HCV, and HBV from a single blood specimen in one run, thus improving the feasibility of routine NAT in donor screening. (In the case of samples from living donors, the pooling could be accepted if the national requirements for the comparable NAT testing of blood donors are fulfilled.)

4th Edition/*Punt* 5.3.2: Although not normal practice, a tissue establishment may accept tissues and cells from a donor with plasma dilution of > 50 %, but only if each required test has been validated appropriately for use with a diluted test specimen. In such cases, to help reduce risk, additional testing should also be performed using molecular tests (i.e. NAT) for the human immunodeficiency virus (HIV), hepatitis B virus (HBV) and hepatitis C virus (HCV), and possibly for other viruses, depending on the donor's travel history, underlying disease or other factors.
[21] COVID-19: Coronavirus Disease 2019

6 Validation of the tests

The approach to verify or validate analytical tests will depend on whether test systems and kits, certified as compliant with the EU In Vitro Diagnostic (IVD) Medical Device Directive, are used or in-house developed analytical tests. In all cases the validation plan should take into account the variety of sample types and materials to be tested, as there may be substances present that interfere.

Verification studies should be done to demonstrate that the performance of the IVD kit or test system, as used in the establishment, meets the expected specification. If using Pharmacopoeia methods, e.g. for sterility testing, the methods should be verified in accordance with the method monograph. For quantitative assays, the acceptance criteria should consider trueness (bias), precision, interferences, linearity, limits of detection, stability and verification of the reference values of the own population. The uncertainty of measurement should be established and quoted with subsequent results. For qualitative tests, then specificity and sensitivity are the key criteria.

In-house developed analytical tests should be validated. The acceptance criteria should consider trueness (bias), precision, analytical sensitivity, analytical specificity, linearity, stability, diagnostic specificity, diagnostic sensitivity and verification of the reference values of the own population.

7 Convention between tissue establishment and lab

As mentioned before, it is very important to have an agreement between the establishment for HBM and the laboratory that performs the serological and NAT testing, (regardless of whether the laboratory is internal to the institution or external to it). It may be useful to also involve the transplant centre in the agreement.
Because of the lack of unambiguous literature data and universal guidelines in this regard, and because of the major operational differences between the clinical labs that perform these tests, it is important that the content of such an agreement is discussed in detail with the lab concerned.
This agreement must include at least the following elements, but should not necessarily be limited to them:

- the type of tissue donor (living vs deceased),
- the fact that it is often not possible to obtain a new sample,
- the minimal sample volume required,
- the priority of the tests to be performed, in the event of reduced volume,
- the collection conditions (centrifugation, type of tubes, labelling (incl. anonymisation, …),
- the transport conditions (interval time, temperature, packaging, time of delivery),
- information about the performed tests, their CE marking, their validation, frequency of performance, time to response, …,
- performance of tests on individual samples, no pooling,
- formatting of the results (SHC 9314; e.g. results of microbiological cultures, etc.),
- the people who are responsible for selecting the tests to be performed,

- the necessity to notify the bank as soon as possible in the event of positive tests,
- the licensing/certification/accreditation of the lab according to the relevant regulatory frame,
- the cost and the billing of the tests,
- the transmission of (positive) tests to the establishment of HBM and/or other involved stakeholders (e.g. transplant center).

8 Reference centers

As mentioned in the advice 9314 of the SHC, non-negative (grey area and reactive) screening results for anti-HIV-1, 2 need to be confirmed.
In Belgium, the recognised Belgian AIDS Reference Laboratories (ARLs) are in charge of interpreting and reporting the confirmatory test results. The final result of the test is that obtained by an ARL on the basis of the confirmatory tests carried out.
Lists of other reference centres and laboratories are available on the website of the Sciensano: https://nrchm.wiv-isp.be/nl/ref_centra_labo/default.aspx.

9 Costs of the tests

No specific costs have been defined for the serological and NAT tests that are performed in allogeneic donors of HBM, and no separate reimbursement has been provided for this particular situation.
It seems appropriate to use the existing NIHDI[22] nomenclatures for similar tests in patients, as a guideline to set the cost in donors, and to explicitly state this cost payable by the tissue establishment in the agreement between the tissue establishment and the lab.

[22] NIHDI: National Institute for Health and Disability Insurance – Belgium

V REFERENCES

Baleriola C, Johal H, Jacka B, Chaverot S, Bowden S, Lacey S et al. Stability of hepatitis C virus, HIV, and hepatitis B virus nucleic acids in plasma samples after long-term storage at -20°C and -70°C. J Clin Microbiol 2011;49:3163-7.

Belgian Kingdom. Royal Decree of 28 September 2009 establishing the quality and safety standards for the donation, removal, acquisition, testing, processing, storage and distribution of human body material, which must be met by human body material banks, human body material intermediary structures and production establishments. BS of 23 October 2009, No 2009018414, p. 69409.

Berger A, Salla S, Keppler OT, Rabenau HF. HCV RNA Testing of Plasma Samples from Cornea Donors: Suitability of Plasma Samples Stored at 4 °C for up to 8 Days. Transfus Med Hemother 2017;44:39-44.

CLSI - Clinical and Laboratory Standards Institute. GP44-A4: Procedures for the Handling and Processing of Blood Specimens for Common Laboratory Tests; Approved Guideline. 2010.

Çuhadar S. Preanalytical variables and factors that interfere with the biochemical parameters: A review. OA Biotechnology 2013;2:19.

EDQM - European Directorate for the Quality of Medicines. Guide to the quality and safety of tissues and cells. 4th edition. 2019

EU – Europese Union - Regulation (EU) 2017/746 of the European Parliament and of the Council of 5 April 2017 on in vitro diagnostic medical devices.
Accessible at: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32017R0746&from=EN

Greenwald MA, Kerby S, Francis K, Noller AC, Gormley WT, Biswas R et al. Detection of human immunodeficiency virus, hepatitis C virus, and hepatitis B virus in postmortem blood specimens using infectious disease assays licensed for cadaveric donor screening. Transpl Infect Dis 2018;20.

Guder WG. The quality of diagnostic samples - Preanalytical aspects. Die Medizinische Welt 2012;63:293-9.

José M, Curtu S, Gajardo R, Jorquera JI. The effect of storage at different temperatures on the stability of Hepatitis C virus RNA in plasma samples. Biologicals 2003;31:1-8.

José M, Gajardo R, Jorquera JI. Stability of HCV, HIV-1 and HBV nucleic acids in plasma samples under long-term storage. Biologicals 2005;33:9-16.

Kitchen AD, Newham JA, Gillan HL. Effective serological and molecular screening of deceased tissue donors. Cell Tissue Bank 2013;14:633-44.

McCaughey EJ, Vecellio E, Lake R, Li L, Burnett L, Chesher D et al. Key factors influencing the incidence of hemolysis: A critical appraisal of current evidence. Crit Rev Clin Lab Sci 2017;54:59-72.

Meyer T, Polywka S, Wulff B, Edler C, Schröder AS, Wilkemeyer I et al. Virus NAT for HIV, HBV, and HCV in Post-Mortal Blood Specimens over 48 h after Death of Infected Patients - First Results. Transfus Med Hemother 2012;39:376-80.

Padalko E. Lagrou K, Delforge ML, Jansens H, Ectors N, Pirnay JP et al. Biological tests carried out on serum/plasma samples from donors of human body material for transplantation: Belgian experience and practical recommendations. Cell Tissue Bank 2018;19:681-95.

Plebani M, Sciacovelli L, Aita A. Chiozza ML. Harmonization of pre-analytical quality indicators. Biochem Med 2014;24:105-13.

Sareen R, Kapil M, Gupta G. Preanalytical variables: Influence on laboratory results and patient care. Int J Clinicopathol Correl 2017;1:31-4.

SHC – Superior Council of Health. Reporting and interpretation of biological tests on samples derived from donors of human body material. Brussel: SHC;2016. Advice n° 9314.

Schmack I, Ballikaya S, Erber B, Voehringer I, Burkhardt U, Auffarth G, Schnitzler D. Validation of spiked postmortem blood samples from cornea donors on the Abbott ARCHITECT and m2000 systems for viral infections. Transfus Med Hemother 2020;47:236-42.

Sotoudeh Anvari M, Gharib A, Abolhasani M, Azari-Yam A, Hossieni Gharalari F, Safavi M et al. Pre-analytical practices in the molecular diagnostic tests, a concise review. Iran J Pathol 2021;16:1-19.

Theodoropoulos N, Nowicki MJ, Chinchilla-Reyes C, Dionne S, Jaramillo A, Mone T et al. Deceased organ donor screening for human immunodeficiency virus, hepatitis B virus and hepatitis C virus: Discordant serology and nucleic acid testing results. Transpl Infect Dis. 2018;20(1).

VI COMPOSITION OF THE WORKING GROUP

The composition of the Committee and that of the Board as well as the list of experts appointed by Royal Decree are available on the following website: About us.

All experts joined the working group ***in a private capacity***. Their general declarations of interests as well as those of the members of the Committee and the Board can be viewed on the SHC website (site: conflicts of interest).

The following experts were involved in drawing up and endorsing this advisory report. The working group was first chaired by **Elizaveta PADALKO and then by Hilde BEELE**; the scientific secretary was Muriel Baltes.

BEELE Hilde	Medicine, dermatology	*UZ Gent*
COLENBIE Luc	Transplant coordinator	*UZ Gent*
DELFORGE Alain	Medicine, cell therapy	ULB Bordet
ECTORS Nadine	Medicine, pathology	*KULeuven*
GUNS Johan	Medical and social sciences	*UZ Brussel*
IMBERT Romain	Gynaecology-obstetrics, reproductive medicine	CHIREC
JANSENS Hilde	Hospital hygiene, medical microbiology	UZA
PADALKO Elizaveta	Clinical biology, virology	*UZ Gent*
PIRNAY Jean-Paul	Medical sciences	MHKA
RODENBACH Marie-Pierre	Pharmacy, biology	*Croix-Rouge*
VAN RIET Ivan	Medicine, cell therapy	*UZ Brussel*
VANSTEENBRUGGE Anne	Medicine, reproductive medicine, embryology	CHIREC
VERBEKEN Gilbert	Biomedical sciences	MHKA

The following experts were involved in the preliminary discussions of this draft, i.e. in early February 2019, but due to the health situation in 2020 - 2022 and new professional orientations did not participate in the approval of the opinion.

GOOSSENS Dominique	Medicine, biology	*Croix-Rouge*
MATTHYS Conny	Medicine, cord blood banking	*UZ Gent*
LATINNE Dominique	Biology, hematology	UCL
TOUNGOUZ NÉVESSIGNSKY Michel	Clinical biology, immunology, transfusion, cell and tissue therapy, clinical research	*Service Francophone du Sang, Croix Rouge*

The following firms/associations/etc. were heard:

Koots Maarten	Sanquin
Inez van Hyfte	Hologic
Joost van Toren	Hologic
Martine Aerts	Abbott
Ann Van Roost	Abbott
Thierry Aelbrecht	Abbott
Peter Branders	Roche
David Muyllaert	Cephied
Wouter Cornette	Cephied

VII APPENDIXES

Appendix 1*: Impact of storage condition prior to fractionation

Data obtained from a master thesis:

1/ Table. Storage conditions (temperature and duration) according to the package insert

Measured property	**Storage until centrifugation**
Treponema pallidum antibodies TP	RT* for up to 24 hours or 2 – 8 °C for up to 7 days
Treponema pallidum antibodies RPR	No guidelines
Hepatitis B core antibodies	15 – 30 °C for up to 3 days or 2 – 8 °C for up to 14 days
Hepatitis B surface antibodies	2 – 8 °C for up to 14 days
Hepatitis B surface antigen	15 – 30 °C for up to 3 days or 2 – 8 °C for up to 14 days
HIV antibodies/Ag	2 – 8 °C for up to 14 days
Hepatitis C antibodies	2 – 8 °C for up to 7 days
Hepatitis B virus DNA	2 – 30 °C for up to 6 hours
Hepatitis C virus RNA	2 – 30 °C for up to 6 hours
HIV-1 RNA	2 – 25 °C for up to 6 hours

*RT: Room Temperature

Based on the following Package inserts:
Package insert: Anti-HBc II (Abbott Laboratories).
Package insert: Anti-HBs (Abbott Laboratories).

Package insert: Anti-HCV (Abbott Laboratories).

Package insert: HBsAg Qualitative (Abbott Laboratories).

Package insert: HIV Ag/Ab Combo (Abbott Laboratories).

Package insert: Syphilis TP (Abbott Laboratories).

Package insert: Macro-Vue™ RPR Card Tests (Becton, Dickinson and Company).

Package insert: Abbott RealTime HBV (Abbott Laboratories).

Package insert: Abbott RealTime HCV (Abbott Laboratories).

Package insert: COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test (Roche Molecular Systems).

2/ A preliminary test protocol for HBV and HCV concerning the pre-analytical conditions (time and temperature before centrifugation) show no clear indications that there is an optimal storage time or temperature before centrifugation of the blood. This would mean that the storage time and temperature before centrifugation do not affect the result for serological testing and PCR and thus the donor evaluation for these parameters. However, supplementary data are needed. Moreover, results for HIV-infected patients and syphilis-infected patients are currently lacking, but these tests will be carried out in the future.

About the Superior Health Council (SHC)

The Superior Health Council is a federal advisory body. Its secretariat is provided by the Federal Public Service Health, Food Chain Safety and Environment. It was founded in 1849 and provides scientific advisory reports on public health issues to the Ministers of Public Health and the Environment, their administration, and a few agencies. These advisory reports are drawn up on request or on the SHC's own initiative. The SHC aims at giving guidance to political decision-makers on public health matters. It does this on the basis of the most recent scientific knowledge.

Apart from its 25-member internal secretariat, the Council draws upon a vast network of over 500 experts (university professors, staff members of scientific institutions, stakeholders in the field, etc.), 300 of whom are appointed experts of the Council by Royal Decree. These experts meet in multidisciplinary working groups in order to write the advisory reports.

As an official body, the Superior Health Council takes the view that it is of key importance to guarantee that the scientific advisory reports it issues are neutral and impartial. In order to do so, it has provided itself with a structure, rules and procedures with which these requirements can be met efficiently at each stage of the coming into being of the advisory reports. The key stages in the latter process are: 1) the preliminary analysis of the request, 2) the appointing of the experts within the working groups, 3) the implementation of the procedures for managing potential conflicts of interest (based on the declaration of interest, the analysis of possible conflicts of interest, and a Committee on Professional Conduct) as well as the final endorsement of the advisory reports by the Board (ultimate decision-making body of the SHC, which consists of 30 members from the pool of appointed experts). This coherent set of procedures aims at allowing the SHC to issue advisory reports that are based on the highest level of scientific expertise available whilst maintaining all possible impartiality.

Once they have been endorsed by the Board, the advisory reports are sent to those who requested them as well as to the Minister of Public Health and are subsequently published on the SHC website (www.hgr-css.be). Some of them are also communicated to the press and to specific target groups (healthcare professionals, universities, politicians, consumer organisations, etc.).

In order to receive notification about the activities and publications of the SHC, please contact: info.hgr-css@health.belgium.be.

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