1. Requirements for the determination of the sensitivity of screening tests used in the manufacture of blood and stem cell preparations for donor testing

Parameters	Seroconversion sensitivity	Diagnostic sensitivity	Analytical sensitivity	Genotype, subtype-, mutant recognition
Anti-HIV-1/2 Ab	For all HIV, HCV and HBsAg screening tests, the sensitivity must be determined during the early infection phase (seroconversion) by testing 30 seroconversion panels with short intervals between the blood collections in the range in which the seroconversion takes place in comparison with a CE marked test with acceptable performance (cf. Annex 3).	The diagnostic sensitivity must be tested on 400 positive samples and for HIV also on 100 anti-HIV-2 Ab positive samples at different stages of the disease and taking into account the pathogen variability pursuant to CTS Table 1.	Not applicable	Sensitivity for HIV-1 subtype group M comparable with subtype B For HIV-1 group 0 and for HIV-2, the test must be positive at least for samples serologically confirmed as positive.
HIV Ag/Ab		The test must show a positive result for all samples confirmed positive in a Western Blot or Line Assay.	HIV-1 p24 antigen ≤ 2 IE/ml referred to the WHO Standard (90/636)	Sensitivity for HIV-1 p24 Ag of subtype group M comparable with subtype B, Reactivity for HIV-1 group 0 must be present Proof must be provided for detection of HIV-2
Anti-HCV Ab **HCV Ag/Ab**	The sensitivity determined must conform to the state of the art determined by the Paul-Ehrlich-Institut (cf. 3). In the case of anti-HCV Ab and HCV Ag/Ab tests, a well-adjusted recognition of anti-core and anti-NS3 must also exist.		Not applicable	Recognition of HCV genotypes 1-6
HBsAg		For HBsAg, the test should show an overall performance in conformity with the state of the art.	< 0.1 IU/ml referred to the WHO Standard (00/588)	Sensitivity for HBV-genotypes and/or HBsAg subtypes must be comparable with genotype A Recognition of known HBsAg mutants.
Anti-HBc Ab	Testing of 10 seroconversion panels minimum with an Anti-HBc Ab course. The sensitivity determined must meet the requirements of the state of the art determined by the Paul-Ehrlich-Institut (cf column 4, analytical sensitivity).	The diagnostic sensitivity must be tested on 400 positive samples pursuant to CTS Table 1. All samples which are simultaneously positive for anti-HBe Ab and/or anti- HBs Ab, must be recognised (100% sensitivity). Isolated anti-HBc Ab positive samples must be examined comparatively for clarification using at least 2 additional anti-HBc Ab tests.	< 1.40 IU/ml referred to the WHO standard (95/522)	Not applicable

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Parameters	Seroconversion sensitivity	Diagnostic sensitivity	Analytical sensitivity	Genotype, subtype-, mutant recognition
HCV-RNA **HIV-1-RNA** **HBV-DNA**	Samples from the pre-seroconversion phase analogous with the requirements laid down in the CTS for qualitative NAT tests (10 seroconversion panels, each beginning with a negative sample and intervals < 7 days between the collections)	Samples from the routine in comparison with another CE marked method. No virus genome positive samples with a concentration above the declared sensitivity limit should be missed. Testing of the influence of potentially NAT inhibiting agents/substances. Regular identification of 5,000 IU HCV-RNA/ml or 10,000 IU HIV1-RNA in a single donation. For the calculation of this sensitivity, triple the 95% LOD is used as a basis.	To be determined as 95% LOD in IU/ml (WHO Standards)	Recognition of prevalent virus genotypes and subtypes with a sensitivity analogues with the appropriate WHO standards

With regard to the specific requirements in the manufacture of blood and stem cell preparations, the Paul-Ehrlich-Institut continuously determines the state of the art on the basis of comparative studies of available testing systems and develops new testing criteria. These criteria are published.
Ag = antigen; Ab = antibody, CTS = Common Technical Specifications

2. Requirements for the batch testing of the screening tests listed in 1

Requirements	Documents to be submitted
Ensuring a consistent quality by a • batch-wise, • manufacturer-independent, and • experimental product testing with the involvement of • a (contract) laboratory of the Notified Body or • an ISO 17025/ISO 15189 accredited or recognized contract laboratory of the manufacturer in conformity with the following testing criteria: • CTS, general principle 3.4	• Ex act description of test methods, samples to be tested and testing criteria • Sensitivity Analytical detection limit (e.g. NAT, Ag tests) or • Accuracy (e.g. in antibody tests) • Precision

3. Requirements for the determination of the seroconversion sensitivity of HIV-1/2, HCV and HBV tests used as screening tests in the manufacture of blood and stem cell preparations

The sensitivity of tests used for the screening of blood donations used in the manufacture of blood and stem cell preparations must be determined using a minimum of 30 suitable seroconversion panels. The minimum sensitivity must conform to the state of the art as laid down by the Paul-Ehrlich-Institut. The panels must be selected pursuant to the requirements of the CTS (Annex 1), and in addition the panels must fulfil the CTS requirements 3.1.8.1, for HIV- also the CTS requirements 3.1.8.3.

3.1. Minimum sensitivity of HIV tests (Anti-HIV-1/2 and HIV-Ag/Ab combination tests) with seroconversions:

Examples of suitable HIV seroconversion panels which can be used to determine the minimum sensitivity:

- SeraCare/BBI PRB927, PRB929, PRB930, PRB932, PRB939 Ext, PRB952, PRB965, PRB966 as well as ZeptoMetrix 6240, 6243, 6244, 6245, 6246, 6247, 6248, 9022, 9010, 9012, 9014, 9017, 9018, 9034, 9033, 9021, 9020, 9023, 9025, 9032, 9030, and 12007.

Altogether, these panels contain 341 single collections. With the exception of Panel PRB930, all panels begin with at least one HIV-1 p24 antigen and HIV antibody negative blood collection.

29 panels contain HIV-1 p24 and/or antibody containing samples and are thus suitable for both HIV Ag/Ab combination tests and Anti-HIV-1/2 tests. Solely Panel 9025 does not contain any antibody-reactive samples. For the panels listed, up to 134 samples can be determined as reactive for HIV-1 p24 antigen and/ or HIV antibodies with very sensitive HIV-Ag/Ab tests. Sensitive anti-HIV-1/2 test can recognize up to 89 single collections.

An Anti-HIV-1/2 test which would still be acceptable would have to recognise at least 65 samples as reactive in the case of this panel selection (this is equivalent to 49% of the samples detectable with HIV Ag/Ab tests or 73% of the anti-HIV-1/2 Ab positive samples).

The requirement for the analytical sensitivity of HIV Ag/Ab combination tests for HIV-1 p24 antigen is described in 1.

3.2. Minimum sensitivity of HBsAg tests with seroconversions:

Examples of suitable HIV seroconversion panels which can be used to determine the minimum sensitivity:

- SeraCare/BBI PHM903, PHM911, PHM914, PHM916, PHM925, PHM926, PHM927, PHM928, PHM929, PHM930, PHM931, PHM932, PHM934, ZeptoMetrix 6271, 6272, 6273, 6274, 6275, 6279, 11000, 11001, 11002, 11003, 11006, 11007, 11008, 11009, 11011, 11012, and 11013.

Altogether, these panels contain 331 single collections. With sensitive HBsAg tests, 184 samples can be determined as reactive with these panels. A test which would still be acceptable would have to recognise at least 107 samples (58%) as positive in the case of this panel selection.

The requirement for the analytical sensitivity of HBsAg tests is described in 1.

3.3. Minimum sensitivity of Anti-HCV Ab tests:

For the determination of the minimum sensitivity of Anti-HCV Ab tests, seroconversion panels must be selected which show different antibody patterns (e.g. anti-NS3 first, anti-core first and mixed antibody profiles). Panels which show exclusively antibodies against NS3 in the early phase of infection should be present in a representative quantity.

Suitable HCV seroconversion panels include:

- HCV panels which show only antibodies against Core:
  SeraCare/ BBI PHV#: 909, 912, 913, 914, 918, ZeptoMetrix 6216, donor 66011
- HCV panels which show only antibodies against NS3
  SeraCare/ BBI PHV#: 904, 905, 915; ZeptoMetrix 6212, 6224, 6225, 6228, 9047, donor 65345, donor 64273
- HCV panels which show mixed antibody profiles in the seroconversion phase:
  SeraCare/ BBI PHV#: 906 (NS3/NS4), 907 (Core/NS3), 908 (NS3/NS4), 910 (Core/NS3/NS4), 916 (NS3/NS4), 919 (Core/NS3), 920 (Core/NS3); ZeptoMetrix 6211 (NS3/NS4), 6213 (NS3/Core), 6214 (NS3/NS4), 6222 (NS3/Core), 6226 (NS3/NS4/Core), 6227 (NS3/Core/NS5/NS4), 6229 (NS3/Core/NS5), 9045 (donor 64150; Core/NS4), 9054 (donor 66626; Core/NS3), 9055 (donor 66732; Core/NS3/NS4), and Donor 77890 (Core/NS3/NS4).

The antibody profiles of the seroconversion panels were determined by means of CHIRON RIBA HCV 3.0 SIA. The order of the antigens indicated in brackets reflects the order of the antibodies which occurred in the course of the seroconversion.

Altogether, all HCV panels contain 337 single collections. With very sensitive Anti-HCV-Ab tests, 128 samples (100%) maximum can be determined as reactive with this panel. An Anti-HCV-Ab test which would still be acceptable would have to recognise at least 90 samples (70%) as positive in the case of this panel selection.

Since there are Anti-HCV tests which exhibit a weakness in recognising samples and seroconversions which only develop antibodies against NS3 in the course, these "NS3 panels" are evaluated again separately. Altogether, the so-called NS3 panels contain 87 single collections of which 37 (100%) can be recognised as positive using the most sensitive Anti-HCV tests. A test acceptable for blood donation should recognise at least 26 (70%) of the anit-NS3 positive samples as reactive.

The definition of analytical sensitivity of HCV Ag/Ab tests is currently not possible.