COMMISSION IMPLEMENTING REGULATION (EU) 2022/1107

of 4 July 2022

laying down common specifications for certain class D *in vitro* diagnostic medical devices in accordance with Regulation (EU) 2017/746 of the European Parliament and of the Council

(Text with EEA relevance)

THE EUROPEAN COMMISSION,

Having regard to the Treaty on the Functioning of the European Union,

Having regard to Regulation (EU) 2017/746 of the European Parliament and of the Council of 5 April 2017 on *in vitro* diagnostic medical devices and repealing Directive 98/79/EC and Commission Decision 2010/227/EU ([1]), and in particular Article 9(1) thereof,

Whereas:

(1) For certain class D *in vitro* diagnostic medical devices falling within the scope of Regulation (EU) 2017/746, harmonised standards do not exist as regards certain requirements of Annex I to that Regulation, and there is a need to address public health concerns as the risk associated with the use of those devices is significant for public health and patient safety. It is therefore appropriate to adopt common specifications for those devices in respect of those requirements.

(2) Regulation (EU) 2017/746 replaces Directive 98/79/EC of the European Parliament and of the Council ([2]). The common technical specifications set out in Commission Decision 2002/364/EC ([3]) for certain devices covered by Directive 98/79/EC remain relevant. Those common technical specifications have therefore been taken into account and where necessary updated to reflect the state of the art.

(3) To allow manufacturers, other economic operators, notified bodies and other actors to adapt to this Regulation, and to ensure its proper application, it is appropriate to defer its application. However, in the interest of public health and patient safety, manufacturers should be allowed to comply with the common specifications laid down in this Regulation on a voluntary basis before its date of application.

(4) To ensure a continuous high level of safety and performance of devices, as a transitional measure it should be provided that devices that are in conformity with Decision 2002/364/EC are to be presumed to be in conformity with the requirements for certain performance characteristics set out in Annex I to Regulation (EU) 2017/746 until the date of application of this Regulation.

(5) The Medical Device Coordination Group has been consulted.

(6) The measures provided for in this Regulation are in accordance with the opinion of the Committee on Medical Devices,

HAS ADOPTED THIS REGULATION:

Article 1

Common specifications

This Regulation lays down common specifications for certain class D *in vitro* diagnostic medical devices in respect of the requirements regarding the performance characteristics set out in Section 9.1, points (a) and (b), Section 9.3 and Section 9.4, point (a), of Annex I to Regulation (EU) 2017/746.

([1]) OJ L 117, 5.5.2017, p. 176.
([2]) Directive 98/79/EC of the European Parliament and of the Council of 27 October 1998 on in vitro diagnostic medical devices (OJ L 331, 7.12.1998, p. 1).
([3]) Commission Decision 2002/364/EC of 7 May 2002 on common technical specifications for in vitro-diagnostic medical devices (OJ L 131, 16.5.2002, p. 17).

Annex I lays down common specifications for devices covered by Annexes II to XIII, as specified in that Annex.

Annex II lays down common specifications for devices intended for detection of blood group antigens in the ABO, Rh, Kell, Duffy and Kidd blood group systems.

Annex III lays down common specifications for devices intended for detection or quantification of markers of human immunodeficiency virus (HIV) infection.

Annex IV lays down common specifications for devices intended for detection or quantification of markers of human T-cell lymphotropic virus (HTLV) infection.

Annex V lays down common specifications for devices intended for detection or quantification of markers of hepatitis C virus (HCV) infection.

Annex VI lays down common specifications for devices intended for detection or quantification of markers of hepatitis B virus (HBV) infection.

Annex VII lays down common specifications for devices intended for detection or quantification of markers of hepatitis D virus (HDV) infection.

Annex VIII lays down common specifications for devices intended for detection of markers of variant Creutzfeldt-Jakob disease (vCJD).

Annex IX lays down common specifications for devices intended for detection or quantification of markers of cytomegalovirus (CMV) infection.

Annex X lays down common specifications for devices intended for detection or quantification of markers of Epstein-Barr virus infection (EBV).

Annex XI lays down common specifications for devices intended for detection of markers of *Treponema pallidum* infection.

Annex XII lays down common specifications for devices intended for detection or quantification of markers of *Trypanosoma cruzi* infection.

Annex XIII lays down common specifications for devices intended for detection or quantification of markers of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection.

Article 2

Definitions

For the purposes of this Regulation, the following definitions apply:

(1) 'true positive' means a specimen known to be positive for the target marker and correctly classified by the device;

(2) 'false negative' means a specimen known to be positive for the target marker and misclassified by the device;

(3) 'false positive' means a specimen known to be negative for the target marker and misclassified by the device;

(4) 'the limit of detection' ('LOD') means the smallest amount of the target marker that can be detected;

(5) 'nucleic acid amplification techniques' ('NAT') means methods of detection and/or quantification of nucleic acids by either amplification of a target sequence, by amplification of a signal or by hybridisation;

(6) 'NAT system' means the combination of devices used for extraction, amplification and detection of nucleic acids;

(7) 'rapid test' means a qualitative or semi-quantitative *in vitro* diagnostic medical device, used singly or in a small series, which involves non-automated procedures (except the reading of results) and has been designed to give a fast result;

(8) 'robustness' means the capacity of an analytical procedure to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage;

(9) 'cross-reactivity' means the ability of non-target analytes or markers to cause false positive results in an assay because of similarity, e.g. the ability of non-specific antibodies binding to a test antigen of an antibody assay, or the ability of non-target nucleic acids to be reactive in a NAT assay;

(10) 'interference' means the ability of unrelated substances to affect the results in an assay;

(11) 'whole system failure rate' means the frequency of failures when the entire process is performed as prescribed by the manufacturer;

(12) 'first-line assay' means a device used to detect a marker or analyte, and the use of which may be followed by the use of a confirmatory assay; devices intended solely to be used to monitor a previously determined marker or analyte are not considered first-line assays;

(13) 'confirmatory assay' means a device used for the confirmation of a reactive result from a first line assay;

(14) 'supplemental assay' means a device that is used to provide further information for the interpretation of the test result of another assay;

(15) 'virus typing device' means a device used for typing with already known positive samples, not used for primary diagnosis of infection or for screening;

(16) '95 % positive cut-off value' means the analyte concentration where 95 % of test runs give positive results following serial dilutions of an international reference material, where available, e.g. a World Health Organisation (WHO) International Standard or reference material calibrated against the WHO International Standard.

Article 3

Transitional provisions

1. From 25 July 2022 until 25 July 2024, devices that are in conformity with the common technical specifications set out in Decision 2002/364/EC shall be presumed to be in conformity with the requirements regarding the performance characteristics set out in Section 9.1, points (a) and (b), Section 9.3 and Section 9.4, point (a), of Annex I to Regulation (EU) 2017/746.

During that period manufacturers of devices that are not in conformity with the common technical specifications set out in Decision 2002/364/EC shall duly justify that they have adopted solutions that ensure a level of safety and performance that is at least equivalent thereto.

2. From 25 July 2022 until 25 July 2024 devices that are in conformity with the common specifications set out in this Regulation shall be presumed to be in conformity with the requirements regarding the performance characteristics set out in Section 9.1, points (a) and (b), Section 9.3 and Section 9.4, point (a), of Annex I to Regulation (EU) 2017/746.

Article 4

Entry into force and date of application

This Regulation shall enter into force on the twentieth day following that of its publication in the *Official Journal of the European Union*.

It shall apply from 25 July 2024.

However, Article 3 shall apply from 25 July 2022.

This Regulation shall be binding in its entirety and directly applicable in all Member States.

Done at Brussels, 4 July 2022.

For the Commission
The President
Ursula VON DER LEYEN

ANNEX I

GENERAL COMMON SPECIFICATIONS

Part I – Requirements for performance characteristics of devices covered by Annexes II to XIII

Performance characteristics	Requirement
All performance characteristics set out in Section 9.1, points (a) and (b), Section 9.3 and Section 9.4, point (a), of Annex I to Regulation (EU) 2017/746	1. The determination of performance characteristics shall be carried out in direct comparison with a state-of-the-art device. The device used for comparison shall be one bearing CE marking, if on the market at the time of the performance evaluation. 2. Devices used for determination of status of specimens used in determination of performance characteristics shall be state-of-the-art devices bearing CE marking. 3. If discrepant results are identified as part of determination of performance characteristics, these results shall be resolved as far as possible, by one or more of the following: — by evaluation of the discrepant specimen in further devices, — by use of an alternative method or marker, — by a review of the clinical status and diagnosis of the patient, — by the testing of follow-up specimens. 4. The determination of performance characteristics shall be performed on a population equivalent to the European population.
Whole system failure rate	5. As part of the required risk analysis the whole system failure rate leading to false negative results shall be determined in repeat assays on low-positive specimens.
Analytical sensitivity and analytical specificity, interference	6. For devices intended for use with plasma the manufacturer shall verify the performance of the device using all anticoagulants which the manufacturer indicates for use with the device, for at least 50 plasma specimens (for devices intended for detection and/or quantification of infectious agents, 25 positive and 25 negative).
Analytical and diagnostic specificity, interference and cross-reactivity	7. The manufacturer shall select the potential interfering substances to be evaluated taking account of the composition of the reagents and configuration of the device.
Batch-to-batch consistency	8. For devices intended to detect antigens and antibodies, the manufacturer's batch testing criteria shall ensure that every batch consistently identifies the relevant antigens, epitopes, and antibodies and is suitable for the claimed specimen types. 9. The manufacturer's batch release testing for first-line assays shall include at least 100 specimens negative for the relevant analyte ([1]).

([1]) This requirement shall not apply to devices covered by Tables 1 and 2 of Annex XIII.

Part II – Requirements for performance characteristics of devices referred to in Annexes III to XIII

Performance characteristic	Requirement
Analytical and diagnostic sensitivity	10. Devices intended by the manufacturer for testing body fluids other than serum or plasma, e.g. urine, saliva, etc., shall meet the same requirements as serum or plasma devices. The manufacturer shall test specimens from the same individuals in both the devices to be approved and in a respective serum or plasma device. ([1]) 11. Devices for self-testing shall meet the same requirements as respective devices for professional use. 12. Positive specimens used in the performance evaluation shall be selected to reflect different stages of the respective disease(s), different antibody patterns, different genotypes, different subtypes, mutants, etc. 13. Seroconversion panels shall start with a negative bleed(s) and shall have narrow bleeding intervals as far as possible. Where this is not possible, manufacturers shall provide a justification in the performance evaluation report. 14. For devices intended by the manufacturer to be used with serum and plasma the performance evaluation must demonstrate serum to plasma equivalency. This shall be demonstrated for at least 25 positive donations. 15. For devices detecting or quantifying antigens or nucleic acids, the target antigen(s) or target nucleic acid region(s) respectively shall be specified in the instructions for use. 16. For devices detecting or quantifying antibodies against an infectious agent, the target antigen(s) of those antibodies shall be specified in the instructions for use.
Analytical and diagnostic specificity	17. Devices intended by the manufacturer for testing body fluids other than serum or plasma, e.g. urine, saliva, etc., shall meet the same requirements as serum or plasma devices. The performance evaluation shall test specimens from the same individuals in both the devices to be approved and in a respective serum or plasma device. ([1]) 18. Devices for self-testing shall meet the same requirements as respective devices for professional use. 19. Negative specimens used in a performance evaluation shall be defined so as to reflect the target population for which the device is intended, such as blood donors, hospitalised patients, pregnant women, etc. 20. Specificity shall be based on repeatedly reactive false positive results in specimens negative for the target marker. 21. For devices intended by the manufacturer to be used with serum and plasma the performance evaluation must demonstrate serum to plasma equivalency. This shall be demonstrated for at least 25 negative donations.

Analytical and diagnostic specificity, interference and cross-reactivity	22. The manufacturer shall include specimens such as, where applicable: — specimens representing related infections, — specimens from multigravida, i.e. women who have had more than one pregnancy, or rheumatoid factor (RF) positive patients, — specimens containing human antibodies to components of the expression system, for example anti-*E. coli*, or anti-yeast.
Performances obtained by lay persons	23. Relevant parts of the performance evaluation shall be carried out (or repeated) by appropriate lay persons to validate the operation of the device and the instructions for use. The lay persons selected for the performance evaluation shall be representative of the intended users groups.

([1]) This requirement shall not apply to devices referred to in Tables 4, 5 and 6 of Annex XIII.

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ANNEX II

COMMON SPECIFICATIONS FOR DEVICES INTENDED FOR DETECTION OF BLOOD GROUP ANTIGENS IN THE ABO, RH, KELL, DUFFY AND KIDD BLOOD GROUP SYSTEMS

Scope

This Annex applies to devices intended for detection of blood group antigens in the ABO, Rh, Kell, Duffy and Kidd blood group systems.

Table 1 applies to performance evaluation of devices detecting blood group antigens in the ABO, Rh, Kell, Duffy and Kidd blood group systems.

Table 2 applies to manufacturer's batch-to-batch consistency testing of reagents and reagent products to determine blood group antigens in the ABO, Rh, Kell, Duffy and Kidd blood group systems (test reagents, control materials).

Table 1. Performance evaluation of devices detecting blood group antigens in the ABO, Rh, Kell, Duffy and Kidd blood group systems

Reagent specificity	Number of tests per method claimed by the manufacturer	Total number of specimens to be tested for a launch device	Total number of specimens to be tested for a new formulation, or use of well-characterised reagents	General qualification criteria	Specific qualification criteria	Acceptance criteria
Anti-ABO1 (Anti-A), Anti-ABO2 (Anti-B), Anti-ABO3 (Anti-A,B)	≥500	≥3 000	≥1 000	Clinical specimens: 10 % of the test population Neonatal specimens: > 2 % of the test population	ABO specimens shall include > 40 % A and B antigen positive specimens which may include specimens from group A, group B and group AB	All of the reagents shall show comparable performance to state-of-the-art CE marked devices with regard to claimed reactivity of the device. For CE marked devices where the application or use has been changed or extended, further testing shall be carried out in accordance with the requirements outlined in column 2 above ("Number of tests per method claimed by the manufacturer").
Anti-RH1 (Anti-D)	≥500	≥3 000	≥1 000		Performance evaluation of Anti-D reagents shall include tests against a range of weak RH1 (D) and partial RH1 (D) specimens, depending on the intended use of the product. Weak and/or partial D cells shall account for > 2 % of RH1 (D) positive specimens.	
Anti-RH2 (Anti-C), Anti-RH4 (Anti-c), Anti- RH3 (Anti-E)	≥100	≥1 000	≥200			
Anti-RH5 (Anti-e)	≥100	≥500	≥200			

Anti-KEL1 (Anti-K)	≥100	≥500	≥200			
Anti-JK1 (Jk[a]), Anti-JK2 (Jk[b])	≥100	≥500	≥200			
Anti-FY1 (Fy[a]), Anti-FY2 (Fy[b])	≥100	≥500	≥200			

Note: Positive specimens used in the performance evaluation shall be selected to reflect variant and weak antigen expression.

Table 2. Manufacturer's batch-to-batch consistency testing of reagents and reagent products to determine blood group antigens in the ABO, Rh, Kell, Duffy and Kidd blood group systems

1. **Test reagents**

Blood group reagents	Minimum number of control cells to be tested as part of specificity testing								Acceptance criteria
	Positive reactions					Negative reactions			Each batch of reagent shall exhibit unequivocal positive or negative results by all techniques claimed by the manufacturer in accordance with the results obtained from the performance evaluation data.
	A1	A2B	Ax			B	O		
Anti-ABO1(Anti-A)	2	2	2 (1)			2	2		
	B	A1B				A1	O		
Anti-ABO2(Anti-B)	2	2				2	2		
	A1	A2	Ax	B		O			
Anti-ABO3(Anti-A,B)	2	2	2 (1)	2		4			
	R1r	R2r	WeakD			r'r	r"r	rr	
Anti-RH1 (Anti-D)	2	2	2 (1)			1	1	1	
	R1R2	R1r	r'r			R2R2	r"r	rr	
Anti-RH2 (Anti-C)	2	1	1			1	1	1	
	R1R2	R1r	r'r			R1R1			
Anti-RH4 (Anti-c)	1	2	1			3			
	R1R2	R2r	r"r			R1R1	r'r	rr	

Anti-RH3 (Anti-E)	2	1	1			1	1	1	
	R1R2	R2r	r"r			R2R2			
Anti-RH5 (Anti-e)	2	1	1			3			
	Kk					kk			
Anti-KEL1 (Anti-K)	4					3			
	Jk(a+b+)					Jk(a–b+)			
Anti-JK1 (Anti-Jk[a])	4					3			
	Jk(a+b+)					Jk(a+b–)			
Anti-JK2 (Anti-Jk[b])	4					3			
	Fy(a+b+)					Fy(a–b+)			
Anti-FY1 (Anti-Fy[a])	4					3			
	Fy(a+b+)					Fy(a+b–)			
Anti-FY2 (Anti-Fy[b])	4					3			

Note: Polyclonal reagents shall be tested against a wider panel of cells to confirm specificity and exclude presence of unwanted contaminating antibodies.
([1]) Only where reactivity against these antigens is claimed.

2. **Control materials (red cells)**

The phenotype of red cells used in the control of blood typing reagents listed above shall be confirmed using an established device(s).

ANNEX III

COMMON SPECIFICATIONS FOR DEVICES INTENDED FOR DETECTION OR QUANTIFICATION OF MARKERS OF HUMAN IMMUNODEFICIENCY VIRUS (HIV) INFECTION

Scope

1. This Annex applies to devices intended for detection or quantification of markers of human immunodeficiency virus (HIV) infection.

   Table 1 applies to first-line assays for HIV-1/2 antibody (anti-HIV-1/2) and first-line combined antigen/antibody assays for HIV-1/2 (HIV-1/2 Ag/Ab) which are not rapid tests.

   Table 2 applies to first-line assays for anti-HIV-1/2 and HIV-1/2 Ag/Ab which are rapid tests.

   Table 3 applies to confirmatory assays for anti-HIV-1/2.

   Table 4 applies to antigen tests for HIV-1 and HIV Ag/Ab assays.

   Table 5 applies to qualitative and quantitative NAT devices for HIV ribonucleic acid (RNA).

   Table 6 applies to HIV-1/2 self-tests.

Definitions

2. For the purposes of this Annex, the following definitions apply:

   (1) 'seroconversion HIV specimen' means:

   — p24 antigen and/or HIV RNA positive, and

   — recognised by the antibody first-line assays, and

   — positive or indeterminate in confirmatory assays.

   (2) 'early seroconversion HIV specimen' means:

   — p24 antigen and/or HIV RNA positive, and

   — not recognised by the antibody first-line assays, and

   — indeterminate or negative in confirmatory assays.

Table 1. First-line assays: anti-HIV-1/2, HIV-1/2 Ag/Ab (requirements for antibody detection)

Performance characteristic	Specimen	Specimen numbers, features, use	Acceptance criteria
Diagnostic sensitivity	Positive specimens	≥400 HIV-1 ≥100 HIV-2 including 40 non-B-subtypes including 25 positive 'same day' fresh serum specimens (≤ 1 day after specimen taking)	all true positive specimens shall be identified as positive

		all available HIV/1 subtypes shall be represented by at least 3 specimens per subtype	
	Seroconversion panels	≥30 panels at least 40 early seroconversion HIV specimens shall be tested	diagnostic sensitivity during seroconversion shall represent the state of the art all seroconversion HIV specimens shall be identified as positive
Diagnostic specificity	Unselected blood donors (including first-time donors) ([1])	≥5 000	≥99,5%
	Hospitalised patients	≥200	Potential limitations for specificity, if any, shall be identified
Cross-reactivity	Potentially cross-reacting specimens	≥100 in total (such as RF+, from related virus infections, from pregnant women, subjects recently vaccinated against any infectious agent)	

([1]) Blood donor populations shall be investigated from at least two blood donation centres and consist of consecutive blood donations, which have not been selected to exclude first-time donors.

Table 2. Rapid tests: anti-HIV-1/2, HIV-1/2 Ag/Ab (requirements for antibody detection)

Performance characteristic	Specimen	Specimen numbers, features, use	Acceptance criteria
Diagnostic sensitivity	Positive specimens	≥400 HIV-1 ≥100 HIV-2 including 40 non-B-subtypes all available HIV/1 subtypes shall be represented by at least 3 specimens per subtype	all true positive specimens shall be identified as positive
	Seroconversion panels	≥30 panels at least 40 early seroconversion HIV specimens shall be tested	diagnostic sensitivity during seroconversion shall represent the state of the art all seroconversion HIV specimens shall be identified as positive
Diagnostic specificity	Unselected blood donors (including first-time donors)	≥1 000	≥ 99 %

	Hospitalised patients	≥200	Potential limitations for specificity, if any, shall be identified
Cross-reactivity	Potentially cross-reacting specimens	≥200 specimens from pregnant women ≥100 other potentially cross-reacting specimens in total (e.g. RF+, from related infections)	

Table 3. Confirmatory assays: anti-HIV-1/2

Performance characteristic	Specimen	Specimen numbers, features, use	Acceptance criteria
Diagnostic sensitivity	Positive specimens	≥200 HIV-1 ≥100 HIV-2 Including different stages of infection and reflecting different antibody patterns	Identification as "confirmed positive" or "indeterminate", not as "negative"
	Seroconversion panels	≥15 seroconversion panels/low titre panels ≥40 early seroconversion HIV specimens	Diagnostic sensitivity during seroconversion shall represent the state of the art All seroconversion HIV specimens shall be identified as positive
Diagnostic specificity	Blood donors	≥200	No false positive results / no neutralisation
	Hospitalised patients	≥200	
Cross-reactivity	Potentially cross-reacting specimens	≥50 in total (including specimens from pregnant women, specimens with indeterminate results in other confirmatory assays)	

Table 4. Antigen tests: HIV-1, HIV Ag/Ab (requirements for antigen detection)

Performance characteristic	Specimen	Specimen numbers, features, use	Acceptance criteria
Diagnostic sensitivity	Positive specimens	≥50 HIV-1 antigen positive ≥50 cell culture supernatants including different HIV-1 subtypes and HIV-2	all true positive specimens shall be identified as positive (after neutralisation if applicable)
	Seroconversion panels	≥20 seroconversion panels/low titre panels ≥40 early seroconversion HIV specimens	diagnostic sensitivity during seroconversion shall represent the state of the art all seroconversion HIV specimens shall be identified as positive

Analytical sensitivity	First International Reference Reagent HIV-1 p24 Antigen, NIBSC code: 90/636		≤ 2 IU/ml
Diagnostic specificity	Blood donors	≥200	≥ 99,5 % after neutralisation or, if no neutralisation test available, after resolution of the specimen status
	Hospitalised patients	≥200	Potential limitations for specificity, if any, shall be identified
Cross-reactivity	Potentially cross-reacting specimens	≥50	

Table 5. Qualitative and quantitative NAT devices for HIV RNA

1. For target sequence amplification devices, a functionality control for each specimen (internal control) shall reflect the state of the art. This control shall as far as possible be used throughout the whole process, i.e. extraction, amplification/hybridisation, detection.

2. Genotype and/or subtype detection shall be demonstrated by appropriate primer or probe design validation and shall also be validated by testing characterised genotyped specimens.

3. Potential cross-reactivity of non-target nucleic acid sequences shall be analysed by appropriate primer or probe design validation and shall also be validated by testing selected specimens.

4. Results of quantitative NAT devices shall be traceable to international standards or calibrated reference materials, if available, and be expressed in international units utilised in the specific field of application.

5. Qualitative HIV NAT devices intended to be used to detect the presence of HIV in blood, blood components, cells, tissues or organs, or in any of their derivatives, in order to assess their suitability for transfusion, transplantation or cell administration shall be designed to detect both HIV-1 and HIV-2.

6. Qualitative HIV NAT devices, other than virus typing devices, shall be designed to compensate for the potential failure of a HIV-1 NAT target region by using two independent target regions.

Performance characteristic	Specimen	Specimen numbers, features, use	Acceptance criteria
Analytical sensitivity	WHO International Standard HIV-1 RNA; WHO International Standard HIV-2 RNA; or calibrated reference materials	NAT sensitivity and NAT LOD shall be validated by dilution series of reference materials, testing of replicates (minimum 24) at different analyte concentrations, including those with transition from positive to negative results with the respective NAT device.	According to the state of the art

		LOD shall be expressed as 95% positive cut-off value (IU/ml) after statistical analysis (e.g. Probit). ([1]) quantitative NAT: definition of lower, upper quantification limit, precision, accuracy, 'linear' measuring range, 'dynamic range'. Reproducibility at different concentration levels	
HIV geno-/subtype sensitivity	all relevant genotypes/subtypes, preferably from international reference materials potential substitutes for rare HIV subtypes (to be quantified by appropriate methods): cell culture supernatants; in vitro transcripts; plasmids	Qualitative NAT: at least 10 specimens/genotype or subtype Quantitative NAT: dilution series for demonstration of quantification efficiencies	According to the state of the art
Diagnostic sensitivity	Positive specimens reflecting the routine conditions of users (e.g. no pre-selection of specimens)	Quantitative NAT: ≥100 Comparative results with another NAT system shall be generated in parallel	According to the state of the art
	Seroconversion panels	Qualitative NAT: ≥10 panels Comparative results with another NAT system shall be generated in parallel	According to the state of the art
Diagnostic specificity	Blood donor specimens	Qualitative NAT: ≥500 Quantitative NAT: ≥100	According to the state of the art
Cross-reactivity	Potentially cross-reacting specimens	≥10 human retrovirus positive specimens (e.g. HTLV)	According to the state of the art
Carry-over	High HIV RNA positive; HIV RNA negative	At least five runs with alternating high-positive and negative specimens shall be performed during robustness studies. The virus titres of the high positive specimens shall be representative of high virus titres occurring naturally.	According to the state of the art
Detection in relation to antibody status	HIV-RNA positives: anti-HIV negative, anti-HIV positive	Pre-seroconversion (anti-HIV negative) and post-seroconversion (anti-HIV positive) specimens	According to the state of the art

Whole system failure rate	HIV RNA low-positive	≥100 HIV RNA low-positive specimens shall be tested. These specimens shall contain a virus concentration equivalent to three times the 95 % positive cut-off virus concentration.	≥99% positive

([1]) Reference: European Pharmacopeia 9.0, 2.6.21 Nucleic acid amplification techniques, Validation.

Table 6. Additional requirements for HIV-1/2 self-tests

Performance characteristic	Specimens ([1])	Number of lay persons
Result interpretation ([2])	Interpretation of results ([3]) by lay persons reflecting the following range of reactivity levels: — non-reactive — reactive — weak reactive ([4]) — invalid	≥ 100
Diagnostic sensitivity	lay persons that are known positive	≥ 200
Diagnostic specificity	lay persons that do not know their status	≥ 400
	Lay persons that are at high risk of acquiring the infection	≥ 200

([1]) For each body fluid claimed for use with the device, e.g. whole blood, urine, saliva, etc., the sensitivity and specificity of the device for self-testing in the hands of lay persons shall be defined against the confirmed patient infectious status.

([2]) The result interpretation study shall include reading and interpretation of the test results by at least 100 lay persons, with each lay person subjected to reading results covering the specified range of result reactivity levels. The manufacturer shall determine the concordance between lay person reading and professional user reading.

([3]) Tests shall be performed prior to the result interpretation study using whenever possible the specimen type intended by the manufacturer. The tests may be performed on contrived specimens based on the natural matrix of the respective specimen type.

([4]) A higher proportion of the specimens shall be in the low-positive range close to the cut-off or LOD of the test.

ANNEX IV

COMMON SPECIFICATIONS FOR DEVICES INTENDED FOR DETECTION OR QUANTIFICATION OF MARKERS OF HUMAN T-CELL LYMPHOTROPIC VIRUS (HTLV) INFECTION

Scope

This Annex applies to devices intended for detection or quantification of markers of human T-cell lymphotropic virus (HTLV) infection.

Table 1 applies to first-line assays for antibodies against HTLV I or II (anti-HTLV I/II) which are not rapid tests.

Table 2 applies to first-line assays for anti-HTLV I/II which are rapid tests.

Table 3 applies to confirmatory assays for anti-HTLV I/II.

Table 4 applies to NAT devices for HTLV I/II.

Table 1. First-line assays: anti-HTLV I/II

Performance characteristic	Specimen	Specimen numbers, features, use	Acceptance criteria
Diagnostic sensitivity	Positive specimens	≥300 HTLV-I ≥100 HTLV-II including 25 positive 'same day' fresh serum specimens (≤ 1 day after specimen taking)	all true positive specimens shall be identified as positive
	Seroconversion panels	To be defined when available	diagnostic sensitivity during seroconversion shall represent the state of the art, if applicable
Diagnostic specificity	Unselected blood donors (including first-time donors) (1)	≥5 000	≥ 99,5%
	Hospitalised patients	≥200	Potential limitations for specificity, if any, shall be identified
Cross-reactivity	Potentially cross-reacting specimens	≥100 in total (e.g. RF+, from related virus infections, from pregnant women)	

(1) Blood donor populations shall be investigated from at least two blood donation centres and consist of consecutive blood donations, which have not been selected to exclude first-time donors.

Table 2. Rapid tests: anti-HTLV I/II

Performance characteristic	Specimen	Specimen numbers, features, use	Acceptance criteria
Diagnostic sensitivity	Positive specimens	≥300 HTLV-I ≥100 HTLV-II	all true positive specimens shall be identified as positive
	Seroconversion panels	To be defined when available	diagnostic sensitivity during seroconversion shall represent the state of the art, if applicable
Diagnostic specificity	Unselected blood donors (including first-time donors)	≥1 000	≥ 99%
	Hospitalised patients	≥200	Potential limitations for specificity, if any, shall be identified
Cross-reactivity	Potentially cross-reacting specimens	≥200 specimens from pregnant women ≥100 other potentially cross-reacting specimens in total (e.g. RF+, from related infections)	

Table 3. Confirmatory assays: anti-HTLV I/II

Performance characteristic	Specimen	Specimen numbers, features, use	Acceptance criteria
Diagnostic sensitivity	Positive specimens	≥200 HTLV I ≥100 HTLV II	Identification as “confirmed positive” or “indeterminate”, not as “negative”
	Seroconversion panels	To be defined when available	diagnostic sensitivity during seroconversion shall represent the state of the art, if applicable
Diagnostic specificity	Blood donors	≥200	No false positive results
	Hospitalised patients	≥200	
Cross-reactivity	Potentially cross-reacting specimens	≥50 in total (including specimens from pregnant women, specimens with indeterminate results in other confirmatory assays)	

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Table 4. NAT devices for HTLV I/II

1. For target sequence amplification devices, a functionality control for each specimen (internal control) shall reflect the state of the art. This control shall as far as possible be used throughout the whole process, i.e. extraction, amplification/hybridisation, detection.

2. Genotype and/or subtype detection shall be demonstrated by appropriate primer or probe design validation and shall also be validated by testing characterised genotyped specimens.

3. Potential cross-reactivity of non-target nucleic acid sequences shall be analysed by appropriate primer or probe design validation and shall also be validated by testing selected specimens.

4. Results of quantitative NAT devices shall be traceable to international standards or calibrated reference materials, if available, and be expressed in international units utilised in the specific field of application.

Performance characteristic	Specimen	Specimen numbers, features, use	Acceptance criteria
Analytical sensitivity	International reference preparations	NAT sensitivity and NAT LOD shall be validated by dilution series of reference materials, testing of replicates (minimum 24) at different analyte concentrations, including those with transition from positive to negative results with the respective NAT device. LOD shall be expressed as 95% positive cut-off value (IU/ml) after statistical analysis (e.g. Probit). ([1]) quantitative NAT: definition of lower, upper quantification limit, precision, accuracy, 'linear' measuring range, 'dynamic range'. Reproducibility at different concentration levels	According to the state of the art
HTLV I and HTLV II genotype sensitivity	all relevant genotypes, preferably from international reference materials potential substitutes for rare HTLV genotypes (to be quantified by appropriate methods): cell culture supernatants; *in vitro* transcripts; plasmids	Qualitative NAT: at least 10 specimens/genotype or subtype Quantitative NAT: dilution series for demonstration of quantification efficiencies	According to the state of the art
Diagnostic specificity	Blood donor specimens	Qualitative NAT: ≥500 Quantitative NAT: ≥100	According to the state of the art

Cross-reactivity	Potentially cross-reacting specimens	≥10 human retrovirus positive specimens (e.g. HIV-1, HIV-2)	According to the state of the art
Carry-over	High HTLV RNA positive; HTLV RNA negative	At least five runs with alternating high-positive and negative specimens shall be performed during robustness studies. The virus titres of the high positive specimens shall be representative of high virus titres occurring naturally.	According to the state of the art
Detection in relation to antibody status	HTLV-RNA positives: anti-HTLV negative, anti-HTLV positive	Pre-seroconversion (anti-HTLV negative) and post-seroconversion (anti-HTLV positive) specimens	According to the state of the art
Whole system failure rate	HTLV RNA low-positive	≥100 HTLV RNA low-positive specimens shall be tested. These specimens shall contain a virus concentration equivalent to three times the 95 % positive cut-off virus concentration.	≥99% positive

([1]) Reference: European Pharmacopeia 9.0, 2.6.21 Nucleic acid amplification techniques, Validation.

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ANNEX V

COMMON SPECIFICATIONS FOR DEVICES INTENDED FOR DETECTION OR QUANTIFICATION OF MARKERS OF HEPATITIS C VIRUS (HCV) INFECTION

Scope

This Annex applies to devices intended for detection or quantification of markers of hepatitis C virus (HCV) infection.

Table 1 applies to first-line assays for anti-HCV antibodies (anti-HCV) and combined antigen/antibody tests for HCV (HCV Ag/Ab) which are not rapid tests.

Table 2 applies to first-line assays for anti-HCV and HCV Ag/Ab which are rapid tests.

Table 3 applies to confirmatory and supplemental assays for anti-HCV.

Table 4 applies to HCV antigen tests and HCV Ag/Ab.

Table 5 applies to qualitative and quantitative NAT devices for HCV RNA.

Table 6 applies to HCV self-tests.

Table 1. First-line assays: anti-HCV, HCV Ag/Ab (requirements for antibody detection)

Performance characteristic	Specimen	Specimen numbers, features, use	Acceptance criteria
Diagnostic sensitivity	Positive specimens	≥400 Including specimens from different stages of infection and reflecting different antibody patterns HCV genotype 1-4: > 20 specimens per genotype (including non-a subtypes of genotype 4); HCV genotypes 5 and 6: > 5 specimens each; including 25 positive 'same day' fresh serum specimens (≤ 1 day after specimen taking)	all true positive specimens shall be identified as positive
	Seroconversion panels	≥30 panels HCV seroconversion panels for the evaluation of HCV antigen and antibody combined tests (HCV Ag/Ab) shall start with one or more negative bleeds and comprise panel members from early HCV infection (HCV core antigen and/or HCV RNA positive but anti-HCV negative).	diagnostic sensitivity during seroconversion shall represent the state of the art HCV Ag/Ab tests shall demonstrate enhanced sensitivity in early HCV infection when compared to HCV antibody only tests.

Diagnostic specificity	Unselected blood donors (including first-time donors) ([1])	≥5 000	≥99,5%
	Hospitalised patients	≥200	Potential limitations for specificity, if any, shall be identified
Cross-reactivity	Potentially cross-reacting specimens	≥100 in total (e.g. RF+, from related virus infections, from pregnant women)	

([1]) Blood donor populations shall be investigated from at least two blood donation centres and consist of consecutive blood donations, which have not been selected to exclude first-time donors.

Table 2. Rapid tests: anti-HCV, HCV Ag/Ab (requirements for antibody detection)

Performance characteristic	Specimen	Specimen numbers, features, use	Acceptance criteria
Diagnostic sensitivity	Positive specimens	≥400 including specimens from different stages of infection and reflecting different antibody patterns. HCV genotype 1-4: > 20 specimens per genotype (including non-a subtypes of genotype 4); HCV genotypes 5 and 6: > 5 specimens each;	all true positive specimens shall be identified as positive
	Seroconversion panels	≥30 panels HCV seroconversion panels for the evaluation of HCV antigen and antibody combined tests (HCV Ag/Ab) shall start with one or more negative bleeds and comprise panel members from early HCV infection (HCV core antigen and/or HCV RNA positive but anti-HCV negative).	diagnostic sensitivity during seroconversion shall represent the state of the art HCV Ag/Ab tests shall demonstrate enhanced sensitivity in early HCV infection when compared to HCV antibody only tests.
Diagnostic specificity	Unselected blood donors (including first-time donors)[1]	≥1 000	≥ 99 %
	Hospitalised patients	≥200	Potential limitations for specificity, if any, shall be identified
Cross-reactivity	Potentially cross-reacting specimens	≥200 specimens from pregnant women ≥100 other potentially cross-reacting specimens in total (e.g. RF+, from related infections)	

Table 3. Confirmatory and supplemental assays: anti-HCV

Performance characteristic	Specimen	Specimen numbers, features, use	Acceptance criteria
Diagnostic sensitivity	Positive specimens	≥300 Including specimens from different stages of infection and reflecting different antibody patterns. HCV genotypes 1 – 4: > 20 specimens (including non-a subtypes of genotype 4; HCV genotype 5: > 5 specimens; HCV genotype 6: as far as available	identification as "confirmed positive" or "indeterminate", not as "negative"
	Seroconversion panels	≥15 seroconversion panels/low titre panels	diagnostic sensitivity during seroconversion shall represent the state of the art
Diagnostic specificity	Blood donors	≥200	No false positive results/ no neutralisation
	Hospitalised patients	≥200	
Cross-reactivity	Potentially cross-reacting specimens	≥50 in total (including specimens from pregnant women, specimens with indeterminate results in other confirmatory assays)	

Table 4. Antigen tests: HCV antigen, HCV Ag/Ab (requirements for antigen detection)

Performance characteristic	Specimen	Specimen numbers, features, use	Acceptance criteria
Diagnostic sensitivity	Positive specimens	≥25 HCV core antigen and/or HCV RNA positive but anti-HCV negative specimens, comprising HCV genotypes 1-6 (if a genotype is not available, a justification shall be made)	all true positive specimens shall be identified as positive
	Seroconversion panels	≥20 seroconversion panels/low titre panels HCV seroconversion panels for the evaluation of HCV antigen and antibody combined tests shall start with one or more negative bleeds and comprise panel members from early HCV infection (HCV core antigen and/or HCV RNA positive but anti-HCV negative).	diagnostic sensitivity during seroconversion shall represent the state of the art HCV antigen and antibody combined tests shall demonstrate enhanced sensitivity in early HCV infection when compared to HCV antibody only tests.

Analytical sensitivity	WHO International Standard HCV core (PEI 129096/12)	Dilution series	
Diagnostic specificity	Blood donors	≥200	≥ 99,5 % after neutralisation or, if no neutralisation test available, after resolution of the specimen status
	Hospitalised patients	≥200	Potential limitations for specificity, if any, shall be identified
Cross-reactivity	Potentially cross-reacting specimens	≥50	

Table 5. Qualitative and quantitative NAT devices for HCV RNA

1. For target sequence amplification devices, a functionality control for each specimen (internal control) shall reflect the state of the art. This control shall as far as possible be used throughout the whole process, i.e. extraction, amplification/hybridisation, detection.

2. Genotype and/or subtype detection shall be demonstrated by appropriate primer or probe design validation and shall also be validated by testing characterised genotyped specimens.

3. Potential cross-reactivity of non-target nucleic acid sequences shall be analysed by appropriate primer or probe design validation and shall also be validated by testing selected specimens.

4. Results of quantitative NAT devices shall be traceable to international standards or calibrated reference materials, if available, and be expressed in international units utilised in the specific field of application.

Performance characteristic	Specimen	Specimen numbers, features, use	Acceptance criteria
Analytical sensitivity	WHO International Standard HCV RNA (or calibrated reference materials)	NAT sensitivity and NAT LOD shall be validated by dilution series of reference materials, testing of replicates (minimum 24) at different analyte concentrations, including those with transition from positive to negative results with the respective NAT device. LOD shall be expressed as 95% positive cut-off value (IU/ml) after statistical analysis (e.g. Probit). ([1]) quantitative NAT: definition of lower, upper quantification limit, precision, accuracy, 'linear' measuring range, 'dynamic range'. Reproducibility at different concentration levels	According to the state of the art

HCV genotype sensitivity	all relevant genotypes/subtypes, preferably from international reference materials potential substitutes for rare HCV genotypes (to be quantified by appropriate methods): in vitro transcripts; plasmids	Qualitative NAT: ≥10 specimens/genotype or subtype Quantitative NAT: dilution series for demonstration of quantification efficiencies	According to the state of the art
Diagnostic sensitivity	Positive specimens reflecting the routine conditions of users (e.g. no pre-selection of specimens)	Quantitative NAT: ≥100 Comparative results with another NAT system shall be generated in parallel	According to the state of the art
	Seroconversion panels	Qualitative NAT: ≥10 panels Comparative results with another NAT system shall be generated in parallel	According to the state of the art
Diagnostic specificity	Blood donor specimens	Qualitative NAT: ≥500 Quantitative NAT: ≥100	According to the state of the art
Cross-reactivity	Potentially cross-reacting specimens	>10 human flavivirus (e.g. HGV, YFV) positive specimens	According to the state of the art
Carry-over	High HCV RNA positive; HCV RNA negative	At least five runs with alternating high-positive and negative specimens shall be performed during robustness studies. The virus titres of the high positive specimens shall be representative of high virus titres occurring naturally.	According to the state of the art
Detection in relation to antibody status	HCV RNA positives: anti-HCV negative, anti-HCV positive	Pre-seroconversion (anti-HCV negative) and post-seroconversion (anti-HCV positive) specimens	According to the state of the art
Whole system failure rate	HCV RNA low-positive	≥100 HCV RNA low-positive specimens shall be tested. These specimens shall contain a virus concentration equivalent to three times the 95 % positive cut-off virus concentration.	≥99% positive

([1]) Reference: European Pharmacopeia 9.0, 2.6.21 Nucleic acid amplification techniques, Validation.

Table 6. Additional requirements for HCV self-tests

Performance characteristic	Specimens ([1])	Number of lay persons
Result interpretation ([2])	Interpretation of results ([3]) by lay persons reflecting the following range of reactivity levels: — non-reactive — reactive — weak reactive ([4]) — invalid	≥ 100
Diagnostic sensitivity	lay persons that are known positive	≥ 200
Diagnostic specificity	lay persons that do not know their status	≥ 400
	lay persons that are at high risk of acquiring the infection	≥ 200

([1]) For each body fluid claimed for use with the device, e.g. whole blood, urine, saliva, etc., the sensitivity and specificity of the device for self-testing in the hands of lay persons shall be defined against the confirmed patient infectious status.

([2]) The result interpretation study shall include reading and interpretation of the test results by at least 100 lay persons with each lay person subjected to reading results covering the specified range of result reactivity levels. The manufacturer shall determine the concordance between lay person reading and professional user reading.

([3]) Tests shall be performed prior to the result interpretation study using whenever possible the specimen type intended by the manufacturer. The tests may be performed on contrived specimens based on the natural matrix of the respective specimen type.

([4]) A higher proportion of the specimens shall be in the weak-positive range close to the cut-off or LOD of the test.

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ANNEX VI

COMMON SPECIFICATIONS FOR DEVICES INTENDED FOR DETECTION OR QUANTIFICATION OF MARKERS OF HEPATITIS B VIRUS (HBV) INFECTION

Scope

This Annex applies to devices intended for detection or quantification of markers of hepatitis B virus (HBV) infection.

Table 1 applies to first-line assays for hepatitis B surface antigen (HBsAg), and for antibodies against hepatitis B core antigen (anti-HBc) which are not rapid tests.

Table 2 applies to first-line assays for HBsAg and anti-HBc which are rapid tests.

Table 3 applies to confirmatory assays for HBsAg.

Table 4 applies to assays for the hepatitis B virus markers: hepatitis B surface antibodies (anti-HBs), IgM antibody against the hepatitis B core antigen (anti-HBc IgM), antibodies against the hepatitis Be antigen (anti-HBe) and hepatitis Be antigen (HBeAg).

Table 5 applies to qualitative and quantitative NAT devices for HBV deoxyribonucleic acid (DNA).

Table 6 applies to HBV self-tests.

Table 1. First-line assays: HBsAg, anti-HBc

Performance characteristic	Specimen	Specimen numbers, features, use	Acceptance criteria
Diagnostic sensitivity	Positive specimens	≥400 anti-HBc: including evaluation of different HBV markers HBsAg: including different HBV genotypes / subtypes / mutants anti-HBc or HBsAg: including 25 positive 'same day' fresh serum (≤ 1 day after specimen taking)	Overall performance shall be at least equivalent to the comparator device
	Seroconversion panels	HBsAg assays: ≥30 panels anti-HBc assays: to be defined when available	diagnostic sensitivity during seroconversion shall represent the state of the art (for anti-HBc this shall be the case if applicable)
Analytical sensitivity	WHO Third International Standard HBsAg (subtypes ayw1/adw2, HBV genotype B4, NIBSC code: 12/226)		For HBsAg assays: <0,130 IU/ml

Diagnostic specificity	Unselected blood donors (including first-time donors) ([1])	≥5 000	≥99,5%
	Hospitalised patients	≥200	Potential limitations for specificity, if any, shall be identified
Cross-reactivity	Potentially cross-reacting specimens	≥100 in total (e.g. RF+, from related virus infections, from pregnant women,)	

([1]) Blood donor populations shall be investigated from at least two blood donation centres and consist of consecutive blood donations, which have not been selected to exclude first-time donor.

Table 2. Rapid tests: HBsAg, anti-HBc

Performance characteristic	Specimen	Specimen numbers, features, use	Acceptance criteria
Diagnostic sensitivity	Positive specimens	≥400 including evaluation of different HBV markers including different HBV genotypes / subtypes / mutants	Overall performance shall be at least equivalent to that of the comparator device
	Seroconversion panels	HBsAg assays: ≥30 panels anti-HBc assays: to be defined when available	Diagnostic sensitivity during seroconversion shall represent the state of the art (for anti-HBc this shall be the case if applicable)
Diagnostic specificity	Unselected blood donors (including first-time donors)	≥1 000	HBsAg assays: ≥ 99 % anti-HBc assays: ≥ 99 %
	Hospitalised patients	≥200	Potential limitations for specificity, if any, shall be identified
Cross-reactivity	Potentially cross-reacting specimens	≥200 specimens from pregnant women ≥100 other potentially cross-reacting specimens in total (e.g. RF+, from related infections)	

Table 3. Confirmatory assays: HBsAg

Performance characteristic	Specimen	Specimen numbers, features, use	Acceptance criteria
Diagnostic sensitivity	Positive specimens	≥300 Including specimens from different stages of infection Including 20 'high positive' specimens (>26 IU/ml); 20 specimens in the cut-off range	Correct identification as positive (or indeterminate), not negative
	Seroconversion panels	≥15 seroconversion panels/low titre panels	Diagnostic sensitivity during seroconversion shall represent the state of the art
Analytical sensitivity	WHO Third International Standard for HBsAg, subtypes ayw1/adw2, HBV genotype B4, NIBSC code: 12/226		
Diagnostic specificity	Negative specimens	≥10 false positives as available from the performance evaluation of the first-line assay	No false positive results/ no neutralisation
Cross-reactivity	Potentially cross-reacting specimens	≥50	

Table 4. Assays for the HBV markers: anti-HBs, anti-HBc IgM, anti-HBe, HBeAg

Performance characteristic		anti-HBs	anti-HBc IgM	anti-HBe	HBeAg	Acceptance criteria
Diagnostic sensitivity	Positive specimens	≥100 vaccinees ≥100 naturally infected persons	≥200 Including specimens from different stages of infection (acute/chronic, etc.)	≥200 Including specimens from different stages of infection (acute/chronic, etc.)	≥200 Including specimens from different stages of infection (acute/chronic, etc.)	≥ 98 % (for anti-HBc IgM: applicable only on specimens from acute infection stage)
	Seroconversion panels	10 anti-HBs seroconversion panels or follow-up series	When available	When available	When available	Diagnostic sensitivity during seroconversion shall represent the state of the art (for anti-HBc IgM, anti-HBe, HBeAg this shall be the case if applicable)

Analytical sensitivity	Standards	WHO Second International Standard for anti-hepatitis B surface antigen (anti-HBs) immunoglobulin, human NIBSC code: 07/164		WHO First International Standard anti-hepatitis B virus e antigen (anti-HBe), PEI code 129095/12	WHO First International Standard for Hepatitis B Virus e Antigen (HBeAg) PEI code 129097/12 HBe	anti-HBs: < 10 mIU/ml
Diagnostic specificity	Negative specimens	≥500 Including clinical specimens ≥50 potentially interfering specimens	≥200 blood donations ≥200 clinical specimens ≥50 potentially interfering specimens	≥200 blood donations ≥200 clinical specimens ≥50 potentially interfering specimens	≥200 blood donations ≥200 clinical specimens ≥50 potentially interfering specimens	≥ 98 %

Table 5. Qualitative and quantitative NAT devices for HBV DNA

1. For target sequence amplification devices, a functionality control for each specimen (internal control) shall reflect the state of the art. This control shall as far as possible be used throughout the whole process, i.e. extraction, amplification/hybridisation, detection.

2. Genotype and/or subtype detection shall be demonstrated by appropriate primer or probe design validation and shall also be validated by testing characterised genotyped specimens.

3. Potential cross-reactivity of non-target nucleic acid sequences shall be analysed by appropriate primer or probe design validation and shall also be validated by testing selected specimens.

4. Results of quantitative NAT devices shall be traceable to international standards or calibrated reference materials, if available, and be expressed in international units utilised in the specific field of application.

Performance characteristic	Specimen	Specimen numbers, features, use	Acceptance criteria
Analytical sensitivity	WHO International Standard HBV DNA (or calibrated reference materials)	NAT sensitivity and NAT LOD shall be validated by dilution series of reference materials, testing of replicates (minimum 24) at different analyte concentrations, including those with transition from positive to negative results with the respective NAT device. LOD shall be expressed as 95% positive cut-off value (IU/ml) after statistical analysis (e.g. Probit). ([1]) quantitative NAT: definition of lower, upper quantification limit, precision, accuracy, 'linear' measuring range, 'dynamic range'. Reproducibility at different concentration levels	According to the state of the art

HBV genotype sensitivity	WHO International Reference Panel HBV DNA (HBV genotypes) all relevant genotypes/subtypes, preferably from international reference materials potential substitutes for rare HBV genotypes (to be quantified by appropriate methods): plasmids; synthetic DNA	Qualitative NAT: at least 10 specimens/genotype or subtype Quantitative NAT: dilution series for demonstration of quantification efficiencies	According to the state of the art
Diagnostic sensitivity	Positive specimens reflecting the routine conditions of users (no pre-selection of specimens)	Quantitative NAT: ≥100 Comparative results with another NAT system shall be generated in parallel	According to the state of the art
	Seroconversion panels	Qualitative NAT: ≥10 panels Comparative results with another NAT system shall be generated in parallel	According to the state of the art
Diagnostic specificity	Blood donor specimens	Qualitative NAT: ≥500 Quantitative NAT: ≥100	According to the state of the art
Cross-reactivity	Potentially cross-reacting specimens		According to the state of the art
Carry-over	High HBV DNA positive; HBV DNA negative	At least five runs with alternating high-positive and negative specimens shall be performed during robustness studies. The virus titres of the high positive specimens shall be representative of high virus titres occurring naturally.	According to the state of the art
Detection in relation to antibody status	HBV DNA positives: anti-HBV negative, anti-HBV positive	Pre-seroconversion (anti-HBV negative) and post-seroconversion (anti-HBV positive) specimens	According to the state of the art
Whole system failure rate	HBV DNA low-positive	≥100 HBV DNA low-positive specimens shall be tested. These specimens shall contain a virus concentration equivalent to three times the 95 % positive cut-off virus concentration.	≥99% positive

([1]) Reference: European Pharmacopeia 9.0, 2.6.21 Nucleic acid amplification techniques, Validation.

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Table 6. Additional requirements for HBV self-tests

Performance characteristic	Specimens ([1])	Number of lay persons
Result interpretation ([2])	Interpretation of results ([3]) by lay persons reflecting the following range of reactivity levels: — non-reactive — reactive — weak reactive ([4]) — invalid	≥100
Diagnostic sensitivity	lay persons that are known positive	≥200
Diagnostic specificity	lay persons that do not know their status	≥400
	lay persons that are at high risk of acquiring the infection	≥200

([1]) For each body fluid claimed for use with the device, e.g. whole blood, urine, saliva, etc., the sensitivity and specificity of the device for self-testing in the hands of lay persons shall be defined against the confirmed patient infectious status.

([2]) The result interpretation study shall include reading and interpretation of the test results by at least 100 lay persons with each lay person subjected to reading results covering the specified range of result reactivity levels. The manufacturer shall determine the concordance between lay person reading and professional user reading.

([3]) Tests shall be performed prior to the result interpretation study using whenever possible the specimen type intended by the manufacturer. The tests may be performed on contrived specimens based on the natural matrix of the respective specimen type.

([4]) A higher proportion of the specimens shall be in the low-positive range close to the cut-off or LOD of the test.

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ANNEX VII

COMMON SPECIFICATIONS FOR DEVICES INTENDED FOR DETECTION OR QUANTIFICATION OF MARKERS OF HEPATITIS D VIRUS (HDV) INFECTION

Scope

This Annex applies to devices intended for detection or quantification of markers of hepatitis D virus (HDV) infection.

Table 1 applies to devices intended for detection (including confirmation) or quantification of the following hepatitis D virus markers: antibodies against hepatitis D virus (anti-HDV), IgM antibodies against hepatitis D virus (anti-HDV IgM), the delta antigen.

Table 2 applies to qualitative and quantitative NAT devices for HDV RNA.

Table 1. Assays for HDV markers: anti-HDV, anti-HDV IgM, delta antigen

Performance characteristic		anti-HDV	anti-HDV IgM	Delta antigen	Acceptance criteria
Diagnostic sensitivity	Positive specimens	≥100 Specifying markers of HBV coinfection	≥50 Specifying markers of HBV coinfection	≥10 Specifying markers of HBV coinfection	≥ 98 %
Diagnostic specificity	Negative specimens	≥200 Including clinical specimens ≥50 potentially interfering specimens	≥200 Including clinical specimens ≥50 potentially interfering specimens	≥200 Including clinical specimens ≥50 potentially interfering specimens	≥ 98 %

Table 2. Qualitative and quantitative NAT devices for HDV RNA

1. For target sequence amplification devices, a functionality control for each specimen (internal control) shall reflect the state of the art. This control shall as far as possible be used throughout the whole process, i.e. extraction, amplification/hybridisation, detection.

2. Genotype and/or subtype detection shall be demonstrated by appropriate primer or probe design validation and shall also be validated by testing characterised genotyped specimens.

3. Potential cross-reactivity of non-target nucleic acid sequences shall be analysed by appropriate primer or probe design validation and shall also be validated by testing selected specimens.

4. Results of quantitative NAT devices shall be traceable to international standards or calibrated reference materials, if available, and be expressed in international units utilised in the specific field of application.

Performance characteristic	Specimen	Specimen numbers, features, use	Acceptance criteria
Analytical sensitivity	WHO First International Standard HDV RNA, PEI code 7657/12	NAT sensitivity and NAT LOD shall be validated by dilution series of reference materials, testing of replicates (minimum 24) at different analyte concentrations, including those with transition from positive to negative results with the respective NAT device. LOD shall be expressed as 95% positive cut-off value (IU/ml) after statistical analysis (e.g. Probit). ([1]) quantitative NAT: definition of lower, upper quantification limit, precision, accuracy, 'linear' measuring range, 'dynamic range'. Reproducibility at different concentration levels	According to the state of the art
HDV genotype sensitivity	all relevant genotypes/subtypes, preferably from international reference materials potential substitutes for rare HDV genotypes (to be quantified by appropriate methods): plasmids; synthetic RNA	Quantitative NAT: dilution series for demonstration of quantification efficiencies	According to the state of the art
Diagnostic specificity	Blood donor specimens	Qualitative NAT: ≥100 Quantitative NAT: ≥100	According to the state of the art
Cross-reactivity	Potentially cross-reacting specimens		According to the state of the art
Carry-over	High HDV RNA positive; HDV RNA negative	At least five runs with alternating high-positive and negative specimens shall be performed during robustness studies. The virus titres of the high positive specimens shall be representative of high virus titres occurring naturally.	According to the state of the art
Whole system failure rate	HDV RNA low-positive	≥100 HDV RNA low-positive specimens shall be tested. These specimens shall contain a virus concentration equivalent to three times the 95 % positive cut-off virus concentration.	≥99% positive

([1]) Reference: European Pharmacopeia 9.0, 2.6.21 Nucleic acid amplification techniques, Validation.

ANNEX VIII

COMMON SPECIFICATIONS FOR DEVICES INTENDED FOR DETECTION OF MARKERS OF VARIANT CREUTZFELDT-JACOB (vCJD) DISEASE

Scope

This Annex applies to devices intended for detection of markers of variant Creutzfeldt-Jakob disease (vCJD).

Table 1 applies to devices intended for detection of markers of vCJD.

Table 1. Devices for detection of markers of vCJD

Performance characteristic	Material	Number of specimens	Acceptance criteria
Analytical sensitivity	vCJD brain spikes in human plasma (WHO reference number NHBY0/0003)	≥24 replicates of each of three dilutions of the material WHO number NHBY0/0003 (1×10^4, 1×10^5, 1×10^6)	23 of the 24 replicates detected at 1×10^4
	vCJD spleen spikes in human plasma (10% spleen homogenate — NIBSC reference number NHSY0/0009)	≥24 replicates of each of three dilutions of the material NIBSC number NHSY0/0009 (1×10, 1×10^2 , 1×10^3)	23 of the 24 replicates detected at 1×10
Diagnostic sensitivity	Specimens from appropriate animal models	As many specimens as reasonably possible and available, and ≥10 specimens	90%
	Specimens from humans with known clinical vCJD	As many specimens as reasonably possible and available, and ≥10 specimens	90%
		Only in cases where 10 specimens are not available: — the number of specimens tested shall be between 6 and 9 — all available specimens shall be tested	max. one false negative result
Analytical specificity	Potentially cross-reacting specimens	≥100	
Diagnostic specificity	Normal human plasma specimens from area of low bovine spongiform encephalopathy (BSE) exposure	≥5 000	≥ 99,5 %

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ANNEX IX

COMMON SPECIFICATIONS FOR DEVICES INTENDED FOR DETECTION OR QUANTIFICATION OF MARKERS OF CYTOMEGALOVIRUS (CMV) INFECTION

Scope

This Annex applies to devices intended for detection or quantification of markers of cytomegalovirus (CMV) infection.

Table 1 applies to first-line assays for total antibodies against CMV (total anti-CMV) and IgG antibodies against CMV (anti-CMV IgG).

Table 2 applies to qualitative and quantitative NAT devices for CMV DNA.

Table 1. First-line assays: total anti-CMV and anti-CMV IgG

Performance characteristic	Specimens	Specimen number, features, use	Acceptance criteria
Diagnostic sensitivity	Positive specimens	≥400 including specimens from recent and past CMV infection, low and high positive titre specimens	≥ 99% sensitivity for confirmable past infection ([1]); overall sensitivity including recent infection ([2]) shall be at least equivalent to the comparator device
	Seroconversion panels	To be tested when available	Diagnostic sensitivity during seroconversion shall represent the state of the art
Analytical sensitivity	Standards	WHO International Standard anti-CMV IgG (PEI-code 136616/17) In case of titre determinations and quantitative statements	
Diagnostic specificity	Negative specimens	≥400 ([3]) CMV negative specimens from unselected donors, as compared to another CMV test.	≥ 99%
	Hospitalised patients ([4])	≥200	Potential limitations for specificity, if any, shall be identified
Cross-reactivity	Potentially cross-reacting ([5]) specimens	≥100 in total (e.g. RF+, related viruses or other infectious agents, pregnant women, etc.)	

([1]) Including testing of other CMV parameters (e.g. CMV-IgM, avidity, immunoblot) or previous / follow-up specimens to assess true specimen status.
([2]) Supplementary testing to confirm recent CMV infection (primary or re-infection): e.g. CMV-IgM, IgG-avidity, immunoblot analysis.
([3]) Corresponding to an initial number of 1000 donors at an assumed CMV prevalence of 60 %.
([4]) Including pre-transplant recipients.
([5]) Including related β-herpes viruses (HHV-6, HHV-7).

Table 2. Qualitative and quantitative NAT devices for CMV DNA

1. For target sequence amplification devices, a functionality control for each specimen (internal control) shall reflect the state of the art. This control shall as far as possible be used throughout the whole process, i.e. extraction, amplification/hybridisation, detection.

2. Genotype and/or subtype detection shall be demonstrated by appropriate primer or probe design validation and shall also be validated by testing characterised genotyped specimens.

3. Potential cross-reactivity of non-target nucleic acid sequences shall be analysed by appropriate primer or probe design validation and shall also be validated by testing selected specimens.

4. Results of quantitative NAT devices shall be traceable to international standards or calibrated reference materials, if available, and be expressed in international units utilised in the specific field of application.

Performance characteristic	Specimens	Specimen numbers, features, use	Acceptance criteria
Analytical sensitivity	WHO First International Standard Human CMV DNA (09/162; 5 000 000 IU/vial) (or calibrated reference materials)	NAT sensitivity and NAT LOD shall be validated by dilution series of reference materials, testing of replicates (minimum 24) at different analyte concentrations, including those with transition from positive to negative results with the respective NAT device. LOD shall be expressed as 95% positive cut-off value (IU/ml) after statistical analysis (e.g. Probit). ([1]) quantitative NAT: definition of lower, upper quantification limit, precision, accuracy, 'linear' measuring range, 'dynamic range'. Reproducibility at different concentration levels	According to the state of the art
Diagnostic sensitivity CMV Strain sensitivity	Patient specimens determined as CMV DNA positive by comparator device Dilution series of CMV positive cell cultures may serve as potential substitutes	Qualitative NAT: ≥100 Quantitative NAT: ≥100 dilution series for demonstration of quantification efficiencies	According to the state of the art
Diagnostic specificity	Blood donor specimens	Qualitative NAT: ≥500 Quantitative NAT: ≥100	According to the state of the art

Cross-reactivity	Potentially cross-reacting specimens	≥20 specimens in total Including human specimens positive for related human herpesviruses, e.g. EBV, HHV6, VZV Herpesvirus positive cell cultures may serve as potential substitutes	According to the state of the art
Carry-over	High CMV DNA positive; CMV DNA negative	At least five runs with alternating high-positive and negative specimens shall be performed during robustness studies. The virus titres of the high positive specimens shall be representative of high virus titres occurring naturally.	According to the state of the art
Whole system failure rate	CMV DNA low-positive	≥100 CMV DNA low-positive specimens shall be tested. These specimens shall contain a virus concentration equivalent to three times the 95 % positive cut-off virus concentration.	≥99% positive

([1]) Reference: European Pharmacopeia 9.0, 2.6.21 Nucleic acid amplification techniques, Validation.

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ANNEX X

COMMON SPECIFICATIONS FOR DEVICES INTENDED FOR DETECTION OR QUANTIFICATION OF MARKERS OF EPSTEIN-BARR VIRUS (EBV) INFECTION

Scope

This Annex applies to devices intended for detection or quantification of markers of Epstein-Barr virus (EBV) infection.

Table 1 applies to first-line assays for IgG antibodies against viral capsid antigen of EBV (anti-EBV VCA IgG).

Table 2 applies to qualitative and quantitative NAT devices for EBV DNA.

Table 1: First-line assays: anti-EBV VCA IgG

Performance characteristic	Specimens	Specimen number, features, use	Acceptance criteria
Diagnostic sensitivity	Positive specimens	≥400 including specimens from recent and past EBV infection, low and high positive titre specimens	≥ 99% for confirmable past infection ([1]); overall sensitivity including recent infection ([2]) shall be at least equivalent to the comparator device
	Seroconversion panels	To be tested when available	diagnostic sensitivity during seroconversion shall represent the state of the art
Analytical sensitivity	Standards	International reference reagents, when available	
Diagnostic specificity	Negative specimens	≥ 200 ([3]) EBV negatives from unselected donors as compared to another EBV device	≥ 99%
	Hospitalised patients ([4])	≥200	Potential limitations for specificity, if any, shall be identified
Cross-reactivity	Potentially cross-reacting specimens	≥100 in total (e.g. RF+, related viruses or other infectious agents, pregnant women, etc.)	

([1]) Including testing of other EBV markers and parameters (e.g. VCA-IgM, EBNA-1 IgG, immunoblot) or previous / follow-up specimens to assess the true specimen status.
([2]) Supplementary testing to confirm recent EBV infection: e.g. VCA-IgM, IgG-avidity, immunoblot analysis.
([3]) At an assumed EBV prevalence of 80 % corresponding to an initial number of 1000 donors.
([4]) Including pre-transplant recipients.

Table 2. Qualitative and quantitative NAT devices for EBV DNA

1. For target sequence amplification devices, a functionality control for each specimen (internal control) shall reflect the state of the art. This control shall as far as possible be used throughout the whole process, i.e. extraction, amplification/hybridisation, detection.

2. Genotype and/or subtype detection shall be demonstrated by appropriate primer or probe design validation and shall also be validated by testing characterised genotyped specimens.

3. Potential cross-reactivity of non-target nucleic acid sequences shall be analysed by appropriate primer or probe design validation and shall also be validated by testing selected specimens.

4. Results of quantitative NAT devices shall be traceable to international standards or calibrated reference materials, if available, and be expressed in international units utilised in the specific field of application.

Performance characteristic	Specimens	Specimen numbers, features, use	Acceptance criteria
Analytical sensitivity	WHO First International Standard Human EBV DNA (09/260; 5 000 000 IU/vial) (or calibrated reference materials)	NAT sensitivity and NAT LOD shall be validated by dilution series of reference materials, testing of replicates (minimum 24) at different analyte concentrations, including those with transition from positive to negative results with the respective NAT device. LOD shall be expressed as 95% positive cut-off value (IU/ml) after statistical analysis (e.g. Probit). (1) quantitative NAT: definition of lower, upper quantification limit, precision, accuracy, 'linear' measuring range, 'dynamic range'. Reproducibility at different concentration levels	According to the state of the art
Diagnostic sensitivity EBV strain sensitivity	Patient specimens determined as EBV DNA positive by comparator device Dilution series of EBV positive cell cultures may serve as potential substitutes	Qualitative NAT: ≥100 Quantitative NAT: ≥100 dilution series for demonstration of quantification efficiencies	
Diagnostic specificity	Negative specimens	Qualitative NAT: ≥500 Quantitative NAT: ≥100	According to the state of the art
Cross-reactivity	Potentially cross-reacting specimens	≥20 specimens in total Including human specimens positive for related human herpesviruses, e.g. CMV, HHV6, VZV Herpesvirus positive cell cultures may serve as potential substitutes	According to the state of the art

Carry-over	High EBV DNA positive; EBV DNA negative	At least five runs with alternating high-positive and negative specimens shall be performed during robustness studies. The virus titres of the high positive specimens shall be representative of high virus titres occurring naturally.	According to the state of the art
Whole system failure rate	EBV DNA low-positive	≥100 EBV DNA low-positive specimens shall be tested. These specimens shall contain a virus concentration equivalent to three times the 95 % positive cut-off virus concentration.	≥99% positive

([1]) Reference: European Pharmacopeia 9.0, 2.6.21 Nucleic acid amplification techniques, Validation.

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ANNEX XI

COMMON SPECIFICATIONS FOR DEVICES INTENDED FOR DETECTION OF MARKERS OF *TREPONEMA PALLIDUM* INFECTION

Scope

This Annex applies to devices intended for detection of markers of *Treponema pallidum* (*T. pallidum*).

Table 1 applies to first-line assays for antibodies against *T. pallidum* (anti-*T.pallidum*).

Table 2 applies to confirmatory and supplemental anti-*T.pallidum* assays.

Table 1. First-line assays: anti-*T.pallidum*

Performance characteristic	Specimens	Specimen number, features, use	Acceptance criteria
Diagnostic sensitivity	Positive specimens	≥200 positive specimens in total, at different stages of the infection if available, including high positive and low positive specimens, identified as positive by at least two different serological tests (one of which is an enzyme immunoassay) for different antibodies to *T.pallidum*	≥99.5% overall sensitivity
	Seroconversion panels	At least 1 seroconversion panel, ≥1 if possible, including individual specimens from the early infection phase	Diagnostic sensitivity during seroconversion shall represent the state of the art.
Analytical sensitivity	Standards	WHO international standards NIBSC code 05/132, when available	
Diagnostic specificity	Unselected blood donors (including first-time donors) ([1])	≥5 000	≥99,5%
	Hospitalised patients	≥200	Potential limitations for specificity, if any, shall be identified
Cross-reactivity	Potentially cross-reacting specimens	≥100 in total including the following specimens: positive for *Borrelia burgdorferi sensu lato* confirmed by IgG immunoblot; anti-HIV positive; RF+; other related microbial/infectious agents; systemic lupus erythematosus (SLE) patients; antiphospholipid antibody positive; pregnant women etc.	

([1]) Blood donor populations shall be investigated from at least two blood donation centres and consist of consecutive blood donations, which have not been selected to exclude first-time donors.

Table 2. Confirmatory and supplemental assays: anti-T.pallidum

Performance characteristic	Specimens	Specimen number, features, use	Acceptance criteria
Diagnostic sensitivity	Positive specimens	≥300 positive specimens at different stages of the infection (early primary syphilis, secondary stage, and during late syphilis) including high positive specimens, 50 low positive specimens, by at least two different serological tests (one of which is an enzyme immunoassay) for different antibodies to *T.pallidum*	99% identification as "confirmed positive" or "indeterminate"
	Seroconversion panels	At least 1 seroconversion panel, ≥1 if possible, including individual specimens from the early infection phase	Diagnostic sensitivity during seroconversion shall represent the state of the art
Analytical sensitivity	Standards	WHO international standards NIBSC code 05/132	
Diagnostic specificity	Blood donors	≥200	≥ 99%;
	Clinical specimens	≥200	Potential limitations for specificity, if any, shall be identified
Cross-reactivity	Potentially cross-reacting specimens	≥50 in total, including specimens from pregnant women and specimens with indeterminate results in other confirmatory assays.	

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ANNEX XII

COMMON SPECIFICATIONS FOR DEVICES INTENDED FOR DETECTION OR QUANTIFICATION OF MARKERS OF *TRYPANOSOMA CRUZI* INFECTION

Scope

This Annex applies for devices intended for detection or quantification of markers of *Trypanosoma cruzi* (*T. cruzi*) infection.

Table 1 applies to first-line assays for antibodies against *T. cruzi* (anti-*T. cruzi*).

Table 2 applies to confirmatory and supplemental anti-*T. cruzi* assays.

Table 3 applies to qualitative and quantitative NAT devices for *T. cruzi* DNA.

Table 1. First-line assays: anti-T. cruzi

Performance characteristic	Specimens	Specimen number, features, use	Acceptance criteria
Diagnostic sensitivity	Positive specimens	≥400 positive specimens, including highly positive confirmed by at least two different serological tests for different antibodies to *T.cruzi*. Of those 400, ≥25 parasite positive specimens which have been confirmed by direct detection.	99.5% overall sensitivity
	Seroconversion panels	To be defined when available	Diagnostic sensitivity during seroconversion shall represent the state of the art
Analytical sensitivity	Standards	WHO international standards NIBSC code: 09/186 NIBSC code: 09/188	
Diagnostic specificity	Unselected donors (including first-time donors) (1)	≥5 000	≥99,5%
	Hospitalised patients	≥200	Potential limitations for specificity, if any, shall be identified
Cross-reactivity	Potentially cross-reacting specimens	≥100 in total including the following specimens: positive for anti-*Toxoplasma gondii*; at least 5 specimens positive for anti-*Leishmania;* RF+; related microbial agents or other infectious agents; SLE patients; antiphospholipid antibody positive patients; pregnant women, etc.	

(1) Blood donor populations shall be investigated from at least two blood donation centres and consist of consecutive blood donations, which have not been selected to exclude first-time donors.

Table 2. Confirmatory and supplemental assays: anti-T. cruzi

Performance characteristic	Specimens	Specimen number, features, use	Acceptance criteria
Diagnostic sensitivity	Positive specimens	≥300 positive specimens, including highly positive confirmed by at least two different serological tests for different antibodies to *T.cruzi*. Of those 300, ≥25 parasite positive specimens, which have been confirmed by direct detection.	≥99% identification as "confirmed positive" or "indeterminate"
	Seroconversion panels	As available	Diagnostic sensitivity during seroconversion shall represent the state of the art, if applicable
Analytical sensitivity	Standards	WHO international standards NIBSC code: 09/186 NIBSC code: 09/188	
Diagnostic specificity	Negative specimens	≥200	≥99%
	Clinical specimens	≥200	Potential limitations for specificity, if any, shall be identified
Cross-reactivity	Potentially cross-reacting specimens	≥50 in total, including specimens from pregnant women and specimens with indeterminate results in other confirmatory assays	

Table 3: NAT devices for T. cruzi DNA

1. For target sequence amplification devices, a functionality control for each specimen (internal control) shall reflect the state of the art. This control shall as far as possible be used throughout the whole process, i.e. extraction, amplification/hybridisation, detection.

2. Genotype and/or subtype detection shall be demonstrated by appropriate primer or probe design validation and shall also be validated by testing characterised genotyped specimens.

3. Potential cross-reactivity of non-target nucleic acid sequences shall be analysed by appropriate primer or probe design validation and shall also be validated by testing selected specimens.

4. Results of quantitative NAT devices shall be traceable to international standards or calibrated reference materials, if available, and be expressed in international units utilised in the specific field of application.

Performance characteristic	Specimens	Specimen number, features, use	Acceptance criteria
Analytical sensitivity	Characterized in-house reference preparation (as long as international reference materials are not available)	NAT sensitivity and NAT LOD shall be validated by dilution series of reference materials, testing of replicates (minimal 24) at different analyte concentrations, including those with transition from positive to negative results with the respective NAT device. LOD shall be expressed as 95% positive cut-off value (IU/ml) after statistical analysis (e.g. Probit). ([1])	According to the state of the art
Diagnostic sensitivity: different *T.cruzi* strains / isolates	Patient specimens from different regions determined as *T.cruzi* DNA positive by comparator device; sequence variants	≥100 Dilution series of *T.cruzi* positive cell cultures (isolates) or *T.cruzi* positive materials from animal models may serve as potential substitutes	According to the state of the art
Diagnostic specificity	Negative specimens	≥100	According to the state of the art
Cross-reactivity	Potentially cross-reacting specimens	≥10 human specimens positive for other parasites, e.g. *Plasmodium* species, *Trypanosoma brucei*. Positive cell cultures may serve as potential substitutes	According to the state of the art
Carry-over		At least five runs with alternating high-positive and negative specimens shall be performed during robustness studies. The *T.cruzi* titres of the high positive specimens shall be representative of high *T.cruzi* titres occurring naturally.	According to the state of the art
Whole system failure rate		≥100 *T.cruzi* DNA low-positive specimens shall be tested. These specimens shall contain a *T.cruzi* concentration equivalent to three times the 95 % positive cut-off *T.cruzi* concentration.	≥99% positive

([1]) Reference: European Pharmacopeia 9.0, 2.6.21 Nucleic acid amplification techniques, Validation.

ANNEX XIII

COMMON SPECIFICATIONS FOR DEVICES INTENDED FOR DETECTION OR QUANTIFICATION OF MARKERS OF SEVERE ACUTE RESPIRATORY SYNDROME CORONAVIRUS 2 INFECTION

Scope

This Annex applies to devices intended for detection or quantification of markers of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection.

Table 1 applies to the following first-line assays (including rapid tests) for antibodies against SARS-CoV-2 (anti-SARS-CoV-2): total antibody, IgG-only, IgG combined with IgM and/or IgA.

Table 2 applies to first-line assays (including rapid tests) for detection of anti-SARS-CoV-2 IgM and/or IgA.

Table 3 applies to confirmatory or supplemental assays for anti-SARS-CoV-2.

Table 4 applies to antigen SARS-CoV-2 tests, including rapid antigen tests.

Table 5 applies to NAT assays for SARS-CoV-2 RNA.

Table 6 applies to SARS-CoV-2 antigen self-tests which have already undergone a performance evaluation for professional use.

Table 7 applies to SARS-CoV-2 antibody self-tests which have already undergone a performance evaluation for professional use.

Table 1: First-line assays (including rapid tests) for anti-SARS-CoV-2: total antibody, IgG-only, IgG combined ([1]) **with IgM and/or IgA**

Performance characteristic	Specimen	Specimen number, features, use	Acceptance criteria
Diagnostic sensitivity	Positive specimens	≥400 including specimens from early infection and post seroconversion ([2]) (within the first 21 days and after 21 days following the onset of symptoms); including specimens from asymptomatic or subclinical and mildly symptomatic (outpatient treatment) individuals; including specimens with low and high titers; including specimens from vaccinated individuals where appropriate ([3]); consideration of genetic variants	≥90% sensitivity ([4]) for specimens taken >21 days after onset of symptoms ([5]); overall sensitivity including the early infection phase shall be at least equivalent to the comparator device ([6])
	Seroconversion panels	As far as available	Seroconversion sensitivity comparable to other CE-marked devices

Analytical sensitivity	Reference preparations	WHO International Standard (IS) for anti- SARS-CoV-2 (NIBSC code 20/136); WHO International Reference Panel (RP) for anti-SARS-CoV-2 antibodies (NIBSC codes 20/140, 20/142, 20/144, 20/148, 20/150)	IS: for titre determinations / quantitative ([7]) result output; RP: all antibody assays
Diagnostic specificity	Negative specimens ([8])	≥400 specimens from non-infected and non-vaccinated individuals ([9])	>99% specificity ([10])
		≥200 hospitalised patients (without SARS-CoV-2 infection)	Potential limitations for specificity, if any, shall beidentified
Cross-reactivity	Potentially cross-reacting specimens	≥100 in total including RF+, pregnant women, specimens with antibodies against endemic human coronaviruses 229E, OC43, NL63, HKU1 and other pathogens of respiratory diseases such as influenza A, B, RSV etc.	

([1]) Performance claim of the combined overall result; for devices with separate claims for IgM and/or IgA, see table 2.
([2]) Details on the time interval between specimen taking and onset of symptoms (or time of infection, if available) shall be provided.
([3]) The manufacturer shall provide a justification of the suitability and timing for sensitivity evaluation of the relevant antibodies in vaccinated individuals.
([4]) Based on confirmed positive SARS-CoV-2 NAT result.
([5]) Claims for sensitivity shall be specified in relation to the time between specimen taking after symptom onset or the initial PCR diagnosis and the test.
([6]) CE marked under Regulation (EU) 2017/746 as class D, if available.
([7]) This applies to quantitative assays if they are also first-line assays.
([8]) Negative specimens shall be from individuals with no history of SARS-CoV-2 infection (if available pre-pandemic).
([9]) Individuals vaccinated against an antigen different from that used in the device may be included, if appropriate.
([10]) False positive results shall be resolved by re-testing in other SARS-CoV-2 serological assays, if necessary with different test design and antigen coating compared to the initial test, and/or confirmatory testing.

Table 2: First-line assays (including rapid tests) for anti-SARS-CoV-2: IgM and/or IgA detection

Performance characteristic	Specimen	Specimen number, features, use	Acceptance criteria
Diagnostic sensitivity	Positive specimens	≥200 ([1]) Specimens ([2]) with a significant proportion from the early phase of the infection (within 21 days after onset of symptoms) compared to specimens past seroconversion (>21 days after onset of symptoms); including specimens from asymptomatic, subclinical, mildly symptomatic (outpatient treatment) individuals; including freshly ([3]) vaccinated individuals if appropriate; consideration of genetic variants	≥80% sensitivity ([4]) for specimens taken during the first 21 days after symptom onset ([5]); overall sensitivity shall be at least equivalent to the comparator device ([6]) of the same type (i.e. IgM and/or IgA)

Seroconversion panels	As far as available	Seroconversion sensitivity comparable to other CE-marked devices	
Analytical sensitivity	Standards	N/A	N/A
Diagnostic specificity	Negative specimens ([7])	≥200 specimens from non-infected and non-vaccinated individuals ([8])	≥98% specificity ([9])
		≥100 from hospitalised patients (without SARS-CoV-2 infection)	Potential limitations for specificity, if any, shall be identified
Cross-reactivity	Potentially cross-reacting specimens	≥100 in total including RF+, pregnant women, specimens with antibodies against endemic human coronaviruses 229E, OC43, NL63, HKU1 and other pathogens of respiratory diseases such as influenza A, B, RSV etc.	

([1]) In case of devices detecting both IgM and IgA, 200 per marker IgM and IgA.
([2]) Details on the time interval between specimen taking and onset of symptoms (or time of infection, if available) shall be provided.
([3]) The manufacturer shall provide a justification of the suitability and timing for sensitivity evaluation of IgM and IgA in vaccinated individuals.
([4]) Diagnosis based on confirmed positive SARS-CoV-2 NAT result.
([5]) Claims for sensitivity shall be specified in relation to the time between specimen taking after symptom onset or the initial PCR diagnosis and the test.
([6]) CE marked under Regulation (EU) 2017/746 as class D, if available.
([7]) Negative specimens shall be from individuals with no history of SARS-CoV-2 infection (if available pre-pandemic).
([8]) Individuals vaccinated against an antigen different from that used in the device may be included, if appropriate.
([9]) False positive results shall be resolved by re-testing in other SARS-CoV-2 serological assays, if necessary with different test design and antigen coating compared to the initial test, and/or confirmatory testing. Clarification of false positive results may additionally include testing for presence of other anti-SARS-CoV-2 antibody types (IgA, IgG, total antibody).

Table 3: Confirmatory or supplemental ([1]) assays for anti-SARS-CoV-2

Performance characteristic	Specimen	Specimen number, features, use	Acceptance criteria
Diagnostic sensitivity	Positive specimens	≥200 including specimens pre and post seroconversion (within the first 21 days and after 21 days following the onset of symptoms)	Correct determination as “positive” (or “indeterminate”)
	Seroconversion panels/low titre panels	as far as available	

Analytical sensitivity	Standards	N/A	N/A
Diagnostic specificity	Negative specimens ([2])	≥200 from non-infected / non-vaccinated population	No false positive results; correct determination as "negative" (or "indeterminate")
		≥200 from hospitalised patients (without SARS-CoV-2 infection)	
Cross-reactivity	Potentially cross-reacting specimens	≥50 in total including specimens with antibodies against endemic human coronaviruses 229E, OC43, NL63, HKU1 and other pathogens of respiratory diseases such as influenza A, B, RSV etc.; including specimens with indeterminate or false positive results in other anti-SARS-CoV-2 assays	

([1]) E.g. immunoblot with antigens different from those used in the initial antibody test.
([2]) Negative specimens shall be from individuals with no history of SARS-CoV-2 infection (if available pre-pandemic).

Table 4: Antigen assays (including rapid tests): SARS-CoV-2

Performance characteristic	Specimen	Specimen number, features, use	Acceptance criteria
Diagnostic sensitivity	Positive specimens	≥100 ([1]) NAT positive specimens ([2]) from early infection within the first 7 days after symptom onset ([3]); specimens shall represent naturally occurring viral loads ([4]); consideration of genetic variants ([5]); consideration of variations in specimen collection and/or specimen handling ([6])	Detection of >80% (rapid tests); detection of >85% (lab-based assays ([7])); relative to SARS-CoV-2-NAT ([8]), ([9])
Analytical sensitivity	Standards	As soon as available	Establishment of a LOD ([10])
Diagnostic specificity	Negative specimens	≥300 from non-infected individuals	Specificity >98% (rapid tests) Specificity >99% (lab-based assays ([7]))
		≥100 from hospitalised patients	Potential limitations for specificity, if any, shall be identified
Cross-reactivity	Potentially cross-reacting specimens	≥50 in total including virus-positive specimens of endemic human coronaviruses 229E, OC43, NL63, HKU1; influenza A, B, RSV, and other pathogens of respiratory diseases, eligible for differential diagnosis; including bacteria ([11]) present in the specimen taking area	

(1) If the device is intended to be used for more than one specimen type, 100 specimens shall be required for each specimen type. If this is not possible in exceptional circumstances (e.g. if specimen collection is very invasive), the manufacturer shall provide a justification and evidence of matrix equivalence.
(2) Specimen taking shall be matched for antigen and NAT testing, e.g., two simultaneous specimens from each individual or optimally NAT- and antigen testing from the same specimen (e.g. from the eluate of one swab); the buffer/transport medium shall be compatible with antigen testing; any volume change in the buffer/medium for specimen uptake between antigen and NAT device shall be clearly communicated.
(3) Or time of infection, if known, taking into account the incubation time.
(4) I.e., without preselection; the viral loads and their distribution shall be shown, e.g. characterized by Ct-values of RT-PCR; or transformed into viral load per ml of specimen, if applicable.
(5) Depending on the design of the device and nature of the genetic variant. For the purpose of evaluation, at least 3 specimens shall be represented for each relevant genetic variant.
(6) Specimen collection and extraction items such as swabs, extraction buffers, etc., shall be part of the evaluation. If proprietary specimen taking/preparation is not included in the device, device performance shall be investigated for an applicable range of specimen taking devices. If the specimen is not tested immediately, e.g. after a certain transport time, stability of the antigen shall be investigated.
(7) Other than rapid tests, i.e. formal laboratory-based devices e.g. enzyme immunoassay, automated tests, etc.
(8) The sensitivity of ≥80%, ≥85% respectively, shall be for all specimen types claimed. All claimed specimen types shall be compared with paired NAT results from nasopharyngeal specimens.
(9) The relationship between the sensitivity of the antigen test and of the NAT shall be demonstrated; sensitivity may be shown relating to different viral load ranges and to the threshold of infectivity. The NAT and extraction method used shall be described.
(10) Unless there is an available international standard, analytical sensitivity may be tested by dilution series of in-house virus preparations, comparatively with other antigen tests and NAT; if inactivated virus is used, the effect of inactivation and freeze/thawing on the antigen shall be investigated.
(11) E.g. staphylococci and streptococci expressing protein A or G.

Table 5: NAT devices for SARS-CoV-2 RNA

Performance characteristic	Specimen	SARS-CoV-2 RNA qualitative	SARS-CoV-2 RNA quantitative
Sensitivity			
Analytical sensitivity: LOD	WHO First International Standard SARS-CoV-2 RNA (NIBSC code 20/146; 7.70 Log10 IU/mL) Secondary standards calibrated against WHO IS	According to Ph. Eur. NAT validation guideline: several dilution series into borderline concentration; statistical analysis (e.g. Probit analysis) on the basis of at least 24 replicates; calculation of 95 % cut-off value	According to Ph. Eur. NAT validation guideline: several dilution series of calibrated reference preparations into borderline concentration; statistical analysis (e.g. Probit analysis) on the basis of at least 24 replicates; calculation of 95 % cut-off value as LOD
Quantification limit; quantification features	WHO First International Standard SARS-CoV-2 RNA (NIBSC code 20/146; 7.70 Log10 IU/mL) Secondary standards calibrated against WHO IS		Dilutions (half-log10 or less) of calibrated reference preparations; determination of lower, upper quantification limit, LOD, precision, accuracy, "linear" measuring range, "dynamic range". Synthetic target nucleic acid may be used as secondary standard to achieve higher concentration levels. Reproducibility at different concentration levels to be shown

Diagnostic sensitivity: different SARS-CoV-2 RNA strains	Patient specimens determined as SARS-CoV-2 RNA positive by comparator device from different regions and outbreak clusters; sequence variants Dilution series of SARS-CoV-2 positive cell cultures (isolates) may serve as potential substitutes	≥100 ([1])	
Quantification efficiency	SARS-CoV-2 RNA positive patient specimens from different regions and outbreak clusters; sequence variants with quantitative values obtained by comparator device Dilution series of SARS-CoV-2 RNA positive cell cultures may serve as potential substitutes		≥100
Inclusivity	*In silico* analysis ([2]); at least two independent target gene regions in one test run (dual-target design)	Evidence of suitable device design: primer/probe sequence alignments with published SARS-CoV-2 sequences	Evidence of suitable device design: primer/probe sequence alignments with published SARS-CoV-2 sequences
Specificity			
Diagnostic specificity	SARS-CoV-2 RNA negative human specimens	≥500	≥100
In silico analysis ([2])		Evidence of suitable device design (sequence alignments); regular check of primer/probe sequences against sequence data bank entries	Evidence of suitable device design evidence (sequence alignments); regular check of primer/probe sequences against sequence data bank entries
Cross-reactivity	specimens positive (various concentrations) for related human coronaviruses 229E, HKU1, OC43, NL63, MERS coronavirus; SARS CoV-1 if available; Influenza virus A, B; RSV; *Legionella pneumophila*; positive cell cultures may serve as potential substitutes	≥20 in total	≥20 in total
Robustness			
Carry-over		At least 5 runs using alternating high positive and negative specimens. The virus titres of the high positive specimens shall be representative of high virus titres occurring naturally.	At least 5 runs using alternating high positive (known to occur naturally) and negative specimens

Inhibition		Internal control preferably to go through the whole NAT procedure	Internal control preferably to go through the whole NAT procedure
Whole system failure rate leading to false negative results: 99/100 assays positive		≥100 specimens virus-spiked with 3 × the 95 % positive cut-off concentration (3 x LOD)	≥100 specimens virus-spiked with 3 × the 95 % positive cut-off concentration (3 x LOD)

(1) If the device is intended to be used for more than one specimen type, 100 specimens shall be required for each specimen type. If this is not possible in exceptional circumstances (e.g. if specimen collection is very invasive), the manufacturer shall provide a justification and evidence of matrix equivalence.
(2) The manufacturer shall document evidence of proactive regular surveillance checks against updated data bank entries in the post-market performance follow-up report.

Table 6: Additional requirements for SARS-CoV-2 antigen self-tests (1)

Performance characteristic	Specimens (2)	Number of lay persons
Result interpretation (3)	Interpretation of results (4) by lay persons reflecting the following range of reactivity levels: — non-reactive — reactive — weak reactive (5) — invalid	≥100
Diagnostic sensitivity (6)	Lay persons that are known antigen positive (7), (8)	≥30
Diagnostic specificity (9)	Lay persons that do not know their status (5)	≥60

(1) It is assumed that the underlying performance of the self-test has already been previously demonstrated with the evaluation/assessment of a professional test of the same design as the respective self-test under evaluation. In case for the self-use specimens in question there is no corresponding professional test variant, comparison shall be made with the standard specimen type (e.g. nasopharyngeal swabs for antigen test, serum or plasma for antibody test) of the corresponding professional test.
(2) For each self-use specimen type claimed with the device (e.g. nasal specimen, sputum, saliva, whole blood, etc.).
(3) The result interpretation study shall include reading and interpretation of the test results by at least 100 lay persons, with each lay person subjected to reading results covering the specified range of result reactivity levels. The manufacturer shall determine the concordance between lay person reading and professional user reading.
(4) Tests shall be performed prior to the result interpretation study using whenever possible the specimen type intended by the manufacturer. Tests may be performed on contrived specimens based on the natural matrix of the respective specimen type.
(5) A higher proportion of the specimens shall be in the low-positive range close to the cut-off or LOD of the test.
(6) In comparison to RT-PCR. The manufacturer shall determine the concordance between lay person reading and professional user reading.
(7) Individuals unaware of the professional diagnostic result prior to self-testing, and performing the entire test procedure from specimen collection and specimen pre-treatment (swab, buffer extraction, etc.) to reading.
(8) Subjects up to about 7 days after symptom onset.
(9) The manufacturer shall determine the concordance between lay person reading and professional user reading.

Table 7: Additional requirements for SARS-CoV-2 antibody self-tests ([1])

Performance characteristic	Specimens ([2])	Number of lay persons
Result interpretation ([3])	Interpretation of results ([4]) by lay persons reflecting the following range of reactivity levels: — non-reactive — reactive — weak reactive ([5]) — invalid	≥100
Diagnostic sensitivity ([6])	Lay persons that are known antibody positive ([7])	≥100
Diagnostic specificity ([8])	Lay persons that do not know their status ([5])	≥100

([1]) It is assumed that the underlying performance of the self-test has already been previously demonstrated with the evaluation/assessment of a professional test of the same design as the respective self-test under evaluation. In case for the self-use specimens in question there is no corresponding professional test variant, comparison shall be made with the standard specimen type (e.g. nasopharyngeal swabs for antigen test, serum or plasma for antibody test) of the corresponding professional test.

([2]) For each self-use specimen type claimed with the device (e.g. nasal specimen, sputum, saliva, whole blood, etc.).

([3]) The result interpretation study shall include reading and interpretation of the test results by at least 100 lay persons, with each lay person subjected to reading results covering the specified range of result reactivity levels. The manufacturer shall determine the concordance between lay person reading and professional user reading.

([4]) Tests shall be performed prior to the result interpretation study using whenever possible the specimen type intended by the manufacturer. Tests may be performed on contrived specimens based on the natural matrix of the respective specimen type.

([5]) A higher proportion of the specimens shall be in the low-positive range close to the cut-off or LOD of the test.

([6]) With previous history of initial RT PCR-confirmed infection for SARS-CoV-2; in comparison to a previous confirmed antibody result. The manufacturer shall determine the concordance between lay person reading and professional user reading.

([7]) Individuals unaware of the professional diagnostic result prior to self-testing, and performing the entire test procedure from specimen collection and specimen pre-treatment (swab, buffer extraction, etc.) to reading.

([8]) The manufacturer shall determine the concordance between lay person reading and professional user reading.