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ANNEX I

Annexes I to XIII are amended as follows:

(1) Annex I is amended as follows:

(a) in Part I, the title is replaced by the following:
'Part I - Requirements for performance characteristics of devices covered by Annexes II to XX';

(b) in Part II, the title is replaced by the following:
'Part II - Requirements for performance characteristics of devices covered by Annexes III to XX';

(2) Annex II is amended as follows:

(a) the title is replaced by the following:
'COMMON SPECIFICATIONS FOR CLASS D DEVICES INTENDED FOR DETECTION OF BLOOD GROUP ANTIGENS IN THE ABO, RH, KELL, DUFFY AND KIDD BLOOD GROUP SYSTEMS';

(b) the first paragraph of the part 'Scope' is replaced by the following:
'This Annex applies to class D devices intended for detection of blood group antigens in the ABO, Rh, Kell, Duffy and Kidd blood group systems.';

(3) Annex III is amended as follows:

(a) the title is replaced by the following:
'COMMON SPECIFICATIONS FOR CLASS D DEVICES INTENDED FOR DETECTION OR QUANTIFICATION OF MARKERS OF HUMAN IMMUNODEFICIENCY VIRUS (HIV) INFECTION';

(b) in point 1, the first sentence is replaced by the following:

‘This Annex applies to class D devices intended for detection or quantification of markers of human immunodeficiency virus (HIV) infection.’;

(c) the title of Table 1, is replaced by the following:
‘Table 1. First-line assays (excluding rapid tests): anti-HIV-1/2, HIV-1/2 Ag/Ab (requirements for antibody detection)’;

the title of Table 2 is replaced by the following:
‘Table 2. First-line assays (rapid tests): anti-HIV-1/2, HIV-1/2 Ag/Ab (requirements for antibody detection)’;

(d) in Table 4, the second row ‘Analytical sensitivity’ is replaced by the following:

‘Analytical sensitivity	First International Reference Reagent HIV-1 p24 Antigen, NIBSC code: 22/230 WHO Reference Panel 1st WHO International Reference Panel for HIV-1 p24 Antigen, NIBSC code: 16/210		≤ 2 IU/ml for the reference reagent and reference panel’

;

(e) Table 5 is amended as follows:

(i) point 4 is replaced by the following:

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

(ii) in the table, the first row ‘Analytical sensitivity’ is replaced by the following:

‘Analytical sensitivity	WHO International Standard HIV-1 RNA; WHO International Standard HIV-2 RNA; or reference materials calibrated against those standards	Analytical 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). (2) 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’

;

(4) Annex IV is amended as follows:

(a) the title is replaced by the following:
‘COMMON SPECIFICATIONS FOR CLASS D DEVICES INTENDED FOR DETECTION OR QUANTIFICATION OF MARKERS OF HUMAN T-CELL LYMPHOTROPIC VIRUS (HTLV) INFECTION’;

(b) the first paragraph of the part ‘Scope’ is replaced by the following:
‘This Annex applies to class D devices intended for detection or quantification of markers of human T-cell lymphotropic virus (HTLV) infection.’;

(c) The title of Table 1 is replaced by the following:
‘Table 1. First-line assays (excluding rapid tests): anti-HTLV I/II’;

(d) in Table 2, the title is replaced by the following:
‘Table 2. First-line assays (rapid tests): anti-HTLV I/II’;

(e) Table 4 is amended as follows:

(i) point 4 is replaced by the following:

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

(ii) in the table, the first row ‘Analytical sensitivity’ is replaced by the following:

‘Analytical sensitivity	International reference preparations	Analytical 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).(2) 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’

;

(5) Annex V is amended as follows:

(a) the title is replaced by the following:
‘COMMON SPECIFICATIONS FOR CLASS D DEVICES INTENDED FOR DETECTION OR QUANTIFICATION OF MARKERS OF HEPATITIS C VIRUS (HCV) INFECTION’;

(b) the first paragraph of the part ‘Scope’ is replaced by the following:
‘This Annex applies to class D devices intended for detection or quantification of markers of hepatitis C virus (HCV) infection.’;

(c) the title of Table 1 is replaced by the following:
‘Table 1. First-line assays (excluding rapid tests): anti-HCV, HCV Ag/Ab (requirements for antibody detection)’;

(d) the title of Table 2, is replaced by the following:

'Table 2. First-line assays (rapid tests): anti-HCV, HCV Ag/Ab (requirements for antibody detection)';

(e) in Table 4, the first row 'Diagnostic sensitivity' and the second row 'Analytical sensitivity' are replaced by the following:

'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	Analytical sensitivity shall be expressed on the basis of the international standard'

(f) Table 5 is amended as follows:

(i) point 4 is replaced by the following:

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

(ii) in the table, the first row 'Analytical sensitivity' is replaced by the following:

‘Analytical sensitivity	WHO International Standard HCV RNA(or reference materials calibrated against that standard)	Analytical 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).(2) 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’

;

(6) Annex VI is amended as follows:

(a) the title is replaced by the following:
‘COMMON SPECIFICATIONS FOR CLASS D DEVICES INTENDED FOR DETECTION OR QUANTIFICATION OF MARKERS OF HEPATITIS B VIRUS (HBV) INFECTION’;

(b) the first paragraph of the part ‘Scope’ is replaced by the following:
‘This Annex applies to class D devices intended for detection or quantification of markers of hepatitis B virus (HBV) infection.’;

(c) Table 1 is amended as follows:

(i) the title is replaced by the following:
‘Table 1. First-line assays (excluding rapid tests): HBsAg, anti-HBc’;

(ii) the first row ‘Diagnostic sensitivity’ and the second row ‘Analytical sensitivity’ are replaced by the following:

‘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	HBsAg: WHO Third International Standard HBsAg (subtypes ayw1/adw2, HBV genotype B4, NIBSC code: 12/226) HBsAg genotype sensitivity: HBV Genotype Panel for HBsAg-Assays, 1st WHO International Reference Panel (PEI 6100/09) anti-HBc: WHO International Standard First International Standard for anti-Hepatitis B core antigen (anti-HBc), plasma, human, NIBSC code: 95/522		HBsAg international standard: <0,130 IU/ml HBsAg genotype panel: sensitivity across genotypes shall be comparable anti-HBc: analytical sensitivity shall be expressed on basis of the international standard’

(d) in Table 2, the title is replaced by the following:
‘Table 2. First-line assays (rapid tests): HBsAg, anti-HBc’;

(e) in Table 3, the second row ‘Analytical sensitivity’ is replaced by the following:

‘Analytical sensitivity	WHO Third International Standard for HBsAg, subtypes ayw1/adw2, HBV genotype B4, NIBSC code: 12/226		Analytical sensitivity shall be expressed on the basis of the international standard’

(f) in Table 4, the second row ‘Analytical sensitivity is replaced by the following:

‘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 in case of titre determinations and quantitative statements	anti-HBs: < 10 mIU/ml anti-HBe: analytical sensitivity shall be expressed on the basis of international standard HBeAg: analytical sensitivity shall be expressed on the basis of the international standard, where applicable’

;

(g) Table 5 is amended as follows:

(i) point 4 is replaced by the following:

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

(ii) in the table, the first row ‘Analytical sensitivity’ and the second row ‘HBVgenotype sensitivity’ are replaced by the following:

‘Analytical sensitivity	WHO International Standard HBV DNA (or reference materials calibrated against that standard)	Analytical 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).([2]) 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	HBV Genotype Panel for NAT-based Assays, 1st WHO International Reference Panel (PEI 5086/08) 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’

;

(7) Annex VII is amended as follows:

(a) the title is replaced by the following:
‘COMMON SPECIFICATIONS FOR CLASS D DEVICES INTENDED FOR DETECTION OR QUANTIFICATION OF MARKERS OF HEPATITIS D VIRUS (HDV) INFECTION’;

(b) the first paragraph of the part ‘Scope’ is replaced by the following:
‘This Annex applies to class D devices intended for detection or quantification of markers of hepatitis D virus (HDV) infection.’;

(c) Table 2 is amended as follows:

(i) point 4 is replaced by the following:

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

(ii) in the table, the first row ‘Analytical sensitivity’ is replaced by the following:

‘Analytical sensitivity	WHO First International Standard HDV RNA, PEI code 7657/12, or reference materials calibrated against that standard	Analytical 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’

;

(8) Annex VIII is amended as follows:

(a) the title is replaced by the following:
‘COMMON SPECIFICATIONS FOR CLASS D DEVICES INTENDED FOR DETECTION OF MARKERS OF VARIANT CREUTZFELDT-JACOB (vCJD) DISEASE’;

(b) the first paragraph of the part ‘Scope’ is replaced by the following:
‘This Annex applies to class D devices intended for detection of markers of variant Creutzfeldt-Jakob disease (vCJD).’;

(9) Annex IX is amended as follows:

(a) The title is replaced by the following:
‘COMMON SPECIFICATIONS FOR CLASS D DEVICES INTENDED FOR DETECTION OR QUANTIFICATION OF MARKERS OF CYTOMEGALOVIRUS (CMV) INFECTION’;

(b) The first paragraph of the part ‘Scope’ is replaced by the following:
‘This Annex applies to class D devices intended for detection or quantification of markers of cytomegalovirus (CMV) infection.’;

(c) in Table 1, the second row ‘Analytical sensitivity’ is replaced by the following:

‘Analytical sensitivity	Standards	WHO International Standard anti-CMV IgG (PEI-code 136616/17) in case of titre determinations and quantitative statements	OR Q, where applicable’

(d) Table 2 is amended as follows:

(i) point 4 is replaced by the following:

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

(ii) in the table, the first row ‘Analytical sensitivity’ is replaced by the following:

‘Analytical sensitivity	WHO First International Standard Human CMV DNA (09/162; 5 000 000 IU/vial) (or	Analytical 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	According to the state of the art’

	reference materials calibrated against that standard)	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).([6]) quantitative NAT: definition of lower, upper quantification limit, precision, accuracy, 'linear' measuring range, 'dynamic range'. Reproducibility at different concentration levels	

;

(10) Annex X is amended as follows:

(a) The title is replaced by the following:
'COMMON SPECIFICATIONS FOR CLASS D DEVICES INTENDED FOR DETECTION OR QUANTIFICATION OF MARKERS OF EPSTEIN-BARR VIRUS (EBV) INFECTION';

(b) the first paragraph of the part 'Scope' is replaced by the following:
'This Annex applies to class D devices intended for detection or quantification of markers of Epstein-Barr virus (EBV) infection.';

(c) Table 2 is amended as follows:

(i) point 4 is replaced by the following:

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

(ii) in the table, the first row 'Analytical sensitivity' is replaced by the following:

'Analytical sensitivity	WHO First International Standard Human EBV DNA (09/260; 5 000 000 IU/vial) (or reference materials calibrated against that standard)	Analytical 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	According to the state of the art'

		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). ([5]) quantitative NAT: definition of lower, upper quantification limit, precision, accuracy, 'linear' measuring range, 'dynamic range'. Reproducibility at different concentration levels	

(11) Annex XI is amended as follows:

(a) the title is replaced by the following:
'COMMON SPECIFICATIONS FOR CLASS D DEVICES INTENDED FOR DETECTION OF MARKERS OF *TREPONEMA PALLIDUM* (*T. PALLIDUM*) INFECTION';

(b) the first paragraph of the part 'Scope' is replaced by the following:
'This Annex applies to class D devices intended for detection of markers of *Treponema pallidum* (*T. pallidum*) infection.';

(c) in Table 1, the second row 'Analytical sensitivity' is replaced by the following:

'Analytical sensitivity	Standards	WHO international standard NIBSC code 05/132, when available	Analytical sensitivity shall be expressed on the basis of the international standard, where applicable'

;

(d) in Table 2, the second row 'Analytical sensitivity' is replaced by the following:

'Analytical sensitivity	Standards	WHO international standard NIBSC code 05/132, when available	Analytical sensitivity shall be expressed on the basis of the international standard'

;

(12) Annex XII is amended as follows:

(a) The title is replaced by the following:
'COMMON SPECIFICATIONS FOR CLASS D DEVICES INTENDED FOR DETECTION OR QUANTIFICATION OF MARKERS OF *TRYPANOSOMA CRUZI* (*T. CRUZI*) INFECTION';

(b) the part 'Scope' is amended as follows:

(i) the first paragraph is replaced by the following:
'This Annex applies to class D devices intended for detection or quantification of markers of *Trypanosoma cruzi* (*T.cruzi*) infection.';

(ii) the fourth paragraph is replaced by the following:

'Table 3 applies to qualitative and quantitative NAT devices for *T. cruzi* nucleic acid.';

(c) in Table 1, the second row 'Analytical sensitivity' is replaced by the following:

'Analytical sensitivity	Standards	WHO 1st International Standard for Chagas antibody (anti-*Trypanosoma cruzi* II), NIBSC code: 09/186WHO 1st International Standard for Chagas antibody (anti-*Trypanosoma cruzi* I), NIBSC code: 09/188 NIBSC codes 09/186 and 09/188 are also provided as Chagas antibody reference panel, NIBSC code: 11/216	Analytical sensitivity shall be expressed on the basis of international standards'

;

(d) in Table 2, the second row 'Analytical sensitivity' is replaced by the following:

'Analytical sensitivity	Standards	WHO 1st International Standard for Chagas antibody (anti-*Trypanosoma cruzi* II), NIBSC code: 09/186 WHO 1st International Standard for Chagas antibody (anti-*Trypanosoma cruzi* I), NIBSC code: 09/188 NIBSC codes 09/186 and 09/188 are also provided as Chagas antibody reference panel, NIBSC code: 11/216	Analytical sensitivity shall be expressed on the basis of international standards'

;

(e) Table 3 is amended as follows:

(i) the title is replaced by the following:

'Table 3: NAT devices for *T. cruzi* nucleic acid';

(ii) point 4 is replaced by the following:

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

(iii) in the table, the first row 'Analytical sensitivity' and the second row 'Diagnostic sensitivity: different *T.cruzi* strains/isolates' are replaced by the following:

'Analytical sensitivity	Characterised in-house reference preparation (as long as international reference materials are not available)	Analytical 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). ([2])	According to the state of the art

Diagnostic sensitivity: different *T.cruzi* strains / isolates	Patient specimens from different regions determined as *T.cruzi* nucleic acid positive by comparator device; sequence variants Dilution series of *T.cruzi* positive cell cultures (isolates) or *T.cruzi* positive materials from animal models may serve as substitutes.	≥100 specimens	According to the state of the art'

(iv) ;in the table, the last row 'Whole system failure rate' is replaced by the following:

'Whole system failure rate		≥100 *T.cruzi* nucleic acid 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'

;

(13) Annex XIII is amended as follows:

(a) the title is replaced by the following:
'COMMON SPECIFICATIONS FOR CLASS D DEVICES INTENDED FOR DETECTION OR QUANTIFICATION OF MARKERS OF SEVERE ACUTE RESPIRATORY SYNDROME CORONAVIRUS 2 (SARS-CoV-2) INFECTION';

(b) the first paragraph of the part 'Scope' is replaced by the following:
'This Annex applies to class D devices intended for detection or quantification of markers of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection.';

(c) in Table 1, the first row 'Diagnostic sensitivity' and the second row 'Analytical sensitivity' are replaced by the following:

‘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 according to the state of the art
Analytical sensitivity	Reference preparations	In case of titre determinations or quantitative ([7]) statements: WHO International Standard (IS) for anti- SARS-CoV-2 (NIBSC code 21/340); All antibody devices: WHO International Reference Panel for antibodies against SARS-CoV-2 variants of concern (NIBSC code22/270)	Analytical sensitivity shall be expressed on the basis of the international standard, where applicable, and on the basis of the reference panel’

;

(d) in Table 2, the first row ‘Diagnostic sensitivity’ is replaced by the following:

‘Diagnostic sensitivity	Positive specimens	≥200 ([11]) Specimens ([12]) 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;	≥80% sensitivity ([14]) for specimens taken during the first 21 days after symptom onset ([15]); overall sensitivity shall be at least equivalent to the comparator device ([16]) of the same type (i.e. IgM and/or IgA)

		including freshly ([13]) vaccinated individuals if appropriate; consideration of genetic variants	
	Seroconversion panels	As far as available	Seroconversion sensitivity according to the state of the art'

;

(e) in Table 5 the first row 'Analytical sensitivity: LOD' and the second row 'Quantification limit; quantification features' are replaced by the following:

'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 reference materials calibrated against the international standard or secondary standards 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 reference materials calibrated against the international standard or secondary standards; 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'

ANNEX II

'ANNEX XIV

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

Scope

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

Table 1 applies to qualitative and quantitative NAT devices for HEV RNA.

Table 1. Qualitative and quantitative NAT devices for HEV 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 reference materials calibrated against those standards, 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 HEV RNA PEI Code: 6329/10 (or reference materials calibrated against that standard)	Qualitative and Quantitative NAT: LOD – analytical NAT sensitivity and NAT limit 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 in the respective NAT assay. LOD shall be expressed as 95% positive cut-off value after statistical analysis (e.g. Probit).([1]) Quantitative NAT: Quantification Limit – definition of lower and upper quantification limit, precision, accuracy, 'linear' measuring range, 'dynamic range'. Reproducibility at different concentration levels	According to the state of the art
HEV genotype sensitivity	HEV RNA genotype panel for NAT-based assays, 1st WHO International Reference Panel (PEI 8578/13) Possible substitutes for rare HEV genotypes (to be quantified by appropriate methods): *in vitro* transcripts; Plasmids. Dilution series of HEV-positive cell cultures may serve as substitutes.	Qualitative NAT: ≥ 5 specimens/genotype, including the different subtypes (at least one specimen each, if available): e.g. HEV subtypes 1a-g; 3a-f, h, I, k-m; 4a-d, f-i; HEV genotype 2 – as many specimens as available 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 test system shall be generated in parallel	According to the state of the art
	Seroconversion panels	Qualitative NAT: ≥5 panels if available Comparative results with another NAT test system shall be generated in parallel	According to the state of the art
Diagnostic specificity	Blood donor specimens (negative specimens)	Qualitative NAT: ≥500 Quantitative NAT: ≥100	According to the state of the art
Cross-reactivity	Potentially cross-reacting specimens	Including blood-borne pathogens, other RNA viruses, enteroviruses and enteric pathogens	According to the state of the art
Carry-over	High HEV RNA positive; HEV RNA negative	≥5 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	HEV RNA positives: anti-HEV negative, anti-HEV positive	Pre-seroconversion (anti-HEV negative) and post- seroconversion (anti-HEV positive) specimens	According to the state of the art
Whole system failure rate	HEV RNA low positive	≥100 HEV 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 XV

COMMON SPECIFICATIONS FOR CLASS D DEVICES INTENDED FOR DETECTION OR QUANTIFICATION OF MARKERS OF *TOXOPLASMA GONDII* (*T. GONDII)* INFECTION

Scope

This Annex applies to class D devices intended for detection of markers of *Toxoplasma gondii* (*T. gondii*) infection.

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

Table 2 applies to first-line assays for anti-*T. gondii* IgM.

Table 3 applies to qualitative and quantitative NAT devices for *T. gondii* nucleic acid.

Table 1. First line assays: total anti-*T. gondii* and anti-*T. gondii* IgG

Performance characteristic	Specimens	Specimen numbers, features, use	Acceptance criteria
Diagnostic sensitivity	Positive specimens	≥400 including specimens from recent([1]) and past([2]) *T. gondii* infection; including low and high positive titre specimens	≥ 99% sensitivity for confirmed past infection; overall sensitivity including recent infection shall represent the state of the art
	Seroconversion panels	To be tested when available	Diagnostic sensitivity during seroconversion shall represent the state of the art

Analytical sensitivity	Standards	WHO International Standard for Antibodies, Human, to *Toxoplasma gondii* (NIBSC-code 13/132, 4th international standard.)	Analytical sensitivity shall be expressed on the basis of the international standard
Diagnostic specificity	Negative specimens	≥400([3]) *T. gondii* negative specimens from unselected donors	≥ 99%
	Hospitalised patients	≥200 including immunocompromised individuals	Potential limitations for specificity, if any, should be identified
Cross-reactivity	Potentially cross-reacting specimens	≥100 (e.g. RF+, other infectious agents, hyper IgG specimens, pregnant women etc.)	

Table 2. First-line assays: anti-*T. gondii* IgM

Performance characteristic	Specimens	Specimen numbers, features, use	Acceptance criteria
Diagnostic sensitivity	Positive specimens	≥100 specimens including ≥ 50 specimens from recent Toxoplasma infection([4]).	≥ 95%
	Seroconversion panels	To be tested when available	Diagnostic sensitivity during seroconversion shall represent the state of the art
Diagnostic specificity	Negative specimens	≥400 specimens from unselected donors	≥ 98%

	Hospitalised patients	≥200 including immunocompromised individuals	Potential limitations for specificity, if any, shall be identified
Cross-reactivity	Potentially cross-reacting specimens	≥100 (e.g. RF+, other infectious agents, hyper IgM specimens, pregnant women etc.)	

Table 3. Qualitative and quantitative NAT devices for *T.gondii* nucleic acid

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. Strain detection shall be demonstrated by appropriate primer or probe design validation and shall also be validated by testing characterised 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 or qualitative NAT devices shall be traceable to international standards or reference materials calibrated against those standards, 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 1st International Standard for *Toxoplasma gondii* DNA Nucleic Acid Amplification Techniques; NIBSC code: 10/242	Analytical NAT sensitivity and NAT LOD shall be validated by dilution series of reference materials calibrated against the WHO International Standard, 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). ([5])	According to the state of the art
Diagnostic sensitivity *Toxoplasma* strain sensitivity	Patient specimens determined as *T. gondii* nucleic acid positive by comparator device, including different types (I, II, III) and different circulating strains Dilution series of *T. gondii* positive cell cultures may serve as substitutes.	Qualitative NAT: ≥100 specimens dilution series including different types and strains for demonstration of detection efficiencies Quantitative NAT: ≥100 specimens dilution series including different types and strains 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	Including *in vitro* and/or *in silico* testing of other genera of *Coccidia* and other *Protozoa*	According to the state of the art
Carry-over	*T.gondii* nucleic acid high positive; *T.gondii* nucleic acid negative	≥5 runs with alternating high-positive and negative specimens shall be performed during robustness studies. The parasite titres of the high positive specimens shall be representative of high parasite titres occurring naturally.	According to the state of the art

Whole system failure rate	*T.gondii* nucleic acid low positive	≥100 *T.gondii* nucleic acid low-positive specimens shall be tested. These specimens shall contain a parasite concentration equivalent to three times the 95 % positive cut-off parasite concentration.	≥99% positive

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[1] Supplementary testing to confirm recent *T.gondii* infection: e.g. anti-*T.gondii* -IgM, low IgG-avidity or follow-up specimens (seroconversion).
[2] Including testing of a combination of other *T.gondii* parameters (e.g. anti-*T. gondii*-IgM, IgG-avidity, confirmatory immunoblot), or previous / follow-up specimens for true specimen status.
[3] Corresponding to an initial number of at least 800 donors at an assumed *T.gondii* prevalence of 50 %.
[4] Supplementary testing to confirm recent *T.gondii* infection: e.g. low IgG-avidity and/or follow-up specimens (seroconversion).
[5] Reference: European Pharmacopeia 9.0, 2.6.21 Nucleic acid amplification techniques, Validation

ANNEX XVI

COMMON SPECIFICATIONS FOR CLASS D DEVICES INTENDED FOR DETECTION OF MARKERS OF *PLASMODIUM* INFECTION

Scope

This Annex applies to class D devices intended for detection of markers of *Plasmodium* infection.

Table 1 applies to assays for IgG and total antibodies against *Plasmodium*.

Table 2 applies to qualitative NAT devices for *Plasmodium* nucleic acid.

Table 1. Anti-*Plasmodium (IgG, total antibodies)*

Performance characteristic	Specimens	Specimen number, features, use	Acceptance criteria
Diagnostic sensitivity	Positive specimens	≥400 positive specimens, including highly positive, confirmed by a diagnosis of *Plasmodium* infection and at least two different serological devices for different antibodies to *Plasmodium* including specimens positive for antibodies against all *Plasmodium* species intended to be detected Of those 400, at least 25 parasite positive specimens which have been confirmed by direct detection (e.g. microscopy or NAT)	≥99,5% overall sensitivity
	Seroconversion panels	To be tested when available	Diagnostic sensitivity during seroconversion shall represent the state of the art.

Analytical sensitivity	Standards	WHO Reference Reagent for Anti-malaria (*Plasmodiumfalciparum*) human serum, NIBSC code: 10/198 (falciparum) WHO Reference Reagent for Anti-Malaria (*Plasmodium vivax*) human plasma, NIBSC code: 19/198 (vivax)	Analytical sensitivity shall be expressed on the basis of the reference reagents
Diagnostic specificity	Unselected blood donors (including first-time donors) ([1])	≥5000	≥99,5%
	Individuals with symptoms (i.e febrile illness) and/or travel history in endemic countries	≥200	Potential limitations for specificity, if any, shall be identified
Cross-reactivity	Potentially cross-reacting specimens	≥100 Including the following specimens: positive for antibodies against HIV, HBV, HCV, *Babesia* sp.; at least 5 specimens positive for antibodies against related microbial agents or other infectious agents; antiphospholipid antibody positive patients.	

Table 2: NAT devices for *Plasmodium* nucleic acid

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 NAT devices shall be traceable to international standards or reference materials calibrated against those standards, if available.

Performance characteristic	**Specimens**	**Specimen number, features, use**	**Acceptance criteria**
Analytical sensitivity	WHO International Standard NIBSC code: 04/176 (*Plasmodium falciparum* DNA for NAT assays)	Analytical NAT sensitivity and NAT LOD should be validated by dilution series of the WHO IS reference materials calibrated against the WHO International Standard, 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). ([2])	According to the state of the art
Diagnostic sensitivity: different *Plasmodium* strains / species	Clinical specimens from different regions determined as *Plasmodium* nucleic acid positive by comparator device, including different strains of *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae* *P. knowlesi* if available	≥100 including dilution series of *Plasmodium* positive clinical specimens; including different strains. Cell cultures may serve as substitutes.	According to the state of the art
Diagnostic specificity	Negative specimens	≥500	According to the state of the art
Cross-reactivity	Potentially cross-reacting specimens	≥10 human specimens positive for other parasites, e.g., *Babesia* species, related microbial agents or other infectious agents. Cell cultures may serve as substitutes	According to the state of the art

Carry-over		≥5 runs with alternating high-positive and negative specimens shall be performed during robustness studies. The *Plasmodium* nucleic acid titres of the high positive specimens shall be representative of high *Plasmodium* nucleic acid titres occurring naturally.	According to the state of the art
Whole system failure rate		≥100 *Plasmodium* nucleic acid low-positive specimens shall be tested. These specimens shall contain a *Plasmodium* nucleic acid concentration equivalent to three times the 95% positive cut-off concentration.	≥99% positive

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[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.

[2] Reference: European Pharmacopeia 9.0, 2.6.21 Nucleic acid amplification techniques, Validation

ANNEX XVII

COMMON SPECIFICATIONS FOR CLASS D DEVICES INTENDED FOR DETECTION OR QUANTIFICATION OF MARKERS OF CHIKUNGUNYA VIRUS (CHIKV) INFECTION

Scope

This Annex applies to class D devices intended for detection or quantification of markers of Chikungunya virus (CHIKV) infection.

Table 1 applies to qualitative and quantitative NAT devices for CHIKV RNA.

Table 1. Qualitative and quantitative NAT devices for CHIKV 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 reference materials calibrated against those standards, 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	1st WHO International Standard for Chikungunya virus RNA for Nucleic Acid Amplification Techniques (NAT)-Based Assays PEI code **11785/16**; 2 500 000 International Units/mL	Analytical NAT sensitivity and NAT LOD shall be validated by dilution series of reference materials calibrated against the WHO International Standard, 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])	According to the state of the art

		quantitative NAT: definition of lower, upper quantification limit, precision, accuracy, 'linear' measuring range, 'dynamic range'. Reproducibility at different concentration levels	
Diagnostic sensitivity CHIKV strain sensitivity	Patient specimens determined as CHIKV RNA positive by comparator device Different CHIKV genotypes, including the lineages of West African, East/Central/South African (ECSA), Asian genotypes Dilution series of CHIKV positive cell cultures may serve as substitutes	Qualitative NAT: ≥ 100 specimens dilution series including different genotypes and lineages for demonstration of detection efficiencies Quantitative NAT : ≥ 100 specimens dilution series including different genotypes and lineages for demonstration of quantificationefficiencies	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	≥ 50 specimens in total Including human specimens positive for related arbovirus, e.g. other *Alphaviruses*, especially Onyong-nyong virus (ONNV); dengue virus (4 types), Zika virus, West Nile virus, yellow fever virus. Positive cell cultures of related arbovirus may serve as substitutes	According to the state of the art
Carry over	High CHIKV RNA positive (positive cell cultures may serve as substitutes); CHIKV 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	CHIKV RNA low-positive (cell cultures may serve as substitutes)	≥ 100 CHIKV 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 XVIII

COMMON SPECIFICATIONS FOR CLASS D DEVICES INTENDED FOR DETECTION OR QUANTIFICATION OF MARKERS OF DENGUE VIRUS (DENV) INFECTION

Scope

This Annex applies to devices intended for detection or quantification of markers of dengue virus (DENV) infection.

Table 1 applies to qualitative and quantitative NAT devices for DENV RNA.

Table 1. Qualitative and quantitative NAT devices for DENV 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 reference materials calibrated against those standards, 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	WHO International Reference Reagents (IRR) for DENV-1 RNA, DENV-2 RNA, DENV-3 RNA, DENV-4 RNA (FDA CBER, USA) with a unitage of 13 500, 69 200, 23 400, and 33 900 units/mL for DENV-1 to 4, respectively	Analytical NAT sensitivity and NAT LOD for the 4 types shall be validated by dilution series of reference materials calibrated against the WHO International Reagents, 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 (units/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	
Diagnostic sensitivity DENV type sensitivity	Patient specimens determined as DENV RNA positive by comparator device Inclusion of DENV types 1-4 Dilution series of DENV positive cell cultures may serve as substitutes	Qualitative NAT: ≥ 100 specimens (four types of DENV) dilution series including different types for demonstration of detection efficiencies Quantitative NAT: ≥ 100 specimens (four types of DENV) dilution series including different types 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	≥50 specimens in total Including human specimens positive for related arbovirus, e.g. Zika virus, chikungunya virus, West Nile virus, yellow fever virus. Positive cell cultures of related arbovirus may serve as substitutes In case of DENV type-specific NAT design, potential reactivity with further DENV types shall be investigated, both in silico and with respective specimens	According to the state of the art
Carry over	High DENV RNA positive (positive cell cultures may serve as substitutes); DENV RNA negative	At least five runs with alternating high-positive and negative specimens shall be performed during robustness studies. The virus titres of the	According to the state of the art

		high positive specimens shall be representative of high virus titres occurring naturally.	
Whole system failure rate	DENV RNA low-positive (cell cultures may serve as substitutes)	≥ 100 DENV 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 XIX

COMMON SPECIFICATIONS FOR CLASS D DEVICES INTENDED FOR DETECTION OR QUANTIFICATION OF MARKERS OF WEST NILE VIRUS (WNV) INFECTION

SCOPE

This Annex applies to devices intended for detection or quantification of markers of West Nile virus (WNV) infection.

Table 1 applies to qualitative and quantitative NAT devices for WNV RNA.

Table 1. Qualitative and quantitative NAT devices for WNV 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 reference materials calibrated against those standards, 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	1st WHO International Standard for West Nile Virus (WNV) RNA Nucleic acid Amplification Techniques NIBSC code: 18/206 (Lineage-1), 7,2 log10 IU/ml	Analytical NAT sensitivity and NAT LOD shall be validated by dilution series of reference materials calibrated against the WHO International Standard, testing of replicates (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

		Quantitative NAT: definition of lower, upper quantification limit, precision, accuracy, 'linear' measuring range, 'dynamic range'. Reproducibility at different concentration levels	
Diagnostic sensitivity WNV strain sensitivity	Patient specimens determined as WNV RNA positive by comparator device Inclusion of Lineage-1 and Lineage-2 ([2]) Dilution series of WNV positive cell cultures may serve as potential substitutes	Qualitative NAT: ≥ 100 specimens dilution series including both lineages for demonstration of detection efficiencies Quantitative NAT: ≥ 100 specimens dilution series including both lineages 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	≥ 50 specimens in total Including human specimens positive for related arboviruses, e.g. Usutu virus, tick-borne encephalitis virus, Japanese encephalitis virus, dengue virus (4 types), Chikungunya virus, Zika virus, yellow fever virus. Positive cell cultures of related arboviruses may serve as substitutes.	According to the state of the art
Carry-over	High WNV RNA positive (positive cell cultures may serve as substitutes); WNV 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	WNV RNA low-positive (cell cultures may serve as substitutes)	≥ 100 WNV 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

[2] Including the WHO International Reference Reagent for West Nile Virus Lineage-2 (WNV-L2) RNA Nucleic acid Amplification Techniques; NIBSC code: 18/208
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ANNEX XX

COMMON SPECIFICATIONS FOR CLASS D DEVICES INTENDED FOR DETECTION OR QUANTIFICATION OF MARKERS OF ZIKA VIRUS (ZIKV) INFECTION

Scope

This Annex applies to devices intended for detection or quantification of markers of Zika virus (ZIKV) infection.

Table 1 applies to qualitative and quantitative NAT devices for ZIKV RNA.

Table 1. Qualitative and quantitative NAT devices for ZIKV 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 reference materials calibrated against those international standards, 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	1st WHO International Standard for Zika virus RNA for Nucleic Acid Amplification Techniques (NAT)-Based Assays, PEI code 11468/16; 50 000 000 IU/ml	Analytical NAT sensitivity and NAT LOD shall be validated by dilution series of reference materials calibrated against the WHO International Standard, 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])	According to the state of the art

		quantitative NAT: definition of lower, upper quantification limit, precision, accuracy, 'linear' measuring range, 'dynamic range'. Reproducibility at different concentration levels	
Diagnostic sensitivity ZIKV strain sensitivity	Patient specimens determined as ZIKV RNA positive by comparator device Inclusion of different ZIKV strains from both Asian and African lineages Dilution series of ZIKV positive cell cultures may serve as substitutes	Qualitative NAT: ≥ 100 specimens dilution series including different lineages and strains for demonstration of detection efficiencies Quantitative NAT: ≥ 100 specimens dilution series including different lineages and strains 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	≥ 50 specimens in total Including human specimens positive for related arbovirus, e.g. Spondweni virus, dengue virus (4 types) chikungunya virus, West Nile virus, yellow fever virus. Positive cell cultures of related arbovirus may serve as substitutes	According to the state of the art
Carry over	High ZIKV RNA positive (positive cell cultures may serve as substitutes); ZIKV 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	ZIKV RNA low-positive (cell cultures may serve as substitutes)	≥ 100 ZIKV 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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