WNV-RNA lineage 1 and 2 reference panels

RUO

The kit insert contains a detailed protocol and should be read carefully before testing the run control to ensure optimal performance

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Overview WNV-RNA panels for sensitivity analysis

This package insert describes the West Nile Virus (WNV)-RNA Lineage 1 and 2 reference panels which can be used to establish the analytical sensitivity of nucleic acid amplification technology (NAT) assays. Table 1 presents an overview of available panels.

Table 1 Product overview: WNV-RNA reference panels

Catalogue nr.	Origin of standard	Product name	number samples
P0360	Italy	P0360 WNV-RNA Lineage 1	8
P0346	Macedonia	P0346 WNV-RNA Lineage 2 inactivated	8

Intended Use

The WNV-RNA Lineage 1 and 2 reference panels provide a consistent standard across NAT methods, enabling blood screening laboratories and *in vitro* diagnostics (IVD) manufacturers to assess the analytical sensitivity of molecular diagnostic test procedures for the qualitative detection of West Nile virus (WNV) in blood samples. This product can be used with amplification methods, including TMA and real-time PCR assays. The WNV-RNA reference panels are useful for establishing the limit of detection (LOD), acceptance testing of reagent lots, NAT system validation and training of operators. The product is for research use only and not for diagnostic use.

Key to Symbols Used

Manufacturer

Lot number

Catalogue number

Store below -65°C

Research use only

Biological substance category B

Date of manufacturing

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Summary and explanation

The WNV-RNA lineage 1 and 2 reference panels help ensure that NAT procedures for WNV-RNA are properly validated, and that test results are consistent across manufacturers, testing laboratories, operators, platforms and assay formats.

The P0360 WNV Lineage 1 reference panel is prepared from the S0164 standard derived from a tissue culture supernatant diluted in plasma. The isolate originated from an Italian patient. The P0346 WNV Lineage 2 reference panel is made from the S0169 WNV-RNA Lineage 2 standard in plasma and is inactivated by betapropiolactone[1,2]. The S0169 standard was prepared from WNV Lineage 2 tissue culture supernatant obtained during the 2010 outbreak in Macedonia (Nea Santa strain kindly provided by Prof. A Papa, Aristotle University of Thessaloniki)[3,4]. The plasma matrix in which the reference panels are diluted is manufactured from plasma units that tested negative for all relevant markers of blood borne viruses. The S0164 and S0169 WNV Lineage 1 and 2 standards have been calibrated in copies/mL against the ISS 0109 (Roche) and ISS 0410 standards[5,6] at the time World Health Organization (WHO) International Standards were not yet available.

For preparation of the reference panels the WNV-RNA standards were diluted in a pool of plasma units that tested negative for viral markers in individual donation NAT and serology testing. The viral concentrations in the reference panel are ensured by gravimetrically recorded dilutions from calibrated viral stock solutions stored at ≤ -65°C.

Traceability to WNV standards

The S0164 and S0169 WNV Lineage 1 and 2 standards were calibrated against the ISS 0410 and ISS 0109 WNV Lineage 1 standards kindly provided by Dr G. Pisani and Dr. K. Cristiano (Instituto Superiore di Sanità (ISS), Rome). These ISS standards have been calibrated in copies/mL and were directly traceable to the Roche standard and the Health Canada standard[7]. The calibration of the two standards against the ISS standards was performed by three laboratories using different real time PCR assays in which dilutions of the WNV Lineage 1 and 2 standards were tested in multiple replicates (n=6-18)[8]. Since one in house assay was not sensitive for WNV Lineage 2, the calibration of the S0169 Lineage 2 standard was based on the data reported by the Roche TaqScreen and GFE Blut PCR assays.

Table 2. Calibration result based multiple replicate tests with three WNV-RNA assays[8]

Standard	BioQ code	Final assigned WNV-RNA concentration to standard	
		methods#	Geomean cp/mL
WNV-RNA lineage 1 (=ISS 0410)^	S0166	3	1.00E+05
WNV-RNA lineage 1 (Italy)	S0164	3	1.02E+06[&]
WNV-RNA lineage 2 (Macedonia)	S0167	2	4.37E+09
WNV-RNA lineage 1 inact. (Italy)	S0168	3	7.56E+03
WNV-RNA lineage 2 inact. (Macedonia)	S0169	2	1,80E+07
WNV-RNA lineage 1 (ISS 0109)[$]	S0165	2	1.01E+03

^reference standard for calibration $ Roche standard (1000 copies/mL)
In house assay was excluded for calibration of WNV L2 standards
& later recalibrated in Roche cobas WNV assay to 2.60E+06 copies/mL

Later dilutions of the native S0164 standard and the inactivated S0169 standard were recalibrated in the Roche cobas WNV assay that was significantly more sensitive for the Health Canada WNV Lineage 1 standard than the previously used TaqScreen assay[9]. According to PCR detectable molecules in limiting dilution analysis and parallel line analysis of Ct values the concentration in the S0164 WNV Lineage 1 standard needed to be adjusted 2.56-fold to 2.60E+06 copies/mL, whereas the original assigned concentration to the S0169 WNV Lineage 2 standard was confirmed[8].

Materials Provided

Table 3 presents the composition of the WNV Lineage 1 and 2 reference panel members.

Table 3. Composition of WNV reference panels. Eight polypropylene tubes (10 mL) with screw caps containing 4.0 mL plasma

Catalogue no and panel name	member-id	copies/mL
P0360 WNV-RNA Lineage 1	B4342-xxx-01	300
	B4342-xxx-02	100
	B4342-xxx-03	30
	B4342-xxx-04	10
	B4342-xxx-05	3
	B4342-xxx-06	1
	B4342-xxx-07	0.3
	B4342-xxx-08	0.1
P0346 WNV-RNA Lineage 2 inactivated	B4319-xxx-01	300
	B4319-xxx-02	100
	B4319-xxx-03	30
	B4319-xxx-04	10
	B4319-xxx-05	3
	B4319-xxx-06	1
	B4319-xxx-07	0.3
	B4319-xxx-08	0.1

The tube identification is B4319- or B4342-xxx-panel member, where xxx is the sequential batch number. The identification is present in the bar-code and further explained on the tube label.

Materials not provided

Test kits and pipettes or pipetting devices for use in IVD test systems.

Storage Instructions

The panel should be stored below –65°C to ensure highest quality. At this temperature the panel is stable. Discard any unused material after the first use. Any panel member that appear cloudy or contain precipitates after thawing should be discarded.

Warning and precautions

Warning: The P0360 WNV-RNA lineage 1 panel members contain infectious virus. The P0346 WNV-RNA Lineage 2 reference panel is made from chemically inactivated virus. However the effectiveness of inactivation has not been established and therefore the panel members should be considered as potentially bio-hazardous. Apply the universal precautions for prevention of transmission of infectious agents when handling these materials[10,11]. Although the normal human plasma used in the production of this panel was negative for blood borne infectious disease markers the reference panel members should be handled as if capable of transmitting (unknown) infectious agents.

- Do not pipette by mouth.
- Use personal protective equipment, including lab coats, gloves and safety glasses.
- Do not eat, drink or smoke in areas where the reference panel is handled.
- Disinfect liquids, materials or spills with a 0.5% sodium hypochlorite solution or equivalent.
- Dispose of all materials and liquids used in the procedure as if they contained pathogenic agents.

Test procedure

- Thaw the panel members quickly in a water bath at 37°C.
- Mix gently during thawing until contents are just thawed.
- Immediately after thawing remove the panel member tube from the water bath.
- Mix the panel member(s).
- Give a short spin in a centrifuge before releasing screw cap from vial.
- Minimise the time period from thawing until usage of the samples.
- When samples are not immediately tested store at 2-8°C not longer than 8 hours.
- The panel member should be handled and tested in a manner identical to that of clinical specimens in the test procedure being evaluated.
- Do not refreeze panel members after thawing.

Interpretation of Results

Limit of detection (LOD)

Establishing the detection limit for screening assays is done by testing the entire panel multiple times. It is recommended to test critical concentrations with intermediate reactivity at least 12 times. The positive or negative results are interpreted using probit analysis[12]. Use results above and below 50 % LOD and apply log transformation on the concentration before performing probit analysis. It is recommended to report both the 50 and 95% LOD for optimal assessment of the analytical sensitivity. The limit of detection is often defined as the 95% LOD, but the 50% LOD can be determined with greater accuracy.

Only limited analytical sensitivity data are available on the P0360 and P0346 WNV Lineage 1 and 2 standard dilutions (table 4)

Table 4. Detection limits on WNV Lineage 1 and 2 standard dilution panels in Grifols Procleix and Roche cobas assays.

standard	Reference panel	NAT method	n	50% LOD (CI) copies/mL	95% LOD (CI) copies/mL
S0164 WNV-RNA Lineage 1	P0360 WNV Lineage 1	Cobas	5	1.1 (0.5-2.5)	6.7 (2.9-26.2)
S0169 WNV-RNA Lineage 2 inactivated	P0346 WNV Lineage 2	Cobas	10	0.9 (0.5-1.6)	5.7 (2.9-18.7)
		Procleix	12	1.7 (0.8-3.0)	15.1 (5.7-340)

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