Bio QControl

# P0346 WNV lineage 2 inactivated

# 1st draft version

RUO



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

Table of contents

Overview WNV-RNA panels for sensitivity analysis ..... 3
Intended Use ..... 3
Key to Symbols Used ..... 3
Summary and explanation ..... 3
Materials Provided ..... 4
Materials not provided ..... 4
Storage Instructions ..... 4
Warning and precautions ..... 4
Test procedure ..... 5
Interpretation of Results ..... 5
References ..... 7

## Overview WNV-RNA panels for sensitivity analysis

This insert describes the P0346 WNV-RNA lineage 2 inact. panel which can be used to establish sensitivity in screening assays and determination of accuracy, precision and lower limit of quantification, detection for quantitative WNV-RNA assays. Table 1 presents an overview of all available panels. All product names provide origin to standard and lineage.

**Table 1** product overview: 16 WNV-RNA reference panels

| Catalogue nr. | Product name | number samples |
|---|---|---|
| P0346 | P0346 WNV-RNA lineage 2 inactivated | 10 |

## Intended Use

The P0346 WNV-RNA lineage 2 inactivated reference panels provide a consistent standard across NAT methods, enabling blood screening laboratories and diagnostic manufacturers to assess the analytical sensitivity and quantification limits of molecular diagnostic test procedures for the qualitative and quantitative 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), limit of quantification (LOQ), batch acceptance, NAT system validation and training. The product is 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


Contents

Caution

Read instructions for use

## Summary and explanation

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

The BioQControl WNV-RNA lineage 2 standard used for preparation of the panel is derived from tissue culture diluted in plasma and chemically inactivated using beta-propriolacton[1,2,3]. The BioQControl WNV-RNA lineage 2 inactivated standard is calibrated against the ISS 0410 standard, and the 1:100 dilution of ISS 0410; ISS 0109[4,5] . The ISS 0410 is a secondary standard which is calibrated against the Health Canada HC-SC WNV Nat. Ref 001/03[6]. All standards were expressed in copies/ml. The calibration on the ISS standards was done using Roche cobas Taqman WNV test[7] and GFEblut WNV PCR kit[8]. The quantification was done using the Ct values found by these tests on standard dilutions series.

For preparation of the reference panel, the WNV-RNA lineage 2 inactivated standard is 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.

## Materials Provided

**Table 2** presents the **preliminary** quantification of the panel members. Ten (10) polypropylene tubes (10 mL) with screw caps (10 members), containing 4.0 mL. An confidence interval is not yet established.

| Catalogue nr. | member-id | copies/ml |
|---|---|---|
| P0346 | B4319-xxx-01 | 3000 |
| | B4319-xxx-02 | 1000 |
| | B4319-xxx-03 | 300 |
| | B4319-xxx-04 | 100 |
| | B4319-xxx-05 | 30 |
| | B4319-xxx-06 | 10 |
| | B4319-xxx-07 | 3 |
| | B4319-xxx-08 | 1 |
| | B4319-xxx-09 | 0.3 |
| | B4319-xxx-10 | 0.1 |

The tube identification is B4319-xxx-number, where xxx is the sequential batch number. The identification is present on 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 at –65°C or lower 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 P0346 WNV-RNA lineage 2 inactivated reference panel members contain infectious West Nile Virus which is chemically inactivated. However in vivo experiments on inactivation efficiency are not available and therefore the members are potentially bio-hazardous. Apply the universal precautions for prevention of transmission of infectious agents when handling these materials[9,10]. 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 members.
- 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. When you intend to test a member multiple times you should organize this within 8 hours duration. When not placed in the robot store at 2-8°C.**

## Interpretation of Results

**Limit of detection (LOD)**
Establishing the detection limit for screening assays is done by testing the whole panel multiple times, we recommend at least 12 times the concentrations with intermediate reactivity. The positive or negative results are interpreted using probit analysis[10]. Apply results above and below 50 % positive hit rate, while at least two concentrations with intermediate reactivity should be available. Apply the log transformation on the concentration before using the probit analysis. We advice to report both the 50 and 95 % hit rate for interpretation by third parties. The limit of detection is often defined as the 95 % hit rate.

**Limit of quantification (LOQ).**
The quantitation limit of an individual analytical procedure is the lowest amount in a sample which can be quantitatively determined with suitable precision and accuracy.

**Checking amplification efficiency.**
For nucleic acid test the relation between $^2$log(concentration) and $^2$log(quantitative results) or Ct value can be judged using linear regression. Ideally the slope of the curve should be -1.00. If the result is different consider to remove lower concentrations with intermittent reactivity. The slope is accepted when the confidence interval on the slope overlaps -1.00

**Calculation of precision.**
The accuracy around the LOQ becomes less. One should calculate for each measurement the - $^2$log(concentration) + $^2$log(result), $^2$log(result) can be replaced by –Ct value. For each concentration determine the average and standard deviation of the sum. The cumulative Chi-square distribution is used to compare the probability the SD of one concentration (s) is significantly different from the SD of all concentrations included:
n is number of measurements
Calculate SD on the log (concentration) or Ct value within one concentration evaluated (n>10): $s^2$, SD on the log (concentration) or Ct value of the reference members: $\sigma^2$
Calculate $X^2=(n-1)\ s^2/\sigma^2$

**Table 3**. Chi-square ($X^2$) values for p=0.05

| n-1 (df) | $X^2$ | n-1 (df) | $X^2$ | n-1 (df) | $X^2$ |
|---|---|---|---|---|---|
| 11 | 19.69 | 21 | 32.67 | 40 | 55.76 |
| 12 | 21.01 | 22 | 33.92 | 50 | 67.51 |
| 13 | 22.36 | 23 | 35.17 | 60 | 79.08 |
| 14 | 23.69 | 24 | 36.42 | 70 | 90.53 |
| 15 | 25.00 | 25 | 37.65 | 80 | 101.88 |
| 16 | 26.30 | 26 | 38.89 | 90 | 113.15 |
| 17 | 27.59 | 27 | 40.11 | 100 | 124.34 |
| 18 | 28.87 | 28 | 41.34 | | |
| 19 | 30.14 | 29 | 42.56 | | |
| 20 | 31.41 | 30 | 43.77 | | |

Interpretation:

Chi-square: $X^2$(Calculated) <$X^2$(p=0.05): SD is not significantly changed.

Chi-square: $X^2$ (Calculated) ≥$X^2$ (p=0.05) : SD has changed significantly.

We advice to report the LOQ as the lowest level having a consistent outcome; SD is not significantly increased.

**Comparison to given concentrations: accuracy**

For concentrations with SD's not significantly different, the average SD on the sum is calculated. When not use the SD for each concentration and report accordingly.

**Table 4**. Relation of Student t value and numbers of measurements (n) to calculate CI's.

| Run (n) | t-value at 95% C.I. | t-value at 99% C.I. |
|---|---|---|
| 10 | 2.306 | 3.355 |
| 20 | 2.101 | 2.878 |
| 30 | 2.048 | 2.763 |
| Infinite | 1.960 | 2.576 |

The lower limit (%) =$10^{(-\text{t-value x SD})}$ and higher limit (%)=$10^{(\text{t-value x SD})}$

**Calculation of accuracy**

Use all concentrations with an equal SD. Calculate delta = Log(concentration assigned) log(concentration measured) for each measurement. The accuracy = $10^{-\text{average delta}}$

Excel spreadsheets for performing the calculations are made available upon request.

## References


1. Stephan W, Dichtelmüller H, Prince AM, Brotman B, Huima T. Inactivation of the Hutchinson strain of hepatitis non-A, non-B virus in intravenous immunoglobulin by beta-propiolactone. J Med Virol. 1988; 26:227-32.
2. Yoshizawa H, Itoh Y, Iwakiri S, Kitajima K, Noguchi Y, Tachibana K, Nakamura T, Miyakawa Y, Mayumi M. Beta-propiolactone for the inactivation of non-A/non-B type 1 hepatitis virus capable of inducing cytoplasmic tubular ultrastructures in chimpanzees. Vox Sang. 1984; 46:86-91.
3. Scheidler A, Rokos, K, Reuter T, Ebermann R and Pauli G. Inactivation of Viruses by beta-propriolactone in Human Cryo Poor Plasma and IgG concentrates. Biologicals 1998; 26:136-144.
4. Pisani G, Pupella S., Marino F., Gaggioli A., Sambri V., Rossini G., Wirz M, Grazzini G and the Interlaboratory study group. Interlaboratory study to evaluate the performance of laboratories involved in West Nile virus RNA screening of blood and blood components by nucleic acid amplification testing in Italy. Blood Transfusion 2001: 9 425-429
5. Detection of West Nile virus RNA (lineages 1 and 2) in an external quality assessment programme for laboratories screening blood and blood components for West Nile virus by nucleic acid amplification testing. Giulio Pisani, Simonetta Pupella, Karen Cristiano, Francesco Marino, Matteo Simeoni, Francesca Luciani, Gabriella Scuderi, Vittorio Sambri, Giada Rossini, Paolo Gaibani, Anna Pierro, Maria Wirz, and Giuliano Grazzini Blood Transfus. 2012 Oct; 10(4): 515–520.
6. Establishment of the Saldanha J, Shead S, Heath A, et al. Collaborative study to evaluate a working reagent for West Nile virus RNA detection by nucleic acid testing. Transfusion 2005; 45: 97-102.
7. Insert Roche cobas Taqman WNV test
8. Insert GFEblut WNV PCR kit
9. Centers for Disease Control (CDC). Guidelines for prevention of transmission of human immunodeficiency virus and hepatitis B virus to health-care and public-safety workers. MMWR 1989; 38(S-6): 1-36.
10. Probit Analysis. [2nd ed.] by D. J. Finney

BioQControl B.V.
De Droogmakerij 31h
1851 LX Heiloo
The Netherlands
Tel: +31 (0)72 20 20 730
Fax: +31 (0)72 20 20 731
Internet: www.bioQControl.com

KI4297
V0.1 Nov 2019