Bio QControl

HCV-RNA 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 HCV-RNA panels for sensitivity analysis

This insert describes the following panels which can be used to establish sensitivity in screening assays and determination of accuracy, precision and lower limit of quantification, detection for quantitative HCV-RNA assays. Table 1 present an overview of all available panels. All product names provide origin to standard and genotype.

Table 1 product overview

Catalogue nr.	Product name	number samples
P0019	P0019 HCV-RNA genotype 1	10
P0020	P0020 HCV-RNA genotype 3 inact.	10
P0035	P0035 HCV-RNA genotype 2	10
P0036	P0036 HCV-RNA genotype 3	10
P0037	P0037 HCV-RNA genotype 4	10
P0038	P0038 HCV-RNA genotype 5	10
P0039	P0039 HCV-RNA genotype 6	10
P0061	P0061 HCV-RNA 3rd WHO standard	10
P0126	P0126 HCV genotype 4	8
P0127	P0127 HCV genotype 5	8
P0128	P0128 HCV genotype 6	8
P0129	P0129 HCV genotype 6n	8
P0130	P0130 HCV genotype 3b	8
P0131	P0131 HCV genotype 1a	8
P0132	P0132 HCV genotype 4a	8

Intended Use

The HCV-RNA 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 Hepatitis C virus (HBV) in blood samples. This product can be used with amplification methods, including TMA and real-time PCR assays and is useful for testing the analytical sensitivity, LOQ, LOD and qualification of new diagnostic kit lots or NAT system validation and training. It also can be used as a calibration panel in quantification of low HCV-RNA concentrations in the window phase. The product are research use only and not for diagnostic use.

Key to Symbols Used


Manufacturer

Lot number

Catalogue number

Store below -70°C

Research use only

Biological substance category B


Date of manufacturing

Contents


Caution


Read instructions for use

Summary and explanation

The HCV-RNA reference panels are designed for testing the analytical sensitivity or quantification limits of NAT methods. The reference panel helps ensure that NAT procedures for HCV-RNA are properly validated, and that test results are consistent across manufacturers, testing laboratories, operators, platforms and assay formats. Figure 1 present the relationship between the different standards which were used for calibration. The quantification is expressed in IU/ml and copies/ml; including confidence intervals.

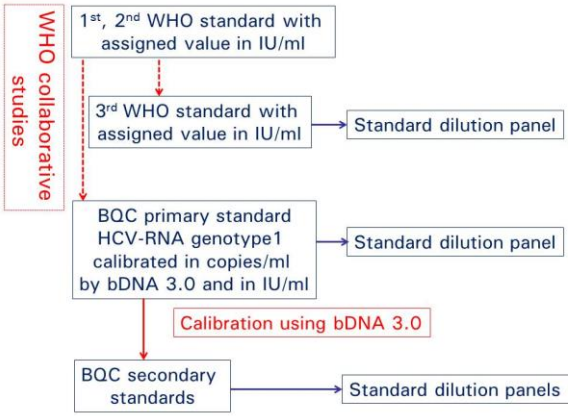

Figure 1; calibration relation between different HIV-RNA reference panels

The HCV-RNA reference panels are designed for testing the analytical sensitivity or quantification limits of NAT methods. The reference panels help ensure NAT procedures for HCV-RNA are properly validated, and test results are consistent across manufacturers, testing laboratories, operators, platforms and assay formats. The HCV-RNA reference panels were prepared from well characterised HCV-RNA plasma standard. The HCV-RNA genotype 1 is characterised in the Eurohep proficiency studies[1]. The HCV-RNA genotype 1 standard was tested multiple times in several dilutions in the Siemens Versant bDNA 3.0 (n=27) assay, which test is calibrated against in vitro RNA transcript copies quantified by physico-chemical methods[2]. When the bDNA results of the WHO calibration study were used for calculation, one IU of the first WHO HCV-RNA standard was found to be equivalent to 2.72 copies of the HCV-RNA genotype 1 standard[3]. The other HCV-RNA plasma standards were calibrated in bDNA 3.0 by multiple parallel testing. For preparation of the reference panels, the HCV-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. During validation studies in which the panels were applied we could confirm the quantification given in copies/ml using limiting dilution analysis[6,7,8,9].

Principles of the Evaluation Procedure

HCV-RNA reference panel members have been carefully formulated to mimic human plasma specimens containing low concentrations of HCV-RNA. The HCV-RNA reference panels are suitable for replicate testing and determination of the 95% and 50% detection limits of the NAT blood screening systems by probit analysis. The panels are also suitable for testing the lower quantification limit of viral load assays, such as real time PCR.

Materials Provided

Table 2 presents the quantification of the panel members, listed in table 1.Ten (10) or eight (8) polypropylene tubes (10 mL) with screw caps (8 or 10 members), containing 4.0 mL.

art_nr	member-id	IU/ml	copies/ml (95 % C.I.)
P0019	B4016-xxx-01	996	2720(2647-2798)
	B4016-xxx-02	363	992(965-1020)
	B4016-xxx-03	100	272(265-280)
	B4016-xxx-04	36.3	99.2(96.5-102)
	B4016-xxx-05	10.0	27.2(26.5-28.0)
	B4016-xxx-06	3.63	9.92(9.65-10.2)
	B4016-xxx-07	1.00	2.72(2.65-2.80)
	B4016-xxx-08	0.36	0.99(0.97-1.02)
	B4016-xxx-09	0.10	0.27(0.26-0.28)
	B4016-xxx-10	0.036	0.099(0.097-0.10)
P0020	B4031-xxx-01	1262	3444(2546-4661)
	B4031-xxx-02	424	1158(856-1567)
	B4031-xxx-03	126	344(255-466)
	B4031-xxx-04	42.4	116(85.6-157)
	B4031-xxx-05	12.6	34.4(25.5-46.6)
	B4031-xxx-06	4.24	11.6(8.56-15.7)
	B4031-xxx-07	1.26	3.44(2.55-4.66)
	B4031-xxx-08	0.42	1.16(0.86-1.57)
	B4031-xxx-09	0.13	0.34(0.25-0.47)
	B4031-xxx-10	0.042	0.12(0.086-0.16)
P0035	B4017-xxx-01	1039	2837(2291-3524)
	B4017-xxx-02	378	1033(834-1282)
	B4017-xxx-03	104	284(229-352)
	B4017-xxx-04	37.8	103(83.4-128)
	B4017-xxx-05	10.4	28.4(22.9-35.2)
	B4017-xxx-06	3.78	10.3(8.34-12.8)
	B4017-xxx-07	1.04	2.84(2.29-3.52)
	B4017-xxx-08	0.38	1.03(0.83-1.28)
	B4017-xxx-09	0.10	0.28(0.23-0.35)
	B4017-xxx-10	0.038	0.10(0.083-0.13)

art_nr	member-id	IU/ml	copies/ml (95 % C.I.)
P0036	B4018-xxx-01	656	1792(1541-2084)
	B4018-xxx-02	218	596(512-693)
	B4018-xxx-03	65.6	179(154-209)
	B4018-xxx-04	21.8	59.6(51.2-69.3)
	B4018-xxx-05	6.56	17.9(15.4-20.8)
	B4018-xxx-06	2.18	5.96(5.12-6.93)
	B4018-xxx-07	0.66	1.79(1.54-2.08)
	B4018-xxx-08	0.22	0.60(0.51-0.69)
	B4018-xxx-09	0	0.18(0.15-0.21)
	B4018-xxx-10	0	0.060(0.051-0.069)
P0037	B4019-xxx-01	726	1982(1503-2621)
	B4019-xxx-02	241	658(499-870)
	B4019-xxx-03	72.6	198(150-262)
	B4019-xxx-04	24.1	65.8(49.9-87.0)
	B4019-xxx-05	7.26	19.8(15.0-26.2)
	B4019-xxx-06	2.41	6.58(4.99-8.70)
	B4019-xxx-07	0.73	1.98(1.50-2.62)
	B4019-xxx-08	0.24	0.66(0.50-0.87)
	B4019-xxx-09	0.07	0.20(0.15-0.26)
	B4019-xxx-10	0.024	0.066(0.050-0.087)
P0038	B4020-xxx-01	903	2465(1905-3190)
	B4020-xxx-02	300	820(634-1062)
	B4020-xxx-03	90.3	246(191-319)
	B4020-xxx-04	30.0	82.0(63.4-106)
	B4020-xxx-05	9.03	24.6(19.1-31.9)
	B4020-xxx-06	3.00	8.20(6.34-10.6)
	B4020-xxx-07	0.90	2.46(1.91-3.19)
	B4020-xxx-08	0.30	0.82(0.63-1.06)
	B4020-xxx-09	0.090	0.25(0.19-0.32)
	B4020-xxx-10	0.030	0.082(0.063-0.11)

art_nr	member-id	IU/ml	copies/ml (95 % C.I.)
P0039	B4021-xxx-01	735	2006(1497-2687)
	B4021-xxx-02	244	665(497-891)
	B4021-xxx-03	73.5	201(150-267)
	B4021-xxx-04	24.4	66.5(49.7-89.1)
	B4021-xxx-05	7.35	20.1(15.0-26.9)
	B4021-xxx-06	2.44	6.65(4.97-8.91)
	B4021-xxx-07	0.73	2.00(1.50-2.69)
	B4021-xxx-08	0.24	0.67(0.50-0.89)
	B4021-xxx-09	0.073	0.20(0.15-0.27)
	B4021-xxx-10	0.024	0.067(0.050-0.089)
P0061	B4055-xxx-01	38700	
	B4055-xxx-02	12900	
	B4055-xxx-03	3870	
	B4055-xxx-04	1290	
	B4055-xxx-05	387	
	B4055-xxx-06	129	
	B4055-xxx-07	38.7	
	B4055-xxx-08	12.9	
	B4055-xxx-09	3.87	
	B4055-xxx-10	1.29	
P0126	B4126-xxx-01	107	293(232-371)
	B4126-xxx-02	35.8	97.6(77.3-123)
	B4126-xxx-03	10.7	29.3(23.2-37.1)
	B4126-xxx-04	3.58	9.76(7.73-12.3)
	B4126-xxx-05	1.07	2.93(2.32-3.71)
	B4126-xxx-06	0.36	0.98(0.77-1.23)
	B4126-xxx-07	0.11	0.29(0.23-0.37)
	B4126-xxx-08	0.036	0.098(0.077-0.12)

art_nr	member-id	IU/ml	copies/ml (95 % C.I.)
P0127	B4127-xxx-01	112	306(230-407)
	B4127-xxx-02	37.4	102(76.8-136)
	B4127-xxx-03	11.2	30.6(23.0-40.7)
	B4127-xxx-04	3.74	10.2(7.68-13.6)
	B4127-xxx-05	1.12	3.06(2.30-4.07)
	B4127-xxx-06	0.37	1.02(0.77-1.36)
	B4127-xxx-07	0.11	0.31(0.23-0.41)
	B4127-xxx-08	0.037	0.10(0.077-0.14)
P0128	B4128-xxx-01	86	234(147-260)
	B4128-xxx-02	28.5	77.9(49.2-86.4)
	B4128-xxx-03	8.58	23.4(14.8-26.0)
	B4128-xxx-04	2.85	7.79(4.92-8.64)
	B4128-xxx-05	0.86	2.34(1.48-2.60)
	B4128-xxx-06	0.29	0.78(0.49-0.86)
	B4128-xxx-07	0.086	0.23(0.15-0.26)
	B4128-xxx-08	0.029	0.078(0.049-0.086)
P0129	B4129-xxx-01	110	300(251-359)
	B4129-xxx-02	36.7	100(83.8-120)
	B4129-xxx-03	11.0	30.0(25.1-35.9)
	B4129-xxx-04	3.67	10.0(8.38-12.0)
	B4129-xxx-05	0.11	3.00(2.51-3.58)
	B4129-xxx-06	0.37	1.00(0.84-1.20)
	B4129-xxx-07	0.011	0.30(0.25-0.36)
	B4129-xxx-08	0.037	0.10(0.084-0.12)
P0130	B4130-xxx-01	110	300(245-369)
	B4130-xxx-02	36.7	100(81.6-123)
	B4130-xxx-03	11.0	30.0(24.5-36.9)
	B4130-xxx-04	2.31	6.31(5.15-7.75)
	B4130-xxx-05	1.10	3.00(2.45-3.69)
	B4130-xxx-06	0.23	0.63(0.51-0.78)
	B4130-xxx-07	0.11	0.30(0.25-0.37)
	B4130-xxx-08	0.023	0.063(0.051-0.077)

art_nr	member-id	IU/ml	copies/ml (95 % C.I.)
P0131	B4131-xxx-01	110	301(256-353)
	B4131-xxx-02	36.7	100(85.4-118)
	B4131-xxx-03	11.0	30.1(25.6-35.4)
	B4131-xxx-04	3.67	10.0(8.54-11.8)
	B4131-xxx-05	1.10	3.00(2.56-3.53)
	B4131-xxx-06	0.37	1.00(0.85-1.18)
	B4131-xxx-07	0.11	0.30(0.26-0.35)
	B4131-xxx-08	0.037	0.10(0.085-0.12)

The tube identification is Byyyy-xxx-number, where yyyy is product specific and xxx the sequential batch number. The identification is present on the bar-code and further explained on the tube label

Materials not provided

Pipettes or pipetting devices for use in IVD test systems.

Storage Instructions

It is recommended that the panel is 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 members that appear cloudy or contain precipitates after thawing should be discarded.

Warning and precautions

Warning: The HCV-RNA reference panel members contain infectious HCV and are potentially bio-hazardous (except P0020 which includes an chemically inactivated preparation). Observe the universal precautions for prevention of transmission of infectious agents when handling these materials. Although the normal human plasma used in the production of this panel was negative for 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.
- Do not refreeze panel members after thawing. In that case we cannot guarantee the claims given.

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.
- Vortex the run control.
- 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.

Expected assay response values

The expected quantitative results are given in table 2. The lowest concentrations are beyond the detection limit, or lower limit of quantification. These concentration will not react in all cases positive, or yield a quantitative result.

Interpretation of Results

Limit of detection

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 intepreted using probit analysis[11]. For an correct outcome both results above and below 50 % positive, and at least two concentrations with intermediate reactivity should be available. Apply the log transformation on the concentration before using the probit analysis. You should report both the 50 and 95 % hit rate for intepretation by third parties. The limit of detection is often defined as the 95 % hit rate.

Limit of quantification; precision and accuracy

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy.

- **Checking amplification efficiency**.
  For nucleic acid test a relation between log(concentration) and log(quantitative results) 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): $s^{2`}$

  Calculate SD on the log (concentration) or Ct value of the reference period: σ^2

  Calculate $X^2=(n-1)\ s^2/\sigma^2$

Table 4. 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) $\geq X^2$ (P=0.05) : SD has changed significantly.

For concentrations with SD's not significantly differing, the average SD on the sum is calculated. When not use the SD per concentration

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

Calculation of accuracy

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

On our website www.biogcontrol.com excell spreadsheets for performing the calculations are made available.

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