# cobas® DPX

## Duplex HAV & parvovirus B19 nucleic acid test for use on the cobas® 6800/8800 Systems

*For in vitro diagnostic use*

| | |
|---|---|
| **cobas® DPX – 96** | P/N: 07001088190 |
| **cobas® DPX Control Kit** | P/N: 07001126190 |
| **cobas® Buffer Negative Control Kit** | P/N: 07002238190 |
| **cobas omni MGP Reagent** | P/N: 06997546190 |
| **cobas omni Specimen Diluent** | P/N: 06997511190 |
| **cobas omni Lysis Reagent** | P/N: 06997538190 |
| **cobas omni Wash Reagent** | P/N: 06997503190 |

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# Intended use

The **cobas**® DPX test is an *in vitro* test for the direct quantitation of parvovirus B19 genotypes 1, 2, and 3 DNA and the direct qualitative detection of Hepatitis A virus (HAV) genotypes I, II, and III RNA in human plasma.

This test is intended for use as an in-process test to quantify parvovirus B19 DNA alone or to simultaneously quantify parvovirus B19 DNA and detect HAV RNA in plasma intended for further manufacture collected from donors of whole blood, blood components, or plasma. Plasma from all donors or manufacturing pools may be tested as individual samples or in pools comprised of aliquots of individual samples.

This test is not intended for use on samples of cord blood.

This test is not intended for use as an aid in diagnosis for parvovirus B19 or HAV.

# Summary and explanation of the test

### Background: Screening of blood for transfusion-transmitted viral infections

Human parvovirus B19 is a small, non-enveloped, single-stranded DNA virus that belongs to the genus *Erythrovirus* of the family *Parvoviridae*.[1] Human erythroviruses are grouped into three distinct genotypes: genotype 1 (B19 strains), genotype 2 (A6 strains), and genotype 3 (V9/D91/1 strains).[2,3] Nearly all virus samples are genotype 1.[1] Genotype 2 is found sporadically in the U.S., Europe, and South America, mostly in patients born before 1940.[1] Genotype 3 occurs primarily in North and West Africa, but it also has been identified in France.[1]

Parvovirus B19 is a common pathogen with a worldwide distribution. The prevalence of circulating B19 IgG antibodies, indicating a past infection, increase with age and ranges from about 20% of 1- to 4-year-olds, to greater than 60% of adults, and to as high as 85% in the geriatric population.[4-6] Although antibody is prevalent in the general population, viremia or the presence of viral DNA is rare.[4] The manifestation and severity of clinical illness depends on the immunologic and hematologic status of the infected individual.[1,7,8] In immunocompetent individuals, the infection is often asymptomatic or may result in mild, self-limiting illness, including erythema infectiosum ("fifth disease") in children or arthropathy in adults.[1, 7,9,10] Parvovirus B19, however, may cause severe disease, such as transient aplastic anemia in patients with hematologic disorders or hydrops fetalis, congenital anemia, and fetal loss in pregnant women.[1,7,11-13] The prevalence of parvovirus B19 in blood and plasma donors ranges from 0.16% to 0.9%, most with very low levels of viral DNA.[14-18] Studies from the plasma manufacturer sector report a lower prevalence.[19]

Parvovirus B19 is normally transmitted via the respiratory route, but transmission via plasma products, as well as red blood cell transfusion, can occur.[1,20] Detection of parvovirus B19 DNA in, as well as transmission to recipients of, plasma products, including Factor VIII concentrate, other coagulation factors, and solvent- detergent treated pooled plasma, are well described in the literature.[20-30] Plasma product-related transmission has been linked to the size of plasma pools; the incidence of acute, inapparent parvovirus B19 infections; high levels of viral DNA (up to $10^{12}$ IU/mL) in viremic donations; and the resistance of parvovirus B19 to most of the common viral inactivation or removal steps, such as solvent/detergent (S/D) treatment or pasteurization.[20,21,27-30] Very few clinical cases of parvovirus B19 transmission from red blood cell transfusion have been reported.[20] Further, transmission to recipients of components with low to moderate levels of parvovirus B19 DNA (< $10^6$ IU/mL) is extremely uncommon.[20]

HAV is a small, non-enveloped, RNA virus that belongs to the Hepatovirus group of the *Picornaviridae* family.[31] HAV has a

global distribution and is transmitted via the oral-fecal route, primarily by close personal contact.[32-34] Several genotypes and subtypes have been identified.[34] Epidemics are common in developing countries, where sanitation standards are low, and the infection is typically acquired early in life and results in a large proportion of the population having protective antibodies to HAV.[31-34] In industrialized countries, the decline in the incidence rate of the virus and the availability of vaccines has led to a shift toward infection in adulthood.[31,34] In Northern Europe, Japan, Canada, and the U.S., the prevalence of the virus in the general population is very low (~0.01%) and outbreaks are mainly associated with risk groups, such as travelers to endemic regions.[32,33]

HAV infections in humans range from asymptomatic infections, mainly seen in young children, to fulminant hepatitis, which in some cases may lead to death.[31,32] HAV causes an acute infection that resolves without a chronic carrier state; consequently, HAV is rarely a transfusion-related infection and blood banks do not test donations for the presence of HAV, relying instead on donor medical history to eliminate donors with a history of hepatitis.[35] Only a few HAV transfusion-transmitted infections, resulting in mild liver disease, have been reported.[36,37] Although infectious HAV can be found in blood during the serologic window period, the risk of transfusion transmission of HAV is very low.[35,38] HAV transmission from donors in an asymptomatic viremic state have also been reported.[35-38] HAV does not have a lipid envelope and, thus, is not easily inactivated via S/D treatment or pasteurization, such as during plasma derivative manufacture.[35] As a result, HAV transmission through plasma products, mainly coagulation factors, have been reported.[36,39,40]

While a single donation may contain both parvovirus B19 DNA and HAV RNA, the prevalence of parvovirus B19/ HAV co-infection in the donor population is not well studied or documented in the literature.[41-43] There are rare reported cases of human parvovirus B19/ HAV co-infection in infants and children, who are not part of the donor population.[41-43] The risk of co-infection may be calculated using the prevalence of each virus. Although the prevalence of HAV is not well characterized in the donor population, the prevalence in the general population is ~0.01% and lower in source plasma donor population (~0.0004%).[32,33,44,45] Using a prevalence of parvovirus B19 of ~0.9%[14-18], the calculated risk of parvovirus B19/HAV co-infection is ~0.0000036% (0.0004% x 0.9%) or 1 in ~28,000,000 donations.

### Rationale for NAT testing

NAT testing may be used to detect HAV and parvovirus B19 contamination. In early 2000, some plasma manufacturers initiated screening of plasma for further manufacture with HAV RNA and parvovirus B19 DNA NAT testing in response to reports of plasma-product transmission of both viruses.[46] The NAT testing, considered in-process testing, was aimed at removing any HAV-contaminated units and reducing the parvovirus B19 burden in plasma pools.[47] In 2004, the European Pharmacopoiea began to require all manufacturers to ensure that parvovirus B19 DNA levels must be below $10^4$ IU/mL in manufacturing pools used for production of human anti-D immunoglobulin and pooled human plasma treated for virus inactivation.[48] Similarly, a 2009 FDA Guidance recommends plasma product manufacturers perform parvovirus B19 NAT on all plasma-derived products to ensure that the viral load of parvovirus B19 DNA in the manufacturing pool does not exceed $10^4$ IU/mL.[47] Neither U.S. nor European regulatory agencies currently require HAV NAT testing on plasma pools used for further manufacture; however, European regulatory requirements provide that, if HAV NAT tests are used on the manufacturing pool as part of in process testing, the test be capable of detecting a control containing 100 IU/mL HAV RNA.[49]

### Explanation of the test

The **cobas**® DPX test is a duplex test that is run on the **cobas**® 6800 System and **cobas**® 8800 System. The **cobas**® DPX test enables the simultaneous quantitation of parvovirus B19 genotypes 1, 2, and 3 DNA and the qualitative detection of hepatitis A virus (HAV) genotypes I, II, and III RNA in human plasma.

### Principles of the procedure

The **cobas**® DPX test is based on real time PCR technology on a fully automated sample preparation (nucleic acid extraction and purification) followed by PCR amplification and detection. The **cobas**® 6800/8800 Systems consist of the sample supply module, the transfer module, the processing module, and the analytic module. Automated data management is performed by the **cobas**® 6800/8800 software which assigns test results for a quantitative value (in IU/mL) for the parvovirus B19 through the use of a Quantitation Standard (QS), directly traceable to the WHO B19 International Standard.[47] The **cobas**® 6800/8800 software also assigns test results for presence of hepatitis A virus as non-reactive, reactive, or invalid. Results can be reviewed directly on the system screen, and printed as a report.

Samples can either be tested individually or, optionally, can be tested in pools consisting of multiple samples. If pooling is to be performed, the **cobas p** 680 instrument, or **cobas**® **Synergy** software with the Hamilton Microlab® STAR IVD (**cobas**® **Synergy** Core), may optionally be used in a pre-analytical step.

Nucleic acid from the donor sample and added armored RNA internal control (IC) as well as DNA QS molecules (which serve as the sample preparation and amplification/detection process control) is simultaneously extracted. Viral nucleic acids are released by addition of proteinase and lysis reagent to the sample. The released nucleic acid binds to the silica surface of the added magnetic glass particles. Unbound substances and impurities, such as denatured protein, cellular debris, and potential PCR inhibitors (such as hemoglobin) are removed with subsequent wash reagent steps and purified nucleic acid is eluted from the magnetic glass particles with elution buffer at elevated temperature.

Selective amplification of target nucleic acid from the donor sample is achieved by the use of virus-specific forward and reverse primers which are selected from highly conserved regions of the viral nucleic acid. A thermostable DNA polymerase enzyme is used for both reverse-transcription and amplification. The master mix includes deoxyuridine triphosphate (dUTP), instead of deoxythimidine triphosphate (dTTP), which is incorporated into the newly synthesized DNA (amplicon).[50-52] Any contaminating amplicon from previous PCR runs are destroyed by the AmpErase enzyme [uracil-N-glycosylase], which is included in the PCR master mix, when heated in the first thermal cycling step. However, newly formed amplicon are not destroyed since the AmpErase enzyme is inactivated once exposed to temperatures above 55°C.

The **cobas**® DPX master mix contains detection probes which are specific for B19 and HAV as well as QS and IC nucleic acid. The specific B19, HAV, IC, and QS detection probes are each labeled with one of four unique fluorescent dyes which act as a reporter. Each probe also has a fifth dye which acts as a quencher. The four reporter dyes are measured at defined wavelengths, thus permitting simultaneous detection and discrimination of the amplified B19, HAV targets, IC, and QS.[53,54] The fluorescent signal of the intact probes is suppressed by the quencher dye. During the PCR amplification step, hybridization of the probes to the specific single-stranded DNA template results in cleavage by the 5' to 3' nuclease activity of the DNA polymerase resulting in separation of the reporter and quencher dyes and the generation of a fluorescent signal. With each PCR cycle, increasing amounts of cleaved probes are generated and the cumulative signal of the reporter dye is concomitantly increased. Since the four specific reporter dyes are measured at defined wavelengths, simultaneous detection and discrimination of the amplified B19 and HAV target and the QS and IC is possible.

# Reagents and materials

## cobas® DPX reagents and controls

All unopened reagents and controls shall be stored as recommended in Table 1 to Table 4.

**Table 1 cobas®** DPX test

**cobas® DPX test**
Store at 2-8°C
96 test cassette (P/N 07001088190)

| Kit components | Reagent ingredients | Quantity per kit |
|---|---|---|
| **Proteinase Solution (PASE)** | Tris buffer, < 0.05% EDTA, calcium chloride, calcium acetate, 8% proteinase<br>EUH210: Safety data sheets available upon request.<br>EUH208: Contains Subtilisin, 9014-01-1. May produce an allergic reaction. | 13 mL |
| **DPX Internal Control and Quantitation Standard (DPX IC/QS)** | Tris buffer, < 0.05% EDTA, < 0.01% internal control armored RNA construct (non-infectious RNA encapsulated in MS2 bacteriophage), < 0.01% non-infectious, synthetic QS B19 DNA encapsulated in lambda bacteriophage coat protein, < 0.002% Poly rA RNA (synthetic), < 0.1% sodium azide | 13 mL |
| **Elution Buffer (EB)** | Tris buffer, 0.2% methyl-4 hydroxybenzoate | 13 mL |
| **Master Mix Reagent 1 (MMX-R1)** | Manganese acetate, potassium hydroxide, < 0.1% sodium azide | 5.5 mL |
| **DPX Master Mix Reagent 2 (DPX MMX-R2)** | Tricine buffer, potassium acetate, glycerol, 18% dimethyl sulfoxide, Tween 20, EDTA, < 0.06% dATP, dGTP, dCTP, < 0.14% dUTP, < 0.01% upstream and downstream parvovirus B19, HAV, internal control and quantitation standard primers, < 0.01% fluorescent-labeled parvovirus B19 and HAV probes, < 0.01% fluorescent-labeled parvovirus B19 QS and HAV IC probes, < 0.01% oligonucleotide aptamer, < 0.01% Z05D DNA polymerase, < 0.01% AmpErase (uracil-N-glycosylase) enzyme (microbial), < 0.1% sodium azide | 6 mL |

**Table 2 cobas**® DPX Control Kit

| **cobas® DPX Control Kit**<br>Store at 2-8°C<br>(P/N 07001126190) | | | |
|---|---|---|---|
| **Kit components** | **Reagent ingredients** | **Quantity per kit** | **Safety symbol and warning*** |
| **DPX Dual Positive Control**<br>(**DPX D(+)C**) | < 0.001% synthetic (armored) HAV RNA encapsulated in MS2 bacteriophage coat protein, < 0.001% synthetic (plasmid) parvovirus B19 DNA encapsulated in Lambda bacteriophage coat protein, normal human plasma, non-reactive by licensed tests for antibody to B19, HAV RNA not detectable by PCR methods, B19 DNA not detectable by PCR methods or below the level affecting the functionality of the control (≤ 5 IU/mL).<br>0.1% ProClin® 300 preservative** | 8 mL<br>(8 x 1 mL) |   <br>**WARNING**<br>H317: May cause an allergic skin reaction.<br>P261: Avoid breathing dust/fume/gas/mist/vapours/spray.<br>P272: Contaminated work clothing should not be allowed out of the workplace.<br>P280: Wear protective gloves.<br>P333 + P313: If skin irritation or rash occurs: Get medical advice/attention.<br>P362 + P364: Take off contaminated clothing and wash it before reuse.<br>P501: Dispose of contents/ container to an approved waste disposal plant.<br>55965-84-9 Mixture of: 5-chloro-2-methyl-4-isothiazolin-3-one [EC no. 247-500-7] and 2-methyl-2H-isothiazol-3-one [EC no. 220-239-6] (3:1) |
| **DPX High Positive Control**<br>(**DPX H(+)C**) | < 0.001% synthetic (plasmid) parvovirus B19 DNA encapsulated in Lambda bacteriophage coat protein, normal human plasma, non-reactive by licensed tests for antibody to B19, HAV RNA not detectable by PCR methods, B19 DNA not detectable by PCR methods or below the level affecting the functionality of the control (≤ 5 IU/mL).<br>0.1% ProClin® 300 preservative** | 8 mL<br>(8 x 1 mL) |   <br>**WARNING**<br>H317: May cause an allergic skin reaction.<br>P261: Avoid breathing dust/fume/gas/mist/vapours/spray.<br>P272: Contaminated work clothing should not be allowed out of the workplace.<br>P280: Wear protective gloves.<br>P333 + P313: If skin irritation or rash occurs: Get medical advice/attention.<br>P362 + P364: Take off contaminated clothing and wash it before reuse.<br>P501: Dispose of contents/ container to an approved waste disposal plant.<br>55965-84-9 Mixture of: 5-chloro-2-methyl-4-isothiazolin-3-one [EC no. 247-500-7] and 2-methyl-2H-isothiazol-3-one [EC no. 220-239-6] (3:1) |

* Product safety labeling primarily follows EU GHS guidance

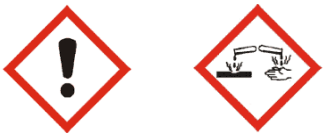
** Hazardous substance

**Table 3 cobas**® Buffer Negative Control Kit

| **cobas® Buffer Negative Control Kit**<br>Store at 2-8°C<br>(P/N 07002238190) | | | |
|---|---|---|---|
| **Kit component** | **Reagent ingredients** | **Quantity per kit** | **Safety symbol and warning** |
| **Buffer Negative Control**<br>(**Buffer-NC**) | Tris buffer, EDTA, 0.002% poly rA RNA (synthetic), < 0.1% sodium azide | 16 mL<br>(16 x 1 mL) | Not applicable |

# cobas omni reagents for sample preparation

**Table 4 cobas omni** reagents for sample preparation*

| Reagents | Reagent ingredients | Quantity per kit | Safety symbol and warning** |
|---|---|---|---|
| **cobas omni MGP Reagent (MGP)**<br>Store at 2–8°C<br>(P/N 06997546190) | Magnetic glass particles, Tris buffer, 0.1% methyl-4 hydroxybenzoate, < 0.1% sodium azide | 480 tests | Not applicable |
| **cobas omni Specimen Diluent (SPEC DIL)**<br>Store at 2–8°C<br>(P/N 06997511190) | Tris buffer, 0.1% methyl-4 hydroxybenzoate, < 0.1% sodium azide | 4 x 875 mL | Not applicable |
| **cobas omni Lysis Reagent (LYS)**<br>Store at 2–8°C<br>(P/N 06997538190) | 42.56% (w/w) guanidine thiocyanate**, 5% (w/v) polydocanol**, 2% (w/v) dithiothreitol, dihydro sodium citrate | 4 x 875 mL |  <br>**DANGER**<br>H302 + H332: Harmful if swallowed or if inhaled.<br>H318: Causes serious eye damage.<br>H412: Harmful to aquatic life with long lasting effects.<br>EUH032: Contact with acids liberates very toxic gas.<br>P261: Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray.<br>P273: Avoid release to the environment.<br>P280: Wear eye protection/ face protection.<br>P304 + P340 + P312: IF INHALED: Remove person to fresh air and keep comfortable for breathing. Call a POISON CENTER/doctor if you feel unwell.<br>P305 + P351 + P338 + P310: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER/doctor.<br>P501: Dispose of contents/ container to an approved waste disposal plant.<br>593-84-0 Guanidinium thiocyanate<br>9002-92-0 Polidocanol |
| **cobas omni Wash Reagent (WASH)**<br>Store at 15–30°C<br>(P/N 06997503190) | Sodium citrate dihydrate, 0.1% methyl-4 hydroxybenzoate | 4.2 L | Not applicable |

* These reagents are not included in the **cobas**® DPX test kit. See listing of additional materials required (Table 7).

**Product safety labeling primarily follows EU GHS guidance

## Reagent storage and handling requirements

Reagents shall be stored and will be handled as specified in Table 5 and Table 6.

When reagents are not loaded on the **cobas**® 6800/8800 Systems, store them at the corresponding temperature specified in Table 5.

**Table 5** Reagent storage (when reagent is not on the system)

| Reagent | Storage temperature |
|---|---|
| **cobas**® DPX – 96 | 2–8°C |
| **cobas**® DPX Control Kit | 2–8°C |
| **cobas**® Buffer Negative Control Kit | 2–8°C |
| **cobas omni** Lysis Reagent | 2–8°C |
| **cobas omni** MGP Reagent | 2–8°C |
| **cobas omni** Specimen Diluent | 2–8°C |
| **cobas omni** Wash Reagent | 15–30°C |

Reagents loaded onto the **cobas**® 6800/8800 Systems are stored at appropriate temperatures and their expiration is monitored by the system. The **s**ystem allows reagents to be used only if all of the conditions shown in Table 6 are met. The system automatically prevents use of expired reagents. Table 6 allows the user to understand the reagent handling conditions enforced by the **cobas**® 6800/8800 Systems.

**Table 6** Reagent expiry conditions enforced by the **cobas**® 6800/8800 Systems

| Reagent | Kit expiration date | Open-kit stability | Number of runs for which this kit can be used | On-board stability (cumulative time on board outside refrigerator) |
|---|---|---|---|---|
| **cobas**® DPX – 96 | Date not passed | 30 days from first usage | Max 10 runs | Max 8 hours |
| **cobas**® DPX Control Kit | Date not passed | Not applicable | Not applicable | Max 8 hours |
| **cobas**® Buffer Negative Control Kit | Date not passed | Not applicable | Not applicable | Max 10 hours |
| **cobas omni** Lysis Reagent | Date not passed | 30 days from loading* | Not applicable | Not applicable |
| **cobas omni** MGP Reagent | Date not passed | 30 days from loading* | Not applicable | Not applicable |
| **cobas omni** Specimen Diluent | Date not passed | 30 days from loading* | Not applicable | Not applicable |
| **cobas omni** Wash Reagent | Date not passed | 30 days from loading* | Not applicable | Not applicable |

* Time is measured from the first time that reagent is loaded onto the **cobas**® 6800/8800 Systems.

## Additional materials required

**Table 7** Material and consumables for use on **cobas**® 6800/8800 Systems

| **Material** | **P/N** |
|---|---|
| **cobas omni** Processing Plate | 05534917001 |
| **cobas omni** Amplification Plate | 05534941001 |
| **cobas omni** Pipette Tips | 05534925001 |
| **cobas omni** Liquid Waste Container | 07094388001 |
| **cobas omni** Lysis Reagent | 06997538190 |
| **cobas omni** MGP Reagent | 06997546190 |
| **cobas omni** Specimen Diluent | 06997511190 |
| **cobas omni** Wash Reagent | 06997503190 |
| Solid Waste Bag | 07435967001 |
| Solid Waste Container | 07094361001 |

## Instrumentation and software required

The **cobas**® 6800/8800 software and **cobas**® DPX analysis package shall be installed on the instrument(s). The Instrument Gateway (IG) server will be provided with the system.

**Table 8** Instrumentation

| **cobas® 6800 / 8800 Systems** | **P/N** |
|---|---|
| **cobas**® 6800 System (Option Moveable) | 05524245001 and 06379672001 |
| **cobas**® 6800 System (Fix) | 05524245001 and 06379664001 |
| **cobas**® 8800 System | 05412722001 |
| Sample Supply Module | 06301037001 |
| **Options for pipetting and pooling** | **P/N** |
| **cobas p** 680 Instrument | 06570577001 |
| **cobas® Synergy** software for use with the Hamilton Microlab® STAR IVD | 07788096001 and 07788339001 |
| Hamilton Microlab® STAR IVD | 04640535001 |

Refer to the **cobas**® 6800/8800 Systems Operator's Manual and **cobas p** 680 instrument Operator's Manual, or to the **cobas**® **Synergy** software User Assistance, for additional information for primary and secondary sample tubes accepted on the instruments.

Note: Contact your local Roche representative for a detailed order list for sample racks, racks for clotted tips and rack trays accepted on the instruments.

# Precautions and handling requirements

## Warnings and precautions

As with any test procedure, good laboratory practice is essential to the proper performance of this assay. Due to the high sensitivity of this test, care should be taken to keep reagents and amplification mixtures free of contamination.

- For *in vitro* diagnostic use only.
- All samples should be handled as if infectious, using good laboratory procedures as outlined in Biosafety in Microbiological and Biomedical Laboratories and in the CLSI Document M29-A4.[55,56] Only personnel proficient in handling infectious materials and the use of the **cobas**® DPX test, the **cobas**® 6800/8800 Systems, and (optionally) the **cobas p** 680 instrument or the Hamilton Microlab® STAR IVD using **cobas**® **Synergy** Core should perform this procedure.
- All human-sourced materials should be considered potentially infectious and should be handled with universal precautions. If spillage occurs, immediately disinfect with a freshly prepared solution of 0.5% sodium hypochlorite in distilled or deionized water (dilute household bleach 1:10) or follow appropriate site procedures.
- **cobas**® DPX Control Kit contains plasma derived from human blood. The source material has been tested by a licensed antibody test and found to be non-reactive for the presence of antibody to B19 IgG and IgM. Testing of normal human plasma by PCR methods showed no detectable HAV RNA and levels of B19 DNA that are either not detectable or low enough to not impact the functionality of the positive DPX controls. No known test method can offer complete assurance that products derived from human blood will not transmit infectious agents.
- Do not freeze whole blood.
- The use of sterile disposable pipettes and nuclease-free pipette tips is recommended. Use only supplied or specified required consumables to ensure optimal test performance.
- Safety Data Sheets (SDS) are available on request from your local Roche representative.
- Closely follow procedures and guidelines provided to ensure that the test is performed correctly. Any deviation from the procedures and guidelines may affect optimal test performance.
- False positive results may occur if carryover of samples is not adequately controlled during sample handling and processing.

## Reagent handling

- Handle all reagents, controls, and samples according to good laboratory practice in order to prevent carryover of samples or controls.
- Before use, visually inspect each reagent cassette, diluent, lysis reagent, and wash reagent to ensure that there are no signs of leakage. If there is any evidence of leakage, do not use that material for testing.
- **cobas omni** Lysis Reagent contains guanidine thiocyanate, a potentially hazardous chemical. Avoid contact of reagents with the skin, eyes, or mucous membranes. If contact does occur, immediately wash with generous amounts of water; otherwise, burns can occur.
- **cobas**® DPX test kits, **cobas omni** MGP Reagent, and **cobas omni** Specimen Diluent contain sodium azide as a preservative. Avoid contact of reagents with the skin, eyes, or mucous membranes. If contact does occur, immediately wash with generous amounts of water; otherwise, burns can occur. If these reagents are spilled, dilute with water before wiping dry.
- Do not allow **cobas omni** Lysis Reagent, which contains guanidine thiocyanate, to contact sodium hypochlorite (bleach) solution. This mixture can produce a highly toxic gas.
- Dispose of all materials that have come in contact with samples and reagents in accordance with country, state, and local regulations.

## Good laboratory practice

- Do not pipette by mouth.
- Do not eat, drink, or smoke in designated work areas.
- Wear laboratory gloves, laboratory coats, and eye protection when handling samples and reagents. Gloves must be changed between handling samples and **cobas**® DPX kits and **cobas omni** reagents to prevent contamination. Avoid contaminating gloves when handling samples and controls.
- Wash hands thoroughly after handling samples and kit reagents, and after removing the gloves.
- Thoroughly clean and disinfect all laboratory work surfaces with a freshly prepared solution of 0.5% sodium hypochlorite in distilled or deionized water (dilute household bleach 1:10). Follow by wiping the surface with 70% ethanol.
- If spills occur on the **cobas**® 6800/8800 instrument, follow the instructions in the **cobas**® 6800/8800 System Operator's Manual to properly clean and decontaminate the surface of instrument(s).

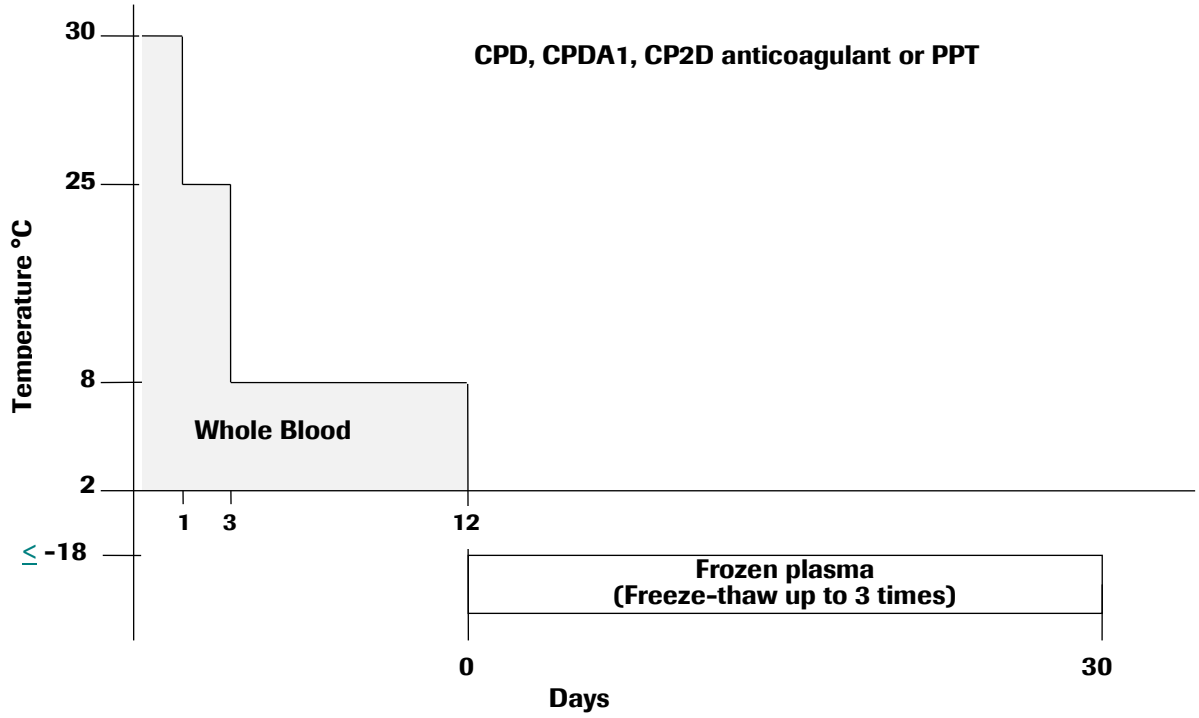
# Sample collection, transport, storage, and pooling

**Note: Handle all samples and controls as if they are capable of transmitting infectious agents.**
Store all donor samples at specified temperatures.
Specimen stability is affected by elevated temperatures.

## Living donor samples

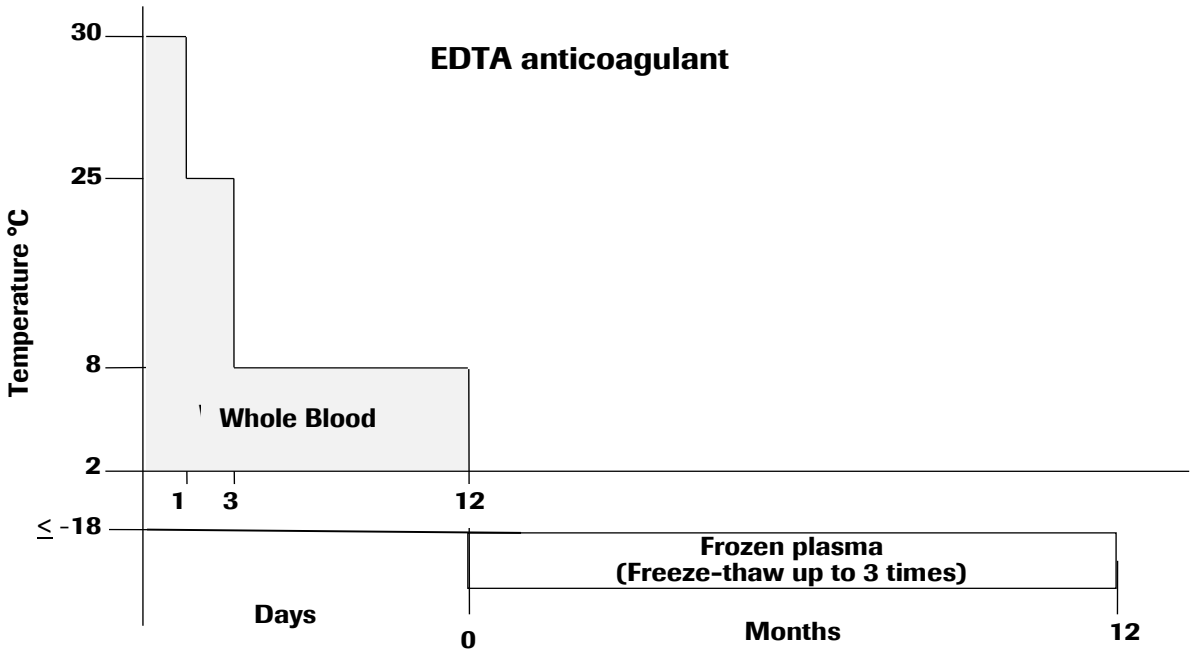
- Plasma collected in EDTA, CPD, CPDA1, CP2D and 4% sodium citrate anticoagulant may be used with the **cobas**® DPX test. Follow the sample collection tube/bag manufacturer instructions for handling and centrifugation.
- Blood collected in CPD, CPDA1, CP2D anticoagulant or Becton-Dickinson EDTA Plasma Preparation Tubes (BD PPT™) may be stored for up to 12 days with the following conditions:
    - Samples must be centrifuged within 72 hours of draw.
    - For storage above 8°C, specimens may be stored for 72 hours at up to 25°C, and up to 30°C for 24 hours during the 72 hours.
    - Other than noted above, samples are stored at 2-8°C. In addition, plasma separated from the cells may be stored for up to 30 days at ≤-18°C with three freeze/thaw cycles. Refer to Figure 1.

**Figure 1** Donor sample storage conditions




- Blood collected in EDTA anticoagulant may be stored for up to 12 days with the following conditions:
  - Samples must be centrifuged within 72 hours of draw.
  - For storage above 8°C, specimens may be stored for 72 hours at up to 25°C, and up to 30°C for 24 hours during the 72 hours.
  - Other than noted above, samples are stored at 2-8°C. In addition, plasma separated from the cells may be stored for up to 12 months at ≤-18°C with three freeze/thaw cycles. Refer to Figure 2.

**Figure 2** Donor sample storage conditions




- Plasma in 4% sodium citrate anticoagulant may be stored for up to 30 days at 2-8°C.
    - For storage above 8°C, samples may be stored for 72 hours at up to 25°C, and up to 30°C for 24 hours during the 72 hours.
    - Other than noted above, samples are stored at 2-8°C. In addition, plasma separated from the cells may be stored for up to 12 months at ≤-18°C with three freeze/thaw cycles.

- If samples are to be shipped, they should be packaged and labeled in compliance with applicable country and/or international regulations covering the transport of samples and etiologic agents.

# Instructions for use

## Automated sample pipetting and pooling (optional)

Either the **cobas p** 680 instrument, or **cobas**® **Synergy** Core can be used as an optional component of the **cobas**® 6800/8800 Systems for automated pipetting and pooling of aliquots of multiple primary samples into one pooled sample.

Refer to the **cobas p** 680 instrument Operator's Manual or to the **cobas**® **Synergy** software User Assistance for more information.

## Setting of cut off value for parvovirus B19

**cobas**® 6800/8800 Systems

The laboratory supervisor determines the B19V titer cut-off by setting the cut-off for pools of 1. The value entered here is used by the software to assign a "B19V < cut off " or "B19V ≥ cut off " result (Table 10). The software automatically calculates the result based on the cut off entered and the pool size.

To assign the B19V titer cut off:

The DPX-B19V cut off value can be found on the user interface (UI) under Administration --> Settings --> Processing settings --> Roche tests. Under "Settings" of the DPX-B19V ASAP the cutoff value can be set using the "Edit" button.

**cobas**® **Synergy** solution

Final B19 and DPX test results are only available in the **cobas**® **Synergy** software, and not on the **cobas**® 6800/8800 Systems.

To assign the B19V titer cut off values (per pool size in IU/mL), follow the description in the **cobas**® **Synergy** software User Assistance. It is recommended to set cutoff in **cobas**® 6800/8800 SW to 1.

## Procedural notes

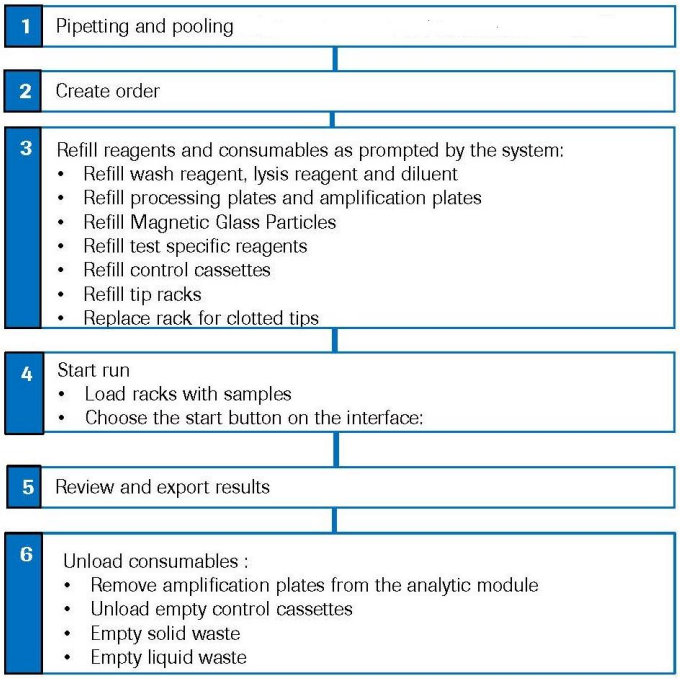
- Do not use **cobas**® DPX test reagents, **cobas**® DPX Control Kit, **cobas**® Buffer Negative Control Kit, or **cobas omni** reagents after their expiry dates.
- Do not reuse consumables. They are for one-time use only.
- Refer to the **cobas**® 6800/8800 Systems Operator's Manual or to the **cobas**® **Synergy** software User Assistance for proper maintenance of instruments.

## Running the cobas® DPX test

The test procedure is described in detail in the **cobas**® 6800/8800 Systems Operator's Manual; refer to the **cobas p** 680 instrument Operator's Manual or to the **cobas**® **Synergy** software User Assistance as applicable for details on optional pooling procedures.

Figure 3 below summarizes the procedure.

**Figure 3 cobas**® DPX test procedure



# Results

The **cobas**® 6800/8800 System automatically determines the parvovirus B19 DNA concentration for donor samples and controls. The parvovirus B19 DNA concentration is expressed in International Units per milliliter (IU/mL). Furthermore, the **cobas**® 6800/8800 Systems automatically detect HAV RNA for the samples and controls.

## Quality control and validity of results

- One negative control [(–)C] and two positive controls, [DPX D(+)C and DPX H(+)C], are processed with each batch.
- In the **cobas**® 6800/8800 software and/or report, check for flags and their associated results to ensure the batch validity.
- The batch is valid if no flags appear for all three controls.

Invalidation of results is performed automatically by the **cobas**® 6800/8800 software based on negative and positive control failures.

### Control flags

**Table 9** Control flags for negative and positive controls

| Negative Control | Flag | Result | Interpretation |
|---|---|---|---|
| (–) C | Q02 | Invalid | The entire batch is assigned invalid if the result for the (–) C is invalid. |
| **Positive Control** | **Flag** | **Result** | **Interpretation** |
| DPX D(+)C | Q02 | Invalid | An invalid result or the calculated titer result for parvovirus B19 is not within the assigned range or the result for HAV is non-reactive.<br>B19 only: An invalid result due to corresponding QS or the calculated titer result for parvovirus B19 is not within the assigned range.<br>HAV only: An invalid result due to corresponding IC or the result for HAV is non-reactive. |
| DPX H(+)C | Q02 | Invalid | An invalid result or the calculated titer result for the high positive control is not within the assigned range. |

If the batch is invalid, repeat testing of the entire batch including samples and controls.

## Interpretation of results

For a valid batch, check each individual sample for flags in the **cobas**® 6800/8800 software and/or report. The result interpretation should be as follows:

- A valid batch may include both valid and invalid donor sample results dependent on flags obtained for the individual samples.
- Sample results are valid only if the respective positive controls and the negative control of the corresponding batch are valid.

Four parameters are measured simultaneously for each sample: one for parvovirus B19, one for HAV, one for the quantitative standard, and the internal control. Final sample results for the **cobas**® DPX test are reported by the software. Donor samples in an invalid pool must be retested. In addition to the overall results, individual target results will be displayed in the **cobas**® 6800/8800 software and should be interpreted as follows:

**Table 10** Target results for individual target result interpretation

| Target results | Interpretation |
|---|---|
| HAV Non-Reactive | No target signal detected for HAV and IC signal detected. |
| HAV Reactive | Target signal detected for HAV and IC signal may be or may not be detected. |
| B19 < cut off value | B19 titer is less than the cut-off as defined by the user; titer is provided.<br>B19 Non-Reactive: no target signal detected for B19 DNA and QS signal detected.<br>B19 < Titer Min: B19 is detected and calculated titer is below the lower limit of quantitation (LLoQ) of the assay.<br>Note:<br>**cobas p** 680 – B19 cut-off value is displayed in **cobas**® 6800/8800 Systems Software.<br>**cobas**® **Synergy** – B19 cut-off value is displayed in **cobas**® **Synergy** software. |
| B19 ≥ cut off value | B19 titer is greater than the cut-off as defined by the user; titer is provided.<br>B19 > Titer Max: calculated titer is above the upper limit of quantitation (ULoQ) of the assay.[a]<br>Note:<br>**cobas p** 680 – B19 cut-off value is displayed in **cobas**® 6800/8800 Systems Software.<br>**cobas**® **Synergy** – B19 cut-off value is displayed in **cobas**® **Synergy** software. |
| Invalid | HAV target and internal control signal not detected.<br>HAV non-reactive results will be reported as Invalid if the B19 titer is > $10^6$ IU/mL.<br>B19 QS signal is not detected and B19 target may be or may be not detected. |

[a] If a quantitative result is desired, the original sample should be diluted with parvovirus B19-negative EDTA plasma and the test should be repeated. Multiply the reported result by the dilution factor.

## Repeat testing of individual sample(s)

Sample tubes with a final result of Invalid for one target require repeat testing regardless of valid results for the other targets. The repeat result of an HAV Invalid due to a high titer B19 (>$10^6$ IU/mL) will remain Invalid.

## Procedural limitations

- The **cobas**® DPX test has been evaluated only for use in combination with the **cobas**® DPX Control Kit, **cobas**® Buffer Negative Control Kit, **cobas omni** MGP Reagent, **cobas omni** Lysis Reagent, **cobas omni** Specimen Diluent, and **cobas omni** Wash Reagent for use on the **cobas**® 6800/8800 Systems.
- Reliable results depend on proper sample collection, storage and handling procedures.
- Do not use heparinized plasma with this test because heparin has been shown to inhibit PCR.
- Though rare, mutations within the highly conserved regions of a viral genome covered by the **cobas**® DPX test may affect primers and/or probe binding resulting in the under-quantitation of virus or failure to detect presence of virus.
- Due to inherent differences between technologies, it is recommended that, prior to switching from one technology to the next, users perform method correlation studies in their laboratory to qualify technology differences. Users should follow their own specific policies/procedures.

# Non-clinical performance evaluation

## Key performance characteristics – Living donor samples

### Limit of Detection (LoD)

#### WHO International Standards

The limits of detection (LoD) of the **cobas**® DPX test for HAV RNA and parvovirus B19 DNA were determined using the WHO International Standards for HAV (NIBSC code 00/560) and parvovirus B19 (NIBSC 99/802).

Three independent dilution series of each viral standard were prepared with pooled, virus negative human plasma collected in EDTA anticoagulant. Each dilution series were tested using three different lots of the **cobas**® DPX reagent kits with approximately 21 replicates per lot, for a total of approximately 189 replicates per concentration. PROBIT analysis on the combined data from all replicates tested for each virus was used to estimate the LoD and two-sided 95% fiducial confidence intervals. Table 11 through Table 13 summarize the overall results of the Limit of Detection study.

**Table 11** Results of PROBIT analysis on LoD data collected with viral standards in EDTA plasma

| Analyte | Measuring units | LoD | Lower 95% confidence limit | Upper 95% confidence limit |
|---|---|---|---|---|
| HAV | IU/mL | 1.1 | 0.9 | 1.3 |
| Parvovirus B19 | IU/mL | 13.9 | 11.7 | 17.4 |

**Table 12** Reactivity rates summary for HAV in EDTA plasma

| HAV RNA concentration (IU/mL) | Number reactive | Number of valid replicates | % Reactive | 95% Lower confidence bound (one-sided) |
|---|---|---|---|---|
| 6 | 189 | 189 | 100% | 98.1% |
| 3 | 189 | 189 | 100% | 98.1% |
| 1.5 | 186 | 189 | 98.4% | 98.9% |
| 0.75 | 165 | 189 | 87.3% | 81.7% |
| 0.375 | 119 | 189 | 63.0% | 55.7% |

**Table 13** Reactivity rates summary for parvovirus B19 in EDTA plasma

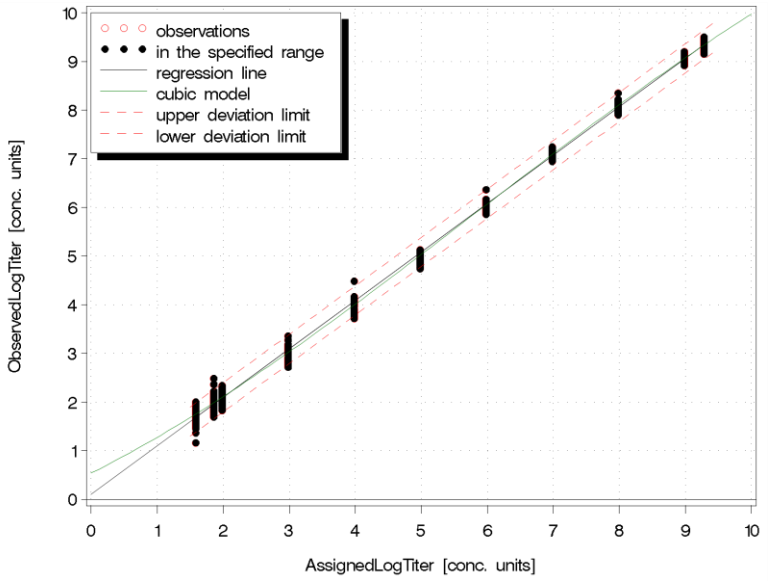
| Parvovirus B19 DNA concentration (IU/mL) | Number reactive | Number of valid replicates | % Reactive | 95% Lower confidence bound (one-sided) |
|---|---|---|---|---|
| 40 | 187 | 189 | 98.9% | 96.2% |
| 20 | 184 | 189 | 97.4% | 93.9% |
| 10 | 175 | 189 | 92.6% | 87.9% |
| 5 | 145 | 189 | 76.7% | 70.0% |
| 2.5 | 91 | 189 | 48.1% | 40.8% |

## Linear range of parvovirus B19 quantitation

Linearity study of parvovirus B19 quantitation of the **cobas**® DPX test was performed with a dilution series consisting of 12 panel members spanning the intended linear range for predominant parvovirus B19 genotype 1. The evaluation was performed according to CLSI Guideline EP6-A. Three reagent lots were analyzed on three **cobas**® 6800/8800 Systems, three operators and in total 16 replicates per panel member and lot across 12 testing days.

The study was performed using three lots of reagents. The linear range was determined to be between 40 IU/mL and 1.00E+09 IU/mL (38.5 IU/mL – 1.93E+09 IU/mL) and shows an absolute deviation from the better fitting non-linear regression of less than ±0.3 $\log_{10}$ in human EDTA plasma (see Figure 4).

**Figure 4** Linear range determination for parvovirus B19 in EDTA plasma



## Reproducibility

The reproducibility of the **cobas**® DPX test was evaluated for three lots of reagents, three different days, four individual systems/operators and run-to run variability. The results for reagent lots are summarized in Table 14.

**Table 14** Reagent lot-to-lot reproducibility

| Analyte | Concentration | Reagent lot | % Reactive (reactive/valid replicates) | Lower limit of 95% confidence interval | Upper limit of 95% confidence interval |
|---|---|---|---|---|---|
| HAV | 2 x LoD | 1 | 100.0% (63/63) | 94.3% | 100.0% |
| | | 2 | 100.0% (63/63) | 94.3% | 100.0% |
| | | 3 | 100.0% (63/63) | 94.3% | 100.0% |
| | 1 x LoD | 1 | 98.4% (62/63) | 91.5% | 100.0% |
| | | 2 | 96.8% (61/63) | 89.0% | 99.6% |
| | | 3 | 100.0% (63/63) | 94.3% | 100.0% |
| | 0.5 x LoD | 1 | 79.4% (50/63) | 67.3% | 88.5% |
| | | 2 | 90.5% (57/63) | 80.4% | 96.4% |
| | | 3 | 92.1% (58/63) | 82.4% | 97.4% |

## Precision

Precision of the **cobas**® DPX was determined for parvovirus B19 by analysis of serial dilutions of a parvovirus B19 positive sample in negative EDTA Plasma. Eight dilution levels were tested in 48 replicates for each level across three lots of **cobas**® DPX test reagents using three instruments and three operators over 12 days. Each sample was carried through the entire **cobas**® DPX test procedure on fully automated **cobas**® 6800/8800 Systems. Therefore, the precision reported here represents all aspects of the test procedure. The results are shown in Table 15.

**cobas**® DPX for parvovirus B19 showed high precision for three lots of reagents tested across concentration range of 1.00 E+03 IU/mL to 2.0 E+09 IU/mL.

**Table 15** Within laboratory precision of **cobas**® DPX test*

| Nominal concentration (IU/mL) | Assigned concentration (IU/mL) | Source material | EDTA plasma | | | |
|---|---|---|---|---|---|---|
| | | | Lot 1 | Lot 2 | Lot 3 | All Lots |
| | | | SD | SD | SD | Pooled SD |
| 2.00E+09 | 1.93E+09 | Clinical Specimen | 0.08 | 0.05 | 0.04 | 0.06 |
| 1.00E+09 | 9.63E+08 | Clinical Specimen | 0.05 | 0.06 | 0.04 | 0.05 |
| 1.00E+08 | 9.63E+07 | Clinical Specimen | 0.04 | 0.07 | 0.04 | 0.05 |
| 1.00E+07 | 9.63E+06 | Clinical Specimen | 0.04 | 0.04 | 0.03 | 0.04 |
| 1.00E+06 | 9.63E+05 | Clinical Specimen | 0.12 | 0.04 | 0.04 | 0.08 |
| 1.00E+05 | 9.63E+04 | Clinical Specimen | 0.06 | 0.05 | 0.02 | 0.05 |
| 1.00E+04 | 9.63E+03 | Clinical Specimen | 0.06 | 0.12 | 0.04 | 0.08 |
| 1.00E+03 | 9.63E+02 | Clinical Specimen | 0.05 | 0.09 | 0.04 | 0.06 |

* Titer data are considered to be log-normally distributed and are analyzed following $\log_{10}$ transformation. Standard deviations (SD) columns present the total of the log-transformed titer for each of the three reagent lots.

## Genotype inclusivity – HAV

The performance of the **cobas**® DPX test to detect three genotypes of HAV was determined by testing a total of 12 unique clinical samples, the HAV WHO standard (NIBSC code 00/560) and eight HAV cultured isolates with known genotypes. All clinical samples and cultured isolates were quantified with the **cobas**® DPX test using the calibrator bracketing method. All clinical samples were tested neat and after dilution with normal, virus negative (HAV) human EDTA-plasma to 3.6 x LoD of the **cobas**® DPX test. All eight cultured isolates and the HAV WHO standard were tested after dilution with normal, virus negative (HAV) human EDTA-plasma to 3.6 x LoD of the **cobas**® DPX test. All clinical samples and cultured isolates were detected at neat and/or at 3.6 x LoD (Table 16).

**Table 16** HAV clinical samples and cultured isolates

| Genotype | Clinical samples | | Cultured isolates |
|---|---|---|---|
| | % Reactive (reactive/samples tested) neat | % Reactive (reactive/samples tested) diluted to 3.6 x LoD | % Reactive (reactive/samples tested) diluted to 3.6 x LoD |
| I A | 100.0% (11/11) | 100.0% (12/12)** | Not tested* |
| I B | 100.0% (1/1) | 100.0% (1/1) | 100.0% (1/1) |
| II A | Not tested* | Not tested* | 100.0% (1/1) |
| II B | Not tested* | Not tested* | 100.0% (1/1) |
| III A | Not tested* | Not tested* | 100.0% (3/3) |
| III B | Not tested* | Not tested* | 100.0% (2/2) |

* Insufficient volume to test at neat/diluted

** Including the HAV WHO standard (NIBSC code 00/560)

## Genotype verification for parvovirus B19

The performance of the **cobas**® DPX test on parvovirus B19 genotypes was evaluated by:

- Verification of the limit of detection for genotypes 1, 2 and 3
- Verification of the linearity for genotypes 2 and 3

### Verification of limit of detection for genotypes 1 through 3

Parvovirus B19 DNA clinical specimens for three different genotypes (1, 2, 3a) were diluted to one concentration level in EDTA plasma. Parvovirus B19 plasmid for genotype 3b was diluted to one concentration level in EDTA plasma. The reactivity rate determination was performed with 21 replicates. Testing was conducted with one lot of **cobas**® DPX reagents. The results from EDTA plasma are shown in Table 17. These results verify that the **cobas**® DPX test detected parvovirus B19 DNA for three different genotypes at concentrations of 10.3 IU/mL-17.4 IU/mL with a reactivity rate of 100%.

**Table 17** Parvovirus B19 genotype inclusivity

| Genotype | Concentration | % Reactive (reactive/valid replicates) | Lower limit of 95% confidence interval | Upper limit of 95% confidence interval |
|---|---|---|---|---|
| 1 | 17.4 IU/mL | 100% (21/21) | 83.9% | 100.0% |
| 2 | 10.3 IU/mL | 100% (21/21) | 83.9% | 100.0% |
| 3a | 10.3 IU/mL | 100% (21/21) | 83.9% | 100.0% |
| 3b | 17.4 IU/mL | 100% (20/20) | 83.2% | 100.0% |

### Verification of the linear range for genotypes 2 and 3a

The dilution series used in the verification of genotypes linearity study of the **cobas**® DPX test consists of seven panel members spanning the intended linear range. High titer panel members were prepared from a high titer plasmid DNA stock whereas the lower titer panel members were made from the 1st WHO International Reference Panel for parvovirus B19 Genotypes (NIBSC 09/110). The linearity panel was designed to have an approximate 2 $\log_{10}$ titer overlap between the two material sources. The linear range of the **cobas**® DPX test spanned from the LLoQ (40 IU/mL) to the ULoQ (1.00E+09 IU/mL) and included one medical decision point. Testing was conducted with one lot of **cobas**® DPX reagent; 11 replicates per level were tested in EDTA plasma. The linear range of the **cobas**® DPX test was verified for both genotypes (2 and 3a). The maximum deviation between the linear regression and the better fitting non-linear regression was equal to or less than 0.3 $\log_{10}$.

## Analytical specificity

The analytical specificity of the **cobas**® DPX test was evaluated for cross-reactivity by testing a panel of 27 microorganisms at $10^6$ particles, IU, copies, or CFU/mL as shown in Table 18. The microorganisms were added to normal, virus-negative, human pooled plasma and tested with and without HAV or parvovirus B19 added to a concentration of approximately 3 x LoD for HAV or 5 x LLoQ for parvovirus B19 of the **cobas**® DPX test. Non-reactive results were obtained with the **cobas**® DPX test on all of the microorganism samples without added HAV or parvovirus B19 and reactive results were obtained on all of the microorganism samples with added HAV or parvovirus B19. Furthermore, the mean $\log_{10}$ titer of each of the positive parvovirus B19 samples containing potentially cross-reacting organism was within ±0.5 $\log_{10}$ of the mean $\log_{10}$ titer of the respective spike control. The tested microorganisms do not cross-react with the **cobas**® DPX test.

The tested microorganisms do not interfere with the **cobas**® DPX test.

**Table 18** Microorganisms tested for analytical specificity

| Viruses | Flavivirus | Bacteria | Yeast |
|---|---|---|---|
| Adenovirus 5 | West Nile Virus (WNV) | *Escherichia coli* | Candida albicans |
| Chikungunya Virus | Dengue Virus type 1 | *Propionibacterium acnes* | |
| Cytomegalovirus (CMV) | Usutu Virus | *Staphylococcus aureus* | |
| Epstein-Barr Virus (EBV) | | *Staphylococcus epidermidis* | |
| Hepatitis B Virus (HBV) | | *Staphylococcus haemolyticus* | |
| Hepatitis C Virus (HCV) | | *Streptococcus viridans* | |
| Hepatitis E Virus (HEV) | | | |
| Hepatitis G Virus (GBV C) | | | |
| Herpes Simplex Virus type 1 (HSV-1) | | | |
| Herpes Simplex Virus type 2 (HSV-2) | | | |
| Human Herpes Virus 6A (HHV-6) | | | |
| Human Immunodeficiency Virus (Subtypes HIV-1M and HIV-2) | | | |
| Human T-cell Lymphotropic Virus type I (HTLV I) | | | |
| Human T-cell Lymphotropic Virus type II (HTLV II) | | | |
| Influenza Virus A | | | |
| Varicella Zoster Virus (VZV) | | | |

Plasma samples from each of the other disease states listed in Table 19 were tested with and without HAV or parvovirus B19 added to a concentration of approximately 3 x LoD for HAV and 5 x LLoQ for parvovirus B19 of the **cobas**® DPX test. The **cobas**® DPX test yielded non-reactive results for all of the disease state samples without added HAV or parvovirus B19. The **cobas**® DPX test yielded reactive results for all of the disease state samples with added HAV or parvovirus B19. Furthermore, the mean $\log_{10}$ titer of each of the positive parvovirus B19 samples containing potentially cross-reacting organism was within ±0.5 $\log_{10}$ of the mean $\log_{10}$ titer of the respective spike control. These disease states did not interfere with the **cobas**® DPX test.

**Table 19** Disease states samples tested for analytical specificity

| Disease state | | |
|---|---|---|
| Adenovirus type 5 | Hepatitis C Virus | Human T-cell Lymphotropic Virus type I |
| Cytomegalovirus | Hepatitis E Virus | Human T-cell Lymphotropic Virus type II |
| Dengue Virus | Herpes Simplex Virus type 1 | West Nile Virus |
| Epstein-Barr Virus | Herpes Simplex Virus type 2 | |
| Hepatitis B Virus | Human Immunodeficiency Virus (HIV-1M) | |

## Analytical specificity – interfering substances

### Endogenous interference substances

Plasma samples with abnormally high levels of triglycerides (up to 33 g/L), hemoglobin (up to 2 g/L), unconjugated bilirubin (up to 0.2 g/L), conjugated bilirubin (up to 0.2 g/L) albumin (up to 60 g/L), or human DNA (up to 1.8 mg/L) were tested with and without HAV or parvovirus B19 added to a concentration of approximately 3 x LoD for HAV and 5 x LLoQ for parvovirus B19 of the **cobas**® DPX test. Samples containing these endogenous substances did not interfere with the sensitivity, quantitation or specificity of **cobas**® DPX test.

### Exogenous interference substances

Normal, virus-negative human EDTA plasma samples containing abnormally high concentrations of drugs (Table 20) were tested with and without HAV and parvovirus B19 added to a concentration of 3 x LoD for HAV and 5 x LLoQ for parvovirus B19 of the **cobas**® DPX. These exogenous substances did not interfere with the sensitivity, quantitation, or specificity of the **cobas**® DPX test.

**Table 20** Clinical samples tested with drugs

| Name of drug tested | Concentration |
|---|---|
| Acetaminophen | 1324 µmol/L |
| Acetylsalicylic Acid | 3620 µmol /L |
| Ascorbic Acid | 342 µmol/L |
| Atorvastatin | 600 µg Eq/L |
| Fluoxetine | 11.2 µmol/L |
| Ibuprofen | 2425 µmol/L |
| Loratadine | 0.78 µmol/L |
| Nadolol | 3.88 µmol/L |
| Naproxen | 2170 µmol/L |
| Paroxetine | 3.04 µmol/L |
| Phenylephrine HCl | 491 µmol/L |
| Sertraline | 1.96 µmol/L |

## Correlation

### Performance evaluation of the cobas® DPX test compared to the cobas® TaqScreen DPX Test

The performance of the **cobas**® DPX test and the **cobas**® TaqScreen DPX Test were compared using 84 HAV NAT-positive plasma samples ,100 parvovirus B19 NAT-positive samples, and 100 HAV and parvovirus B19-negative samples.

The negative samples demonstrated 100% specificity by generating 100 out of 100 non-reactive results with both methods.

For HAV positive specimens the **cobas**® DPX test and **cobas**® TaqScreen DPX Test were in agreement for 84 out of 84 samples (Table 21). This results in a positive percent agreement of 100%.

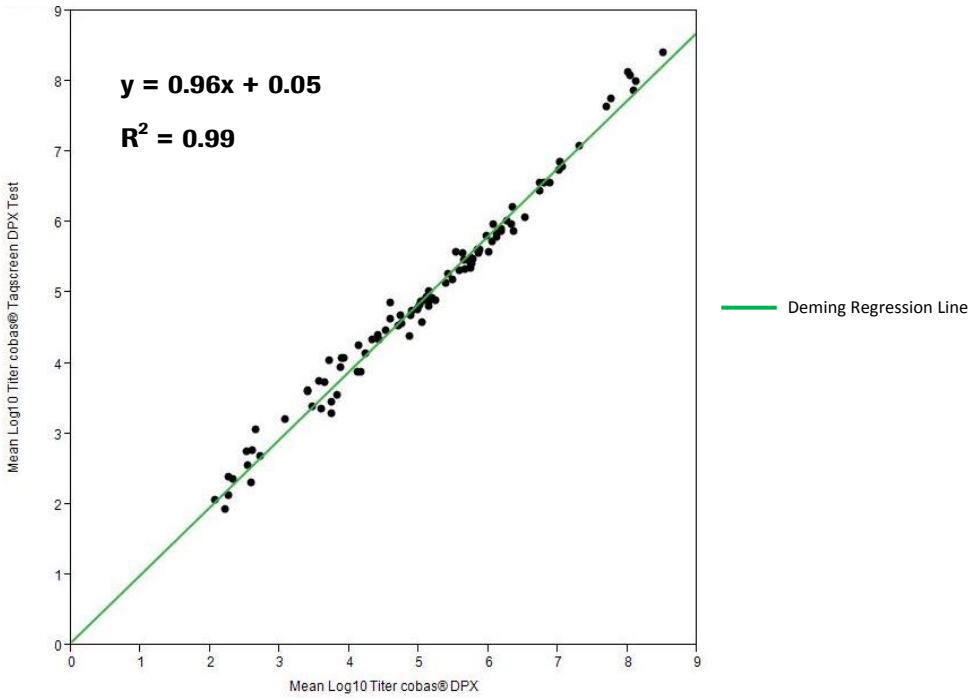
**Table 21** Correlation of HAV positive / negative samples

| Methods | | HAV results | |
|---|---|---|---|
| **cobas® TaqScreen DPX** | **cobas® DPX** | **Positive samples** | **Negative samples** |
| Non-reactive | Non-reactive | 0 | 100 |
| Reactive | Non-Reactive | 0 | 0 |
| Non-reactive | Reactive | 0 | 0 |
| Reactive | Reactive | 84 | 0 |
| Total | | 84 | 100 |
| McNemar's Test, p-value (two-sided, $\alpha = 0.05$) | | 1.00 | 1.00 |

The parvovirus B19 positive specimens were tested with the **cobas**® DPX test and **cobas**® TaqScreen DPX Test in duplicates. Deming regression analysis was performed. The mean titer deviation of the samples tested with the two tests was 0.15 $\log_{10}$. Furthermore within the titer range of 1.0E+03 – 1.0E+06 IU/mL the mean titer deviation with the two tests was 0.14 $\log_{10}$.

The Deming regression results are shown in Figure 5.

**Figure 5** Regression analysis of **cobas**® DPX vs **cobas**® TaqScreen DPX Test, 100 positive parvovirus B19 samples




## Whole system failure

The whole system failure rate for the **cobas**® DPX test was determined by testing 100 replicates of EDTA plasma spiked with HAV and parvovirus B19. These samples were tested at a target concentration of approximately 3 x LoD and were run in pools of one (undiluted). The study was performed using the **cobas**® 8800 System with **cobas p** 680 Instrument (pipetting and pooling).

The results of this study determined that all replicates were reactive for parvovirus B19, resulting in a whole system failure rate of 0%. The two-sided 95% exact confidence interval was 0% for the lower bound and 3.62% for the upper bound [0%: 3.62%].

The results of this study determined that 99 of 100 replicates were reactive for HAV, resulting in a whole system failure rate of 1%. The two-sided 95% exact confidence interval was 0% for the lower bound and 5.45% for the upper bound [0%: 5.45%].

## Cross contamination

The cross-contamination rate for the **cobas**® DPX test was determined by testing 239 replicates of negative control buffer and 223 replicates of a high titer parvovirus B19 sample at 1.00E+08 IU/mL. The study was performed using the **cobas**® 8800 System. In total, five runs were performed with positive and negative samples in a checkerboard configuration.

All 239 replicates of the negative control buffer were non-reactive, resulting in a cross-contamination rate of 0%. The two-sided 95% exact confidence interval was 0% for the lower bound and 1.53% for the upper bound [0%: 1.53%].

# Clinical performance

## Reproducibility

The reproducibility of **cobas**® DPX was established by testing a 16-member panel composed of two plasma panel members negative for HAV and less than the lower limit of quantitation (LLOQ) for parvovirus B19, and 14 positive plasma samples, which were comprised of two samples positive for HAV at each of 3 different concentrations (approximately 0.5 x, 1.0 x, and 3.0 x the LoD of **cobas**® DPX for HAV) and two samples of parvovirus B19 at each of 4 different concentrations (ranging from $10^3$ to $10^6$ IU/mL).

Operators at each of the three **cobas**® DPX sites performed five days of testing, using 3 lots of **cobas**® DPX reagents to obtain two valid batches per day. Two replicates per concentration were tested to yield up to 180 tests per panel member virus at each of the three concentrations of HAV and each of the four concentrations for parvovirus B19.

For HAV, all valid batches and test results were analyzed by calculating the percentage of reactive test results for each panel member and the percentage of non-reactive results for the negative control panel member (Table 22). This study demonstrated that **cobas**® DPX shows reproducible performance across the variables assessed (lot, site/instrument, day, batch, and within batch) at each of the three different HAV concentrations tested.

**Table 22** HAV test results summarized by site/instrument, lot, day, and batch (positive panel members)

| | | | | Number of Reactive Tests / Total Number of Valid Results | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| HAV Concen-tration | Mean Ct | Ct SD | Ct CV% | Lot | | | Site/Instrument | | | Day | | | Batch | | |
| | | | | ID # | Reactive/ Valid | % | ID # | Reactive/ Valid | % | ID # | Reactive/ Valid | % | ID # | Reactive/ Valid | % |
| 0.5 x LoD | 37.50 | 0.799 | 2.1 | 1 | 53/60 | 88.3 | 1 | 48/60 | 80.0 | 1 | 30/36 | 83.3 | 1 | 76/90 | 84.4 |
| | | | | 2 | 48/60 | 80.0 | 2 | 51/60 | 85.0 | 2 | 33/36 | 91.7 | 2 | 77/90 | 85.6 |
| | | | | 3 | 52/60 | 86.7 | 3 | 54/60 | 90.0 | 3 | 31/36 | 86.1 | | | |
| | | | | | | | | | | 4 | 26/36 | 72.2 | | | |
| | | | | | | | | | | 5 | 33/36 | 91.7 | | | |
| 1.0 x LoD | 37.04 | 0.763 | 2.1 | 1 | 57/59 | 96.6 | 1 | 55/60 | 91.7 | 1 | 34/36 | 94.4 | 1 | 88/89 | 98.9 |
| | | | | 2 | 58/60 | 96.7 | 2 | 59/59 | 100.0 | 2 | 35/35 | 100.0 | 2 | 85/90 | 94.4 |
| | | | | 3 | 58/60 | 96.7 | 3 | 59/60 | 98.3 | 3 | 36/36 | 100.0 | | | |
| | | | | | | | | | | 4 | 34/36 | 94.4 | | | |
| | | | | | | | | | | 5 | 34/36 | 94.4 | | | |
| 3.0 x LoD | 35.95 | 0.725 | 2.0 | 1 | 60/60 | 100.0 | 1 | 60/60 | 100.0 | 1 | 36/36 | 100.0 | 1 | 90/90 | 100.0 |
| | | | | 2 | 60/60 | 100.0 | 2 | 60/60 | 100.0 | 2 | 36/36 | 100.0 | 2 | 90/90 | 100.0 |
| | | | | 3 | 60/60 | 100.0 | 3 | 60/60 | 100.0 | 3 | 36/36 | 100.0 | | | |
| | | | | | | | | | | 4 | 36/36 | 100.0 | | | |
| | | | | | | | | | | 5 | 36/36 | 100.0 | | | |

Note: Ct = Cycle Threshold

For parvovirus B19, all valid batches and test results were analyzed by calculating the standard deviation for each of the variables (lot, site/instrument, day, batch, and within batch) and the total precision standard deviation for each B19 concentration (Table 23). This study demonstrated that **cobas**® DPX shows reproducible performance across the variables assessed (lot, site/instrument, day, batch, and within batch) at each of the four different parvovirus B19 concentrations tested.

**Table 23** Parvovirus B19 test results summarized by site/instrument, lot, day, and batch (positive panel members)

| Expected B19 DNA Concentration ($log_{10}$ IU/mL) | Expected B19 DNA Concentration (IU/mL) | Mean B19 DNA Concentration ($log_{10}$ IU/mL) | Lognormal Mean B19 DNA Concentration (IU/mL)[a] | No. of Tests[b] | Lot | Site/ Inst | Day | Batch | Within-Batch | Total Standard Deviation of $Log_{10}$ B19 DNA Concentration |
|---|---|---|---|---|---|---|---|---|---|---|
| 3.000 | 1,000 | 3.09 | 1252 | 176 | 0.0444 | 0.0092 | 0.0000 | 0.0000 | 0.0559 | 0.072 |
| 4.000 | 10,000 | 4.04 | 11008 | 178 | 0.0348 | 0.0141 | 0.0248 | 0.0135 | 0.0543 | 0.072 |
| 5.000 | 100,000 | 5.04 | 111745 | 179 | 0.0305 | 0.0221 | 0.0000 | 0.0265 | 0.0663 | 0.081 |
| 6.000 | 1,000,000 | 6.08 | 1216471 | 179 | 0.0248 | 0.0181 | 0.0166 | 0.0141 | 0.0718 | 0.081 |

[a] Lognormal mean = $10^{\hat{\mu}+\hat{\sigma}^2 \bullet 1.151}$ where the mean and standard deviation are estimates from the random effects variance component models.

[b] Number of tests with detectable viral load. At least 180 tests/panel member were planned. Invalid tests were not repeated.

# Additional information

## Key test features

| | |
|---|---|
| **Sample type** | Plasma |
| **Amount of sample required** | 1000 µL |
| **Amount of sample processed** | 850 µL |
| **Test duration** | Results are available within less than 3.5 hours after loading the sample on the system. |

## Symbols

**The following symbols are used in labeling for Roche PCR diagnostic products.**

**Table 24** Symbols used in labeling for Roche PCR diagnostics products

| | | | |
|---|---|---|---|
|  | Ancillary Software |  | *In Vitro* diagnostic medical device |
|  | Authorized representative in the European community |  | Lower Limit of Assigned Range |
|  | Barcode Data Sheet |  | Manufacturer |
|  | Batch code |  | Store in the dark |
|  | Biological risks |  | Contains sufficient for <*n*> tests |
|  | Catalogue number |  | Temperature limit |
|  | Consult instructions for use |  | Test Definition File |
|  | Contents of kit |  | Upper Limit of Assigned Range |
|  | Distributed by |  | Use-by date |
|  | For IVD performance evaluation only |  | Global Trade Item Number |
| **Rx Only** | US Only: Federal law restricts this device to sale by or on the order of a physician. | | |

 This product fulfills the requirements of the European Directive 98/79 EC for *in vitro* diagnostic medical devices.

US Customer Technical Support 1-800-526-1247

## Manufacturer and distributors

**Table 25** Manufacturer and distributors

Manufactured in the United States

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim, Germany
www.roche.com

Roche Diagnostics (Schweiz) AG
Industriestrasse 7
6343 Rotkreuz, Switzerland

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim, Germany

Roche Diagnostics, SL
Avda. Generalitat, 171-173
E-08174 Sant Cugat del Vallès
Barcelona, Spain

Roche Diagnostica Brasil Ltda.
Av. Engenheiro Billings, 1729
Jaguaré, Building 10
05321-010 São Paulo, SP Brazil

Roche Diagnostics
201, boulevard Armand-Frappier
H7V 4A2 Laval, Québec, Canada
(For Technical Assistance call:
Pour toute assistance technique,
appeler le: 1-877-273-3433)

Roche Diagnostics
2, Avenue du Vercors
38240 Meylan, France

Distributore in Italia:
Roche Diagnostics S.p.A.
Viale G. B. Stucchi 110
20052 Monza, Milano, Italy

Distribuidor em Portugal:
Roche Sistemas de Diagnósticos Lda.
Estrada Nacional, 249-1
2720-413 Amadora, Portugal

## Trademarks and patents

See http://www.roche-diagnostics.us/patents

## Copyright



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# Document revision

| Document Revision Information | |
|---|---|
| Doc Rev. 4.0<br>01/2019 | Updated description of screening tests used for NHP screening in **Table 2** and in the **Warnings and precautions** section.<br>Added Rx Only symbol and description to and updated descriptions of the harmonized symbol page.<br>Updated hazard warnings.<br>Please contact your local Roche Representative if you have any questions. |
| Doc Rev. 5.0<br>05/2019 | Add genotype 3b data to **Table 17**.<br>Clarified genotype 3a used in Linear Range Verification.<br>Corrected typographical error in the name of WHO International Standard.<br>Corrected the **Total** number of samples tested listed in **Table 21**.<br>Please contact your local Roche Representative if you have any questions. |