**TITLE: BuBc Vitros Microslide Assay 7180-CH-185**

**Principle:**

The VITROS BuBc Slide method is performed using the VITROS BuBc Slides and the VITROS Chemistry Products Calibrator Kit 4 on the VITROS 350.

The VITROS BuBc Slide is a multilayered, analytical element coated on a polyester support.

A drop of patient sample is deposited on the slide and is evenly distributed by the spreading layer to the underlying layers. Aided by caffeine and sodium benzoate in the spreading layer, Bu dissociates from albumin and migrates with Bc through the masking layer to the reagent layer. Proteins (including the albumin-bound delta bilirubin and hemoglobin), as well as lipids and lipochromes, are retained in the spreading layer. The masking layer optically blocks potentially interfering compounds trapped in the spreading layer, thereby preventing them from being measured.

In the reagent layer, Bu and Bc bind to a cationic mordant. As a result, the absorbance peaks of the bilirubin fractions are shifted, and molar extinction coefficients are significantly increased.

Bilirubin mono- and di-glucuronides, when bound to the mordant, have identical spectra and are quantitated together as Bc. In the vicinity of 400 to 420 nm, Bu and Bc have similar molar absorptivities; at 460 nm, Bu has a higher molar absorptivity than Bc. Because of these unique spectral characteristics, the reflection densities at two wavelengths, 400 and 460 nm, are used to determine the concentrations of Bu and Bc.

**Test Type and Conditions**

| **Test Type** | **VITROS System** | **Approximate Incubation Time** | **Temperature** | **Wavelength** | **Reaction Sample Volume** |
| --- | --- | --- | --- | --- | --- |
| End-point colorimetric (dual‑wavelength) | 350 | 5 minutes | 37 °C (98.6 °F) | 400 and 460 nm | 10 µL |

**Reaction Scheme**

|  |  |  |
| --- | --- | --- |
| bilirubin complexes | 2 | bilirubin (Bu + Bc) |
| bilirubin + mordant | 3 | bilirubin-mordant complex |

**Warnings and Precautions**

 For *in vitro* diagnostic use only

|  |  |
| --- | --- |
| **WARNING:** | ***Take care when handling materials and samples of human origin. Since no test method can offer complete assurance that infectious agents are absent, consider all clinical specimens, controls, and calibrators potentially infectious. Handle specimens, solid and liquid waste, and test components in accordance with local regulations and CLSI Guideline M29*** [***2***](#d6e4061)  ***or other published biohazard safety guidelines.*** |

For specific warnings and precautions for calibrators, quality control materials, and other components, refer to the Instructions for Use for the appropriate VITROS product, or to other manufacturer’s product literature

**Clinical Significance:**

Jaundice has been classified as unconjugated and conjugated hyperbilirubinemia. Increased plasma-unconjugated bilirubin is commonly seen in hemolytic disorders, Gilbert’s syndrome, Crigler-Najjar syndrome, neonatal jaundice, and ineffective erythropoiesis and in the presence of drugs competing for glucuronide. Increased plasma-conjugated bilirubin occurs with hepatobiliary disorders, including intrahepatic and extrahepatic biliary tree obstruction, liver cell damage, Dubin-Johnson syndrome, and Rotor syndrome.

Neonatal bilirubin, the sum of Bu and Bc, is increased in erythroblastosis fetalis (hemolytic disease of the newborn), which causes jaundice in the first two days of life. Other causes of neonatal jaundice include physiologic jaundice, hematoma/hemorrhage, hypothyroidism, and obstructive jaundice. [1](#d6e4054)

**Specimen Collection, Preparation and Storage:**

 **Specimens Recommended**

* + - * + Serum
				+ Plasma: Heparin

|  |  |
| --- | --- |
| **IMPORTANT:** | *Certain collection devices have been reported to affect other analytes and tests.* [*3*](#d6e4068)  *Owing to the variety of specimen collection devices available, Ortho-Clinical Diagnostics is unable to provide a definitive statement on the performance of its products with these devices. Confirm that your collection devices are compatible with this test.* |

 **Serum and Plasma**

 ***Specimen Collection and Preparation***

 Collect specimens using standard laboratory procedures. [4](#d6e4075)  ,  [5](#d6e4082)

|  |  |
| --- | --- |
| **Note:** | For details on minimum fill volume requirements, refer to the operating instructions for your system. |

 **Patient Preparation**

 No special patient preparation is necessary.

 **Special Precautions**

* + - Protect specimens from light. Refer to “Limitations of the Procedure.”
		- For the effect of sample hemolysis on test results, refer to “Limitations of the Procedure.”
		- Centrifuge specimens and remove the serum or plasma from the cellular material within 4 hours of collection. [6](#d6e4089)

 **Specimen Handling and Storage**

1. Handle and store specimens in stoppered containers to avoid contamination and evaporation.
* Mix samples by gentle inversion and bring to room temperature, 18–28 °C (64–82 °F), prior to analysis

 **Specimen Storage and Stability**  [**6**](#d6e4089)

| **Storage** | **Temperature** | **Stability** |
| --- | --- | --- |
| Room temperature | 18–28 °C (64–82 °F) | ≤ 4 hours |
| Refrigerated | 2–8 °C (36–46 °F) | ≤ 7 days |
| Frozen | ≤-18 °C (≤0 °F) | ≤ 6 months |

**Reagents:**

|  |  |  |
| --- | --- | --- |
| **Slide Ingredients** | 4 | 1. **Upper slide mount**
2. **Spreading layer (TiO2)**
* caffeine
* sodium benzoate
1. **Masking layer**
2. **Reagent layer**
* buffer, pH 8.0
* mordant
1. **Support Layer**
2. **Lower slide mount**
 |
| ***Reactive Ingredients per cm2*** |
| Caffeine 560 µg, sodium benzoate 530 µg; and mordant 180 µg. |
| ***Other Ingredients*** |
| Binders, pigment, buffer, surfactants, stabilizer and cross-linking agent. |

**Reagent Handling**

|  |  |
| --- | --- |
| **Caution:** | **Do not use slide cartridges with damaged or incompletely sealed packaging.** |

* Inspect the packaging for signs of damage.
* Be careful when opening the outer packaging with a sharp instrument so as to avoid damage to the individual product packaging.

**Reagent Preparation**

|  |  |
| --- | --- |
| **IMPORTANT:** | *The slide cartridge must reach room temperature, 18–28 °C (64–82 °F), before it is unwrapped and loaded into the slide supply.* |

1. Remove the slide cartridges from storage.
2. Warm the wrapped cartridge at room temperature for 30 minutes when taken from the

 refrigerator or 60 minutes from the freezer.

1. Unwrap and load the cartridge into the slide supply.

**Reagent Storage and Stability**

VITROS BuBc Slides are stable until the expiration date on the carton when they are stored and handled as specified. Do not use beyond the expiration date

| **Reagent** | **Storage Condition** | **Stability** |
| --- | --- | --- |
| Unopened | Refrigerated | 2–8 °C (36–46 °F) | Until expiration date |
| Frozen | ≤-18 °C (≤0 °F) | Until expiration date |
| Opened | On-analyzer | System turned on | ≤ 2 weeks |
| On-analyzer | System turned off | ≤ 2 hours |

Verify performance with quality control materials:

* If the system is turned off for more than 2 hours.
* After reloading cartridges that have been removed from the slide supply and stored for later use.

**Calibration**

 **Required Calibrators**

 VITROS Chemistry Products Calibrator Kit 4

 **Calibrator Preparation, Handling, and Storage**

 Refer to the Instructions for Use for VITROS Calibrator Kit 4.

 **Calibration Procedure**

 Refer to the operating instructions for your system

 **When to Calibrate**

 Calibrate:

* When the slide lot number changes.
* When critical system parts are replaced due to service or maintenance.
* When government regulations require.

 For example, in the USA, CLIA regulations require calibration or calibration verification at least once every six months.

 The VITROS BuBc test may also need to be calibrated:

* If quality control results are consistently outside acceptable range.
* After certain service procedures have been performed.

 For additional information, refer to the operating instructions for your system.

 **Calculations**

 Reflectance from the slide is measured at two distinct wavelengths, 400 and 460 nm, after a fixed incubation period. Once a calibration has been performed for each slide lot, unconjugated and conjugated bilirubin concentration in unknown samples can be determined using the software-resident endpoint colorimetric, dual-wavelength math model and the responses obtained from each unknown test slide at each wavelength.

 **Validity of a Calibration**

 Calibration parameters are automatically assessed by the system against a set of quality parameters detailed in the Coefficients and Limits screen on VITROS 250/350/950 Systems. Failure to meet any of the pre-defined quality parameters results in a failed calibration. The calibration report should be used in conjunction with quality control results to determine the validity of a calibration.

 **Measuring (Reportable or Dynamic) Range**

|  | **Conventional Units (mg/dL)** | **SI Units (µmol/L)** | **Alternate Units (mg/L)** |
| --- | --- | --- | --- |
| **Bu** | 0.0–27.0 | 0–462 | 0–270 |
| **Bc** | 0.0–27.0 | 0–462 | 0–270 |

For out-of-range samples, refer to “Sample Dilution.”

 **Traceability of Calibration**

 Values assigned to the VITROS Chemistry Products Calibrator Kit 4 for unconjugated and conjugated bilirubin are traceable to internal Master Lots of BuBc slides and Calibrator Kit 4. Performance of the Master Lot of BuBc slides was initially established by comparison to the High Performance Liquid Chromatography (HPLC) method described by Lauff et al. [7](#d6e4095)  The Ortho-Clinical Diagnostics calibration laboratory uses the Master Lot of BuBc slides and Master Lot of Calibrator Kit 4 for unconjugated and conjugated bilirubin value assignment for new lots of VITROS Calibrator Kit 4.

**Quality Control**

Refer to Chemistry Quality Control Procedure for Specifics

At least once each day of use, analyze two levels of a quality control material with known BuBc concentrations. If the results fall outside of the laboratory’s acceptable limits, follow the Chemistry Quality Control Procedure.

 **Quality Control Material Selection**

|  |  |
| --- | --- |
| **IMPORTANT:** | *VITROS Performance Verifiers are recommended for use with the VITROS Chemistry and Integrated Systems. Evaluate the performance of other commercial control fluids for compatibility with this test before using for quality control.* |

* Control materials other than VITROS Performance Verifiers may show a difference when compared with other methods if they:

 – Depart from a true human matrix.

 – Contain high concentrations of preservatives, stabilizers, or other nonphysiological additives.

* + - Use controls that contain both Bu and Bc (or surrogate Bc materials such as di-tauro bilirubin). Monitor both measured components (Bu and Bc).
		- Do not use control materials stabilized with ethylene glycol.

**Quality Control Procedure Recommendations**

* Choose control levels that check the clinically relevant range.
* Analyze quality control materials in the same manner as patient samples, before or during patient sample processing.
* To verify system performance, analyze control materials:

– After calibration.

– According to local regulations or at least once each day that the test is being performed.

– After specified service procedures are performed. Refer to the operating instructions for your system.

* If control results fall outside your acceptable range, investigate the cause before deciding whether to report patient results.
* For general quality control recommendations, refer to *Statistical Quality Control for Quantitative Measurements: Principles and Definitions; Approved Guideline-Third Edition*  [8](#d6e4102)  or other published guidelines.
* For additional information, refer to the operating instructions for your system.

 **Quality Control Material Preparation, Handling, and Storage**

Refer to the Instructions for Use for VITROS Chemistry Products Performance Verifier I and II or to other manufacturer's product literature.

**Testing Procedure**

**Materials Provided**

VITROS Chemistry Products BuBc Slides

**Materials Required but Not Provided**

* VITROS Chemistry Products Calibrator Kit 4
* Quality control materials, such as VITROS Chemistry Products Performance Verifier I and II
* VITROS Chemistry Products 7% BSA

**Operating Instructions**

* Check reagent inventories at least daily to ensure that quantities are sufficient for the planned workload.
* For additional information, refer to the operating instructions for your system.

|  |  |
| --- | --- |
| **IMPORTANT:** | *Bring all fluids and samples to room temperature, 18–28 °C (64–82 °F), prior to analysis.* |

**Sample Dilution**

 If Bu or Bc concentrations exceed the system’s measuring (reportable or dynamic) range

 ***Manual Sample Dilution***

1. Dilute the sample with VITROS 7% BSA or normal patient sample.
2. Reanalyze.
3. Multiply the results by the dilution factor to obtain an estimate of the original sample’s Bu or Bc concentration.

***On-Analyzer Sample Dilution***

Refer to the operating instructions for your system for more information on the On-Analyzer Dilution Procedure.

**Results**

Orders do not cross into interface. Must manually order a Bu and Bc on the Vitros for babies 14 days and younger. Enter results manually using “Resulting Worklist” ychm. Calculate total bilirubin and enter as well.

NBILD= Bc (direct)

NBILI=Bu (indirect)

NBILT= Calculation of Bu+Bc (total)

 **Reporting Units and Unit Conversion**

 The VITROS Chemistry and Integrated Systems may be programmed to report BuBc results in conventional, SI, and alternate units.

| **Conventional Units** | **SI Units** | **Alternate Units** |
| --- | --- | --- |
| mg/dL | µmol/L (mg/dL x 17.1) | mg/L (mg/dL x 10.0) |

**Expected Values**

 **Reference Interval**

The BuBc reference intervals are the central 95% of results from an internal study of 110 apparently healthy adults from a working population (85 females and 25 males) with normal liver enzymes and 40 neonates with an average age of 1.2 days.

 **Reference Interval for BuBc**

| Neonatal Bilirubin-Direct -Bc | 0-30 days | 0.0-0.6 mg/dL |
| --- | --- | --- |
| Neonatal Bilirubin – Indirect –Bu | 0-24 hours | 0.0-7.4 mg/dL |
| Bu | 24 hours-48 hours | 0.0-10.4 mg/dL |
| Bu | 48 hours – 30 days | 0.0-11.4 mg/dL |
| Bu | 30 days – 3 months | 0.0-0.7 mg/dL |

 **Reference Interval for NBIL**

| Neonatal Bilirubin Total  NBIL | 0-24 hours | 1.0-8.0 mg/dL |
| --- | --- | --- |
| NBIL | 24 hours-48 hours | 1.0-11.0 mg/dL |
| NBIL | 48 hours-1 month | 1.0-12.0 mg/dL |
| NBIL | 1month-3 months | 0.0-1.3 mg/dL |

**Procedure Notes**

 **Limitations of the Procedure**

The VITROS BuBc Slide method was screened for interfering substances following NCCLS Protocol EP7. [9](#d6e4109)  The substances listed in the table, when tested at the concentrations indicated, caused the bias shown.

For substances that were tested and did not interfere, refer to “Specificity.”

 **Known Interfering Substances for Bu**

| Interferent**[\*](#d6e1036)** | **Interferent** | **Average Bias** |
| --- | --- | --- |
| **Concentration** | **Conv. (mg/dL)** | **SI (µmol/L)** |
| Hemoglobin | 200 mg/dL | (2 g/L) | -0.4 | -7 |
| 800 mg/dL | (8 g/L) | -0.8 | -14 |
| Amphotericin B | 4 µg/mL | (4.3 µmol/L) | -0.2 | -3 |
| Biliverdin**[\*\*](#d6e1040)** | 4 mg/dL | (68.6 µmol/L) | -1.8 | -31 |
| Levodopa**[\*\*\*](#d6e1044)** | 300 µg/mL | (1.52 mmol/L) | -0.3 | -5 |
| Methotrexate | 50 µg/mL | (0.11 mmol/L) | -0.7 | -12 |
| Nitrofurantoin | 20 µg/mL | (84 µmol/L) | -1.7 | -31 |
| Phenazopyridine | 80 µg/mL | (0.32 mmol/L) | +0.3 | +5 |
| Piroxicam | 10 µg/mL | (30 µmol/L) | -0.4 | -7 |
| Sulfasalazine | 40 µg/mL | (0.10 mmol/L) | -2.3 | -39 |
| Triamterene | 60 µg/mL | (237 µmol/L) | +0.3 | +5 |

**\*** It is possible that other interfering substances may be encountered. These results are representative; however, your results may differ somewhat due to test-to-test variation. The degree of interference at concentrations other than those listed might not be predictable.

**\*\*** The upper limit of high biliverdin concentration is approximately 2.0 mg/dL.

**\*\*\*** 300 µg/mL is the concentration of levodopa found in patients treated for Parkinson’s disease.

 **Known Interfering Substances for Bc**

| Interferent**[\*](#d6e1306)** | **Interferent** | **Average Bias** |
| --- | --- | --- |
| **Concentration** | **Conv. (mg/dL)** | **SI (µmol/L)** |
| Hemoglobin | 200 mg/dL | (2 g/L) | +0.4 | +7 |
| 800 mg/dL | (8 g/L) | +0.8 | +14 |
| Amphotericin B | 4 µg/mL | (4.3 µmol/L) | +0.4 | +6 |
| Biliverdin**[\*\*](#d6e1310)** | 4 mg/dL | (68.6 µmol/L) | +3.2 | +51 |
| Levodopa**[\*\*\*](#d6e1314)** | 300 µg/mL | (1.52 mmol/L) | +0.8 | +14 |
| Methotrexate | 50 µg/mL | (0.11 mmol/L) | +1.1 | +19 |
| Nitrofurantoin | 20 µg/mL | (84 µmol/L) | +3.2 | +58 |
| Phenazopyridine | 80 µg/mL | (0.32 mmol/L) | +2.1 | +34 |
| Piroxicam | 10 µg/mL | (30 µmol/L) | +0.8 | +12 |
| Sulfasalazine | 40 µg/mL | (0.10 mmol/L) | +4.0 | +77 |
| Triamterene | 60 µg/mL | (237 µmol/L) | -0.5 | -9 |

**\*** It is possible that other interfering substances may be encountered. These results are representative; however, your results may differ somewhat due to test-to-test variation. The degree of interference at concentrations other than those listed might not be predictable.

**\*\*** The upper limit of high biliverdin concentration is approximately 2.0 mg/dL.

**\*\*\*** 300 µg/mL is the concentration of levodopa found in patients treated for Parkinson’s disease.

**Other Limitations**

* *In vitro* exposure to light may alter bilirubin chemical and spectral properties because of the formation of photobilirubin. Specimens from patients receiving intensive light therapy may also exhibit an increase in the measured Bc because of *in vivo* formation of photobilirubin. [10](#d6e4116)
* Bc results flagged with a Potential Interferent (PI) code should be repeated with the VITROS TBIL Slide, which is not sensitive to the same spectral interferents.
* Bu results flagged with a Potential Interferent (PI) code should be diluted with a normal patient sample or VITROS 7% BSA and rerun on the BuBc Slide.
* Certain drugs and clinical conditions are known to alter Bu and Bc concentrations *in vivo*. For additional information, refer to one of the published summaries. [11](#d6e4123)  ,  [12](#d6e4131)

**Performance Characteristics**

 **Method Comparison**

The Bu plots and table show the results of a comparison of serum samples analyzed on the VITROS 750 System with those analyzed using the Jendrassik-Grof/Lauff HPLC comparative method. [7](#d6e4095)

The table also shows the results of comparisons of serum samples analyzed on the VITROS 250 and 950 Systems and the VITROS 750 System, and comparisons of serum samples on the VITROS 5,1 FS System and the VITROS 950 System.

In addition, the table shows the results of a comparison of serum and plasma samples on the VITROS 5600 Integrated System and the VITROS 5,1 Chemistry System. The testing followed NCCLS Protocol EP9. [13](#d6e4138)

 **Method Comparison for Bu**





**†** Analytical processing hardware and software algorithms on the VITROS 4600 Chemistry System are designed to the same specifications as those applied to the VITROS 5,1 FS Chemistry System. Assay performance on the VITROS 4600 System has been demonstrated to be comparable to that on the VITROS 5,1 FS System. All performance characteristics for VITROS 5,1 FS System are therefore applicable to the VITROS 4600 System.

The Bc plots and table show the results of a comparison of serum samples analyzed on the VITROS 950 System with those analyzed using the Jendrassik-Grof/Lauff HPLC comparative method. [7](#d6e4095)

 The table also show the results of comparisons of serum samples on the VITROS 250 and 950 Systems with the VITROS 750 System and comparisons of serum samples on the VITROS 5,1 FS System with the VITROS 950 System.

 In addition, the table shows the results of a comparison of serum and plasma samples on the VITROS 5600 Integrated System and the VITROS 5,1 Chemistry System. The testing followed NCCLS Protocol EP9. [13](#d6e4138)

 **Method Comparison for Bc**

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**†** Analytical processing hardware and software algorithms on the VITROS 4600 Chemistry System are designed to the same specifications as those applied to the VITROS 5,1 FS Chemistry System. Assay performance on the VITROS 4600 System has been demonstrated to be comparable to that on the VITROS 5,1 FS System. All performance characteristics for VITROS 5,1 FS System are therefore applicable to the VITROS 4600 System.

 **Precision**

 Precision was evaluated with quality control materials on VITROS 250, 950 and 5,1 FS Systems following NCCLS Protocol EP5. [14](#d6e4145)  Precision was also evaluated with quality control materials on VITROS 5600 Integrated System following NCCLS protocol EP5. [15](#d6e4152)

 The data presented are a representation of test performance and are provided as a guideline. Variables such as sample handling and storage, reagent handling and storage, laboratory environment, and system maintenance can affect reproducibility of test results.

 **Bu**

|  | **Conventional Units (mg/dL)** | **SI Units (µmol/L)** | **Within Lab CV%** [**\*\***](#d6e3097)  | **No. Observ.** | **No. Days** |
| --- | --- | --- | --- | --- | --- |
| **Mean Conc.** | **Within Day SD** [**\***](#d6e3093)  | **Within Lab SD** [**\*\***](#d6e3097)  | **Mean Conc.** | **Within Day SD** [**\***](#d6e3093)  | **Within Lab SD** [**\*\***](#d6e3097)  |
| **250** | 0.4 | 0.01 | 0.03 | 7 | 0.2 | 0.5 | 8.5 | 79 | 20 |
| 9.7 | 0.11 | 0.15 | 166 | 1.9 | 2.6 | 1.6 | 78 | 20 |
| **750** | 0.2 | 0.01 | 0.06 | 4 | 0.2 | 1.0 | 23.7 | 90 | 23 |
| 9.6 | 0.09 | 0.11 | 164 | 1.5 | 1.9 | 1.1 | 91 | 23 |
| **950** | 0.1 | 0.01 | 0.06 | 2 | 0.3 | 1.0 | 43.6 | 90 | 23 |
| 9.6 | 0.10 | 0.13 | 165 | 1.7 | 2.3 | 1.4 | 91 | 23 |
| **5,1 FS** [**†**](#d6e2619)  | 0.6 | 0.01 | 0.03 | 11 | 0.2 | 0.5 | 4.9 | 90 | 22 |
| 9.7 | 0.09 | 0.18 | 166 | 1.6 | 3.1 | 1.9 | 90 | 22 |
| **5600** | 0.7 | 0.02 | 0.04 | 12 | 0.3 | 0.7 | 5.7 | 88 | 22 |
| 9.7 | 0.12 | 0.19 | 166 | 2.1 | 3.2 | 2.0 | 88 | 22 |

**†** Analytical processing hardware and software algorithms on the VITROS 4600 Chemistry System are designed to the same specifications as those applied to the VITROS 5,1 FS Chemistry System. Assay performance on the VITROS 4600 System has been demonstrated to be comparable to that on the VITROS 5,1 FS System. All performance characteristics for VITROS 5,1 FS System are therefore applicable to the

VITROS 4600 System.

 **Bc**

|  | **Conventional Units (mg/dL)** | **SI Units (µmol/L)** | **Within Lab CV%** [**\*\***](#d6e3097)  | **No. Observ.** | **No. Days** |
| --- | --- | --- | --- | --- | --- |
| **Mean Conc.** | **Within Day SD** [**\***](#d6e3093)  | **Within Lab SD** [**\*\***](#d6e3097)  | **Mean Conc.** | **Within Day SD** [**\***](#d6e3093)  | **Within Lab SD** [**\*\***](#d6e3097)  |
| **250** | 0.5 | 0.03 | 0.06 | 8 | 0.5 | 1.0 | 12.9 | 79 | 20 |
| 3.9 | 0.08 | 0.13 | 67 | 1.4 | 2.2 | 3.3 | 78 | 20 |
| **750** | 0.3 | 0.03 | 0.05 | 5 | 0.5 | 0.9 | 18.5 | 90 | 23 |
| 3.6 | 0.04 | 0.09 | 61 | 0.7 | 1.5 | 2.5 | 91 | 23 |
| **950** | 0.4 | 0.03 | 0.07 | 7 | 0.5 | 1.3 | 18.3 | 90 | 23 |
| 3.4 | 0.04 | 0.06 | 59 | 0.7 | 1.1 | 1.8 | 91 | 23 |
| **5,1 FS** [**†**](#d6e3101)  | 0.4 | 0.02 | 0.05 | 7 | 0.4 | 0.8 | 10.3 | 90 | 22 |
| 4.1 | 0.04 | 0.07 | 71 | 0.7 | 1.2 | 1.7 | 86 | 22 |
| **5600** | 0.5 | 0.04 | 0.06 | 9 | 0.7 | 1.0 | 12.0 | 88 | 22 |
| 4.5 | 0.06 | 0.09 | 77 | 1.0 | 1.5 | 2.0 | 88 | 22 |

**\*** Within Day precision was determined using two runs/day with two to three replications.

**\*\*** Within Lab precision was determined using a single lot of slides and calibrating weekly.

**†** Analytical processing hardware and software algorithms on the VITROS 4600 Chemistry System are designed to the same specifications as those applied to the VITROS 5,1 FS Chemistry System. Assay performance on the VITROS 4600 System has been demonstrated to be comparable to that on the VITROS 5,1 FS System. All performance characteristics for VITROS 5,1 FS System are therefore applicable to the VITROS 4600 System.

 **Specificity**

**Substances that Do Not Interfere**

 The substances listed in the table were tested with VITROS BuBc Slides at an approximate bilirubin concentration of 1.0 mg/dL (17 µmol/L) following NCCLS Protocol EP7 [9](#d6e4109)  and found not to interfere, bias <0.2 mg/dL (<3.4 µmol/L), at the concentration shown.

| **Compound** | **Concentration** |  | **Compound** | **Concentration** |
| --- | --- | --- | --- | --- |
| Acetaminophen | 20 mg/dL | 1323 µmol/L |  | Ibuprofen | 40 mg/dL | 1.9 mmol/L |
| Acetate | 25 mg/dL | 4.2 mmol/L |  | Indomethacin | 1 mg/dL | 28 µmol/L |
| Acetoacetic acid | 20 mg/dL | 2 mmol/L |  | Intralipid | 800 mg/dL | 8 g/L |
| Acyclovir | 25 µg/mL | 11 µmol/L |  | Lactulose | 65 µg/mL | 190 µmol/L |
| Amiloride | 0.1µg/mL | 435 nmol/L |  | Lidocaine | 6 mg/dL | 256 µmol/L |
| Amphotericin B | 2 µg/mL | 2.2 µmol/L |  | Mannitol | 100 mg/dL | 5.5 mmol/L |
| Ampicillin | 200 µg/mL | 572 µmol/L |  | Menadione Na bisulfite (Vitamin K3) | 2 µg/mL | 12 µmol/L |
| Ascorbic acid | 6 mg/dL | 341 µmol/L |  | N-Acetylcysteine | 200 mg/dL | 12.3 mmol/L |
| Azathioprine | 1 µg/mL | 4 µmol/L |  | Neomycin | 12 µg/mL | 20 µmol/L |
| B-Hydroxybutyrate | 20 mg/dL | 1921 µmol/L |  | Phenobarbital | 15 mg/dL | 646 µmol/L |
| Bromocriptine | 5 ηg/mL | 7.6 nmol/L |  | Phenylpropanolamine | 1.25 mg/dL | 83 µmol/L |
| Caffeine | 10 mg/dL | 515 µmol/L |  | Phenytoin | 10 mg/dL | 396 µmol/L |
| Beta-carotene | 0.6 mg/dL | 11 µmol/L |  | Prednisone | 0.1 µg/mL | 279 nmol/L |
| Cefoperazone (Cefobid) | 400 µg/mL | 620 µmol/L |  | Prednisolone | 1 µg/mL | 2.8 µmol/L |
| Chloramphenicol | 25 mg/dL | 774 µmol/L |  | Procainamide | 10 mg/dL | 425 µmol/L |
| Cholesterol | 450 mg/dL | 11.6 mmol/L |  | Propranolol | 5 µg/mL | 19 µmol/L |
| Cholic acid | 6 mg/dL | 147 µmol/L |  | Propylthiouracil | 10 µg/mL | 59 µmol/L |
| Codeine | 25 mg/dL | 835 µmol/L |  | Pseudoephedrine | 3 mg/dL | 181 µmol/L |
| Colchicine | 35 ng/mL | 87.6 nmol/L |  | Retinol | 1885 U/mL | 1885 U/mL |
| Cyclosporine | 3.5 µg/mL | 3 µmol/L |  | Salicylate | 50 mg/dL | 3.6 mmol/L |
| Dextran 40 | 3000 mg/dL | 750 µmol/L |  | Spironolactone | 0.5 µg/mL | 1.2 µmol/L |
| Diphenhydramine hydrochloride | 0.2 µg/mL | 781 nmol/L |  | Sulfamethoxazole | 350 µg/mL | 1.4 mmol/L |
| Doxorubicin | 0.55 µg/mL | 1.0 µmol/L |  | Sulfisoxazole | 100 mg/dL | 3.7 mmol/L |
| Erythromycin | 20 mg/dL | 173 µmol/L |  | Theophylline | 25 mg/dL | 1.4 mmol/L |
| Ethanol | 350 mg/dL | 76.1 mmol/L |  | Alpha-tocopherol | 0.24 U/mL | 0.24 U/mL |
| 5-Fluorouracil | 15 mg/dL | 1153 µmol/L |  | Tolbutamide | 100 mg/dL | 3.7 mmol/L |
| Furosemide | 2 mg/mL | 6 mmol/L |  | Total protein | 4.0 g/dL | 40 g/L |
| Gentamicin | 12 mg/dL | 256 µmol/L |  | Total protein | 9.0 g/dL | 90 g/L |
| Hydrochlorothiazide | 2 µg/mL | 7 µmol/L |  | Triamterene | 15 µg/mL | 59 µmol/L |
| Hydroxyurea | 250 µg/mL | 3.3 mmol/L |  | Triglycerides | 900 mg/dL | 10.2 mmol/L |
| Hydroxyzine | 1 µg/mL | 2.7 µmol/L |  | Trimethoprim | 25 µg/mL | 86 µmol/L |
| Hypaque | 500 mg/dL | 8.2 mmol/L |  | Urea nitrogen | 100 mg/dL | 35.7 mmol/L |

**References**

1. Tietz NW (ed). *Fundamentals of Clinical Chemistry*. ed. 3. Philadelphia: WB Saunders; 730–736; 1987.
2. CLSI. *Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline – Third Edition.* CLSI document M29-A3 (ISBN 1-56238-567-4). CLSI, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898 USA; 2005.
3. Calam RR. Specimen Processing Separator Gels: An Update. *J Clin Immunoassay*. 11:86–90; 1988.
4. CLSI. *Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture; Approved Standard – Sixth Edition.*  CLSI document H3-A6 (ISBN 1-56238-650-6). CLSI, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA; 2007.
5. NCCLS. *Procedures and Devices for the Collection of Diagnostic Capillary Blood Specimens; Approved Standard – Fifth Edition.* NCCLS document H4-A5 [ISBN 1-56238-538-0]. CLSI, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2004.
6. *Clinical Laboratory Handbook for Patient Preparation and Specimen Handling*. Fascicle VI: Chemistry/Clinical Microscopy. Northfield, IL: College of American Pathologists; 1992.
7. Lauff JJ, Kasper ME, Wu T.W, Ambrose RT. Isolation and preliminary characterization of a fraction of bilirubin in serum that is firmly bound to protein. *Clin. Chem.* 28:629–637, 1982.
8. NCCLS. *Statistical Quality Control for Quantitative Measurements: Principles and Definitions; Approved Guideline – Third Edition.* CLSI document C24-A3 (ISBN 1-56238-613-1). CLSI, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898 USA; 2006.
9. NCCLS.*Interference Testing in Clinical Chemistry*. NCCLS Document EP7. CLSI, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898 USA; 1986.
10. Schlebusch H, Grunn U, Liappis N. *Bilirubin-Fractions in Sera of Newborns Undergoing Phototherapy, as Measured by HPLC and Kodak Ektachem Analyzer*, July 31, 1991 presented at the AACC National Meeting.
11. Young DS. *Effects of Drugs on Clinical Laboratory Tests*. ed. 4. Washington D.C.: AACC Press; 1995.
12. Friedman RB, Young DS. *Effects of Disease on Clinical Laboratory Tests*. Washington, D.C.: AACC Press; 1990.
13. NCCLS. *Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline.* NCCLS document EP9-A2 [ISBN 1-56238-472-4]. CLSI, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA; 2002.
14. NCCLS. *User Evaluation of Precision Performance with Clinical Chemistry Devices*. NCCLS Document EP5. CLSI, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898 USA; 1992.
15. NCCLS. *Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline – Second Edition.* NCCLS document EP5-A2 [ISBN 1-56238-542-9]. CLSI, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898 USA; 2004.