CHEM.VITROS.ASSAY.26.0 BILIRUBIN, TOTAL, BY VITROS (TBIL)

STATEMENT OF PURPOSE

The Vitros TBIL slides are an in vitro diagnostic test to quantitatively measure total bilirubin (TBIL) concentration in serum and plasma. Total bilirubin in serum and plasma is the sum of unconjugated bilirubin (Bu), mono- and di-glucuronide conjugated bilirubin (Bc), and delta bilirubin (DELB), a bilirubin fraction covalently bound to albumin. With the exception of anicteric jaundice, total serum bilirubin is invariably increased in jaundice. Causes of jaundice are prehepatic, resulting from various hemolytic diseases; hepatic, resulting from hepatocellular injury or obstruction; and posthepatic, resulting from obstruction of the hepatic or common bile ducts.

PRINCIPLE

The VITROS TBIL Slide is a multilayered, analytical element coated on a polyester support. The analysis is based on a modification of the classic diazo reaction. A drop of patient sample is deposited on the slide and is evenly distributed by the spreading layer to the underlying layers. This layer provides a reflective background for measuring the diazo products of bilirubin and contains all reagents necessary to determine total bilirubin. The method uses dyphylline to dissociate unconjugated bilirubin from albumin. Unconjugated bilirubin, conjugated bilirubin, and albumin-linked bilirubin (delta) subsequently react with the diazonium salt 4-(N carboxymethylsulfonyl) benzenediazonium hexafluorophosphate to produce azobilirubin chromophores that have similar molar absorptivities and absorbance maxima around 520 nm. The concentration of total bilirubin is determined by measuring the azobilirubin chromophores at two wavelengths through the transparent support. The reflectance measurement at 460 nm corrects for spectral interferences.

OWNERS

Hospital Based Laboratories

RELATED DOCUMENTS

CHEM.VITROS.1.0 *Operation of the 250 and 350 Vitros Analyzer Systems*
CHEM.VITROS.2.0 *Vitros 250 and 350 Calibration Procedure*

SPECIMEN

A. Normal procedures for collecting and storing serum and plasma may be used for samples to be analyzed by this method.
B. No patient preparation required.
C. Serum or heparinized plasma is acceptable.
D. Protect specimens from light.
E. Centrifuge specimens and remove the serum or plasma from the cellular material ≤ 4 hours of collection.
F. Handle and store specimens in stoppered containers to avoid contamination and evaporation.
G. Specimen stability
    1. Room temperature: ≤ 4 hours

2. Refrigerated: ≤ 7 days
3. Frozen: ≤ 6 months

H. Mix samples by gentle inversion and bring to room temperature (18–28°C) prior to analysis.

REAGENTS

A. TBIL Vitros Slides:
1. Remove the slide cartridges from storage.
   a. Inspect the packaging for signs of damage.
   b. Do not use slide cartridges with damaged or incompletely sealed packaging.
2. Warm the wrapped cartridge at room temperature for 60 minutes if removed from Freezer or 30 minutes if removed from refrigerator.
3. Unwrap and load the cartridge into the slide supply.
4. Be careful when opening the outer packaging with a sharp instrument so as to avoid damage to the individual product packaging.
5. Storage & Stability:
   a. Unopened Frozen: ≤-18°C or Refrigerated 2–8 °C until expiration date on package
   b. Opened On-analyzer: ≤ 2 weeks.

EQUIPMENT

Vitros Chemistry Analyzer

CALIBRATION

A. VITROS Chemistry Products Calibrator Kit 4.
1. Refer to the Instructions for Use for VITROS Calibrator Kit 4.
2. Refer to Vitros Calibration Procedure

B. Calibration Required when:
1. When the slide lot number changes.
2. When critical system parts are replaced due to service or maintenance
3. Calibration or calibration verification at least once every six months
4. As needed.

QUALITY CONTROL

A. Analyze two levels of quality control material each day of patient testing.

B. Refer to Vitros Operation Procedure

PROCEDURE

A. TBIL Vitros slides are required to perform the Total Bilirubin (TBIL) test. This test is performed after the slides are calibrated and QC ran.

B. Load specimen on Vitros per Vitros Operation procedure.

CALCULATIONS

Reflectance from the slide is measured at two distinct wavelengths, 540 and 460 nm, after a fixed incubation time, and net density is determined from the difference between the two measurements. Once a calibration has been performed for each slide lot, total bilirubin concentration in unknown samples can be determined using the software-resident blank-corrected (dualwavelength) colorimetric math model and the responses obtained from each unknown test slide at each wavelength.

REPORTING RESULTS

A. The instrument automatically calculates and prints the concentration of total bilirubin in mg/dL.

B. Measurement Range: 0.10 – 27.0 mg/dL.

C. If samples show total bilirubin concentrations that exceed the system's measuring (reportable or dynamic) range:
   1. Dilute 1 part sample with 1 part reagent-grade water or normal patient sample.
   2. Reanalyze.
   3. Multiply the results by 2 to obtain an estimate of the original sample's total bilirubin concentration.
   4. If necessary, correct for the bilirubin concentration in the diluent sample.

D. Reference Range: 0.2 – 1.3 mg/dL

E. Critical Values:

< 1 day old:	>12.0 mg/dL
1 day old:	>13.0 mg/dL
2 days old:	> 15.0 mg/dL
3 days - < 15 days:	>18.0 mg/dL
15 days – 2 years:	>20.0 mg/dL

NOTE: All hospital registered outpatient bilirubin results on babies <5 days old will be called to the ordering physician regardless of the bilirubin result

LIMITATIONS

A. Do not use this slide for specimens from neonatal patients less than 14 days old.

B. Known Interfering Substances

1. Cefotiam (Pansporin)
2. Levodopa
3. 4-Aminosalicylic acid
4. Phenazopyridine
5. Biliverdin
6. Hemoglobin

REFERENCES

Vitros Chemistry Products Online Guide