VITROS Products Chemistry

INSTRUCTIONS FOR USE SUPPLEMENT

Bilirubin Supplement for the
VITROS 250/350/5,1 FS/4600/XT 3400 Chemistry Systems and the
VITROS 5600/XT 7600 Integrated Systems

Bilirubin

Summary and Background Information

Traditionally, both laboratorians and clinicians have used the term **direct bilirubin** to mean bilirubin conjugated to glucuronic acid (Bc) and the term **indirect bilirubin** to mean unconjugated bilirubin (Bu).1 The concentration of each of the different forms of serum bilirubin provides important additional diagnostic information for clinicians when compared to the measurement of **total bilirubin** (TBIL) alone. This information can assist clinicians in diagnosing, treating, and monitoring certain disease states.

Depending upon the analytical method used, particular bilirubin fractions may be measured either directly, or calculated from the results of direct measurements of other bilirubin fractions. With VITROS Chemistry and Integrated Systems, TBIL, Bu, and Bc are directly measured. Other, more conventional bilirubin methods, especially those employing diazo chemistry, directly measure both total bilirubin and the direct bilirubin fraction (approximately equivalent to Bc), but cannot directly measure unconjugated bilirubin (Bu).

For these diazo chemistry methods, unconjugated bilirubin (Bu) is determined by using a calculation, once the total bilirubin and the direct bilirubin measurements are known (Indirect bilirubin = Total Bilirubin - Direct Bilirubin). While this approach is certainly a viable alternative, it is important to recognize that variation and errors in determination of either of the measured fractions may contribute significantly to errors in the estimation of the indirect bilirubin. Because unconjugated bilirubin (Bu) is directly measured on VITROS Chemistry and Integrated Systems, lower analytical variation in the determination of unconjugated bilirubin (Bu) is achievable when using the VITROS BuBc Slides.

Bilirubin Fractions on VITROS Chemistry and Integrated Systems*

Abbreviation	Analyte	Reference Interval mg/dL	Reference Interval µmol/L	Measured / Calculated by VITROS System	Bilirubin Fractions Included
TBIL	Total Bilirubin	0.2-1.3	3-22	Measured	All bilirubin fractions (not recommended for neonates)
Bu	Unconjugated Bilirubin	Adults: 0.0-1.1 Neonates: 0.6-10.5	Adults: 0-19 Neonates: 10-180	Measured	Unconjugated bilirubin
Bc	Conjugated Bilirubin	Adults: 0.0-0.3 Neonates: 0.0-0.6	Adults: 0-5 Neonates: 0-10	Measured	Bilirubin mono- and di-glucuronides
DBIL	Direct Bilirubin	0.0-0.4	0-7	Calculated Calculation: DBIL = TBIL–Bu	Conjugated bilirubin and delta bilirubin
DELB	Delta Bilirubin	0.0-0.2	0-3	Calculated Calculation: DELB = TBIL – (Bu + Bc)	Bilirubin covalently bound to albumin
NBIL	Neonatal Bilirubin	1.0-10.5	17-180	Calculated Calculation: NBIL = Bu + Bc	Unconjugated bilirubin and Conjugated bilirubin

*Not all products and systems are available in all countries

Relationship Between TBIL and BuBc Measurements

Since TBIL, Bu, and Bc are each measured independently on VITROS Chemistry and Integrated Systems, expected uncertainties of each measurement can mean that the sum of the results of (Bu + Bc) may not be exactly equal to the TBIL result.

Although each of these assays is quite precise with VITROS Chemistry and Integrated Systems, the normally expected variability in the independent measurements of TBIL, Bu, and Bc, will occasionally produce a sum of (Bu + Bc) that may be slightly greater or slightly less than [up to ± 0.3 mg/dL (5 µmol/L)] the TBIL result, even when the true concentrations of (Bu + Bc) and TBIL are identical.

NOTE: To minimize variability due to calibrator reconstitution, time of calibration, and instrument maintenance, you may wish to calibrate VITROS TBIL Slides and VITROS BuBc Slides at the same time. Calibration alone will not eliminate the (Bu + Bc) vs. TBIL differences due to random measurement variation, but will minimize any systematic differences which you may encounter.

- A negative DBIL result with the VITROS Chemistry and Integrated Systems can occur in normal subjects, when Bu is the only bilirubin species actually present. A negative result is due to an artifact of the calculation (VITROS calculated DBIL = TBIL - Bu), when the measured Bu result is slightly higher than the TBIL measurement.
- Direct bilirubin measurements have been shown to be variable, depending on the diazo method used. At low or normal concentrations of serum bilirubin, these methods tend to overestimate conjugated bilirubin. However, in subjects with higher concentrations of direct bilirubin, as typically observed in patients with obstructive jaundice, many of these same methods do not recover the direct-reacting bilirubin fractions completely (the conjugated and delta species). These analytical errors also affect the calculated Indirect bilirubin; underestimating concentrations at normal total bilirubin levels, and overestimating it for patients with elevated total bilirubin.[2, 3, 4, 5]
- Delta bilirubin (DELB) is not expected to be present in healthy adults or neonates. In the absence of liver pathology, if the sum of (Bu + Bc) is less than the TBIL result, and both values are within normal ranges, a positive calculated DELB value should *not* be interpreted as an increase in Delta Bilirubin.
- Differences in the VITROS TBIL and VITROS BuBc methods due to effects of bilirubin photodegradation and/or presence of interferences such as hemolysis, may also contribute to instances where the sum of (Bu + Bc) does not equal the TBIL result.

Clinical Utility of Bilirubin Fractions

If the Unconjugated Bilirubin fraction (Bu) is increased

Mechanism	Disease State
Impaired conjugation	Physiological jaundice Crigler- Najjar Syndrome
Increased production	Hemolytic jaundice (kernicterus in neonates)
Decreased uptake	Gilbert's disease

If the Conjugated Bilirubin fraction (Bc) is increased

Mechanism	Disease State
Biliary obstruction	Biliary calculi
Impaired secretion	Dubin-Johnson Syndrome

Bilirubin in Neonatal Specimens

Recommended Approach

The VITROS BuBc Slide should be used to measure bilirubin in neonatal specimens. Reporting the results for Bu, Bc, and Neonatal Bilirubin (NBIL) provides clinicians with a complete clinical picture.

It is important to maintain consistency in bilirubin methodology. Regardless of the infant's age, if BuBc is used to monitor a patient initially, then continue to monitor the patient using BuBc. Do not switch to TBIL regardless of the patient's age. TBIL slides are *not* to be used for neonatal samples. Biases of up to ±10% have been observed with these samples when using the VITROS TBIL Slides.

Rationale for Recommending BuBc

- Increased bilirubin loads, due to catabolism of fetal hemoglobin, and a deficiency in glucuronidase, result in a reduced capacity to conjugate bilirubin. Therefore, the major bilirubin fraction in neonatal specimens is Bu. In a healthy neonate, the conjugated bilirubin result is expected to be close to 0 mg/dL (0 µmol/L). Delta bilirubin is negligible in neonates less than 14-21 days old; however, if present, it is associated with an elevated Bc result.[6] No clinical utility for Delta-bilirubin has been reported.
- In vivo exposure to light may alter bilirubin chemical and spectral properties because of the formation of photobilirubin. Specimens from patients receiving intensive phototherapy may also exhibit an increase in the measured Bc because of the *in vivo* formation of photobilirubin.

Bilirubin in Adult Specimens

Recommended Approach

The VITROS BuBc Slide or a combination of the VITROS BuBc and VITROS TBIL Slides should be used to measure bilirubin and its fractions in adult specimens. BuBc and TBIL results provide information to help clinicians better diagnose, treat, and monitor patient's disease states. Typically, Bu is the only bilirubin fraction present in normal adult specimens.

How Laboratories Report Bilirubin Results

Laboratories report a variety of combinations of bilirubin results, which are dependent upon the unique laboratory setting and the needs of the clinicians they serve. Some examples of reporting combinations using the VITROS bilirubin methods are:

- Reporting TBIL, Bu, and Bc results provide complete clinical information on all measured results. Bc results have been considered more useful than calculated Direct Bilirubin (DBIL) because Bc is a better indicator of relief from hepatobiliary obstruction than DBIL.[7] Calculated Direct bilirubin results include the Delta bilirubin fraction that may remain elevated longer than the Bc in an otherwise improving clinical picture.
- Reporting TBIL and DBIL most closely approximates historical practice. DBIL results are calculated on the VITROS Chemistry and Integrated Systems (DBIL= TBIL- Bu).
- Reporting TBIL, Bu, Bc, DBIL and DELB provides all measured and calculated results from the VITROS Chemistry and Integrated Systems.

References

1. Lott JA. New Concepts in Serum Bilirubin Measurement. *Laboratory Management.* 1987.
2. Lo DH, Wu Tai-Wing. Assessment of the Fundamental Accuracy of the Jendrassik-Grof Total and Direct Bilirubin Assays. *Clin.Chem.* 29/1:31-36; 1983.
3. Ambrose RT, Kasper ME, and Lauff JJ. Quantitative Liquid-Chromatographic Estimation of Bilirubin Species in Pathological Serum. *Clin. Chem.* 29/5:800-805; 1983
4. Powers DM, Lasky FD, Ferris RE, Shirey DJ, and O'Connor JE. *Measurement of "Direct-Reacting" Bilirubin by Diazo Methods, HPLC, and KODAK EKTACHEM Fractionated Bilirubin Slides: Clinical Impact*. Presented October 1, 1985 at 6ème Colloque International Biologie Perspective, Pont-à-Mousson, France.
5. Alpers J, Riddle K, Chan D, Gerhardt G, Graham G, Williams J, Hicks J, Mueller K, Lasky F, and Day B, 1985. Interlaboratory Comparison of Direct Bilirubin Methods, July 25, presented at the AACC National Meeting, Atlanta, Georgia.
6. Brett EM, Hicks JM, Powers DM, and Rand RN. Delta Bilirubin in Serum of Pediatric Patients: Correlations with Age and Disease. *Clin. Chem.* 30: 1561-1564; 1984.
7. Arvan D, Shirey TL. Conjugated Bilirubin: A Better Indicator of Impaired Hepatobiliary Excretion than Direct Bilirubin. *Ann Clin Lab Sci.* 15: 252-259; 1985.

Revision History

Date of Revision	Version	Description of Technical Changes*
2019-09-06	8.0	• Added information for the VITROS XT 3400 Chemistry System • Updated EC Representative address • Statement Added: "Not all products and systems are available in all countries"

* The change bars indicate the position of a technical amendment to the text with respect to the previous version of the document.

When this Instructions For Use is replaced, sign and date below and retain as specified by local regulations or laboratory policies, as appropriate.

_____ _____

Signature Obsolete Date

CE

EC REP

Ortho-Clinical Diagnostics
1500 Boulevard Sébastien Brant
B.P. 30335
67411 Illkirch
CEDEX, France

Ortho-Clinical Diagnostics, Inc.
100 Indigo Creek Drive
Rochester, NY 14626
USA

Ortho Clinical Diagnostics