ALL CHILDREN'S HOSPITAL
PATHOLOGY AND LABORATORY MEDICINE

Bilirubin Testing on New Chemistry System

All Children's Hospital Department of Pathology and Laboratory Medicine will soon be replacing their chemistry analyzer with the Ortho Vitros 5600 chemistry integrated system. This system compliments our service to our customers by providing clinicians with a direct detection of conjugated and unconjugated bilirubin. For pediatricians, this means true neonatal bilirubin determinations as opposed to common calculated methods (indirect bilirubin).

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). 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) 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) Systems, lower analytical variation in the determination of unconjugated bilirubin (Bu) is achievable when using the VITROS BuBc test.

For more information on how this new testing method impacts neonatal and adult patient testing, please refer to the following pages.

Bilirubin in Neonatal Specimens

Recommended Approach

The VITROS BuBc test is used to measure bilirubin in neonatal specimens. The total bilirubin is then calculated and referred to as neonatal bilirubin (NBIL). 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. Bu (unconjugated) and Bc (conjugated) will be measured in neonates (0-30 days) and total bilirubin will be calculated. After thirty days Bu, Bc and total bilirubin will be measured, however, if BuBc has been used to monitor a neonate, it is recommended that BuBc monitoring is continued. TBIL measured should *not* be used for neonatal samples. Biases of up to ±10% have been observed with these samples when using measured TBIL.

Rationale for Recommending BuBc

• Increased bilirubin, 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 (0μmol/L). Delta bilirubin is negligible in neonates less than 14-21 days old; however, if present, it is associated with an elevated Bc result. No clinical utility for Delta-bilirubin has been reported.

• In vivo exposure to light may alter bilirubin's 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). The VITROS Bc method is less susceptible to the photodegradation of the sample and, thus gives a more exact value of the bilirubin level of the patient.

Bilirubin in Adult Specimens

Recommended Approach

A combination of the BuBc and TBIL tests will be used to measure bilirubin and its fraction 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:

Bilirubin Fractions on VITROS Chemistry Systems

Abbreviations	Analyte	Reference Interval Mg/dL	Measured/Calculated by VITROS system	Bilirubin Fractions Included
TBIL	Total Bilirubin	0.2-1.3	Measured	All bilirubin fractions (not recommended for neonates)
Bu	Unconjugated Bilirubin	Adults: 0.0 – 1.1 Neonates: 0.5 – 10.5	Measured	Unconjugated bilirubin
Bc	Conjugated Bilirubin	Adults: 0.0 – 0.3 Neonates: 0.0 – 0.6	Measured	Bilirubin mono- and di-glucuronides
DBIL	Direct Bilirubin	0.0 – 0.4	Calculated Calculation: DBIL = TBIL – Bu	Conjugated bilirubin And delta bilirubin
NBIL	Neonatal Bilirubin	1.0 – 10.5	Calculated Calculation: NBIL = Bu + Bc	Unconjugated bilirubin and conjugated bilirubin

• The new method for measuring direct bilirubin, Bc, is a more accurate method of determining only conjugated bilirubin because it does not include other bilirubin fractions (Delta) that may remain elevated longer than the Bc. This is particularly important in hepatobiliary obstruction.

Enzyme Testing on New Chemistry System

All Children's Hospital Department and Laboratory Medicine is in the process of replacing the Beckman chemistry analyzers LX20/CX5 with the Ortho Vitros 5600 (Chemistry) integrated chemistry analyzer. Clients will notice some reference range changes with enzymes. LDH and lipase demonstrate the most significant changes in reference values. For more information on how this new testing method impacts enzyme testing and reference ranges, please refer to the following pages.

ALT

ALT Reference Range:

Age	**Male** Conventional Units (U/L)	**Female** Conventional Units (U/L)
0-7 days	6-40	7-40
8-30 days	10-40	8-32
1-3 months	13-39	12-47
4-6 months	12-42	12-37
7-11 months	13-45	12-41
1-3 years	5-45	5-45
4-6 years	10-25	10-25
7-9 years	10-35	10-35
10-11 years	10-35	10-30
12-13 years	10-55	10-30
14-15 years	10-45	5-30
16-19 years	10-40	5-35
>19 years	21-72	9-52

AST

AST Reference Range:

Age	**Male** Conventional Units (U/L)	**Female** Conventional Units (U/L)
0-7 days	30-100	24-95
8-30 days	20-70	24-72
1-3 months	22-63	20-64
4-6 months	13-65	20-63
7-11 months	25-55	22-63
1-3 years	20-60	20-60
4-6 years	15-50	15-50
7-9 years	15-40	15-40
10-11 years	10-60	10-40
12-13 years	15-40	10-30
14-15 years	15-40	10-30
16-19 years	15-45	5-30
>19 years	17-59	14-36

Both methods utilize P-5-P (pyridoxal-5-phosphate), an essential coenzyme also known as Vitamin B6. P-5-P is naturally in serum and plasma, however, in renal dialysis patients and older specimens it can be depleted. The VITROS dry slide format allows for the incorporation of P-5-P in high levels. Other methods may not use P-5-P because of the high background it causes. The addition of P-5-P causes the enzyme values for AST and ALT to be higher.

ALKP

ALKP Reference Range:

Age	**Male** Conventional Units (U/L)	**Female** Conventional Units (U/L)
0-7 days	77-265	65-270
8-30 days	91-375	65-365
1-3 months	60-360	80-425
4-6 months	55-325	80-345
7-11 months	60-300	60-330
1-3 years	129-291	129-291
4-6 years	134-346	134-346
7-9 years	156-386	156-386
10-11 years	120-488	116-515
12-13 years	178-455	93-386
14-15 years	116-483	62-209
16-19 years	58-237	45-116
>19 years	38-126	38-126

This method used p-Nitrophenyl phosphatase substrate and 2-amino-2-methyl-1-propanol (AMP) as the phosphate acceptor buffer. The reference range is 38 – 126 U/L in adults. Difference seen may be attributed to the fact that ALKP is very temperature sensitive. Samples should be analyzed within 4 hours. A 3-6% increase in ALKP activity can occur when samples are stored between 1-4 days at room temperature and as much as 2%/day when the sera is refrigerated.

AMYLASE

AMY Reference Range:

Age	**Male and Female Conventional and SI Units (U/L)**
0.0 – 0.2 months	< 30
0.3 – 0.5 months	< 50
0.6 – 11 months	< 80
≥ 1.0 year	30 - 100

The VITROS uses a dyed starch with a high molecular weight, which is hydrolyzed by the sample amylase to produce dyed saccharides with a low molecular weight. Comparison of reference ranges, 30-100 U/L for VITROS System, can be used to determine how different these methods may be.

GGT

GGT Reference Range:

Age	**Male** Conventional Units (U/L)	**Female** Conventional Units (U/L)
1-7 days	25-148	19-131
8-30 days	23-153	17-124
1-3 months	17-130	17-124
4-6 months	8-83	15-109
7-12 months	10-35	10-54
1-3 years	5-16	5-16
4-6 years	8-18	8-18
7-9 years	11-21	11-21
10-11 years	14-25	14-23
12-13 years	14-37	12-21
14-15 years	10-28	12-22
16-19 years	9-29	9-23
>19 years	15-73	12-43

The method employed by the VITROS uses a L-y-glytamyl-p-nitroanilide substrate. This method is also used by other closed systems like Baxter Paramax and DuPont ACA. Other methods use L-y-glytamyl-3-carboxy-4-nitroanilide as the substrate. The bias between these two methods is also demonstrated by the different normal ranges. The VITROS %CV for CAP survey samples is one of the lowest and is the most widely used method for GGT.

LDH

LDH Reference Range:

Age	**Male** Conventional Units (U/L)	**Female** Conventional Units (U/L)
0-30 days	550-2100	580-2000
1-3 months	480-1220	460-1150
4-6 months	400-1230	480-1150
7-11 months	380-1200	460-1060
1-3 years	500-920	470-900
4-6 years	470-900	470-900
7-9 years	420-750	420-750
10-11 years	432-700	380-700
12-13 years	470-750	380-640
14-15 years	360-730	390-580
16-19 years	340-670	340-670
>19 years	313-618	313-618

VITROS slide utilizes the Pyruvate to Lactate reaction sequence, the sequence that naturally occurs in the body. This scheme allows the reaction to run faster, has better precision and higher rates (sensitivity). It is also the recommended reaction scheme of the International Federation of Clinical Chemistry. Values for LDH will be approximately three (3) times higher.

LIPASE

Lipase Reference Range:

Age	Male Conventional and SI Units (U/L)	Female Conventional and Units (U/L)
0-90 days	10-85	10-85
3-11 months	13-95	9-128
12-23 months	15-135	15-150
2-6 years	15-175	10-150
7-10 years	10-175	13-150
11-14 years	10-195	10-180
15-18 years	10-195	10-220
≥ 19 years	23-300	23-300

Lipase elevations usually parallel those of amylase, but increases in lipase activity may occur sooner or persist longer than increases in amylase activity and lipase may rise to a greater extent. The diagnosis of acute pancreatitis is sometimes difficult since this disorder must be differentiated from other acute intra-abdominal disorders with similar clinical findings. Elevation of serum lipase activity is probably a more specific diagnostic test in these cases than serum amylase activity, because many of these conditions are less likely to cause increases in lipase activity than in amylase activity. To improve the diagnostic efficiency of this assay, most studies have suggested utilizing a 2-5 times the Upper Limit of Normal as an indication of acute pancreatitis when colipase is incorporated.

Advantages of Vitros Method:

1. Addition of COLIPASE

The optimal catalytic activity of lipase requires calcium, low sodium chloride concentration, bile salts and colipase at A pH between 8.5 and 9.4. When colipase and bile salts are added to the assay, the reaction rate and analytic sensitivity of pancreatic lipase is increased whereas that for lipoprotein lipase is eliminated. Colipase aided by the addition of bile salts bind to lipase which creates a configurational change resulting in greater binding efficiency to the substrate. Older lipase methods do not contain colipase, thus rendering it a less effective assay in diagnosing pancreatitis.

2. No interference from HEMOLYSIS, BILIRUBIN, LIPEMIA or CARBOXYLESTERASE.
3. Quicker Assay time.

* Reference: Tietz Clinical Chemistry and Molecular Diagnostics, 4th Edition.