**BILIRUBIN TOTAL & DIRECT - TAB** (Part-2: Bilirubin Total - TAB)

4 x 50 mL
51003004

## Intended Use

This reagent is intended for *in vitro* quantitative determination of Bilirubin in serum or plasma.

- Modified TAB Method
- Linear up to 25 mg/dL
- Fast incubation 5 minutes at room temperature
- Sample volume only 50 µL
- Without sample blank procedure also included

## Clinical Significance

Bilirubin is formed by the breakdown of RBC's in the spleen, liver & bone marrow. Small amount of bilirubin circulates in the plasma loosely bound to albumin, which is not water soluble. This is referred to as indirect or unconjugated bilirubin. In the liver bilirubin is conjugated with glucuronic acid, which forms a soluble compound. This is referred as direct bilirubin.
Elevated levels are found in Hepatitis, Cirrhosis, Haemolytic jaundice, obstruction of biliary tract & drug induced reactions.

## Principle

Sulfanilic acid reacts with sodium nitrite to form diazotized sulfanilic acid.Total Bilirubin reacts with diazotized sulfanilic acid in the presence of TAB to form azobilirubin.

## Kit Components

| Reagent/ Component | Product Code 51003004 | Description |
|---|---|---|
| Total Bilirubin Reagent | 2 x 50 mL | Sulfanilic Acid 28.9 mmol/L<br>TAB 9 mmol/L<br>Preservatives and Stabiizers |
| Total Bilirubin Activator | 1 x 4 mL | |

## Risk & Safety

Material safety data sheets (MSDS) will be provided on request.

## Reagent Preparation

Total Bilirubin Reagent and Activator are ready to use.

## Reagent Storage and Stability

The sealed reagents are stable up to the expiry date stated on the label, when stored at room temperature. The activator should be stored at 2 - 8°C.

## Open Vial Stability

Once opened, the reagent is stable up to 4 weeks, if contamination is avoided.

## Onboard Calibration Stability

On board Calibration stability is 15 days.

## Reagent Deterioration

Turbidity or precipitation in any kit component indicates deterioration and the component must be discarded. Values outside the recommended acceptable range for the Agappe Qualichek Norm & Path control may also be an indication of reagent instability and associated results are invalid. Sample should be retested, using a fresh vial of reagent.

## Precaution

To avoid contamination, use clean laboratory wares. Use clean, dry disposable pipette tips for dispensing. Close reagent bottles immediately after use.

Avoid direct exposure of reagent to light.

Do not blow into the reagent bottles.

This reagent is only for IVD use and follow the normal precaution required for handling all laboratory reagents.

## Waste Management

Reagents must be disposed off in accordance with local regulations.

## Sample

Serum / Plasma (free of hemolysis)

## Interferences

No interference for

| | |
|---|---|
| Ascorbic Acid up to | 50 mg/dL |
| Hemoglobin up to | 1000 mg/dL |

## Materials Provided

Total Bilirubin Reagent and Total Bilirubin Activator

## Materials Required but Not Provided

- Pipettes & Tips
- Test Tubes & racks
- Timer
- Incubator
- Analyzer

## Test Parameters

| Without Sample Blank Mode | |
|---|---|
| Mode of Reaction | End Point |
| Slope of Reaction | Increasing |
| Wavelength I | 546 nm |
| Wavelength II | 630 nm |
| Temperature | 30°C |
| Factor (Total) | 29 |
| Blank | Reagent Blank |
| Linearity | 25 mg/dL |
| Reaction Time | 5 min |
| Sample Volume | 50 µL |
| Reagent Volume | 1000 µL |
| Activator | 20 µL |
| Cuvette | 1 cm light path |

1. Application parameters for various instrument are available on request.

2. Sample Blank procedure available on request. Please contact Customer Support.

## Unit Conversion

| Traditional Unit | SI Unit | Conversion from Traditional to SI |
|---|---|---|
| mg/dL | µmol/L | x 17.1 |

## Calibration

Agappe Multicalibrator (51610002) is recommended for calibration of this assay on fully auto analyzers.

Use provided factor for estimation of this assay on semi auto analzers.

## Procedure Notes

| Laboratory Procedure for Without Sample Blank Mode | | |
|---|---|---|
| | **Reagent Blank** | **Test** |
| Total Bilirubin Reagent | 1000 µL | 1000 µL |
| Activator Total | 20 µL | 20 µL |
| Serum / Calibrator | - | 50 µL |
| Mix well and incubate for 5 minute at room temperature. Measure the absorbance of calibrator and test against respective blank at 546/630 nm | | |

## Calculation

**With Factor:**

Total Bilirubin = OD of Test – OD of Reagent Blank X 29

**With Calibrator:**

$$\text{Bilirubin Concentration} = \frac{\text{OD of Test} - \text{OD of Sample Blank}}{\text{OD of Calibrator} - \text{OD of Reagent Blank}} \times \text{Conc. of Calib.}$$

**SYMBOLS USED ON THE LABELS**

IVD IN VITRO DIAGNOSTIC USE | SEE PACKAGE INSERT FOR PROCEDURE | LOT LOT NUMBER | MANUFACTURER'S ADDRESS | MANUFACTURING DATE | EXPIRY DATE | TEMPERATURE LIMIT

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REV. NO.: ADS/IFU/TDTB/CHEM/R02

CE ISO 9001:2008
EN ISO 13485:2012

## Quality Control

It is recommended to use Agappe Qualicheck Norm & Path (51601001) to verify the performance of the assay. Each laboratory has to establish its own internal quality control scheme and procedure for corrective action, if control do not recover within the acceptable range.

## Reference Range

It is recommended that each laboratory should establish its own reference values.

The following value may be used as guide line.

Adults: upto 1.2 mg/dL.

Infants: 0.2 - 8 mg/dL

Results obtained for patient samples are to be correlated with clinical findings of patient for interpretation and diagnosis.

## Performance

**1. Linearity**

The procedure is linear upto 25 mg/dL

If the concentration is greater than linearity (25 mg/dL), dilute the sample with normal saline and repeat the assay. Multiply the result with dilution factor.

**2. Comparison**

A comparison study has been performed between Agappe reagent and another internationally available reagent yielded a correlation coefficient of $r^2 = 0.991$ and a regression equation of y = 1.1791x.

**3. Precision**

| Intra Run | | |
|---|---|---|
| | Control Level 1 | Control Level 2 |
| n | 20 | 20 |
| Mean (mg/dL) | 1.1 | 4.0 |
| SD | 0.04 | 0.15 |
| CV(%) | 4.13 | 3.66 |

| Inter Run | | |
|---|---|---|
| | Control Level 1 | Control Level 2 |
| n | 20 | 20 |
| Mean (mg/dL) | 1.2 | 4.16 |
| SD | 0.05 | 0.15 |
| CV(%) | 4.46 | 3.70 |

| Accuracy (mg/dL) | | |
|---|---|---|
| Control | Expected Value | Measured Value |
| Control Level 1 | 1.2 ± 0.65 | 1.5 |
| Control Level 2 | 4.5 ± 0.9 | 4.7 |
| Qualichek Norm | 1.10 ± 0.29 | 1.0 |
| Qualichek Path | 4.1 ± 0.90 | 4.2 |

**4. Sensitivity**

Lower detection Limit is 0.05 mg/dL

## Bibliography

1. Walter, M., Gerard, H.; MICROCHEM JM 15, 231.(1980)
2. Annino J. S.; C.C. Principles and procedure,1960
3. A.A. A.C.C.: Clin. Chem. 8 : 405,196

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