

| REF          | CONTENT                        | Analyzer(s) on which <b>cobas c</b> pack(s) can be used            |
|--------------|--------------------------------|--|
| 04489365 190 | Serum Index Gen.2 (2750 tests) | System-ID 07 6870 7<br>COBAS INTEGRA 400 plus<br>COBAS INTEGRA 800 |

## English

## System information

Multitest SI2, test ID 0-435

Test LIP2, test ID 0-436 (serum, plasma)

Test HAEM2, test ID 0-437 (serum, plasma)

Test ICT2, test ID 0-438 (serum, plasma)

Ratio L2, ratio ID 0-439

Ratio H2, ratio ID 0-440

Ratio I2, ratio ID 0-441

Profile SI2P, profile ID 0-442

## Intended use

In vitro test for the semi-quantitative determination of the lipemia index, hemolysis index and icterus index in human serum and plasma on COBAS INTEGRA systems.

Summary<sup>1</sup>

Medical laboratory tests can be affected by endogenous and exogenous constituents in the sample matrix. Some of these potentially interfering factors can be recognized in the pre-analytical phase by a coloured appearance of the sample, whereas others are detected only by receiving additional information and/or by direct analysis. Interference due to lipemia (turbidity), hemolysis and icterus (bilirubin) is difficult to predict because of their strong method-dependence. The limits at which the analysis can be made are described for each method subject to that interference. The European directive for in vitro diagnostics (IVDD) states that providers of reagents must define the appropriate limitations. Each report on laboratory findings should contain a notation characterising the sample's "appearance". If lipemia or a relevant colour is found, the type of finding is characterised in each case, e.g. "lipemic", "hemolytic" or "icteric". A quantification of these interferants is possible with the Serum Index Gen.2 (SI2) application which can be applied on all COBAS INTEGRA systems. All analyzers are capable of semi-quantitative measurement and reporting of the tests Lipaemia Index Gen.2 (L2), Haemolysis Index Gen.2 (H2) and Icterus Index Gen.2 (I2).

Serum index results are very useful for monitoring the degree of potential interference due to lipemia (turbidity), hemolysis and icterus (bilirubin).

## Lipemia

Lipemia is defined as turbidity in serum and plasma samples which is visible to the naked eye. The most frequent cause of lipemia is an elevated triglyceride concentration in plasma and serum. This can be caused by food intake, a disturbance of lipoprotein metabolism or an infusion of lipids.

## Hemolysis

Hemolysis is defined as the release of intracellular components of erythrocytes and other blood cells into the extracellular space of blood. It can appear in vivo (e.g. due to a transfusion reaction or during malaria parasite infection) as well as in vitro in all components of the preanalytical phase (sampling, sample transport and storage). After the separation of blood cells, hemolysis is detected in serum and plasma by its red colour caused by hemoglobin.

## Icterus

Icterus is defined as an elevated level of different bilirubin species (conjugated and unconjugated) in serum and plasma. Increased levels of bilirubin can be caused by diseases or conditions which, through hemolytic processes, produce bilirubin faster than the liver can metabolize it. Liver immaturity and several other diseases in which the bilirubin conjugation mechanism is impaired cause similar elevations of circulating unconjugated bilirubin. Bile duct obstruction or damage to hepatocellular structure causes increases in the levels of both conjugated (direct) and unconjugated (indirect) bilirubin in the circulation.

## IMPORTANT NOTE

The Serum Index Gen.2 test should not be used for the quantitative determination of triglycerides, hemoglobin or bilirubin.

## Test principle

The COBAS INTEGRA Serum Index Gen.2 assay (multitest SI2, 0-435) is based on calculations of absorbance measurements of diluted samples at different bichromatic wavelength pairs to provide a semi-quantitative representation of levels of lipemia, hemolysis and icterus present in serum and plasma samples.

The COBAS INTEGRA analyzers take an aliquot of the patient specimen and dilutes it in saline (0.9 % sodium chloride) to measure the absorbances for lipemia at 659 nm (primary wavelength) and 800 nm (secondary wavelength), for hemolysis at 583 nm (primary wavelength) and 629 nm (secondary wavelength), and for icterus at 480 nm (primary wavelength) and 512 nm (secondary wavelength). These individual test results are displayed as Lipaemia Gen.2 (LIP2, 0-436), Haemolysis Gen.2 (HAEM2, 0-437) and Icterus Gen.2 (ICT2, 0-438). The unit of these results is just "Abs".

The Serum Index Gen.2 values for the Lipaemia Index Gen.2 (L2, 0-439), Haemolysis Index Gen.2 (H2, 0-440) and Icterus Index Gen.2 (I2, 0-441) are generated as calculated results by multiplying the individual test results for LIP2, HAEM2 and ICT2 with the relevant factors as follows:

$$L2 = (7867.8 \times LIP2)$$

$$H2 = (-5332.0 \times LIP2) + (9124.2 \times HAEM2)$$

$$I2 = (-67.2 \times LIP2) + (-26.1 \times HAEM2) + (101.9 \times ICT2)$$

## Reagents - working solutions

**R1** Sodium chloride: 9 %

R1 is in position B and in position C.

## Precautions and warnings

Pay attention to all precautions and warnings listed in Section 1 / Introduction of this Method Manual.

## Reagent handling

Ready for use

## Storage and stability

Shelf life at 2-8 °C See expiration date on **cobas c** pack label

COBAS INTEGRA 400 plus system

On-board in use at 10-15 °C 12 weeks

COBAS INTEGRA 800 system

On-board in use at 8 °C 12 weeks

## Specimen collection and preparation

For specimen collection and preparation only use suitable tubes or collection containers.

Only the specimens listed below were tested and found acceptable.

Serum

Plasma: Li-heparin, K<sub>2</sub>-EDTA, K<sub>3</sub>-EDTA, citrated, NaF/Na-heparin, NaF/K-oxalate plasma

Separate serum and plasma from the clot or cells within one hour.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

**Note:** Measure the Serum Index Gen.2 in parallel to the respective parameters.

#### Materials provided

See "Reagents – working solutions" section for reagents.

#### Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

The performance of applications not validated by Roche is not warranted and must be defined by the user.

#### Applications for LIP2, HAEM2 and ICT2

##### COBAS INTEGRA 400 plus/800 test definition

|                       | LIP2       | HAEM2      | ICT2       |
|-----------------------|------------|------------|------------|
| Measuring mode        | Absorbance | Absorbance | Absorbance |
| Abs. calculation mode | Endpoint   | Endpoint   | Endpoint   |
| Reaction mode         | R1-S       | R1-S       | R1-S       |
| Reaction direction    | Increase   | Increase   | Increase   |
| Wavelength A/B        | 659/800 nm | 583/629 nm | 480/512 nm |
| Calc. first/last      | 17/20      | 17/20      | 17/20      |
| Predilution factor    | No         | No         | No         |
| Unit                  | Abs        | Abs        | Abs        |

#### Pipetting parameters

| LIP2 / HAEM2 / ICT2 | Diluent (H <sub>2</sub> O) |        |
|---------------------|----------------------------|--------|
| R1                  | 12 µL                      | 108 µL |
| Sample              | 5 µL                       | 5 µL   |
| Total volume        | 130 µL                     |        |

#### Ratio definition for Lipaemia Index Gen.2 calculation

|                        |                               |
|------------------------|-------------------------------|
| Abbreviated ratio name | L2 (0-439)                    |
| Equation               | $(7867.8 \times \text{LIP2})$ |
| Unit <sup>a)</sup>     | No                            |

#### Ratio definition for Haemolysis Index Gen.2 calculation

|                        |   |
|------------------------|---|
| Abbreviated ratio name | H2 (0-440)  |
| Equation               | $(-5332.0 \times \text{LIP2}) + (9124.2 \times \text{HAEM2})$ |
| Unit <sup>a)</sup>     | No  |

#### Ratio definition for Icterus Index Gen.2 calculation

|                        |   |
|------------------------|---|
| Abbreviated ratio name | I2 (0-441)  |
| Equation               | $(-67.2 \times \text{LIP2}) + (-26.1 \times \text{HAEM2}) + (101.9 \times \text{ICT2})$ |
| Unit <sup>a)</sup>     | No  |

a) With the use of these factors, the displayed and printed out L2, H2 and I2 values correspond to an approximate concentration in mg/dL. If the displayed and printed out H2 and I2 values shall correspond to a concentration in µmol/L, please use the established conversion factors 17.1 for bilirubin and 0.621 for hemoglobin (according to the literature).

There is poor correlation between the Lipaemia Index Gen.2 (ratio L2, 0-439; corresponds to turbidity) and triglycerides concentration.

Use the predefined SI Gen.2 Profile (SI2P, 0-442) for simultaneous order entry of Lipaemia Gen.2 (LIP2, 0-436), Haemolysis Gen.2 (HAEM2, 0-437) and Icterus Gen.2 (ICT2, 0-438) tests from the same sample. The ratios for L2, H2 and I2 will be automatically calculated after result output of the 3 tests.

#### IMPORTANT:

To ensure the correct pipetting sequence of LIP2 followed by HAEM2 followed by ICT2, do not enter any of these tests in the Processing Sequence List. Please also ensure that the test number entered is the

same as the test-ID. We recommend using the predefined SI Gen.2 Profile SI2P (profile-ID 0-442).

#### Calibration

##### LIP2, HAEM2, ICT2

|                      |                   |
|----------------------|-------------------|
| Calibration factor   | 1                 |
| Calibration offset   | -0.001            |
| Calibration mode     | Linear regression |
| Calibration interval | No                |

Lipaemia Gen.2, Haemolysis Gen.2 and Icterus Gen.2 are calibrated using a fixed factor and offset.

#### Calculation

##### LIP2, HAEM2, ICT2

COBAS INTEGRA analyzers automatically calculate the analyte concentration of each sample. For more details, please refer to Data Analysis in the Online Help (COBAS INTEGRA 400 plus/800 analyzers).

##### L2, H2, I2

For calculation of the L2, H2 and I2 values, refer to the Test principle and Ratio definition sections in this method sheet. The displayed and printed out L2, H2 and I2 values correspond to an approximate concentration in mg/dL.

#### Limits and ranges

##### Measuring range

**L2:** 11-2000<sup>b)</sup>      **H2:** 5-1200<sup>b)</sup>      **I2:** 0-60<sup>b)</sup>

b) The measuring range corresponds to an approximate concentration in mg/dL

##### Lower limits of measurement

Lower detection limit of the test:

**L2:** 11<sup>c)</sup>      **H2:** 5<sup>c)</sup>      **I2:** 0<sup>c)</sup>

c) The lower detection limit corresponds to an approximate concentration in mg/dL

The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying 3 standard deviations above that of the lowest standard (standard 1 + 3 SD, repeatability, n = 21).

#### Specific performance data

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

#### Precision

Repeatability was determined using human samples and controls in an internal protocol (n = 21). The following results were obtained on a COBAS INTEGRA 800 analyzer:

##### Lipaemia Index Gen.2 (L2)

| Repeatability | Mean <sup>d)</sup> | SD <sup>d)</sup> | CV % |
|---------------|--------------------|------------------|------|
| Sample low    | 78.5               | 1.4              | 1.8  |
| Sample high   | 1048               | 16               | 1.5  |

##### Haemolysis Index Gen.2 (H2)

| Repeatability | Mean <sup>d)</sup> | SD <sup>d)</sup> | CV % |
|---------------|--------------------|------------------|------|
| Sample low    | 5.48               | 0.98             | 17.9 |
| Sample high   | 366                | 2                | 0.5  |

##### Icterus Index Gen.2 (I2)

| Repeatability | Mean <sup>d)</sup> | SD <sup>d)</sup> | CV % |
|---------------|--------------------|------------------|------|
| Sample low    | 1.00               | 0.00             | 0.0  |
| Sample high   | 35.0               | 0.0              | 0.0  |

d) Values correspond to an approximate concentration in mg/dL

**Method comparison**

L2, H2 and I2 values for human serum and plasma samples obtained on COBAS INTEGRA analyzers with the COBAS INTEGRA Serum Index Gen.2 assay (y) were compared with those determined using the Serum Index assay on Roche/Hitachi 917 analyzers (x).



Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim  
www.roche.com

**Lipaemia Index Gen.2**

Sample size (n) = 484

| Passing/Bablok <sup>2</sup> | Linear regression   |
|-----------------------------|---------------------|
| $y = 0.987x + 6.55$         | $y = 0.983x + 4.57$ |
| $r = 0.975$                 | $r = 0.999$         |
| SD (md 95) = 24.5           | Sy.x = 9.88         |

The values were between 22 and 1640 (values correspond to an approximate concentration in mg/dL).

**Haemolysis Index Gen.2**

Sample size (n) = 727

| Passing/Bablok <sup>2</sup> | Linear regression   |
|-----------------------------|---------------------|
| $y = 0.980x + 1.33$         | $y = 0.990x + 1.07$ |
| $r = 0.925$                 | $r = 0.994$         |
| SD (md 95) = 47.1           | Sy.x = 19.2         |

The values were between 7 and 1002 (values correspond to an approximate concentration in mg/dL).

**Icterus Index Gen.2**

Sample size (n) = 390

| Passing/Bablok <sup>2</sup> | Linear regression    |
|-----------------------------|----------------------|
| $y = 1.00x + 0.000$         | $y = 0.995x + 0.145$ |
| $r = 0.974$                 | $r = 0.997$          |
| SD (md 95) = 2.12           | Sy.x = 0.982         |

The values were between 1 and 60 (values correspond to an approximate concentration in mg/dL).

**References**

- 1 Guder WG, da Fonseca-Wolheim F, Heil W, et al. The Haemolytic, Icteric and Lipemic Sample Recommendations Regarding their Recognition and Prevention of Clinically Relevant Interferences. J Lab Med 2000;24:357-364.
- 2 Bablok W, Passing H, Bender R, et al. A general regression procedure for method transformation. Application of linear regression procedures for method comparison studies in clinical chemistry, Part III. J Clin Chem Clin Biochem 1988 Nov;26(11):783-790.

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

**Symbols**

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard.

|  |                                       |
|--|---------------------------------------|
|  | Contents of kit                       |
|  | Volume after reconstitution or mixing |
|  | Global Trade Item Number              |

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Significant additions or changes are indicated by a change bar in the margin.

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