# Rheumatoid Factor (FR) – Latex Immunoturbidimetric method

Instructions for Use (IFU)

IVD CE

## ORDERING INFORMATION

| | Code | Composition |
|---|---|---|
| OPEN KONELAB INDIKO | REF B78182262 | n° 2 vials x 40 mL (R.A)<br>n° 2 vials x 5 mL (R.B) |
| CHEMILAB | REF B82181021 | n° 2 vial x 26 mL (R.A)<br>n° 1 vial x 6 mL (R.B) |
| | REF B82181022 | n° 4 vials x 33 mL (R.A)<br>n° 1 vial x 13 mL (R.B) |

## INTENDED USE

Diagnostic immunoturbidimetric test for the quantitative determination of Rheumatoid factor in human serum and plasma. All results must be interpreted in conjunction with the clinical context. FOR PROFESSIONAL USE ONLY.

## CLINICAL SIGNIFICANCE

Rheumatoid Factor (RF) is an auto-antibody which reacts with fragment Fc of human IgG and is present, in particular, in patients affected by rheumatoid arthritis (RA), and can be considered as an indication of the inflammatory process. High RF concentrations often indicate a marked degeneration of the disease.
The classical test methods are based on the agglutination of erythrocytes or latex particles coated with human IgG. These methods are suitable for qualitative or semi-quantitative evaluations, while the immunoturbidimetric technique adopted in the present test allows the reproducible, quantitative determination of the rheumatoid factor concentration. Determination of the RF is therefore of clinical importance to establish the diagnosis and prognosis, and to monitor therapeutic efficacy in rheumatoid arthritis.

## PRINCIPLE OF THE METHOD

Turbidimetric method. Latex particles are activated with human, denatured IgG, by means of a covalent bond to increase sensitivity and stability of the reagent. The suspension of coated particles agglutinates in the presence of RF, causing a degree of turbidity which can be detected photometrically and is proportional to the RF concentration in the sample. The quantitative analysis is obtained by interpolation of this photometric value with those found by testing known concentrations of RF.

### Storage and Stability

= **Storage temperature 2-8 °C**

If stored at 2-8°C avoiding direct light, the intact reagents remain stable until the expiration date, printed on the label. Do not freeze. Slight variations in composition among batches will not affect test results.

### Concentrazione

| | | Conc. | U.M. |
|---|---|---|---|
| **Reagent A** | | | |
| RF Buffer | Sodium-phosphate | 30 | mmol/L |
| | EDTA | 10 | mmol/L |
| | $NaN_3$ | < 0.1 | % |
| **Reagent B** | | | |
| RF latex | Polystyrene particles coated with denatured human IgG | | |
| | Glycine buffer | 20 | mmol/L |
| | $NaN_3$ | < 0.1 | % |

### Materials included in the kit

Reagent as described above.

### Necessary materials not included in the kit

Controls and calibrators

## PRECAUTIONS and WARNINGS

1. Reagents and waste materials shall be disposed of in accordance with Community waste provisions or national or regional provisions.
2. Reagents may contain non-active components such as preservatives and detergents. The total concentration of these components is below the limits set out in Regulation 1272/2008 EC and subsequent amendments and additions.
3. It is recommended that the reagent be handled in accordance with the rules of good laboratory practice and that appropriate personal protective equipment be used.
4. Do not use the reagent if it is visibly degraded (e.g. presence of corpuscles).
5. All human samples shall be handled and disposed of as potentially infectious material.
6. The kit should only be used by qualified and properly trained technical personnel.
7. Diagnoses shall be carried out exclusively by authorised and qualified personnel.
8. Comply with national directives on occupational safety and quality assurance.
9. Use equipment that complies with current regulations.

### Reporting of serious incidents

Please inform the manufacturer (through your distributor) and the competent authority of the member state of the European Union in which the user and/or patient is established, of cases of serious incident that has occurred in relation to the device. For other jurisdictions, reports of serious incidents must be made in accordance with the regulatory requirements of the home Member State. By reporting serious incidents, you help provide more information about the safety of your in vitro medical diagnostic device.

## PROCEDURE

### Quality Control

Use the Sclavo Diagnostics Int. ASO - CRP - RF Control Low B47182278 and High B47182279 for quality control purposes at least once a day. Repeat the analysis also after calibration. Obtained values must be within the range of acceptability.

### Calibration

For calibration use Single Level Calibrator FR B47182292 (for dilutions to be used refer to the Instructions for Use of the calibrator).

### Traceability

The RF value has been harmonised according to the *International Reference Preparation of Rheumatoid Arthritis Serum, WHO 1st British Standard, NIBSC 64/2 (1970).*

## SAMPLE

### Sample types and storage

Serum or plasma obtained by normal medical techniques can be used. No special preparation of the patient is necessary. Test samples can be stored for three days at 2-8°C or 12 months if frozen. Defrost samples at room temperature and mix carefully by turning upside down before testing. Avoid repeated freezing and thawing of samples.
**The samples do not require predilution before analysis.**
Strongly lipemic samples, or those with significant turbidity or precipitates, must be clarified by centrifugation (10 min. to 15,000xg), prior to analysis.

## PREPARATION OF THE REAGENT

The Reagents are liquid, ready for use. After opening, the Reagents are stable until the expiry date if kept as indicated in “Storage and stability”.

### Test Procedure

This kit, although developed and produced for use as a manual test and with Sclavo analyzers, can also be used with other analyzers that can meet the specifications indicated in the "General Procedure" section. Detailed instructions for using this kit with automated clinical chemistry analyzers are available on request. All applications not explicitly approved by Sclavo Diagnostics International, Siena, cannot be guaranteed in terms of performance and must therefore be established by the operator.

### Automation

The kit can be used with all automatic analyzers that can meet the operating conditions of the reagent while maintaining the volumetric ratios R1/ R2/C. Validated applications are available for Sclavo Konelab® - Indiko® and CHEMILAB instruments. Applications not approved by Sclavo Diagnostics do not guarantee the performance of the reagent and must therefore be approved under the responsibility of the user.

## MANUAL METHOD

The kit, in Open format, can be used manually through the use of spectrophotometer or photometer with the following parameters:

### Reaction conditions

| | |
|---|---|
| Wavelength: | 600 nm |
| Temperature: | 37°C |
| Reaction | Fixed-time |

Dispense as follows for the blank, for each calibrator and unknown samples

| | Volume (µL) |
|---|---|
| Buffer R1 (RA) | 250 |
| Test Sample | 3 |
| Mix and incubate at 37°C for at least 1 minute, then add: | |
| RF Latex (RB) | 40 |
| Mix and incubate at 37°C.<br>Read the absorbance (Abs-1). After 6 minutes read the absorbance (Abs-2) | |

Note: **The reaction volumes can be varies proportioning without altering the results.**

### Results

The concentration of Rheumatoid Factor is obtained as following: Generate the calibration curve with the ΔAbs values and concentration of the single calibrators. Calculate the analytical result expressed in “UI/mL”, from the calibration curve. All

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samples with a Rheumatoid Factor concentration higher than the highest calibration point and/or giving a signal denoting an excess of antigen (in the automated instruments) must be diluted and retested. It is advisable to use doubling dilutions in saline.

**Control of the calibration curve**

The calibration curve is valid for at least one month. However, its validity should be checked periodically using the SCLAVO Diagnostics Control ASO-CRP-RF Low B47182278 High B47182279. The validity is confirmed if the values obtained are within the concentration range of the controls reported on the Instructions for use.

**Automation**

The results are automatically calculated by the analyzer based on the calibration line. The analyzer automatically performs calibration in accordance with the method protocol. The calibration line is calculated automatically by the different instruments.

## REFERENCE RANGE

The typical reference range is ≤ 20 UI/mL.

As sex, age, geographical location and other factors can influence the normal values found in the population, each laboratory should determine its own normal, medium and pathological values for its own population.

## CHARACTERISTICS/PERFORMANCE

**Analytical Range – Antigen excess**

The analytical range was tested using a strongly positive sample and serial dilutions of this serum in saline solution. The method guarantees a correct response throughout the minimal detectable measurement range and the calibrator higher concentration. The present method does not show Antigen Excess until 800 UI/mL.

**Accuracy**

Commercial control sera were tested with the present kit and the data obtained with the Konelab analyzer are reported in the table below (mean of three tests).

| NIBSC 64/2 | | | Sclavo |
|---|---|---|---|
| Sample | Mean value (mg/L) | Intervallo | Mean (mg/L) |
| Low | 23 | 17 – 29 | 23.8 |
| High | 66 | 49 – 82 | 65.1 |

**Specificity**

The method is 100% specific for human Rheumatoid factor.

**Interferences**

The influence of the following substances on the analytical response was tested up to the concentrations reported below:

Bilirubin 30 mg/dL, Ascorbic Acid 50 mg/dL, EDTA 10 mM, Hemoglobin 500 mg/dL, Sodium citrate 1000 mg/dL, Sodium Heparin 40 mg/mL, Triglycerides 2%. No appreciable interference was found in any case, and the variations observed were within the expected precision range. Higher concentrations were not tested.

However, in view of the wide heterogeneity of potentially interfering substances and pharmaceuticals, for diagnostic purposes the results of this test must always be taken into consideration in conjunction with the clinical history of the patient, other clinical tests and medical investigations.

**Precision of the method**

The Precision of the analytical results has been determined as Repeatability and Total Precision according to the CLSI EP15-A2 guideline, using commercial control sera. The data obtained are shown in the following table (confidence interval 95%).

| Within-run Precision – Repeatability | | | | |
|---|---|---|---|---|
| **Level** | **Replicates** | **Mean (mg/L)** | **DS** | **CV%** |
| Low | 10 | 32,2 | 0,4216 | 1,31 |
| High | 10 | 70,3 | 1,7129 | 2,44 |
| **Total Precision (Within-lab Precision)** | | | | |
| **Level** | **Replicates** | **Mean (g/L)** | **DS** | **CV%** |
| Low | 10 | 23,2 | 0,7004 | 3,02 |
| High | 10 | 66,6 | 1,0143 | 1,55 |

**Limits of sensitivity**

The Sensitivity limit has been measured using serial dilutions of a high concentrated serum. The smallest measurable concentration is 10 UI/mL.

**Comparison between methods**

The present method was compared with another commercially available, analyzing 99 human sera. The correlation data between the two methods are reported in the table below.

| Parameter | Estimation |
|---|---|
| Intercept | 1.5778 |
| Slope | 0.6852 |
| Correlation Coeff. (R) | 0.9724 |

| Symbols used in IFU and Packaging | |
|---|---|
| IVD In vitro diagnostic medical device vitro | Manufacturer |
| REF Catalogue Number | Instruction for use |
| LOT Lot Number | Temperature limitation |
| Expiration date | |

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