

# INSTRUCTIONS FOR USE

# TRFRN

VITROS Chemistry Products TRFRN Reagent

Transferrin

REF 6801767

## Intended Use

For *in vitro* diagnostic use only.

VITROS Chemistry Products TRFRN Reagent is used on the VITROS 5,1 FS Chemistry System, the VITROS 4600 Chemistry System and the VITROS 5600 Integrated System to quantitatively measure transferrin (TRFRN) concentration in human serum and plasma.

## Summary and Explanation of the Test

Transferrin is the principal plasma protein for transport of iron. Transferrin levels are used as an aid in the diagnosis of malnutrition, acute inflammation, infection, and red blood cell disorders, such as iron deficiency anemia.<sup>1</sup>

## Principles of the Procedure

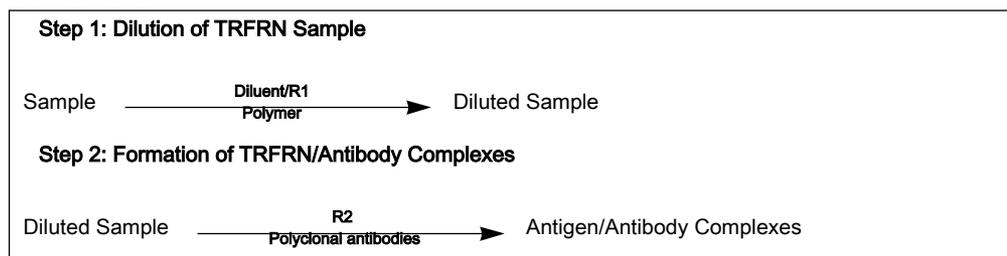
The quantitative measurement of transferrin is performed using the VITROS Chemistry Products TRFRN Reagent in conjunction with the VITROS Chemistry Products Calibrator Kit 20 and VITROS Chemistry Products FS Diluent Pack 2 on the VITROS 5,1 FS/4600 Chemistry Systems and the VITROS 5600 Integrated System. The VITROS Chemistry Products TRFRN Reagent is a dual chambered package containing ready-to-use liquid reagents. Samples, calibrators and controls are automatically diluted in saline and mixed with Reagent 1 containing a polymer. Addition of antisera specific for human transferrin (Reagent 2) produces an immunochemical reaction yielding antibody/antigen complexes. The light scattering properties of the antibody/antigen complexes increase solution turbidity proportional to transferrin concentration in the sample. The turbidity is measured spectrophotometrically at 340 nm. Once a calibration has been performed for each reagent lot, the transferrin concentration in each unknown sample can be determined using the stored calibration curve and the measured absorbance obtained in the assay of the sample.

## Test Type and Conditions

| Test Type        | VITROS System      | Approximate Incubation Time | Temperature     | Wavelength | Reaction Sample Volume |
|------------------|--------------------|-----------------------------|-----------------|------------|------------------------|
| Blanked Endpoint | 5600, 4600, 5,1 FS | Incubation 1: 5 minutes     | 37 °C (98.6 °F) | 340 nm     | 4.8 µL                 |
|                  |                    | Incubation 2: 5 minutes     |                 |            |                        |

Not all products and systems are available in all countries.

## Reaction Scheme



## Warnings and Precautions

For *in vitro* diagnostic use only.

**WARNING:** *This product contains sodium azide. Disposal of reagents into sinks with copper or lead plumbing should be followed with plenty of water to prevent formation of potentially explosive metallic azides.*

**WARNING:** *Take care when handling materials and samples of human origin. Since no test method can offer complete assurance that infectious agents are absent, consider all clinical specimens, controls, and calibrators potentially infectious. Handle specimens, solid and liquid waste, and test components in accordance with local regulations and CLSI Guideline M29<sup>2</sup> or other published biohazard safety guidelines.*

For specific warnings and precautions for calibrators, quality control materials, and other components, refer to the Instructions for Use for the appropriate VITROS product, or to other manufacturer's product literature.

## Reagents

### Reactive Ingredients

**Reagent 1 (R1):** None

**Reagent 2 (R2):** Goat antisera to human transferrin 1 mL/mL

### Other Ingredients

**Reagent 1 (R1):** Preservative, polymer, buffers, inorganic salt

**Reagent 2 (R2):** Preservative, buffer, inorganic salt

### Reagent Handling

**Caution:** Do not use reagent packs with damaged or incompletely sealed packaging.

- Inspect the packaging for signs of damage.
- Be careful when opening the outer packaging with a sharp instrument so as to avoid damage to the individual product packaging.
- The reagents are supplied ready for use.
- Avoid agitation, which may cause foaming or the formation of bubbles.

### Reagent Preparation

1. Remove from refrigerated storage.
2. Immediately load into Supply 3.

**IMPORTANT:** *Do not loosen or remove caps prior to loading.*

### Reagent Storage and Stability

VITROS Chemistry Products TRFRN Reagent is stable until the expiration date on the carton when it is stored and handled as specified. Do not use beyond the expiration date.

| Reagent  | Storage Condition |                   | Stability             |
|----------|-------------------|-------------------|-----------------------|
| Unopened | Refrigerated      | 2–8 °C (36–46 °F) | Until expiration date |
| Opened   | On-analyzer       | System turned on  | ≤ 4 weeks             |

Verify performance with quality control materials:

- If the system is turned off for more than 30 minutes.
- After reloading reagents that have been removed from Supply 3 and stored for later use.

## Specimen Collection, Preparation and Storage

### Specimens Recommended

- Serum
- Plasma:
  - Heparin
  - EDTA

**IMPORTANT:** *Certain collection devices have been reported to affect other analytes and tests<sup>3</sup>. Owing to the variety of specimen collection devices available, Ortho-Clinical*

*Diagnostics is unable to provide a definitive statement on the performance of its products with these devices. Confirm that your collection devices are compatible with this test.*

### Specimens Not Recommended

None

### Serum and Plasma

#### **Specimen Collection and Preparation**

Collect specimens using standard laboratory procedures.<sup>4, 5</sup>

**Note:** For details on minimum fill volume requirements, refer to the operating instructions for your system.

#### **Patient Preparation**

No special patient preparation is necessary.

#### **Special Precautions**

- Refer to "Sample Dilution" for dilution instructions.
- Centrifuge specimens and remove the serum or plasma from the cellular material within two hours of collection.<sup>6</sup>

#### **Specimen Handling and Storage**

- Handle and store specimens in stoppered containers to avoid contamination and evaporation.
- Mix samples by gentle inversion and bring to room temperature, 18–28 °C (64–82 °F), prior to analysis.

#### **Specimen Storage and Stability**

| Storage             | Temperature         | Stability    |
|---------------------|---------------------|--------------|
| Room Temperature    | 18–28 °C (64–82 °F) | ≤ 1 day      |
| Refrigerated        | 2–8 °C (36–46 °F)   | ≤ 3 days     |
| Frozen <sup>6</sup> | ≤ -20 °C (≤ -4 °F)  | Indefinitely |

**IMPORTANT:** Avoid repeated freeze-thaw cycles.

## Testing Procedure

### Materials Provided

VITROS Chemistry Products TRFRN Reagent

### Materials Required but Not Provided

- VITROS Chemistry Products Calibrator Kit 20
- Quality control materials, such as VITROS Chemistry Products Protein Performance Verifiers I, II, and III
- VITROS Chemistry Products FS Diluent Pack 2 (BSA/Saline)

### Operating Instructions

- Check reagent inventories at least daily to ensure that quantities are sufficient for the planned workload.
- For additional information, refer to the operating instructions for system.

**IMPORTANT:** Bring all fluids and samples to room temperature, 18–28 °C (64–82 °F), prior to analysis.

### Sample Dilution

#### **On-Analyzer Sample Dilution:**

Refer to the operating instructions for your system for more information on the On-Analyzer Dilution Procedure. Use VITROS Chemistry Products FS Diluent Pack 2 (BSA/Saline) for the dilution.

#### **Manual Sample Dilution:**

If TRFRN concentrations exceed the system's measuring (reportable) range:

1. Dilute sample using saline from VITROS Chemistry Products FS Diluent Pack 2 (BSA/saline). Samples with values greater than the reportable range may be diluted up to 1 part sample with 1 part diluent (2X dilution).
2. Reanalyze
3. Multiply the results by the dilution factor to obtain an estimate of the original sample's TRFRN concentration.

**IMPORTANT:** *If using the system in On-Analyzer Dilution Mode, do not manually dilute samples for analysis. Refer to the system operating instructions for more information on the On-Analyzer Dilution Procedure*

## Calibration

### Required Calibrators

VITROS Chemistry Products Calibrator Kit 20

### Calibrator Preparation, Handling, and Storage

Refer to the Instructions for Use for VITROS Chemistry Products Calibrator 20.

### Calibration Procedure

Refer to the operating instructions for your system.

### When to Calibrate

Calibrate:

- When the reagent lot number changes.
- When critical system parts are replaced due to service or maintenance.
- When government regulations require.

For example, in the USA, CLIA regulations require calibration or calibration verification at least once every six months.

The VITROS TRFRN assay may also need to be calibrated:

- If quality control results are consistently outside acceptable range.
- After certain service procedures have been performed.

For additional information, refer to the operating instructions for your system.

### Calculations

Absorbance is measured at 340 nm after each incubation step (blank, endpoint) and the response calculated from the difference in absorbance values. Once a calibration has been performed for each reagent lot, transferrin concentration in the unknown samples can be determined using the stored calibration curve and the calculated response obtained in the assay of each sample.

### Validity of a Calibration

Calibration parameters are automatically assessed by the system against a set of quality parameters detailed in the Review Assay Data screen (found via Options → Review/Edit Calibrations → Review Assay Data). Failure to meet any of the pre-defined quality parameters results in a failed calibration. The calibration report should be used in conjunction with quality control results to determine the validity of a calibration.

### Measuring (Reportable) Range

| Conventional Units<br>(mg/dL) | SI Units<br>(g/L) |
|-------------------------------|-------------------|
| 80.00–750.00                  | 0.80–7.50         |

For out-of-range samples, refer to "Sample Dilution"

### Traceability of Calibration

Values assigned to the VITROS Chemistry Products Calibrator Kit 20 for transferrin are traceable to ERM-DA470.<sup>7</sup>

## Quality Control

### Quality Control Material Selection

**IMPORTANT:** *VITROS Chemistry Products Protein Performance Verifiers are recommended for use with the VITROS 5,1 FS/4600 Chemistry and VITROS 5600 Integrated*

*Systems. Evaluate the performance of other commercial control fluids for compatibility with this assay before using for quality control.*

Control materials other than VITROS Chemistry Products Protein Performance Verifiers may show a difference when compared with other transferrin methods if they:

- Depart from a true human matrix.
- Contain high concentrations of preservatives, stabilizers, or other nonphysiological additives.

## Quality Control Procedure Recommendations

- Choose control levels that check the clinically relevant range.
- Analyze quality control materials in the same manner as patient samples, before or during patient sample processing.
- To verify system performance, analyze control materials:
  - After calibration.
  - According to local regulations or at least once each day that the assay is being performed.
  - After specified service procedures are performed. Refer to the operating instructions for your system.
- If control results fall outside your acceptable range, investigate the cause before deciding whether to report patient results.
- For general quality control recommendations, refer to CLSI *Statistical Quality Control for Quantitative Measurements: Principles and Definitions; Approved Guideline-Third Edition*<sup>8</sup> or other published guidelines.
- For additional information, refer to the operating instructions for your system.

## Quality Control Material Preparation, Handling, and Storage

Refer to the Instructions for Use for VITROS Chemistry Products Protein Performance Verifier I, II, and III or to other manufacturer's product literature.

## Results

### Reporting Units and Unit Conversion

The VITROS 5,1 FS/4600 Chemistry and VITROS Integrated Systems may be programmed to report transferrin results in conventional or SI units.

| Conventional Units | SI Units           |
|--------------------|--------------------|
| mg/dL              | g/L (mg/dL x 0.01) |

## Limitations of the Procedure

No antigen excess effect was observed for samples with transferrin concentrations up to 1500 mg/dL.

### Known Interferences

None identified.

### Other Limitations

Certain drugs and clinical conditions are known to alter transferrin concentration *in vivo*. For additional information, refer to one of the published summaries.<sup>9, 10</sup>

## Expected Values

### Reference Interval

The reference interval is the central 95% of results from an internal study of 121 apparently healthy adults.

| Conventional Units<br>(mg/dL) | SI Units<br>(g/L) |
|-------------------------------|-------------------|
| 206–381                       | 2.1–3.8           |

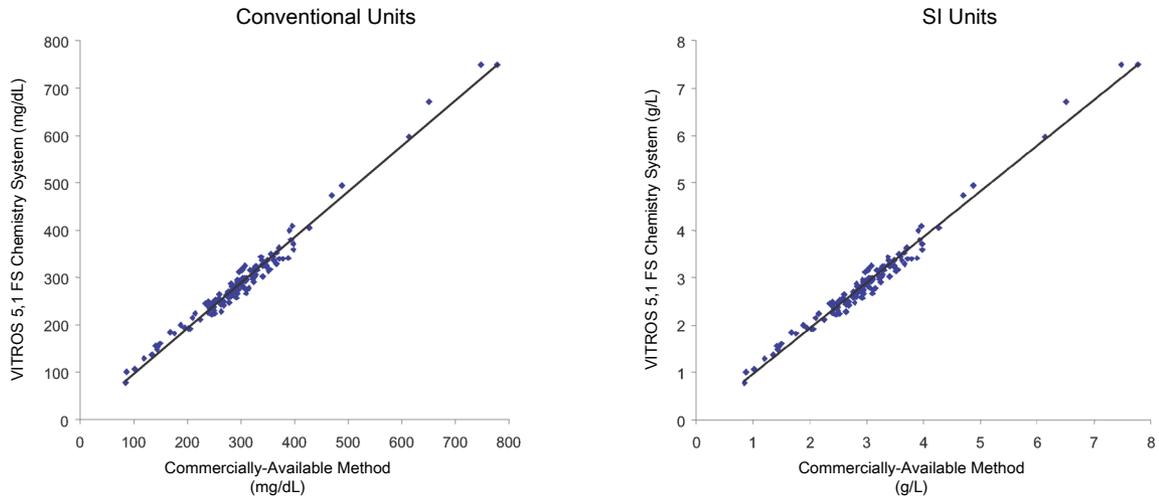
Each laboratory should confirm the validity of these intervals for the population it serves.

## Performance Characteristics

### Method Comparison

The plots and data below show the results of a method comparison study with serum samples analyzed on the VITROS 5,1 FS Chemistry System and a commercially available system, based on NCCLS Protocol EP9.<sup>11</sup>

The table also shows the results of comparisons with serum and plasma samples on the VITROS 5600 Integrated System and the VITROS 5,1 FS Chemistry System. The testing followed NCCLS Protocol EP9.<sup>11</sup>



|   | n   | Slope | Correlation Coefficient | Conventional Units (mg/dL) |           |       | SI Units (g/L)        |           |      |
|---|-----|-------|-------------------------|----------------------------|-----------|-------|-----------------------|-----------|------|
|   |     |       |                         | Range of Sample Conc.      | Intercept | Sy.x  | Range of Sample Conc. | Intercept | Sy.x |
| <b>5,1 FS<sup>†</sup> vs. commercially available method<sup>*</sup></b> | 137 | 0.96  | 0.986                   | 104.74–749.91              | -0.24     | 14.48 | 1.05–7.50             | 0.00      | 0.14 |
| <b>5600 vs. 5,1 FS<sup>†</sup></b>                                      | 108 | 0.99  | 0.999                   | 88.46–729.06               | +3.81     | 8.60  | 0.88–7.29             | 0.04      | 0.09 |

<sup>\*</sup> Beckman IMMAGE Transferrin assay

<sup>†</sup> Analytical processing hardware and software algorithms on the VITROS 4600 Chemistry System are designed to the same specifications as those applied to the VITROS 5,1 FS Chemistry System. Assay performance on the VITROS 4600 System has been demonstrated to be comparable to that on the VITROS 5,1 FS System. All performance characteristics for VITROS 5,1 FS System are therefore applicable to the VITROS 4600 System.

### Precision

Precision was evaluated with quality control materials on the VITROS 5,1 FS System following NCCLS Protocol EP5.<sup>12</sup> Precision was also evaluated with quality control materials on the VITROS 5600 Integrated System following NCCLS Protocol EP5.<sup>13</sup>

These results are guidelines. Variables such as instrument maintenance, environment, reagent storage/ handling, control material reconstitution, and sample handling can affect the reproducibility of test results.

|                     | Conventional Units (mg/dL) |                            |                             | SI Units (g/L) |                            |                             | Within Lab CV% <sup>**</sup> | No. Observ. | No. Days |
|---------------------|----------------------------|----------------------------|-----------------------------|----------------|----------------------------|-----------------------------|------------------------------|-------------|----------|
|                     | Mean Conc.                 | Within Day SD <sup>*</sup> | Within Lab SD <sup>**</sup> | Mean Conc.     | Within Day SD <sup>*</sup> | Within Lab SD <sup>**</sup> |                              |             |          |
| 5,1 FS <sup>†</sup> | 138.08                     | 2.555                      | 6.354                       | 1.38           | 0.026                      | 0.063                       | 4.6                          | 91          | 23       |
|                     | 269.88                     | 4.183                      | 7.077                       | 2.70           | 0.042                      | 0.072                       | 2.6                          | 92          | 23       |
|                     | 547.07                     | 11.569                     | 24.491                      | 5.47           | 0.116                      | 0.245                       | 4.5                          | 92          | 23       |
| 5600                | 133.45                     | 2.057                      | 2.534                       | 1.33           | 0.021                      | 0.025                       | 1.9                          | 88          | 22       |
|                     | 266.40                     | 3.141                      | 3.623                       | 2.66           | 0.031                      | 0.036                       | 1.4                          | 88          | 22       |
|                     | 519.46                     | 9.818                      | 10.676                      | 5.19           | 0.098                      | 0.107                       | 2.1                          | 88          | 22       |

<sup>\*</sup> Within Day precision was determined using two runs per day with two replications per run.

<sup>\*\*</sup> Within Lab precision was determined using a single lot of reagent and at least 4 calibrations.

<sup>†</sup> Analytical processing hardware and software algorithms on the VITROS 4600 Chemistry System are designed to the same specifications as those applied to the VITROS 5,1 FS Chemistry System. Assay performance on the VITROS 4600 System has been demonstrated to be comparable to that on the VITROS 5,1 FS System. All performance characteristics for VITROS 5,1 FS System are therefore applicable to the VITROS 4600 System.

## Specificity

### Substances that Do Not Interfere

The substances listed in this table were tested on the VITROS Chemistry Products TRFRN Reagent at transferrin concentration of approximately 183 mg/dL (1.8 g/L) using protocols based on NCCLS Protocol EP7<sup>14</sup>, and found not to interfere, bias < 16.5 mg/dL (1.7 g/L), at the concentrations shown. Hemoglobin and bilirubin were tested on the VITROS Chemistry Products TRFRN Reagent at transferrin concentration of approximately 134 mg/dL (1.3 g/L) using protocols based on NCCLS Protocol EP7 and found not to interfere, bias < 13.5 mg/dL (1.3 g/L), at the concentrations shown.

| Compound             | Concentration |              |
|----------------------|---------------|--------------|
| Acetaminophen        | 200 µg/mL     | 1324 µmol/L  |
| Acetyl-L-cystein (N) | 100 mg/dL     | 6.13 mmol/L  |
| Amoxicillin          | 20 µg/mL      | 55 µmol/L    |
| Ascorbic acid        | 3 mg/dL       | 0.17 mmol/L  |
| Bilirubin            | 60 mg/dL      | 1.03 mmol/L  |
| Carbamazepine        | 120 µg/mL     | 507.6 µmol/L |
| Dipyron              | 30 mg/dL      | 0.85 mmol/L  |
| Ethamsylate          | 3 mg/dL       | 0.11 mmol/L  |
| Gentamicin sulfate   | 120 µg/mL     | 251 µmol/L   |
| Hemoglobin           | 1000 mg/dL    | 10 g/L       |
| Ibuprofen            | 400 µg/mL     | 1940 µmol/L  |
| Intralipid           | 1000 mg/dL    | 10 g/L       |
| Lidocaine            | 60 µg/mL      | 256 µmol/L   |
| Methotrexate         | 2 mmol/L      | 2 mmol/L     |
| Procainamide         | 100 µg/mL     | 368 µmol/L   |
| Propranolol          | 5000 ng/mL    | 19.3 µmol/L  |
| Rantidine            | 200 µg/mL     | 637 µmol/L   |
| Salicylic acid       | 500 µg/mL     | 3.6 mmol/L   |
| Simvastatin          | 16 µg/mL      | 1.2 mmol/L   |
| Theophylline         | 250 µg/mL     | 1388 µmol/L  |
| Triglycerides        | 1000 mg/dL    | 11.3 mmol/L  |
| Valproic acid        | 500 µg/mL     | 3465 µmol/L  |

## References

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13. NCCLS. *Evaluation of Precision Performance of Clinical Chemistry Devices; Approved Guideline- Second Edition*. NCCLS document EP5-A2 [ISBN 1-56238-542-9]. CLSI, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898 USA, 2004.
14. NCCLS. *Interference Testing In Clinical Chemistry*; Proposed Guideline. NCCLS document EP7-A [ISBN 1-56238-480-5]. NCCLS, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898, USA, 2002.

## Glossary of Symbols

The following symbols may have been used in the labeling of this product.

|  |   |  |  |  |                         |
|--|---|--|--|--|-------------------------|
|  | Do Not Reuse  |  | Upper Limit of Temperature   |  | Range                   |
|  | Use by or Expiration Date (Year-Month-Day)          |  | Lower Limit of Temperature   |  | Range of Means          |
|  | Batch Code or Lot Number                            |  | Temperature Limitation   |  | Midpoint                |
|  | Serial Number                                       |  | Consult Instructions for Use   |  | Revised                 |
|  | Catalog Number or Product Code                      |  | Attention: The Instructions for Use (IFU) has been updated   |  | Supersedes              |
|  | Caution   |  | For use in Slide Supply 1  |  | Irritant                |
|  | Manufacturer  |  | For use in Slide Supply 2  |  | Harmful                 |
|  | Date of Manufacture                                 |  | SI Units   |  | Toxic                   |
|  | Authorized Representative in the European Community |  | Conventional Units   |  | Corrosive               |
|  | Contains Sufficient for "n" Tests                   |  | Value  |  | Flammable               |
|  | In vitro Diagnostic Medical Device                  |  | Der Grüne Punkt (the Green Dot). Manufacturer follows certain packaging material waste disposal management regulations |  | Estimated within-lab SD |

## Revision History

| Date of Revision | Version | Description of Technical Changes*              |
|------------------|---------|--|
| 2014-09-05       | 5.0     | Glossary of Symbols: added Date of Manufacture |

# INSTRUCTIONS FOR USE

Revision History

**TRFRN**

Transferrin

| Date of Revision | Version | Description of Technical Changes*  |
|------------------|---------|--|
| 2012-02-28       | 4.0     | Glossary of Symbols: updated   |
| 2010-11-01       | 3.0     | Added information for the VITROS 4600 Chemistry System   |
| 2008-10-24       | 2.0     | <ul style="list-style-type: none"> <li>• Added information for the VITROS 5600 Integrated System</li> <li>• Test Type and Conditions – Added statement</li> <li>• Traceability of the Calibration – Updated</li> <li>• Method Comparison – Added information on sample types</li> <li>• Precision – 5,1 FS: Updated</li> <li>• References – Updated</li> <li>• Glossary of Symbols– Updated</li> <li>• Minor wording and formatting changes</li> </ul> |
| 2004-09-09       | 1.0     | First release of document  |

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

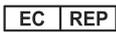
When this Instructions For Use is replaced, sign and date below and retain as specified by local regulations or laboratory policies, as appropriate.

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Signature

\_\_\_\_\_

Obsolete Date



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