Immunoturbidimetric determination of serum transferrin on a Kone Progress autoanalyser

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The Kone Progress, a multiparametric discrete analyser, was used to determine serum transferrin with a kit supplied by Kone. Assays recommended by the French Society of Clinical Chemistry were performed in order to assess the suitability of the test. Repeatability was assessed using serum pools with low (L), medium (M) and high (H) concentrations of transferrin. The coefficients of variation (CV) were 5.4%, 3.2% and 2.0% respectively for 30 determinations (within-batch). Reproducibility on 15 consecutive days (between-batch) was also satisfactory (CV for L = 7.3%, M = 6.3% and H = 3.8%). There was no serum-to-serum contamination. Results correlated closely with those obtained using radial immunodiffusion (RID) (r = 0.942) and total iron-binding capacity (r = 0.954) for 90 determinations.

Transferrin measurement by immunoturbidimetry on the Kone Progress emerges as a well-suited, rapid and inexpensive alternative to other time-consuming (RID) and sophisticated (laser immunonephelometer) techniques.

Materials and methods

Immunoturbidimetry was performed using the transferrin test kit (Kone, Finland) containing the anti-transferrin anti-serum and polyethylene glycol to enhance the immunoprecipitation. The apparatus (Progress, Kone, Finland) [9] was used under the conditions described in table 1.

Table 1. Immunoturbidimetric determination.

| Sample      | 5 µl (1/11 diluted) |
|-------------|---------------------|
| Buffer      | 100 µl              |
| Incubation  | 130 s               |
| Antibody    | 30 µl               |
| Incubation  | 120 s               |
| Measurement | end point           |
| λ           | 340 nm              |
| T°C         | +37°C               |

The non-linear calibration curve was obtained using the protein calibration set (supplied by Kone) containing transferrin at five known concentrations (from 1 to 8 g/l). Samples of serum and calibrators were diluted at 1/11 before use.

Radial immunodiffusion: nor-partigen plates were used (Behring, Marburg, FR Germany) with the appropriate serum control (serum RDT, Behring).

Total iron-binding capacity was measured by the classic addition of FeCl₃ to serum, elimination of excess iron by MgCO₃ and measurement of total bound iron by the bathophenanthrolin reaction. Results are expressed in µmol iron/l.

Results

Within-run and between-run precision was tested with pools of serum from patients at the hospital: low (L₁), medium (M) and high (H) transferrin concentrations (different pools were used for within-run and between-run assays). The within-run assay consisted of 30 consecutive measurements for each pool; and the between-run of measurements on 15 consecutive days. Results are given in table 2.

Sample dispenser carry-over assessment was performed measuring (10 times) a sample with low transferrin (L₁), and (10 times) a sample with high transferrin (H₁) and then performing the sequence H₂H₃L₂L₃ six times where H₂ is the sample H₁ measured in first position, H₃ is the

† This work is dedicated to Marie-Thé Gaubert (1962–1988).
Figure 1. Correlations between immunoturbidimetry (IT), radial immunodiffusion (RID) and total iron binding capacity (TIBC), on 90 samples from hospital patients.

Table 2. Precision assay.

|        | Within-run | Mean | SD  | CV % |
|--------|------------|------|-----|------|
| L      | 0.32       | 0.04 | 5.4 |
| M      | 2.33       | 0.07 | 3.2 |
| H      | 3.60       | 0.07 | 2.0 |

Table 3. Comparison of serum transferrin measured by immunoturbidimetry (IT) and radial immunodiffusion (RID) or assessed by total iron binding capacity (TIBC).

|        | N  | Slope and intercept (y = ax + b) | r   |
|--------|----|----------------------------------|-----|
| IT/TIBC| 90 | 0.057 -0.46                      | 0.96|
| RID/IT | 90 | 0.977 0.36                       | 0.95|
| RID/TIBC|90 | 0.059 -0.27                       | 0.97|

Discussion

Transferrin has been assessed indirectly by the measurement of TIBC for some years. However, there is now a general consensus [1] that advances in clinical biochemistry make the direct determination of transferrin preferable to TIBC. The most common methods are IDR and laser nephelometry; but the former takes 48 hours and the latter requires expensive, dedicated apparatus. Immunoturbidimetry thus appears as an attractive alternative, especially when automatized on a routine analyser. Immunoturbidimetric determination of transferrin on the Kone Progress proved to be accurate, reliable and inexpensive. It is interesting to note that immunoturbidimetry gave significantly lower values than RID (T = 27-4; p < 0.001). There is no clear explanation for this difference but this has previously been reported in this work, as in our own, there is a systematic and constant difference in values (intercept different from 0, slope near 1). In the same way, immunoturbidimetry gives lower values than laser nephelometry [4]. These differences requires that the values obtained be compared to normal values for a given technique: normal values of serum transferrin range from 2.50 to 3.50 g/l using immunoturbidimetry [4 and 7].

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