Analysis of glutathione peroxidase in erythrocytes and plasma

**Principe** : In the presence of reduced glutathione (GSH), glutathione peroxidase reduces hydroperoxide (ROOH), while GSH is oxidized to glutathione disulfide (GSSG).

\[
\text{GSH-px} \\
2 \text{GSH} + \text{ROOH} \rightarrow \text{ROH} + \text{GSSG} + \text{H}_2\text{O}
\]

The rate of oxidation of GSH is measured by following the decrease of NADPH consumed for the reduction of GSSG by the glutathione reductase in excess (GR).

\[
\text{GR} \\
\text{GSSG} + \text{NADPH} + \text{H}^+ \rightarrow 2 \text{GSH} + \text{NADP}^+
\]
Preparation of reagents, buffers, substrates

Ransel kit (Randox) is used for assays on an automated biochemistry analyser.
The plasma is decanted and analyzed directly on the analyser for the determination of GSH-pxp. Red blood cells are then decanted and washed with a washing centrifuge for determination of GSH-pxe in blood cells.
Once the red cells are washed with NaCl, centrifuged and decanted, they are again centrifuged, using a blood-bank centrifuge.

Then, a precise volume of blood cell is added to a precise volume of dilution buffer, then vortexed.
The standards are reconstituted on the morning of the assay. Two controls (internal and commercial) frame the series of samples and validate the calibration.
- **Matrix**: plasma, erythrocytes
- **Range**: 31 - 1170 U/l or 20 - 740 U/gHb
- **Uncertainty**: 12%

- **Validated method**:
  - **Regression**: linear model
  - **Limit of quantification**: 31 U/l or 20 U/gHb
  - **Accuracy**:
    - CV_r = 2% (160 U/l)  _  1% (680 U/l)
    - CV_r = 5% (140 U/gHb)  _  7% (500U/gHb)
    - CV_R = 5% (160 U/l)  _  6% (680 U/l)
    - CV_R = 7% (140 U/gHb)  _  6% (500U/gHb)
  - **Trueness**: Bias of -11U/l at 393 U/l
    - Bias of -7U/gHb at 248 U/gHb