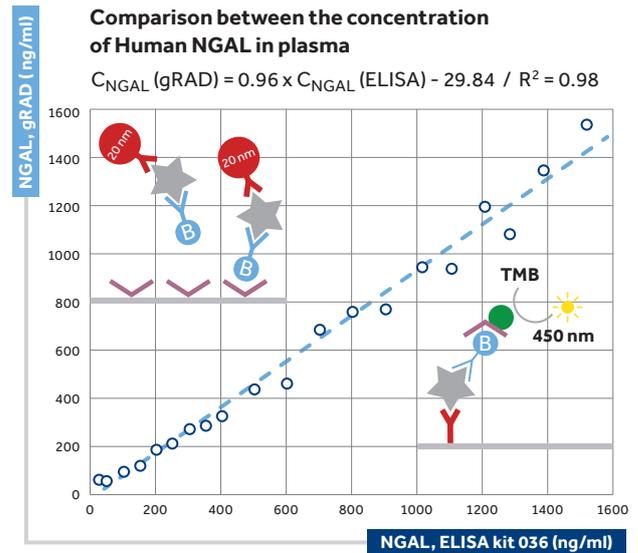


gRAD TECHNICAL NOTE

Comparison between the performance of a gRAD test and a ELISA assay

What does this study show?

The concentration of **Human NGAL** was measured using two different immunoassays: a validated and CE-marked¹ **ELISA Kit (KIT 036, BioPorto Diagnostics)** and a lateral-flow test developed using **gRAD (generic Rapid Assay Device)**, - the new ready-to-use generic lateral flow platform from BioPorto. The study shows a good correlation (slope = 0.96, R² = 0.98) between the concentration of NGAL measured by the two methods, by **single measurements of human plasma samples**.



Comparison between the concentration of Human NGAL in plasma, measured using BioPorto's ELISA Kit (KIT 036) and measured with a NGAL-gRAD (generic Rapid Assay Device) test. The top-left inset in the figure shows schematically what happens in a gRAD strip, where the biotinylated antibodies bind to a biotin-binding protein. The bottom-right inset shows a representation of the ELISA sandwich assay, with all the components involved.

1) In selected countries. For Research Use Only in all other territories.

How was this study performed?

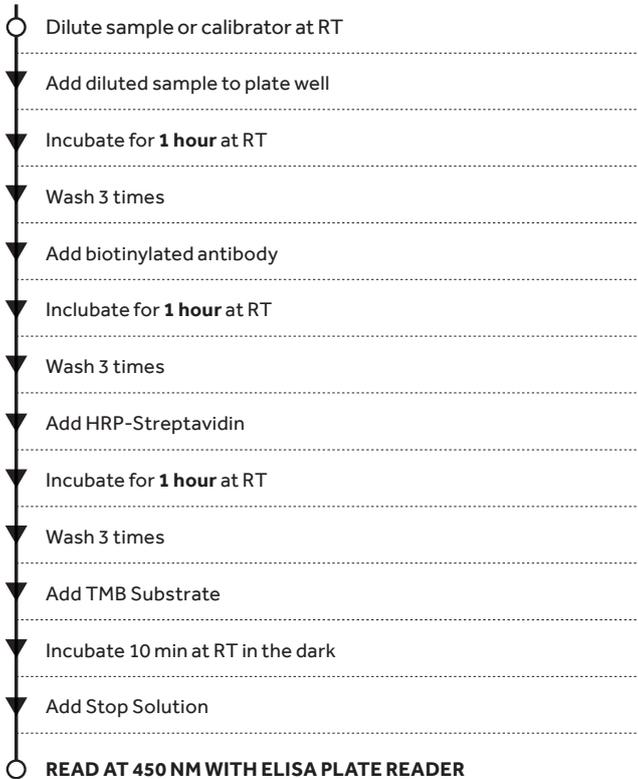
Measurement with NGAL ELISA Kit 036

Data was collected from internal clinical plasma samples measured with KIT 036 at a dilution of 1/500, as recommended by the IFU of the kit. Concentrations were calculated using the calibration curve obtained with the calibrators provided in the kit.

Measurement with NGAL-gRAD Test

A NGAL-gRAD Test was developed using the same **matched antibody pair used in KIT 036**. In this example, a freeze-dried and ready to use test reagent was prepared. The test reagent contains the detection antibody conjugated to **20 nm** gold particles and the biotinylated capture antibody in optimized concentrations. Full development of the test took **3 days**. The concentration of NGAL in plasma was measured by adding 120 μl of Sample Dilution Buffer (SDB) and 10 μl of sample to the test reagent. The concentrations were calculated using a calibration curve obtained with internal calibrator solutions. A summary of the measurement procedure, in comparison with ELISA can be seen in the figure on the next page.

ELISA

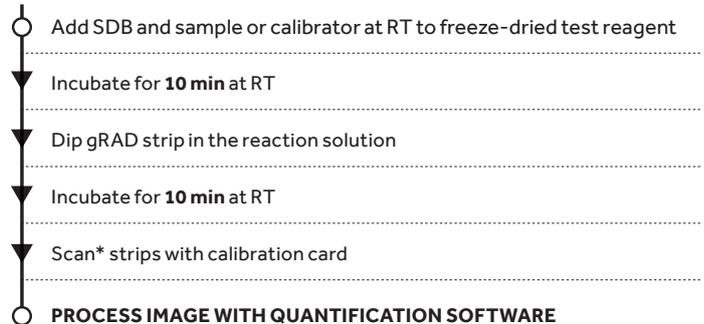


TOTAL ASSAY TIME = 4 HOURS

About KIT 036

KIT 036 is a sandwich ELISA assay built with a matched Human Anti-NGAL antibody pair. The ELISA plates of KIT 036 are coated with one of the antibodies from the pair – this is, in the ELISA, the capture antibody, which specifically recognizes Human NGAL. The second antibody from the pair is biotinylated and works as detection antibody. The biotinylated antibody binds to HRP-Streptavidin. A quantifiable signal is obtained by measuring the intensity of the color produced after reaction of the HRP with TMB at 450 nm, using an ELISA plate reader.

gRAD



TOTAL ASSAY TIME = 30 MIN FOR 20 SAMPLES

About gRAD

*gRAD is a generic Rapid Assay Device based on proprietary lateral-flow technology. The gRAD lateral-flow strip is composed by a **test line**, made of a **biotin-binding protein**, and a **control line** that captures any antibody from **mouse, rabbit** or **goat**. The reaction between the antibodies and the antigen, in this case NGAL, takes place in **the solution** where the strip is dipped. The biotinylated antibody from the pair (the capture antibody, in gRAD), - both free and in complex - binds to the test line. The control line captures uncomplexed gold-conjugated antibodies. The ratio between the free and the complexed biotinylated antibodies in a saturated test line correlates with the concentration of NGAL in the sample and can be reliably **quantified**.*

* CanoScan LiDE 220 S/N KJRP49424, (JPEG, Color, 300 DPI), in this example.

For further information go to
www.bioporto.com