ABSTRACT
This work presents the conversion of a clinical liquid-phase GHB detection enzymatic assay into a user-friendly solid-phase GBL/GHB detection assay. The test depends on the activity of GHB dehydrogenase from Ralstonia eutropha for sensitivity and specificity. Transfer of the assay to the new format required several modifications: coupling of detection reaction with a color generating enzymatic reaction involving diaphorase, design of an adequate paper support and optimization of buffer and reagents in order to limit matrix interferences. These interferences consist primarily in ethanol-dependent low activity and reduction of pro-dye substrate by vitamin C and have been minimized in order to fit application which consist in testing drinks for drugs of abuse, GHB and GBL.

INTRODUCTION
Chemical approaches for detection of drugs of abuse γ-hydroxybutyrate (GHB, KO drops, liquid ecstasy) and its precursor γ-butyrolactone (GBL) in drinks have failed because of the challenging diversity of matrices. To solve this problem, current efforts focus on the optimization of specificity and sensitivity by using one or multiple enzyme reactions. [3, 4, 5]

We have formerly developed a rapid enzymatic assay for detection of γ-hydroxybutyrate (GHB) in blood and urine based on the activity of GHB dehydrogenase.[1,2] The test is patented for in vitro diagnostic applications and currently available on the market. We now aim the development of an enzymatic assay for drink testing based on the same enzyme reaction.

RESULTS
In a first step, we developed and tested a basic device design (not detailed), that already intrinsically solves several problems.
1. Background coloration does not generate false positive.
2. Insoluble matter does not affect test.
3. GBL converts to GHB.
4. Ethanol excess partially evaporates.

The basic paper-based device yields adequate specificity in application-relevant conditions (demonstrative results in figure below).

![Tested solutions (both pictures). G: GBL 0.5%, corresponds to 1 mL pure substance in 200 mL drink, E: Ethanol 20%, corresponds to typical strong cocktail concentration. C: Vitamin C 1 mg/mL. For information, fresh orange juice contains about 0.25 mg/mL vitamin C. Ø: Water.](image)

In a next step, we improved design and procedure in order to emphasize the signal intensity. The semi-optimized devices, partly illustrated in the picture beside, present a typical end results. These hand-made device prototypes allow an indisputable analysis and should further benefit on production technique (spray application for example) for their readability.

Vitamin C interferences were reduced by adjusting buffer conditions and reagents concentrations. In our current prototypes, even high vitamin C concentrations (1 mg/mL) do not significantly affect the assay.

Specificity of assay was improved in the same manner in order to reduce ethanol-dependent activity. The ratio between signal at reference GBL/EtOH concentrations has been emphasized about a factor 5 toward our commercial blood/urine assay on which this development is based. As shown in picture, this enables us to obtain evident signal for GBL low doses (0.5%) without generating ethanol-dependent false positives.

CONCLUSION AND FURTHER STEPS
We dispose on a functional and flexible low-cost prototype measuring GBL and GHB in drinks, that overcomes the major difficulties caused by the matrices. The next steps will consist in screening more exhaustively further matrices and solve occurring interferences or exclude their sources from application list. We intend to improve assay rapidly (aiming less than 3 min) and improve handling design. Eventually we have to define the production process, conditioning and storage conditions of the product which warrant long term stability at room temperature.

REFERENCES