

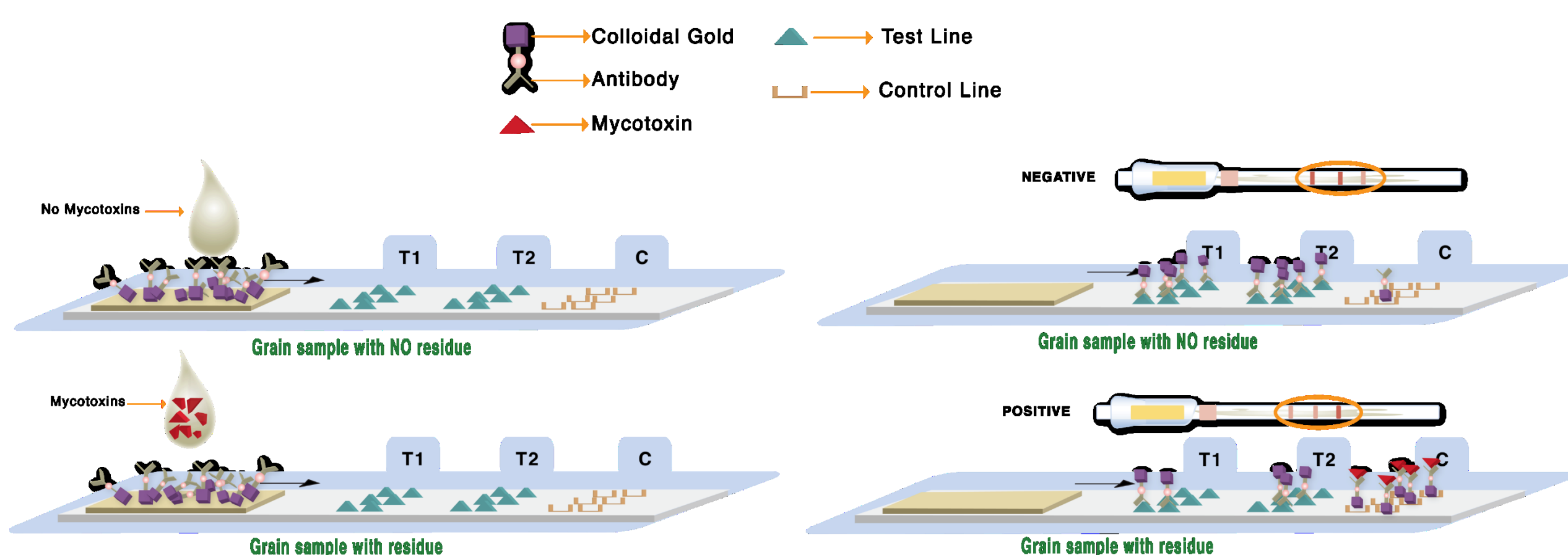
Introduction

The fungal pathogen *Fusarium head blight* (FHB) affects barley, causing loss of yield, kernel damage and negatively affects the quality of finished malt and beer. The fungal species *Fusarium graminearum* thrives in malting conditions and produces metabolites that are able to survive malting and brewing processes. Deoxynivalenol (DON), part of the tricothecene mycotoxin family, is a toxin most commonly produced by the fungi *Fusarium* and DON can be detected in kernels that do not show FHB symptoms. The accurate measurement of the levels of DON is important in monitoring the quality of food and beverages.

Rapid methods are required to screen for DON in grain samples at intake facilities for selection and segregation. Recently a class of rapid test kits based on an immunochromatographic principle, also called Lateral Flow Assays (LFA), has become available which allows for rapid and sensitive detection of DON in a variety of matrices.

Lateral flow immunochromatographic assays are based on a competitive immunoassay format. The diluted extract is wicked through a reagent zone containing antibodies conjugated to a visualizing particle specific for DON. If DON is present then the antibodies will bind to form a complex. The complex then is wicked onto a membrane containing a zone of DON conjugated to a protein carrier, which captures any un-complexed DON antibodies. This creates visible lines which can be quantified. As concentrations of DON increase, the line density decreases. The reader then converts the line densities into a quantitative result.

Figure 1. Principle of Lateral Flow Assay.



Methodology

In this study, two different commercially available lateral flow assay kits, ROSA DONQ2 with the Charm EZ system (Charm Sciences) and Reveal Q+ with AccuScan Gold Reader (Neogen Corporation) were used to quantify DON levels in 30 samples of naturally contaminated barley and malt. Results were compared with traditional ELISA (Neogen Veratox 2/3) and gas chromatography (ASBC Barley-11A).

Bland-Altman plots (Difference plots) were used to quantify agreement between methods by constructing limits of agreement. These statistical limits are calculated by using the mean and the standard deviation of the differences between two measurements.

Results

Figure 2. Difference plot analysis between LFA and ELISA methods for barley

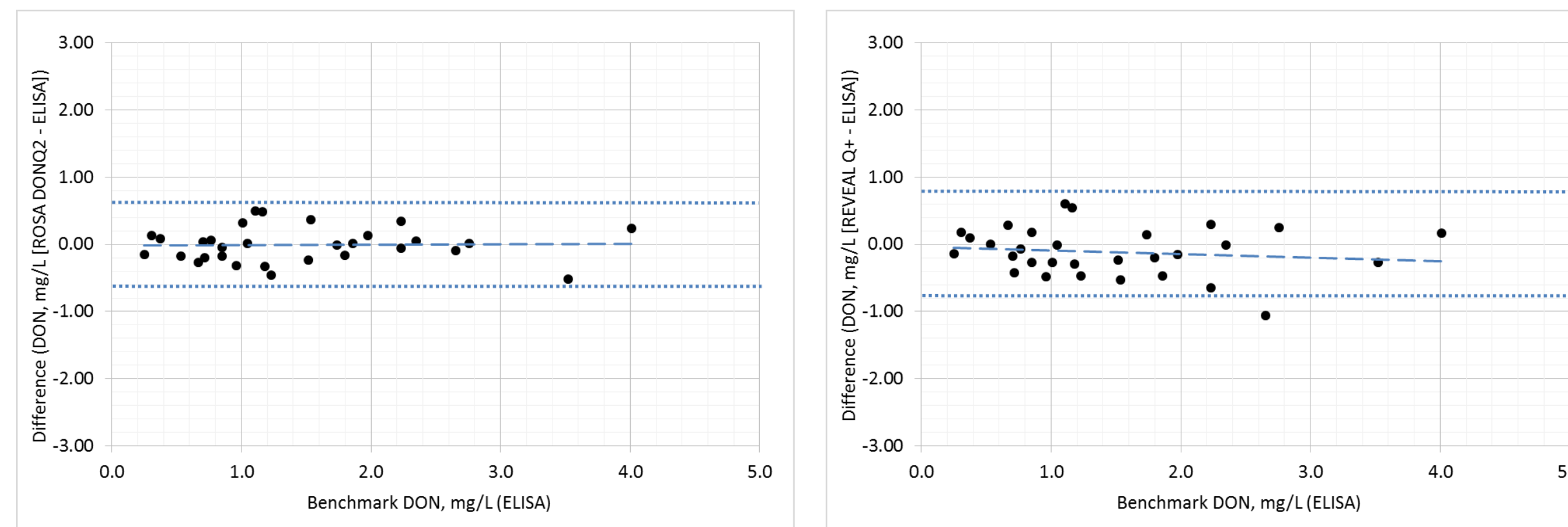
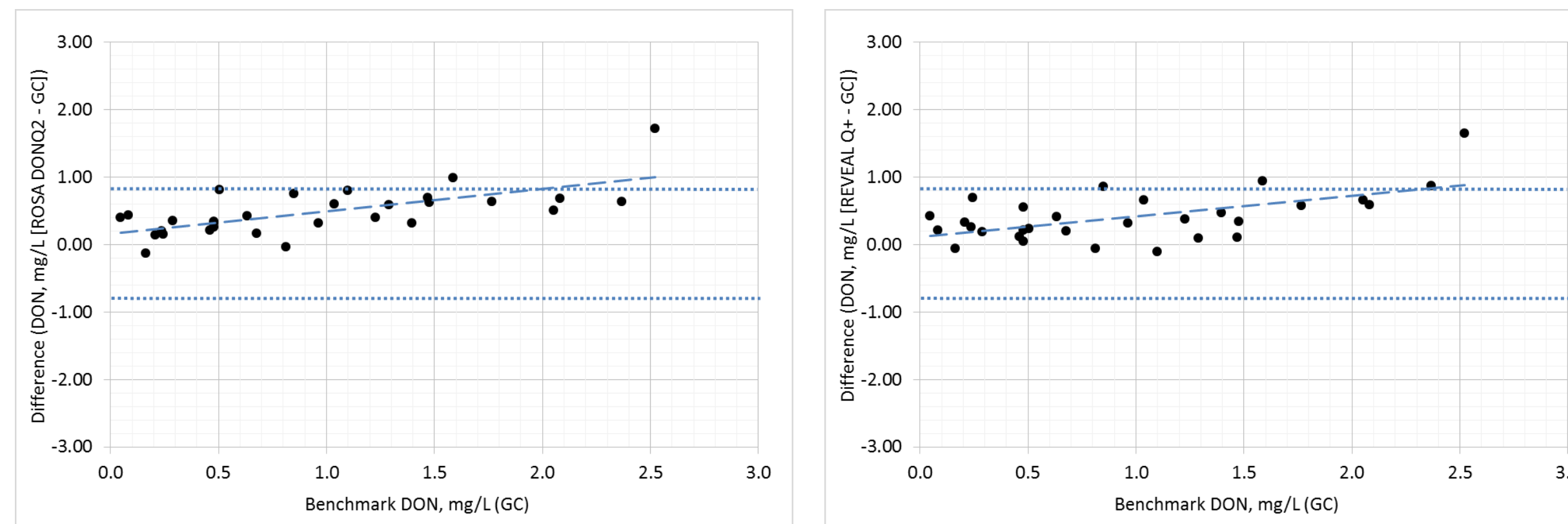


Figure 3. Difference plot analysis between LFA and GC methods for barley



Discussion

Difference plots are useful to reveal relationships between the differences and the magnitude of measurements to identify systematic or proportional bias between two methods.

Observation of the Difference plots reveals that the lateral flow assay overestimates the DON level compared to gas chromatography, especially at higher DON levels. This overestimation is commonly attributed to cross reactions between the antibodies and structurally related metabolites and is greater in malt.

Table 1. Mean (Standard Deviation) of differences between methods

	Barley		Malt	
	ROSA DON Q2	Reveal Q+	ROSA DON Q2	Reveal Q+
ELISA	-0.02 (0.3)	-0.12 (0.4)	-0.04 (0.5)	-0.08 (0.4)
GC	0.48 (0.3)	0.37 (0.4)	0.87 (0.7)	0.84 (0.8)

Figure 4. Difference plot analysis between LFA and ELISA methods for malt

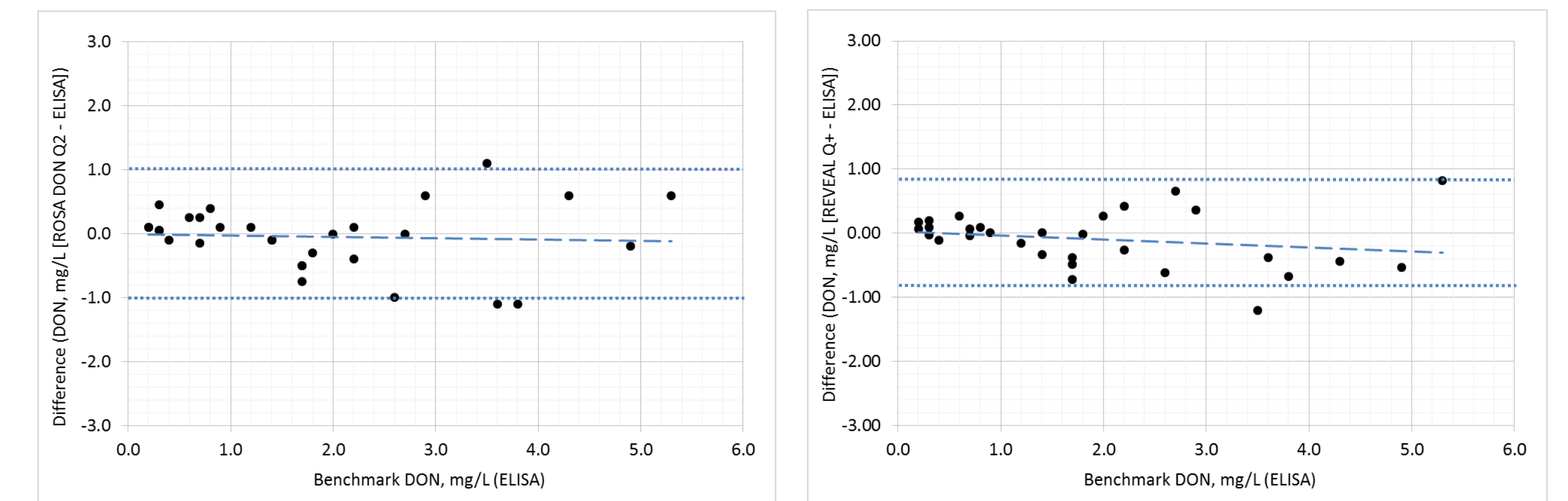
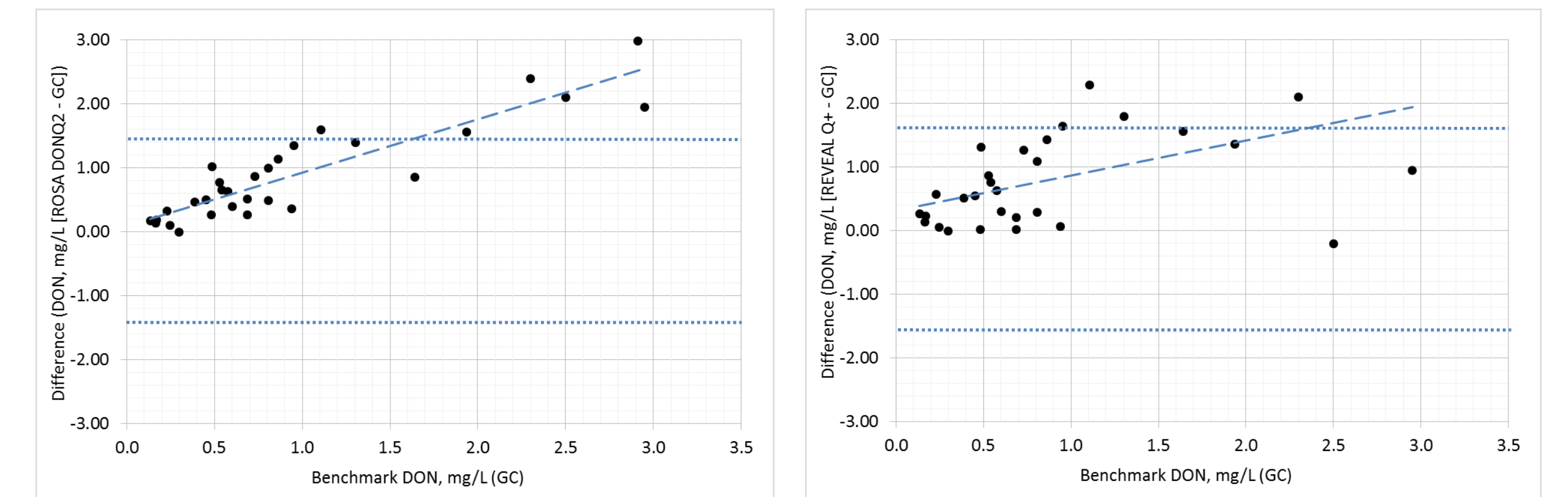


Figure 5. Difference plot analysis between LFA and GC methods for malt



Conclusions

Results for Lateral Flow Assays correlate favorably to the ELISA method for the determination of DON in both barley and malt. Immunoassays are valuable screening tools to quantify DON in large sample sizes that can be conducted in a short time period with minimal effort while avoiding costly labor and expensive laboratory equipment. These kits were found to be well suited for screening and possess the sensitivity to adequately screen un-malted grain samples. For accurate determination, it is advisable to analyze samples using chromatography when the sample result identified by a rapid method is near the tolerance level.

References

Bland JM, Altman DG (1986) Statistical methods for assessing agreement between two methods of measurement. *Lancet*. 76:307-310