Evaluation of qualitative lateral flow immunoassay for detecting gluten immunogenic peptides in celiac patients following a habitual gluten-free diet.

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Background: Detection of Gluten Immunogenic Peptides (GIP) in stool as assessed by ELISA, has been shown to be sensitive and specific to detect gluten exposure in the context of studies exploring adherence to gluten-free diet (GFD). A lateral flow immunoassay (LFIA) test has also been developed as a qualitative point-of-care test which potentially could simplify GFD adherence monitoring. LFIA performance has not been thoroughly investigated especially in real life scenario context. **AIM**: Our aim was to assess the performance of a stool LFIA test compared with the quantitative determination of stool GIP by ELISA, used as the gold standard, in a series of samples from treated celiac disease (CeD) patients. **Methods**: 53 patients collected stool samples over four consecutive weeks. Samples were kept frozen until testing. To measure stool GIP (µg/g of stool) an ELISA kit (iVYLISA GIP S®, Biomedal SL) was used and positive tests were categorized as ≥0.156 µg/g (lowest limit of detection), ≥0.32, and ≥0.64. For the qualitative evaluation of stool GIP, we employed an LFIA test (GlutenDetect®; Biomedal S.L) that was reported as positive or negative. An expert in the reading of LFIA tests participated in the evaluation. The statistical performance of the LFIA tests was determined using the above mentioned levels of stool GIP concentration. **Results**: A total of 299 stool samples were collected during the study period. ELISA for stool GIP was >0.156 µg/g in 40.6%, ≥0.32 in 39.1%, and ≥0.64 in 23.4% of samples (Table 1). The sensitivity of LFIA for detecting GIP at the three different cut-offs varied from 43.9 to 53.3%. In contrast, specificities were high (from 83.7% to 88.8%). Agreement between ELISA and LFIA tests ranged from 70.9% and 76.7%. Finally, *Cohen's kappa* statistics evidenced a weak concordance between tests (Table 1). **Conclusion**: Our study found that the LFIA test has lower sensitivity but adequate specificity and a weak concordance with quantitative ELISA. We hypothesize that performance differences between the two tests could be related different combination of monoclonal antibodies and extraction procedures in LFIA vs. ELISA. Furthermore, the higher detection limit and the requirement of higher levels of gluten exposure for a positive LFIA test seem to be factors contributing to its low sensitivity. Although less sensitive than ELISA, LFIA is a simple tool that can be used by patients for self-monitoring of gluten contaminations.

Table 1

Stool GIP µg/g (ELISA)	**N of patients** (%)	**LFIA Sensitivity** % (95% CI)	**LFIA Specificity** % (95% CI)	**LFIA PPV** % (95%IC)	**AUR curve** (95% IC)	**Agreement** %	**Cohen´s *Kappa***
≥0.156 µg/g of GIP	121/299 (40.5)	43.9 (34.6-53.5)	88.2 (82.5-92.5)	70.4 (58.4-80.7)	0.66 (0.61-0.71)	70.9	0.34
≥0.32 µg/g of GIP	119/299 (39.1)	44.5 (35.1-54.3)	87.9 (82.3-92.3)	69 (56.9-79.5)	0.66 (0.61-0.71)	71.6	0.35
≥0.64 µg/g of GIP	70/299 (23.4)	53.3 (39.5-64.9)	83.7 (78.2-88.3)	47.9 (35.9-60.1)	0.68 (0.61-0.75)	76.7	0.35

Table 1: Statistical performance of the lateral flow immunologic assay (LFIA) tests for detection of stool GIP (positive or negative test) compared with the quantitative determination of GIP concentration (µg/g) by ELISA in the same stool samples. PPV: positive predictive value; 95% CI: 95% confidence interval. AUR: area under the rock curve.