A multiplexed chemiluminescent screening assay for determination of IgG and IgA antibodies to tissue transglutaminase and recombinant gliadin

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Background

The detection of autoantibody levels to tissue transglutaminase (tTG) and deamidated gliadin peptide (dGP) are used, together with clinical information, to aid in the diagnosis of patients with celiac disease and to monitor adherence to gluten-free diets. Multiplexing with Dynex® Technologies' SmartPLEX™ technology allows for the simultaneous detection of these targets with advantages in terms of time and cost. The Dynex Technologies Multiplier® system fully automates these assays. Antigen-coated SmartPLEX beads are embedded in the base of a 96-well SmartPLEX plate and serve as individual targets for IgG and IgA binding from the test sample. The assays are based on standard direct solid phase assay principles with a chemiluminescent end point. If antibodies to any of the targets are present, a luminescent signal is produced that is proportional to the antibody concentration and is detected by an integrated CCD camera. See Figures 1a-d for pictures of the SmartPLEX assay plate and the Multiplier instrument.

The IgA assay uniquely incorporates a bead that detects total serum IgA. Approximately 1:400 celiac patients is IgA deficient; a negative IgA tTG or dGP result could be a consequence of this deficiency rather than the absence of specific IgA antibodies. IgA concentrations <0.07 mg/mL are indicative of IgA deficiency.

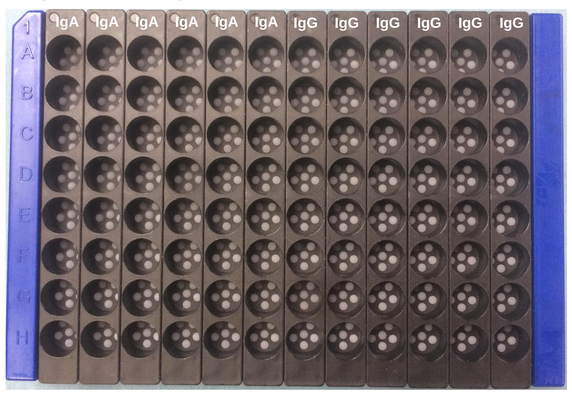

Figure 1a. Celiac SmartPLEX assay plate with combined IgA and IgG SmartPLEX test strips

Figure 1b. IgA well

Figure 1c. IgG well

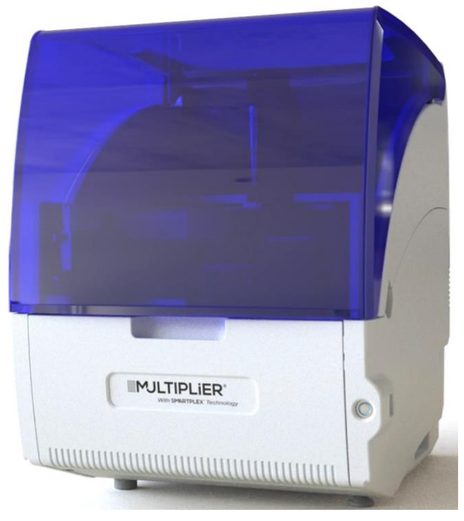

Figure 1d. Multiplier instrument

Method

Concordance was assessed by comparing the results for each assay to a respective 510(k) cleared ELISA using 40 samples collected for routine tTG IgG/IgA and dGP IgG/IgA testing. Duplicate precision was calculated as the percentage coefficient of variation (%CV) using the 40 concordance samples for each assay.

Results

Precision: The mean within assay %CV for each assay based on 40 duplicate results is summarized in Figure 2 below:

	tTG	dGP
IgG	3.8%	3.9%
IgA	5.5%	4.9%

Figure 2. Celiac SmartPLEX assay precision mean

Concordance: The positive and negative predictive values (PPV and NPV) were calculated, applying a 95% confidence interval for each assay, to give an indication of the assay concordance in Figure 3. Borderline results were scored as positive:

		tTG	dGP
IgG	PPV	95%	92%
	NPV	100%	85%
IgA	PPV	100%	94%
	NPV	100%	90%

Figure 3. Celiac SmartPLEX assay concordance vs. commercial 510(k) cleared ELISA's

There was one discrepant sample for tTG IgG that tested positive in the SmartPLEX assay. This sample also tested positive in an alternative commercial assay. The total IgA assay detected one sample that had an undetectable level of total IgA. This sample was confirmed negative for both tTG IgA and dGP IgA.

Conclusion

Based on these data, the Multiplier instrument and celiac multiplexed assay combination offers comparable performance to existing ELISA assays. Total assay time for a full celiac SmartPLEX assay kit is 4 hours 30 minutes, for a maximum of 92 sample results for both IgA and IgG.

Disclaimer: This assay is currently for INVESTIGATIONAL USE and is NOT AVAILABLE FOR COMMERCIAL SALE.