# A whole blood cytokine release assay employing short-term gluten challenge identifies patients with celiac disease on a gluten free diet

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## Background and aim

Current diagnostic criteria for celiac disease (CD) require small bowel histology showing villous atrophy and crypt hyperplasia while gluten is consumed (Husby S, *et al.* JPGN. 2012)

Patients following gluten-free diet without a definite CD diagnosis present a challenge. They need reintroduction of gluten for weeks, but time to disease relapse and reappearance of typical markers is unpredictable (Catassi C, *et al*. Am J Clin Nutr 2007; Lahdeaho ML, *et al.* BMC Gastroenterol 2011)

T cells specific for certain "dominant" gluten peptides circulate in blood 6 days after commencing 3-day oral gluten challenge in patients with CD on strict gluten free diet (Anderson RP, *et al.* Nat Med 2000, Tye-Din JA, *et al*. Sci Transl Med 2010)

The aim was to design and test a simple blood test to detect gluten specific T cells after a brief 3-day wheat challenge in treated CD patients carrying genes encoding HLA-DQ2.5.

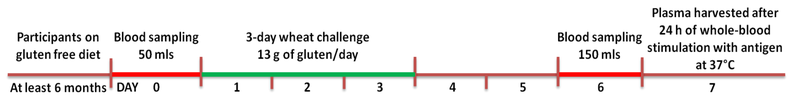
## Subjects and methods

Participants: 27 HLA-DQ2.5 treated CD and 16 non-CD control subjects without immune-suppressant medication. All participants completed 3-day wheat bread challenge. Blood from 3 DQ2.5 untreated CD subjects on a normal diet (no gluten challenge) was also studied. Chymotrypsin-digested wheat gliadin deamidated by transglutaminase, or T-cell stimulatory gluten peptides were incubated with PBMC freshly isolated from heparinized blood, or fresh whole blood. IFN-γ secreting T cells specific for gliadin or peptides were enumerated in PBMC by overnight ELISpot assay (spot forming cells) using fresh PBMC (16h incubation). IFN-γ (secreted by T cells) and IFN-γ inducible protein-10 (secreted by monocytes) in plasma after whole blood was incubated for 24h were quantified by ELISA. Cytokine data were compared by Kruskal-Wallis test and Spearman Rank test. Response to medium was subtracted. Cut-off and performance were calculated by ROC analysis utilizing MedCalc. Symptoms were compared by Fisher's exact test.

Peptides tested were:
1. pELQPFPQPELPYPQPQ (overlapping DQ2.5-gli-α1/α2 epitopes)
2. pEQPFPQPEQPFPWQP (overlapping DQ2.5-gli-ω1/ω2 epitopes)
3. pEEQPIPEQPQPYPQQ (DQ2.5-hor-3 epitope)

Equimolar cocktail 1 (C1): peptides 1+2; cocktail 2 (C2): peptides 1+2+3.

## Results

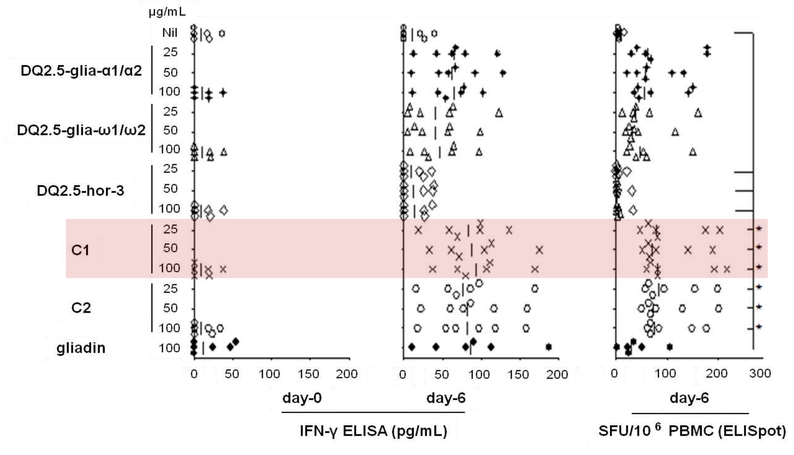



### Symptoms after 3-day wheat challenge: No difference between CD and non-CD

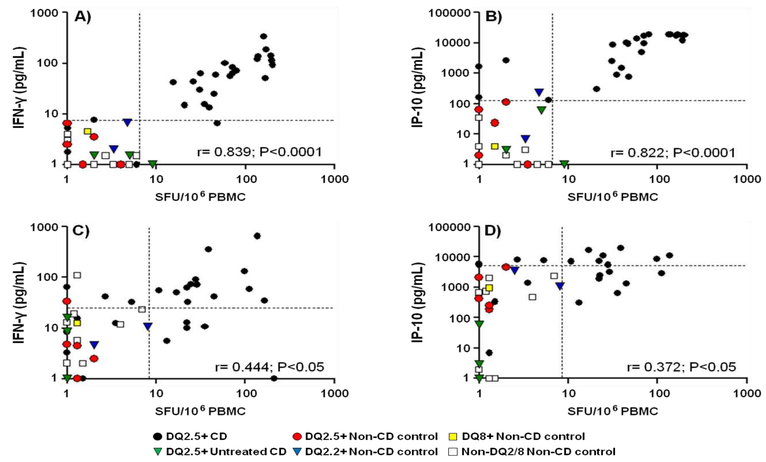
| Symptom | HLA-DQ2.5 Treated CD n=27 (%) | Non-CD controls [1] n=16 (%) | P-value (Fisher's exact test) |
|---|---|---|---|
| Asymptomatic | 6 (22.2) | 2 (12.5) | 0.688 |
| Nausea | 4 (14.8) | 2 (12.5) | 1.0 |
| Constipation | 2 (7.4) | 4 (25.0) | 0.174 |
| Vomiting | 2 (7.4) | 1 (6.3) | 1.0 |
| Lethargy | 6 (22.2) | 7 (43.8) | 0.178 |
| Diarrhea | 7 (25.9) | 5 (31.3) | 0.737 |
| Bloating | 5 (18.5) | 6 (37.5) | 0.278 |
| Abdominal pain | 7 (25.9) | 4 (25.0) | 1.0 |
| Headache | 2 (7.4) | 1 (6.3) | 1.0 |
| Irritability | 0 | 3 (18.8) | 0.045 |
| Flatulence | 2 (7.4) | 1 (6.3) | 1.0 |
| Arthralgia | 0 | 2 (12.5) | 0.133 |
| Insomnia | 0 | 1 (6.3) | 0.372 |
| Mouth ulcers | 0 | 2 (12.5) | 0.133 |
| "Swollen glands" in neck | 0 | 1 (6.3) | 0.372 |

Lethargy, diarrhea, bloating and abdominal pain were the most common symptoms in the two groups. All participants completed the 3-day wheat challenge.

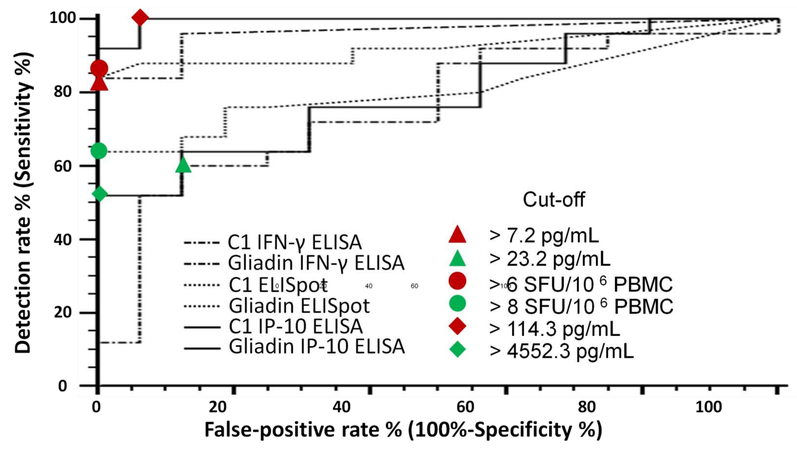
### IFN-γ responses to gliadin and gliadin peptides: Whole blood ELISA and ELISpot yield similar results Day-6 responses after oral wheat challenge evelated to peptides 1 & 2, and gliadin




### Peptides 1+2 (C1) IFN-γ ELISpot and whole-blood IFN-γ and IP-10 ELISAs closely correlated




### Peptides 1+2 (C1) IFN-γ and IP-10 release assays highly specific and sensitive for CD




## Conclusions

**The whole-blood assay shows advantages over current diagnostic modalities for patients on strict gluten free diet:**

- **Highly sensitive and specific for CD**
- **Requires just 3 days gluten exposure**
- **Simple blood test using established platform**

**The assay offers the first potential diagnostic for CD in HLA-DQ2.5 patients already following gluten free diet.**

JT-D and RPA are co-inventors of patents pertaining to the use gluten peptides in therapeutics, diagnostics, and non-toxic gluten; both are shareholders of Nexpep Pty Ltd, and RPA of ImmusanT, Inc. (USA). RPA is Chief Scientist and JT-D is a consultant to ImmusanT, Inc. RPA is a Director of Nexpep Pty Ltd. Full disclosure was provided to all study participants.