DEVELOPMENT OF A NEW AND RAPID SEMIQUANTITATIVE METHOD FOR GLIADIN DETECTION USING R5 ANTIBODY

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INTRODUCTION

The Codex Alimentarius Commission of the FAO (Food and Agricultural Organization of the United Nations) and the WHO (World Health Organization) has stated that products containing more than 0.3% protein from wheat, rye, barley and oats should be excluded from the coeliac diet. A wheat starch with a 0.3% protein level has an actual gluten content of around 200 ppm (mg/kg) and is declared "gluten-free".

In order to guarantee gluten-free food, the gluten content should be controlled in raw material and finished products. A new maximum level for gluten-free foods has been proposed. In this proposal it is specified that gluten detection in foods and ingredients must be based on an immunological method, offering a good sensitivity between 20 and 100 ppm of gluten.

At this moment, there are some products available in the market, using R5 Mab which detects gluten of wheat, rye and barley showing high sensitivity and reproducibility. This kind of products is endorsed temporarily as Typ IV – Method on the twenty-fifth session of the Committee on methods of analysis and sampling, Budapest 8 – 12 March 2004.

One of these products is INGEZIM GLUTEN, a quantitative method with a detection limit (1.5 ppm of gliadin) that is well under the forthcoming requirements of the Codex Alimentarius Commission and which was presented in the 18th Prolamin Working Group on Prolamin Analysis and Toxicity. The R5 antibody used in the detection step shows a supreme specificity to the toxic peptide motif. Ready-to-use, European-certified reference standards, recommended by the *Working Group on Prolamin Analysis and Toxicity* (WGPAT)), are included in the kit.

During the last year INGENASA has continued in the study and improving of gluten detection methods, developing a rapid and reliable immunoenzimatic assay for semi quantitative detection of gluten, INGEZIM SEMIQ which advantages are based principally in short periods of incubation (total time 35 min), easy handling (it is no necessary the use of a standards curve), use of an unique sample dilution, and unnecessary use of controls.

DESCRIPTION OF THE ASSAY

IINGEZIM SEMIQ is an enzyme immunoassay based on the Double Antibody Sandwich ELISA (DAS) technique which uses two key reagents:

- ✓ R5 MAb, both for capture and as conjugate.
- ✓ "SemiQ point" which allows establishing equality between numerical value of sample dilution and ppm.

Briefly, after a extraction step, one or two sample dilutions depending on the objective of the analysis (higher/lower than a value, approximate quantification of ppm, bounding into a range, etc) are added to the wells. "SemiQ point" is added to other wells. After incubation of 15min at Room Temperature (RT) and washing, a conjugate is added and incubated 15 min at RT. After a new washing step, substrate is added to react for 5 min. The gluten content in the sample is determined depending on the dilution used.

- ✓ If the sample OD is higher than the OD semiQ point, the sample will have more gluten ppm than the dilution used.
- ✓ If the sample OD is lower than the OD of semiQ point, the sample will have less gluten ppm than the dilution used.
- ✓ If two dilutions have been used and the OD semiQ point value is between the two OD values obtained, the sample will have a concentration of gluten between the two dilutions used.

TECHNICAL CHARACTERISTICS OF THE ASSAY

The assay has a sensitivity of 10 ppm of gluten, just limited because of the quantity of ethanol in the sample once extracted.

The accuracy of the assay was determined by comparing with INGEZIM GLUTEN. A set of 133 samples were analysed with INGEZIM SEMIQ and the results were compared with the ones obtained with INGEZIM GLUTEN. The study showed that the coincidence between both assays was near 94%. Regarding the 6% of no matching results, it was determined that all of them gave values near the expected ones.

Moreover, the assay is highly repetitive. In order to determine the reproducibility of the assay, a set of samples was analysed. The study consisted of checking these samples in 3 different assays, one of them using more concentrated conjugate (analysis 3). Table III