Immundiagnostik AG • Stubenwald-Allee 8a • D-64625 Bensheim

To Whom It May Concern

Bensheim, 21.3.2018

Dear customers:

The IDK zonulin ELISA kit manufactured by Immundiagnostik is a well-established kit used to detect and monitor a disruption of the intestinal barrier (also known as leaky gut). The ELISA was developed in response to the ground-breaking work of Prof Fasano's group during the late 1990ies until 2001 when he and his co-workers identified zonulin as the human equivalent to the permeability-increasing *vibrio cholerae* enterotoxin, ZOT. The polyclonal anti-body used in our ELISA is based on the zonulin sequence as published by Wang (Journal of Cell Science, 2000) and di Pierro (Journal of Biological Chemistry, 2001).

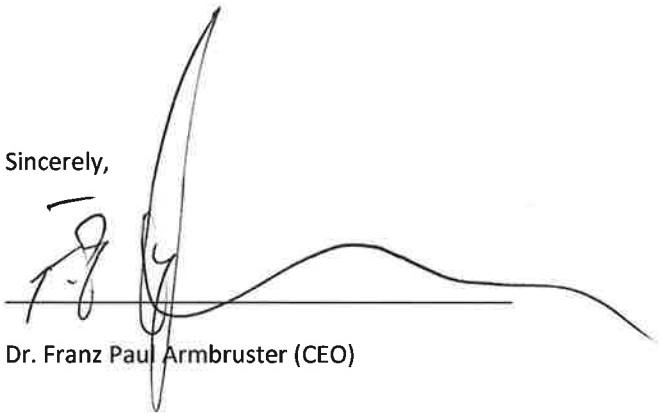
In 2009, Tripathi and co-workers from Fasano's group published findings pointing to zonulin's identity with pre-haptoglobin-2. Along with the advent of more and more haptoglobin genotyping – driven by the remarkable finding that Hp-2 homozygosity represented an additional risk factor for cardiovascular events in diabetic individuals – this report eventually confronted the scientific community with an observational challenge: At least in patients homozygous for Hp-1 (15 – 20 % of Caucasians), there should be no "zonulin" detectable at all. This, however, did not seem to be the case.

In response to this, Immundiagnostik established a cooperation with Prof Fasano's group in Boston, USA, and with the group of Prof Kovacs in Leipzig, Germany, to explain this observation. The publication by Scheffler et al. (Frontiers in Endocrinology, Feb 2018) incorporates relevant parts of this work. According to this, our ELISA does not detect recombinant pre-Hp-2, but properdin, the currently known activator of the alternative complement pathway. According to a personal communication with Prof Fasano, he is convinced and is about to publish corroborating data substantiating properdin as functionally and structurally belonging to a "zonulin family" of permeability-increasing mediators. This view is also supported by clinical and experimental work (see attached files) showing the involvement of the alternative complement pathway in bowel inflammation and other auto-immune disorders. Along the same line though still preliminary, larazotide – the octapeptide which acts as zonulin antagonist and contains the different epitopes that are recognised by our anti-body – also blocks the effects of properdin (Prof A. Fasano, personal communication). Correspondingly, the readings of our ELISA correlate well – as already found in many previous papers – with established metabolic traits linked to increased gut permeability, such as insulin resistance and obesity.

In the Discussion section of the paper by Scheffler et al., "sequence discrepancies" regarding the Wang publication of 2000 (showing a zonulin sequence including the octapeptide GGVLVQPG – the peptide later named larazotide epitopes of which are detected by our polyclonal anti-body) and the Tripathi publication of 2009 are clearly stated and possible reasons given.

We feel we owe you, our valued customers, an explanation as to how Immundiagnostik is going to act from here on with regard to measuring "zonulin family" proteins to determine barrier function. First, we will help explore the role and function of properdin in this clinical setting, and we take these findings as confirmatory for the clinical usefulness of our current ELISA. Second, with these new facts at hand, we will set out to develop new methods to detect pre-Hp-2 and properdin – and possibly even more members of a zonulin family as hypothesized by Prof Fasano - in order to enable you to measure alterations linked to the leaky gut syndrome more comprehensively than ever before.

Sincerely,



Dr. Franz Paul Armbruster (CEO)

Attachments:

- 5 papers showing properdin/properdin factors in the context of IBD or experimental gut inflammation
- 2 properdin reviews