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**Summary**

*van Brummelen EMJ, Boesten LSM, de Vooght KMK. Use of the Precision Xceed Pro Point of Care glucose system in neonates. Ned Tijdschr Klin Chem Labgeneesk. 2013; 38: 190-193.*
It is important to use a point-of-care (POC) glucose system in neonates that is specifically validated for these subjects. Glucose test strip measurements can result in false results due to increased hematocrits in neonates. In this study, we performed Precision Xceed Pro glucose measurements on capillary neonatal blood samples (obtained directly from the heel of the neonate) and compared the results with a well validated routine glucose assay.
Results with the Precision Xceed Pro correlated well with those by the ABL blood gas analyzer and with those by the iSTAT glucose cartridge (R = 1.00 [95% CI 0.96, 1.13] and R = 1.00 [95% CI 0.87; 1 , 16], respectively). When using a capillary to obtain a blood sample, results from the Precision Xceed Pro were significantly higher compared to the iSTAT cartridge glucose test results ($\Delta$ = 0.19 ± 0.37 mmol/L). In conclusion: the Precision Xceed Pro is suitable for use in neonates if a blood sample is directly applied from the heel onto the strip (not using a capillary).

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# Confirmation of high levels of transglutaminase-2 antibodies by deamidated gliadin antibodies in the diagnosis of celiac disease in children: a laboratory perspective

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**According to the ESPGHAN guidelines for celiac disease diagnosis, biopsies in symptomatic children can be omitted when transglutaminase-2 antibody (TGA) levels exceed 10xULN, the child is HLA-DQ2.5/8 positive and TGA is confirmed by endomysium antibodies (EmA).**
**We prospectively explored if deamidated-gliadin-peptide antibodies (DGPA) could replace EmA-confirmation of >10xULN TGA, since this would be preferred in laboratories not performing EmA. 136 sera, with >10xULN TGA were received from Dutch labs that participate in the interlaboratory quality control program within the Netherlands (SKML, section HIM). EmA confirmed strong positive TGA in 100% (n=136) of the sera. DGPA were measured with three different tests (EliA; Thermofisher Scientific, QUANTA Flash; INOVA, GAF-3X ELISA; Euroimmun); not all samples were DGPA-positive (IgG 89.7-97.1%; IgA 86.0-95.6%). DGPA can be used to confirm TGA instead of EmA, but depending on the assay used, 1.5-15% of the 136 children would still have to be biopsied to diagnose celiac disease. DGPA provide a good alternative for EmA in this diagnostic process, for laboratories that do not perform EmA on a routine basis.**

*Keywords: Celiac disease; Children; DGPA; biopsy; diagnostics*

The updated ESPGHAN guidelines for diagnosis of celiac disease (CD) recommend that in children with typical symptoms of celiac disease and a serum concentration of antibodies directed against transglutaminase-2 (TGA) exceeding 10 times the upper limit of normal (ULN), the clinician follows a diagnostic pathway in which a duodenal biopsy can be omitted (1). Confirmation of such high TGA titers by a positive endomysium antibody (EmA) test (in an independent sample) and positivity for HLA-DQ2.5 or DQ8 is now sufficient to establish the diagnosis CD, without the previous need of confirmation by typical findings in duodenal biopsies like villous atrophy and epithelial lymphocytosis.

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*Abbreviations:* TGA, transglutaminase antibodies; EmA, endomysium antibodies; DGPA, deamidated gliadin peptide antibodies; CD, celiac disease; ULN, upper limit of normal; TG2, transglutaminase-2

Determination of EmA is not performed by all laboratories; it is laborious and requires extensive experience in the technique itself but especially in interpretation. For laboratories not performing EmA tests, it would be convenient to replace confirmation of high TGA by EmA with confirmation by another ELISA-based test. As an alternative for TGA confirmation by EmA, deamidated gliadin peptide antibodies (DGPA) may be used. This test is available for various automated systems and as ELISA, like the TGA test. Another advantage could be that the antigens recognized by DGPA differ from those recognized by TGA, whereas EmA recognize essentially the same antigen as TGA, i.e. transglutaminase-2.

In this study, we compared sensitivity of EmA and DGPA in 136 sera with >10xULN TGA and observed that DGPA is a good candidate to replace EmA as confirmatory test. DGPA are, however, less sensitive than EmA. Replacing EmA with DGPA will thus result in a slightly higher number of children that should be biopsied. This study may serve as a lead for laboratories in conjunction with their clinic in deciding which tests to use for diagnosing celiac disease in children with high TGA levels.

## Methods

*Samples*

All laboratories participating in the interlaboratory quality control program within the Netherlands (SKML, section HIM) were invited to participate in this prospective study (n=58); seventeen labs joined the study. From November 2011 untill May 2012, 136 eligible sera from 136 patients, were consecutively collected in a prospective fashion. Sera were included on basis of the TGA values as determined by the collaborating labs, using their cut-off value. All subjects were younger than 18 years. From 97 subjects it was known if duodenal biopsy took place or not: this was the case in 16 cases (17%), all of which were Marsh IIIa-c. Of the TGA results, 79.7 % was obtained with EliA on the immunocap (Phadia; Thermofisher Scientific), the remaining 20.3% was obtained with Orgentec TGA ELISA (7.2%), Phadia VarELISA (8.0%) or an in-house ELISA (5.1%). The following cut-off levels were used: for EliA 7 or 10 U/mL depending on the lab (58% and 42%, respectively), for Orgentec ELISA 10 U/mL, for VarELISA 4 U/mL, for the VUmc in-house ELISA 6 U/mL. The 10xULN used for inclusion, thus were 70 or 100, 40 and 60 U/mL, respectively.

*DGPA, TGA and EmA tests*

Within the Dutch interlaboratory quality control program for celiac disease, organized by SKML/VUmc, three platforms were used for DGPA testing. We decided to use these platforms to test all sera for DGPA IgG and IgA, as well as retest all samples with the TGA tests of these companies. The companies and tests involved were Thermofisher Scientific (MA): EliA/Phadia; INOVA diagnostics (CA): BIO-Flash/QUANTA Flash and Euroimmun (Lübeck, Germany): GAF-3X ELISA. The cut-offs as provided by the companies were used: for DGPA IgG and IgA: Phadia EliA 10 U/mL, INOVA QUANTA Flash 20 U/mL, Euroimmun 25 U/mL; for TGA Phadia EliA 10 U/mL, INOVA QUANTA Flash 20 U/mL, Euroimmun 20 U/mL; equivocal results were considered negative. In addition, all sera were tested with the QUANTA Flash DGPA IgG/IgA combination test, with a cut-off of 20 U/mL. EmA tests were performed directly upon receipt on primate oesophagus, using a two-fold serum dilution. Fluorescence was evaluated qualitatively by at least two independent observers. Sera were stored at -20°C until DGPA testing.

## Results

*EmA confirmation*

136 serum samples with TGA exceeding 10xULN, according to locally used cut offs, were included. All sera were tested in the VUmc in-house EmA IF test and all 136 confirmed as positive. For the purpose of this study, for all 136 patients, these test results were considered sufficient proof of celiac disease.

*DGPA and TGA tests*

After receipt and inclusion of all sera, they were collectively tested for DGPA and TGA with the different tests. For the EliA and QUANTA Flash platforms, DGPA IgG test was most sensitive, yielding 92.6% and 97.1% positivity, whereas IgA DGPA were positive in 84.6% and 94.1% of the sera, respectively (table 1). The Euroimmun DGPA IgG ELISA was positive for 89.7% of the sera whereas a higher level of positivity (95.6%) was reached for IgA. The QUANTA Flash screen (DGPA IgG/A combination) gave in 97.8% of the cases a positive result. Highest sensitivity was reached when positivity of IgG and/or IgA was observed (EliA 94.1%, QUANTA Flash 98.5% and GAF-3X 97.8% positive samples), so when DGPA IgG and IgA were both performed.

Additionally, TGA were determined in all sera by Euroimmun ELISA and INOVA QUANTA Flash. For all samples not yet tested for EliA TGA by the laboratory

**Table 1.** Numbers (#) and percentages of 136 transglutaminase antibody (TGA)$^{high}$ sera with positive deamidated gliadin peptide antibody (DGPA) IgG and/or IgA for the performed tests.

| Assay | # pos | % pos |
|---|---|---|
| *IgG pos* | | |
| EliA | 126 | 92.6 |
| Quanta-flash | 132 | 97.1 |
| GAF-3X | 122 | 89.7 |
| *IgA pos* | | |
| EliA | 115 | 84.6 |
| Quanta-flash | 128 | 94.1 |
| GAF-3X | 130 | 95.6 |
| *IgG and/or A pos* | | |
| EliA | 128 | 94.1 |
| Quanta-flash | 134 | 98.5 |
| GAF-3X | 133 | 97.8 |
| *IgA/G screen* | | |
| Quanta-flash | 133 | 97.8 |

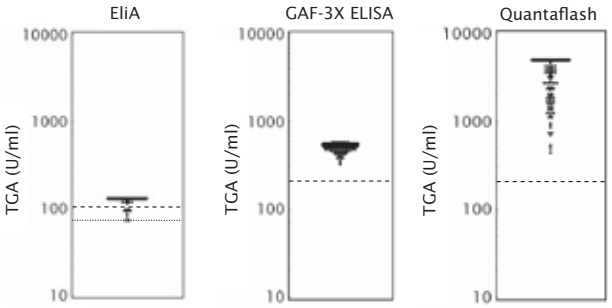


**Figure 1.** Results of different TGA tests. Dashed lines represent 10xULN as provided by the companies. 80% of the EliA results were results obtained by the collaborating labs, 20% were obtained with other TGA tests and re-measured with EliA at VUMC. Some labs used 70 U/mL as 10xULN for EliA TGA test (dotted line). All values exceeding the upper test limit due to dilution by the collaborating lab are shown as the upper test limit value. GAF-3X results were extrapolated by Euroimmun.

**Table 2.** Relation between EliA TGA levels and DGPA IgG outcome. $\chi^2$ test was used to calculate significant differences between the groups.

| EliA TGA | 70-100 U/mL (n=10) | >100 U/mL (n=127) | $\chi^2$ test |
|---|---|---|---|
| *EliA* | | | |
| DGPA IgG neg | 4 (40%) | 7 (6%) | |
| DGPA IgG pos | 6 (60%) | 120 (95%) | P< 0.05 |
| *Quanta-flash* | | | |
| DGPA IgG neg | 4 (40%) | 11 (9%) | |
| DGPA IgG pos | 6 (60%) | 116 (92%) | P< 0.05 |
| *GAF-3X* | | | |
| DGPA IgG neg | 3 (30%) | 2 (2%) | |
| DGPA IgG pos | 7 (70%) | 125 (99%) | P< 0.05 |

of origin, EliA TGA was determined as well (Fig 1). None of the sera yielded a lower than 10xULN level of TGA for INOVA and Euroimmun TGA tests. For EliA, 10 sera yielded levels between 70 and 100 U/mL TGA, thus depending on the ULN used, these samples do or do not fulfill the criteria. Of these 10 samples, 6 (60%) were negative for one or more DGPA tests. These 6 sera represent 27% (6/22) of all sera negative for one or more of the seven DGPA tests. More specifically, sera with an EliA TGA concentration between 70 and 100 U/mL, had a higher percentage of DGPA IgG negativity (as being the most sensitive DGPA test) as compared with EliA TGA levels >100 U/mL (table 2). Sera with the lowest TGA concentrations in the EliA test were also among the sera with the lowest TGA levels in the INOVA and Euroimmun tests (data not shown).

## Discussion

We found that out of 136 children's sera with a TGA concentration higher than 10xULN (ULN as chosen by the collaborating lab) between 84.6 and 98.5% were positive for DGPA, depending on the test platform and whether IgG, IgA or both antibodies were measured. EmA, on the other hand, were positive in all samples, using a 1 in 2 dilution. DGPA can therefore indeed safely be used to confirm TGA in stead of EmA. However, even when testing for *both* IgG and IgA DGPA (either test positive) 8 (for EliA), 2 (for Quantaflash) or 3 (for GAF-3-X) out of 136 children would require a biopsy for diagnosis or EmA confirmation by another laboratory, whereas none needed biopsy to diagnose CD when EmA would be used as confirmatory test (2). We support the conclusion made in the paper of Egner et al (3), that results yielded with different TGA tests may not be comparable and that using 10xULN may not be suitable for all TGA tests. Accordingly, we show here, that the choice of ULN for the TGA test determines if a child will be biopsied or not. For example, a child with EliA TGA levels of 80 U/mL will be biopsied when 7 U/mL is used as ULN, but not when 10 U/mL is used as ULN, although in both cases EmA may confirm the high TGA levels. Interestingly, we show here for EliA TGA that sera with levels between 70 and 100 U/mL were all positive for EmA, but that the percentage of sera negative for one or more DGPA tests was significantly higher in this group as compared to the group with a TGA concentration >100 U/mL. Remarkably, TGA levels obtained with Orgentec and Euroimmun assay all exceeded the 10xULN far more than with the Elia TGA assay. Future studies should reveal if confirmation of >10xULN TGA by DGPA IgG is more specific than confirmation by EmA. Although a previous study showed that TGA is superior to DGPA for diagnosis (4), specificity of the combination of these two tests being positive was not investigated.

We conclude that DGPA can be used as confirmatory test when TGA exceeds 10xULN, in laboratories not performing EmA. It would, however, result in a slightly increased number of biopsies to be performed. The choice of TGA test and the choice of ULN (lower or upper limit of equivocal area) critically determine if biopsies are taken or if diagnosis is made without biopsy. These tests thus need extensive validation in a relevant population of children.

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