Journal No. 7. 2013

Journal ImmunoDiagnostics

Scientific news, opinions and reports

8th EliA Symposium: Autoimmune Gastrointestinal Diseases

Our 8th EliA Symposium in Freiburg took place from May 26th - 27th, 2013.
More than 220 guests came to this symposium where eight excellent speakers held presentations on inflammatory bowel diseases and celiac disease, their clinical presentations, therapy and diagnostic tools. Additionally, we invited scientists from Europe to present their studies and received 15 posters on very diverse topics such as the comparison of different calprotectin stool tests, cost effectiveness of calprotectin or HLA testing in celiac disease. These posters are reprinted in this issue of the Immuno-Diagnostics Journal.

Thermo SCIENTIFIC

Autoimmune Gastrointestinal Diseases discussed in Freiburg at the 8th EliA Symposium

From May 26 to 27 Phadia GmbH, now part of Thermo Fisher Scientific, organized the 8th scientific symposium in Freiburg. More than 200 guests from Europe, Asia and North America came to listen to a full program of lectures which were held by eight internationally renowned speakers, all experts in the field.

The chairman Prof. Ingvar Bjarnason from the King's College Hospital in London led through the morning session which was dedicated to inflammatory bowel diseases and the diagnostic marker fecal calprotectin. In the afternoon, celiac disease was the main discussed topic. This session was led by the chairman Prof. Riccardo Troncone from the University Hospital of Naples. After presentations on the clinical picture of celiac disease and the diagnostic tools, new insights from the big European study PreventCD were given. The chairman himself talked about non-celiac gluten sensitivity.

As EliA Calprotectin is the first fully automated test for fecal calprotectin. It has recently been introduced to the market and therefore most posters at the symposium focussed on the detection of fecal calprotectin, e.g., on the comparison of EliA with other commercial tests, comparison of different stool extraction kits or the level of fecal calprotectin in children.

In this journal, we summarize all 14 posters which were presented at the symposium. Additionally on page 19, we put our internal validation studies on EliA Calprotectin in a comparable format to inform you about our own data.

Enjoy reading,

CONTENTS

ImmunoDiagnostics Journal is the Journal of
Thermo Fisher Scientific

This issue is published by Thermo Fisher Scientific - Phadia GmbH
Munzinger Straße 7, D-79111 Freiburg

Editor
Nina Olschowka

Summary of Posters
Huntingtree Bioscience Supplies, New Zealand

Layout
Bernhard-Layout, bernhard-layout.de

Numbers printed
2,000

Analytical and clinical evaluation of fecal calprotectin as marker of inflammatory bowel disease

Van der Linden IJM[1,2], Joosen A[1], Bozkurt Z[1], Broos H[1], van Heerde M[3], de Groot MJM[1]
[1] Dept. of Clinical Chemistry and Haematology, Amphia Hospital Breda, [2] Department of Clinical Chemistry and Haematology, Franciscus Hospital Roosendaal, [3] Department of Internal Medicine, Amphia Hospital, Breda, The Netherlands.

Objective: To compare a recently launched method (EliA Phadia AB, Uppsala, Sweden) with current methods of measuring fecal calprotectin (Calpro AS) and to evaluate the discriminative power of the various methods to safely exclude inflammatory bowel disease.

Patients and Methods: From an initial pool of 78 patients suspected of inflammatory bowel disease, 39 fecal samples were selected, which had been routinely analysed for calprotectin and showed values across the entire measuring range. Feces extraction was performed by a Fecal Extraction Device (Roche) and three methods
1. CALPRO ELISA CAL0100 (CALPRO AS)
2. CALPRO ELISA CALP0170 (CALPRO AS) and
3. Phadia 250 EliA (Thermo Fisher Scientific)
were compared and evaluated against clinical findings.

Results:

Comparison of	Slope	Intercept	R
CAL0100 vs CALP0170	0.655 (0.492-0.845)	5.2 (2.3-7.6)	0.9217
CAL0100 vs EliA	0.548 (0.174-0.818)	6.8 (2.7-12.4)	0.5834
CALP0170 vs EliA	0.713 (0.349-1.545)	4.3 (-8.2-9.8)	0.6060

Table 1: Comparison of the methods.

Method	Mean calprotectin conc. (µg/g)	CV %
CAL0100 ELISA	27	33.0
	156	7.6
	178*	1.9
CALP0170 ELISA	44	13.6
	204	11.5
	93*	16.0
	542*	23.5
EliA ImmunoCAP	62	8.5
	116	4.2
	223*	4.2

Table 2: Precision.
*** Control sample of the method**

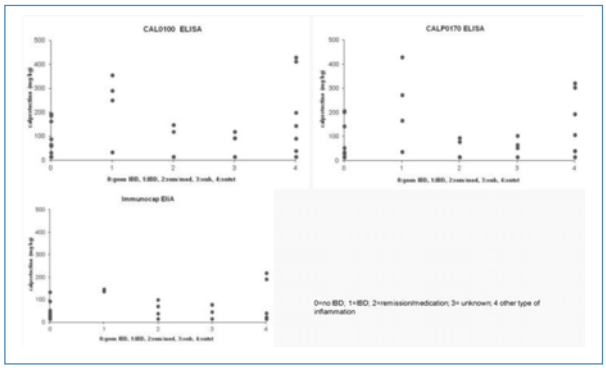

Figure 1: Clinical Evaluation.

Conclusion:

- There is no good correlation between the various methods measuring fecal calprotectin.
- This can be explained by sampling variation and different antibodies used in the methods.
- The EliA method has the best discriminative power between patients with and without inflammatory bowel disease.

Evaluation of a new method for calprotectin analysis in feces with Phadia 250

Zafirova T, Linnarsson B and Thuden I
Kemilaboratoriet, Laboratoriemedicin, Länssjukhuset Ryhov, Jönköping, Sweden

Objective: To compare the clinical performance, precision and practical use of the new EliA Calprotectin assay (Thermo Fisher Scientific) and Calprotectin ELISA (Bühlmann)

Patients and Methods: 198 routine samples sent to the Chemical Laboratory (Jönköping, Sweden) for fecal calprotectin analysis were assessed by both systems according to the manufacturer's directions for use. Precision, variation in weights of samples, differences due to buffers and association to clinical diagnosis were all studied.

F-Calprotectin, mg/kg		
	Calprotectin ELISA kit > 50	Calprotectin ELISA kit < 50
EliA Calprotectin kit >50	93	2
EliA Calprotectin kit <50	27	76

Table 1: Concentration of F-Calprotectin of EliA and ELISA methods.

Results:

- Intra-run CV for EliA was 5.2% (n=10)
- Inter-run CV at two levels for EliA was 5.7% and 6.2% (n=20)
- Correlation between ELISA and EliA showed a positive correlation of F-calprotectin 0.9 (Passing-Bablok)
- The weight of fecal sample tubes had a variation of 5% (n=15)
- Using the EliA method, samples prepared with the ELISA buffer gave significantly different results from those prepared with the EliA-specific buffer.

Conclusion:

- EliA Calprotectin method showed good precision
- The EliA method may be more reliable in excluding irritable bowel syndrome – provided the EliA reagent kit and buffer are used
- Weighing of fecal sample tubes can be excluded
- EliA reduces the time for analytic procedure due to its wider measuring range, simplified sample preparation and greater convenience

F-Calprotectin, mg/kg					
Diagnosis	**No. of patients**	**EliA Calprotectin**		**Calprotectin, ELISA**	
		> 50	< 50	> 50	< 50
Unspecific gastro intestinal disorder	3	1	2	2	1
Crohn's Disease	2	1	1	1	1
Haemorrhoids	2	0	2	2	0
IBS ?	7	0	7	7	0
No information	10	0	10	10	0
ulcerative colitis during remission	5	0	5	5	0

Table 2: Final diagnosis of 29 discrepant results with EliA and ELISA methods.

Fecal calprotectin in healthy children from 0 to 4 years

Povlsen J, Samson M, Hornung N
Department of Clinical Biochemistry, Regional Hospital Randers, Denmark.

Objective: To determine a cut-off level of fecal calprotectin (FC) in children under the age of 4 as this age group typically have higher FC values compared to older children and adults.

Patients and Methods: 34 girls and 33 boys in apparent good health and with no signs of recent infection or stomach problems provided stool samples. Samples were extracted with extraction buffer in the ratio 1:50 (weight/volume) and were measured using the EliA method from Thermo Fisher Scientific.

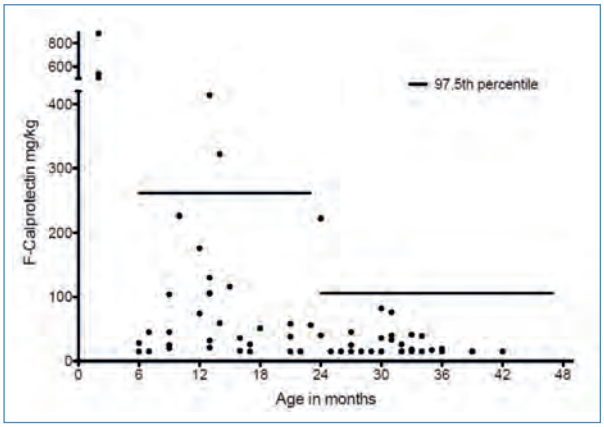

Figure 1: Concentrations of FC in healthy children aged 0-4 years. The 97.5th percentile is shown for the age groups 6-23 months and 24-48 months.

Results:

- FC levels decreased with increasing age
- Children younger than 6 months had the highest values of FC
- From 6 months to 2 years the variation was much less
- After 2 years of age, the FC values approached the values of children older than 4 years

	< 6 months	6 months < 2 years	2 < 4 years
N	3	31	33
Minimum	508	15	15
Median	541	45	15
Maximum	883	414	222
Mean	644	78	31
Std. Deviation	170	92	38
97,5th Percentile	976	258	105
Cut-off	-	250	100

Table 1: Statistical analysis of F-Calprotectin levels (mg/kg) in healthy children aged 0-4 years.

Conclusion: We suggest cut-off values for fecal calprotectin in healthy children based on measurements in feces from 67 healthy children. To our knowledge, these represent the best available levels when using the FC test from Thermo Fisher.

The New Assay Fecal Calprotectin in Random Access: What Changes for the Laboratory?

Alpini C, Valaperta S, Merlilni G
S.C. Medicina di Laboratorio, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy.

Objective: We evaluated the EliA Calprotectin from Phadia, a new random access assay for the measurement of fecal calprotectin and compared it to Phical ELISA (also called Calprest), Eurospital. We also evaluated the performance of the two commercial sample extraction devices (ScheBo Biotech for Eurospital and EliA Stool extraction kit) against the manual weighing method.

Patients and Methods: 27 random stool samples were collected from patients presenting with abdominal discomfort. The samples were evaluated by both methods using the appropriate sampling system and the manual weighing method.

Results: Results showed a good agreement in the classification of patients with only one patient negative with Calprest Europsital and positive with the new assay.

Conclusion: While assaying Calprotectin, laboratories should be aware of the lack of an international standardisation (as demonstrated by the between- assay variability), of the high biological variation and of the criticality of the extraction.
In our experience, the possibility to have a random access assay allowed us to improve the diagnostic accuracy of the test as well as patient clinical management.

	Eurospital Calprest		EliA Calprotectin	
mg/Kg	Kit Device	Manual	Kit Device	Manual
Average	220	300	671	531
SD	231	348	1269	963

Table 1: Average and SD of sample system results.

Group		Eurospital	EliA
		mg/Kg	mg/Kg
1	negative	<50	<50
2	very low positive	50-100	50-200
3	low positive	100-200	200-500
4	positive	200-500	500-3000
5	high positive	>500	>3000

Table 2: Patient classification in groups.

Group	Eurospital Calprest		EliA Calprotectin	
	Patient #	%	Patient #	%
1	8	30	7	26
2	2	7	6	22
3	6	22	5	19
4	8	30	7	26
5	3	11	2	7

Table 3: Distribution of patients in groups.

First results on project “Calprotectin analysed with two methods held against clinical data”

Hoffman-Lücke E, Povlsen J and Hornung N
Department of Clinical Biochemistry, Randers Regional Hospital, Denmark.

Objective: To characterize and to define inflammatory bowel disease progression by analyzing clinical scores, endoscopic investigations, biochemical parameters as well as fecal calprotectin concentrations.

Patients and Methods: 651 feces samples were extracted using the weight/volume method and analysed with EK-CAL, Bühlmann Laboratories and EliA Calprotectin, Thermo Fisher. 150 samples were selected to investigate the confirmed diagnosis and the data from 91 patients is presented in the table below.

Results:

Population Data	n
Number of subjects	91
Gender	57 females, 34 males
Age	20-80 years, median 47
Ulcerative colitis	28
Crohn’s disease	28
Inflammatory, not defined	1
Infectious	7
Irritable colon	3
Unclassified abdominal pain	12
Other diagnosis	10
Abdominal cancer	2

Figure 1: Population data for subjects.

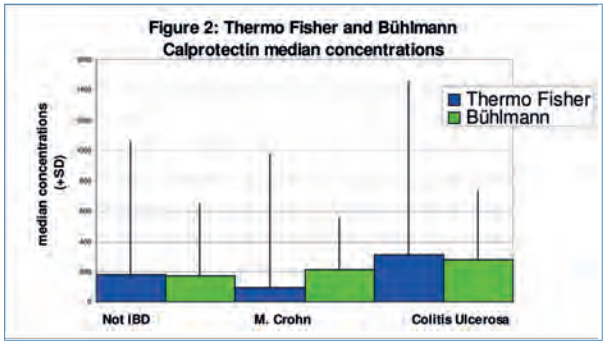

Figure 2: Median concentrations for non-inflammatory bowel disease (IBD) patients are around 169-180 mg/kg (n=34), Crohn’s Disease results show similar median calprotectin concentrations 93-211 mg/kg. Patients diagnosed with ulcerative colitis exhibit higher median values (279-311 mg/kg), but much more obvious, this patient population reveals a higher standard deviation for calprotectin concentration measured with the Thermo Fisher kit.

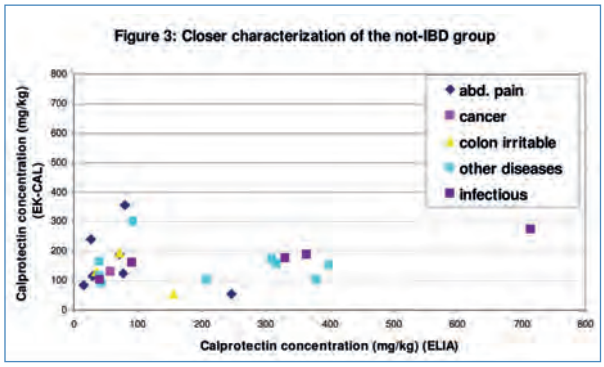

Figure 3: Closer differentiation of the non-IBD group shows that patients with irritable colon and other abdominal diseases have calprotectin concentrations around 250 mg/kg. Very high calprotectin concentrations are related to infectious conditions. The number of analysed individuals for each IBD subgroup is very low and allows only limited conclusions. Seven samples with very high calprotectin concentrations (>1000 mg/kg) are not shown in this figure. These samples belong to infectious and abdominal pain subgroups, containing abdominal abscess, lymphoma and diarrhea.

Conclusion: Calprotectin concentrations, measured with two immunological methods, were related to the patients’ diagnosis. The “non-IBD” subgroup contains diagnoses like irritable colon with concentrations around 35-196 mg/kg; three very heterogenic groups with infectious conditions, abdominal pain and other diseases show results between 26-3000 mg/kg. Median concentrations for all three categories (non-IBD, Crohn’s Disease and Ulcerative Colitis) cluster around 200-300 mg/kg. The project is designed to relate inflammatory disease activity and confirmed diagnosis to calprotectin results measured with the two methods. Concentrations measured with EliA cover a broader value spectrum in all three patient groups compared to results obtained with the EK-CAL method.

Evaluation of EliA Test for the measure of fecal calprotectin levels in the diagnosis of chronic inflammatory bowel diseases

Bonneau J[1]*, Noyel P[1]*, Laurent D[1], Genin C[1], Roblin X[2], Rinaudo-Gaujous M[1], Paul S[1]

[1] Immunology Laboratory, University Hospital of Saint-Etienne, France. (* the authors contribute equally to this work)

[2] Gastroenterology Department, University Hospital of Saint-Etienne, France.

Objective: To evaluate the EliA Calprotectin test (Thermo Fisher Scientific) in comparison with our routine immunochromatographic method (Quantum Blue, Bühlmann) for the determination of fecal calprotectin.

Patients and Methods: Stools were collected in 54 patients (30 women, 24 men, median age 39 years, 0.9–87 years): 22 with Crohn's disease, 13 with ulcerative colitis, 9 with undetermined inflammatory colitis and 10 with chronic organic diarrhea.

Stool extraction: fecal sample preparation kit ScheBo Quick-Prep (Bühlmann) or EliA Stool Extraction kit for solid stool and sample preparation kit Bühlmann Smart-Prep for liquid stool. Fecal calprotectin was determined by the immunochromatographic Quantum Blue assay and the immunoenzymatic EliA Calprotectin test on Phadia 250. Cut-off for > 18 years old: 50 µg/g; for 1-18 years old: 275 µg/g; and for < 1 year old: 350 µg/g.

Results:

		Quantum Blue		
		Positive	Negative	Total
EliA Calprotectin	Positive	31	3	34
	Negative	2	18	20
	Total	33	21	54

Table 1: Agreement of the two fecal calprotectin assays. The correlation is 82.7%.

	Age	Diagnosis	Quantum Blue (µg/g)	EliA Calprotectin (mg/kg)
1	39	undetermined inflammatory colitis	28	206
2	64	Crohn's disease	43	288
3	60	Crohn's disease	16	76
4	21	undetermined inflammatory colitis	50	36
5	23	Crohn's disease	614	33

Table 2: Details of the 5 discordant results.

	Quantum Blue	EliA Calprotectin
Sensitivity	68.2 %	70.5 %
Specificity	70 %	70 %
Positive predictive value	90.9 %	91.2 %
Negative predictive value	33.3 %	35 %

Table 3: Diagnostic performances of the two assays. Real positives were considered as patients with diagnosis of Crohn's disease, or ulcerative Colitis or undetermined inflammatory colitis, and calprotectin ≥ cut-off.

Conclusion: There is a very good agreement between the two tests with a better sensitivity for the EliA test. The positive predictive value of both tests is very good but we found a very poor negative predictive value. For easier handling, a kit for extraction without weighing would be needed. With the EliA test, higher levels of Calprotectin were found (need to establish new cut-off value for mucosal healing).

Clinical validation of two assays for measuring calprotectin

Kok M, Blondel J, van Haaster F, van Pelt J
Medical Center Alkmaar, Laboratory for Clinical Chemistry, Haematology and Immunology, Alkmaar, The Netherlands.

Objective: To compare the clinical validation for two calprotectin assays, Bühlmann ELISA (Alere) and Phadia 250 EliA Calprotectin (Thermo Fisher Scientific).

Patients and Methods: 58 samples were selected to cover a broad concentration range (6-2300 mg/kg) based on results provided by Medlon, Twente, The Netherlands. Samples were extracted according to the method-specific protocol. Using a cut-off for positivity of 50 mg/kg, results were compared to the known diagnosis, colonoscopy and/or pathology.

Results:

Concordance = 89.7%		EliA (ThermoFisher Scientific (Phadia))	
		-	+
Clinical Validation	-	40	3
	+	3	12

Concordance = 79.3%		Bühlmann ELISA (Alere)	
		-	+
Clinical Validation	-	31	12
	+	0	15

Table1: Concordance of the two tests with clinical validation data.

EliA: A concordance of 89.7% was found in comparing the test results with the clinical validation. The false negative results are most likely caused by the presence of fecal residue in the samples. This gives the samples a dark brown colour which is easily distinguishable from the non-contaminated orange extracts.

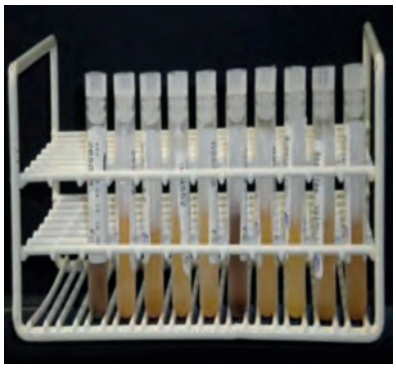

Figure 1: False negative samples are easily recognized by their dark brown colour (samples 1 and 6) after freezing of the extract.

ELISA: A concordance of 79.3% was found. 12 false positive results were found and no false negative result was observed.

Direct comparison of assays: A very weak linear relationship was present when comparing the results of EliA and ELISA (Figure 2)

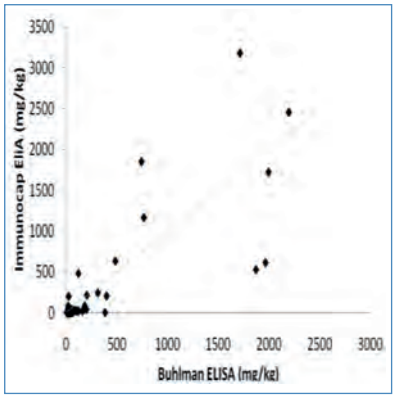

Figure 2: Phadia EliA versus Bühlmann ELISA. Unity (x=y) is represented by the solid grey line.

Conclusion:

- The Thermo Fisher EliA method showed a higher clinical concordance than the Bühlmann ELISA (Alere).
- False negatives can be prevented by visual inspection of the extracts before analysis.
- EliA required less time to perform since the analysis is fully automated on the Phadia 250 platform.
- We have chosen the EliA system for the measurement of calprotectin in our lab.

Comparison of two calprotectin extraction methods: “weight/volume” versus device extraction

Hoffman-Lücke E, Povisen J and Hornung N
Department of Clinical Biochemistry, Randers Regional Hospital, Denmark.

Objective: To simplify the “gold-standard” extraction method (weight/volume) fast insert extraction devices have been introduced. We compared the performance of a device extraction method to the standard method.

Patients and Methods: Three different feces textures: thin (n=10), normal (n=11) and solid (n=9) were analysed with the different extraction methods. A single insertion of the devices resulted in similar amounts attached to the device for thin (mean weight 28 mg (range 13-67 mg)) and normal (mean weight 28 mg (range 12- 89 mg)) feces, but solid (mean weight 16 mg (range 8-33 mg)) consistency seems to be a greater challenge for the device. Two controls were used to determine the cv% of the analysis method. A negative control showed a negative result of all analyses and a positive control (calprotectin level around 231 mg/kg) showed a CV% of 4.9% (n=22).

Results:

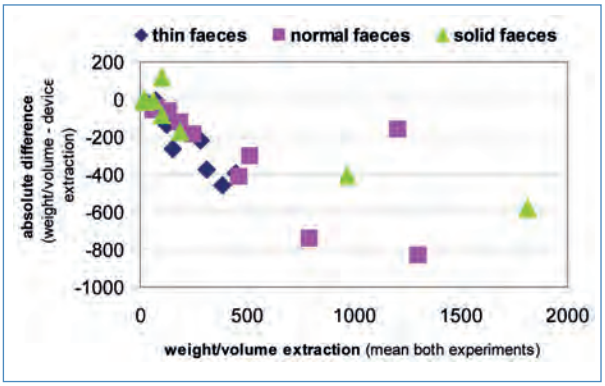

Figure 1: The absolute differences of calprotectin in samples extracted by the two different methods are shown on the y axis and mean levels are shown on the X-axis. Two samples are not shown in the figure, reaching very high differences between both extraction methods, 770 (830) mg/kg for device extraction detected in thin (normal) feces and over 3000 mg/kg for weight/volume extraction.

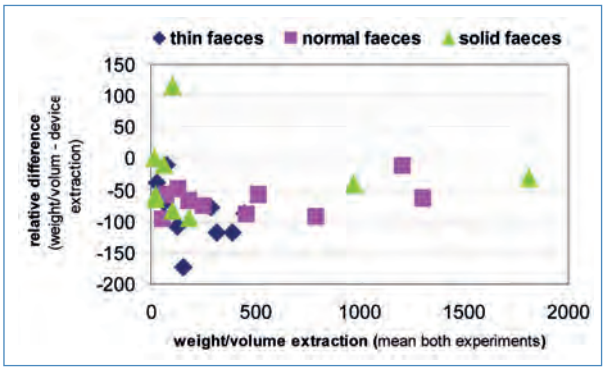

Figure 2: The figure shows that the relative difference is constant over the mean of calprotectin concentrations and the relative mean differences are -66 to -87% for both thin and normal texture and around -42% for solid samples.

Conclusion: Weight/volume extraction ensures a stable and reproducible ratio between sample and extraction buffer independent from feces texture. Device extraction varies strongly in the amount of feces attached to the device which may partly explain the differences in calprotectin results obtained with the two methods.

Comparison of two extraction devices for detection of fecal calprotectin on Phadia 250

Oyaert M[1], Trouvé C[2], Langlois M[2], Vanpoucke H[1]
[1]Department of Laboratory Medicine, H. Hart Hospital Roeaselare-Menen, Belgium
[2]Department of Laboratory Medicine, AZ. Sint Jan Hospital Brugge-Oostende, Belgium.

Objective: To evaluate the performance of two different extraction devices (Smart Prep Device; Roche vs EliA Calprotectin Extraction Device; Thermo Fisher) for detection of fecal calprotectin on the Phadia 250.

Patients and Methods: We analysed 105 clinical samples with a great diversity of stool textures and covering a broad range of calprotectin levels (0->3000 µg/g). Within and between-run precision was evaluated for both devices. Correlation of both devices over the whole measuring range (0-3000 µg/g) as well as in the low range (0-500 µg/g) was performed.

Results:

- Within-run coefficients of variation for the Roche (2.69%) and Thermo Fisher (3.01%) devices were excellent. Acceptable between-run precisions were obtained; 14.25% (Roche) and 10.77% (Thermo Fisher)
- Correlation coefficient of calprotectin levels obtained in high and low range with both devices was 0.93 (intercept -2.17; slope 0.91) and 0.89 (intercept 1.96; slope 0.99) respectively.
- Comparison of measured and calculated calprotectin values for each device showed a better correlation for the SmartPrep Extraction Device than for the EliA Stool Extraction Device; 0.99 (intercept -6.93; slope 1.07) and 0.80 (intercept -3.82; slope 1.99) respectively.
- Correlations between calculated and measured calprotectin levels in the low measuring range were 0.99 (intercept 1.15; slope 0.99) with Roche and 0.37 (intercept 33.26; slope 1.15) with Thermo Fisher devices.
- For fluid stool samples, measured calprotectin values are lower for the Thermo Fisher device in comparison to the Roche device. These values suggest that Thermo Fisher devices underestimate fecal calprotectin levels when compared to the Roche device.

Conclusion: We found discrepant results for the Thermo Fisher extraction device between measured and expected (weight-adjusted) values on Phadia 250. Nevertheless, correlations for the Roche device were excellent. Enormous variations in weight with the Thermo Fisher device resulted in poor correlations between measured and calculated calprotectin levels, more specifically in the low range and attributable to the different stool consistencies. Fluid samples are not captured with the Thermo Fisher device due to the grooves on the dosing tips. We therefore do not recommend the Thermo Fisher device to extract calprotectin for measurement on Phadia 250 for fluid samples. Additionally, weight correction with the Thermo Fisher device is not reliable.

Cost-effectiveness in diagnostic tests: comparison of the IBD pre-endoscopic screening F-calprotectin test versus serologic markers in the United Kingdom

Mascialino B[1], Hermansson L[1], Larsson A[2]

[1] Thermo Fisher Scientific, Uppsala Sweden. [2] Department of Medical Sciences, Uppsala University, Sweden.

Objective: To evaluate the economic impact of F-calprotectin tests compared to the standard pre-endoscopic tests currently used to distinguish IBD from IBS in the United Kingdom. We propose a refinement of an economic evaluation of NHS (Table 1) using new sensitivity and specificity values for F-calprotectin from a meta-analysis including published and new manufacturer's data (6, 8-12) and an updated Markov simulation model (13).

	Sensitivity (%)	Specificity (%)	Source
F-Calprotectin	90	80	[6]
CRP+ESR	35	73	[6]
	Correctly diagnosed IBS	Correctly diagnosed IBD	Total costs (£) / patient
F-Calprotectin	720	90	312.14
CRP+ESR	657	35	325.61
	Additional correctly diagnosed IBS	Additional correctly diagnosed IBS	Incremental costs (£) / patient
	63	55	-13.46
	Cost per correctly diagnosed IBS	Cost per correctly diagnosed IBD	

Table 1: Summary results of F-calprotectin versus CRP + ESR published in the NHS report (see reference 1).

The NHS report conceded that the cost-savings deriving from their model might be an underestimation of reality. Using a threshold for F-calprotectin of 50 µg/g,

- Patients with a result below the threshold are suspected of having IBS, follow a special diet and if not feeling better, repeat the F-calprotectin test and eventually take medications. Non-respondents will be referred to a specialist for further investigations, including endoscopy.
- Patients with a result >250 µg/g are IBD suspected and are referred directly to colonoscopy.
- Patients with a result between 5 µg/g and 250 µg/g are usually re-tested before referral for colonoscopy. The NHS model takes into account this second test but then treats the subjects as if all were negative to the test. Our experience suggests that 58% of these patients are still test-positive after the second test and this needs to be included in the model.

Patients and Methods: An 18-week Markov simulation was implemented for each diagnostic strategy (Figures 1 and 2). Each model represents a hypothetical situation in which 1000 symptomatic patients under 45 years visit a GP and are examined with different approaches. In the model illustrated in Figure 2, different sensitivity and specificity figures are used from a systematic meta-analysis (6, 8-12) and manufacturer's data on EliA Calprotectin (13). The costs included here are the same as in the NHS report (see reference 1).

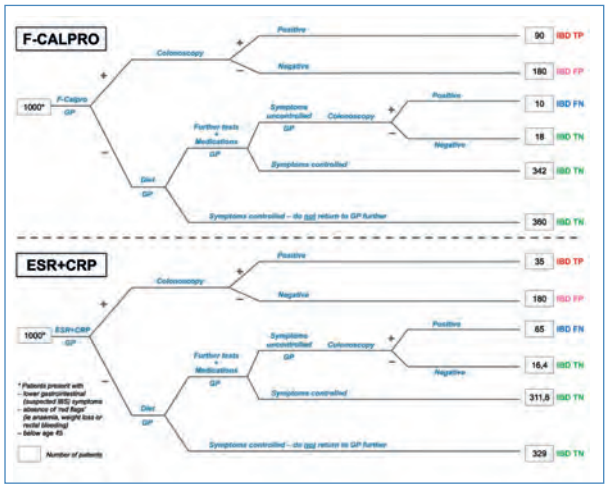

Figure 1: The NHS proposed models for F-calprotectin (top) and ESR+CRP (bottom) (see reference 1).

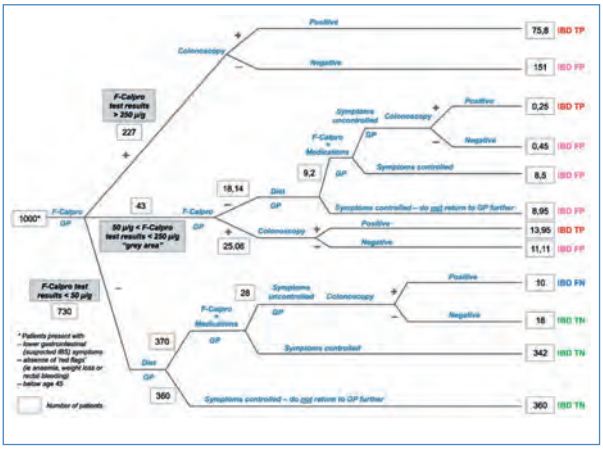

Figure 2: Refined model for F-calprotectin.

Results:

	Sensitivity (%)	Specificity (%)	Source
F-Calprotectin	90	80	[6]
CRP+ESR	35	73	[6]
	Correctly diagnosed IBS	Correctly diagnosed IBD	Total costs in UK (£) / patient
F-Calprotectin	720	90	274.01
CRP+ESR	657	35	325.61
	Additional correctly diagnosed IBS	Additional correctly diagnosed IBD	Incremental costs in UK (£) / patient
	63	55	**-51.6**
	Cost per correctly diagnosed IBS	Cost per correctly diagnosed IBD	

Table 2: Summary results of F-calprotectin versus CRP+ESR using the refined Markov model: F-calprotectin's sensitivity and specificity are the same used in the NHS report.

	Sensitivity (%)	Specificity (%)	Source
F-Calprotectin	94,06	94,61	[6, 8-13]
CRP+ESR	35	73	[6]
	Correctly diagnosed IBS	Correctly diagnosed IBD	Total costs in UK (£) / patient
F-Calprotectin	851,49	94,06	216.13
CRP+ESR	657	35	325.61
	Additional correctly diagnosed IBS	Additional correctly diagnosed IBD	Incremental costs in UK (£) / patient
	194,49	59,06	**-109.47**
	Cost per correctly diagnosed IBS	Cost per correctly diagnosed IBD	

Table 3: Summary results of F-calprotectin versus CRP+ESR using the refined Markov model: F-calprotectin's sensitivity and specificity are calculated with a meta-analysis.

Conclusion:

- The use of F-calprotectin is a cost-effective method to rule out IBD at the primary care level
- F-calprotectin has a higher diagnostic accuracy than CRP + ESR
- It results in more correct IBD/IBS diagnoses at a lower price
- It reduces the number of unnecessary endoscopies via a lower false positive rate
- F-calprotectin's cost-effectiveness is below the usually accepted threshold and thus could be recommended for reimbursement in the United Kingdom.

We are convinced that this cost-effectiveness analysis would concretely help clinical practitioners in making decisions for the best health care of their IBD/IBS patients.

References:

1. NHS, CEP09041, February 2010.
2. Molodecky N.A., et al, Gastroenterology (2012), 142: 46-54.
3. Bellini M., et al, World Journal of Gastroenterology (2011), 17 (46): 5089-5096.
4. NICE clinical guideline 61, London (2008).
5. von Roon A.C., et al, Am J Gastroenterol (2007), 102: 803-813.
6. Tibble J.A., et al, Gastroenterology (2002), 123: 450-460.
7. Prof. Larsson A., Uppsala University (2012), unpublished data.
8. Limburg P.J., et al, Am J Gastroenterol (2000), 95: 2831-2837.
9. Schroder O., et al, Aliment Pharmacol Ther (2007), 26: 1035-1042.
10. Schoepfer A.M., et al, Dis Colon Rectum (2007), 50: 1697-1706.
11. Otten C.M., et al, Clin Chem Lab Med (2008), 46: 1275-1280.
12. Schoepfer A.M., et al, Inflamm Bowel Dis (2008), 14: 32-39.
13. Zafirova T., et al, "Utvärdering av en ny metod för analys av kalprotektin i feces på Phadia 250", Kemilaboratoriet, Jönköping. Poster presented at: Spring Meeting 2012 for Swedish Society of Clinical Chemistry
14. van Rheenen P., et al, BMJ (2010), Jul 15: c3369-c3380.

Cost-effectiveness in diagnostic tests: comparison of the IBD pre-endoscopic screening F-calprotectin test versus serologic markers in selected European markets

Mascialino B[1], Hermansson L[1], Larsson A[2]
[1] Thermo Fisher Scientific, Uppsala Sweden. [2] Department of Medical Sciences, Uppsala University, Sweden.

Objective: To evaluate the economic impact of F-calprotectin tests compared to the standard pre-endoscopic tests currently used to distinguish IBD from IBS in selected European markets (Sweden, France and Italy). We propose a refinement of an economic evaluation of NHS (Figure 1) using new sensitivity and specificity values for F-calprotectin from a meta-analysis including published and new manufacturer's data (6, 8-12) and an updated Markov simulation model (13).

	Sensitivity (%)	Specificity (%)	Source
F-Calprotectin	90	80	[6]
CRP+ESR	35	73	[6]
	Correctly diagnosed IBS	Correctly diagnosed IBD	Total costs (£) / patient
F-Calprotectin	720	90	312.14
CRP+ESR	657	35	325.61
	Additional correctly diagnosed IBS	Additional correctly diagnosed IBS	Incremental costs (£) / patient
	63	55	-13.46
	Cost per correctly diagnosed IBS	Cost per correctly diagnosed IBD	

Table 1: Summary results of F-calprotectin versus CRP + ESR published in NHS report (see reference 1).

The NHS report conceded that the cost-savings deriving from their model might be an underestimation of reality. Using a threshold for F-calprotectin of 50 µg/g,

- Patients with a result below the threshold are suspected of having IBS, follow a special diet and if not feeling better, repeat the F-calprotectin test and eventually take medications. Non-respondents will be referred to a specialist for further investigations, including endoscopy.
- Patients with a result >250 µg/g are IBD suspected and are referred directly to colonoscopy
- Patients with a result between 5 µg/g and 250 µg/g are usually re-tested before referral for colonoscopy. The NHS model takes into account this second test but then treats the subjects as if all were negative to the test. Our experience suggests that 58% of these patients are still test-positive after the second test and this needs to be included in the model.

Patients and Methods: An 18-week Markov simulation was implemented for each diagnostic strategy (Figures 1 and 2). Each model represents a hypothetical situation in which 1000 symptomatic patients under 45 years visit a GP and are examined with different approaches. In the model illustrated in Figure 2, different sensitivity and specificity figures are used from a systematic meta-analysis (6, 8-12) and manufacturer's data on EliA Calprotectin (13). The costs included here are the same as in reference 1.

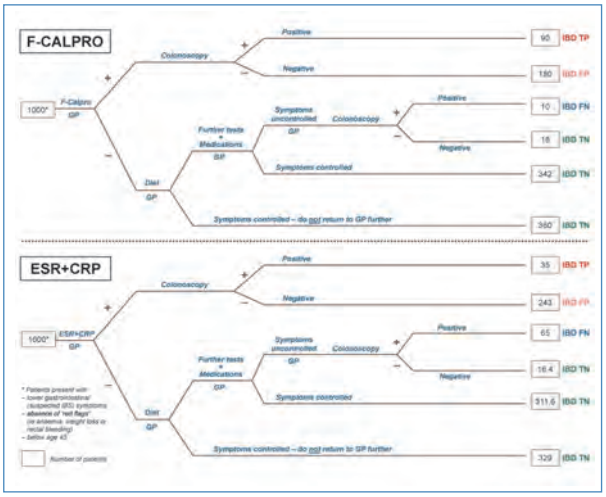

Figure 1: The NHS proposed models for F-calprotectin (top) and ESR + CRP (bottom) (see reference 1).

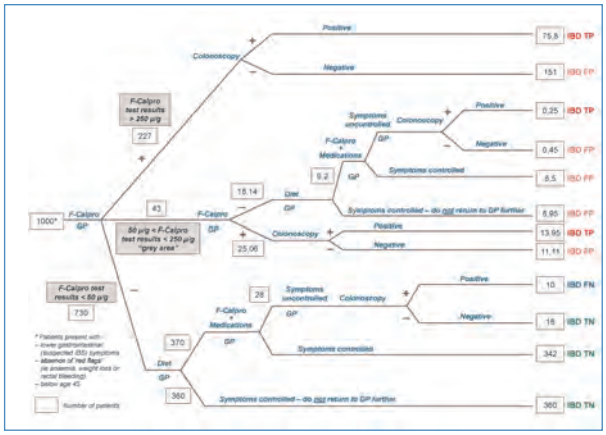

Figure 2: Refined model for F-calprotectin.

Conclusion:

- The use of F-calprotectin is a cost-effective method to rule out IBD at the primary care level in all the European countries considered.
- F-calprotectin has a higher diagnostic accuracy than CRP+ESR
- It results in more correct IBD/IBS diagnoses at a lower price
- It reduces the number of unnecessary endoscopies via a lower false positive rate
- F-calprotectin's cost-effectiveness is below the usually accepted threshold and thus could be recommended for reimbursement in all the European countries considered.

We are convinced that this cost-effectiveness analysis would concretely help clinical practitioners in making decisions for the best health care of their IBD/IBS patients.

Results:

a)	Sensitivity (%)	Specificity (%)	Source			
F-Calprotectin	90	80	[6]	NHS REPORT		
CRP+ESR	35	73	[6]			
	Correctly diagnosed IBS	Correctly diagnosed IBD	Total costs in UK (£) / patient	Total costs in SW (SEK) / patient	Total costs in FR (EUR) / patient	Total costs in IT (EUR) / patient
F-Calprotectin	720	90	274.01	4275.9	178.2	117.1
CRP+ESR	657	35	325.61	5861.4	180.3	127.3
	Additional correctly diagnosed IBS	Additional correctly diagnosed IBD	Incremental costs in UK (£) / patient	Incremental costs in SW (SEK) / patient	Incremental costs in FR (EUR) / patient	Incremental costs in IT (EUR) / patient
	63	55	**-51.6**	**-1585,6**	**-2,1**	**-10,17**

b)	Sensitivity (%)	Specificity (%)	Source			
F-Calprotectin	94,06	94,61	[6, 8-13]	META-ANALYSIS		
CRP+ESR	35	73	[6]			
	Correctly diagnosed IBS	Correctly diagnosed IBD	Total costs in UK (£) / patient	Total costs in SW (SEK) / patient	Total costs in FR (EUR) / patient	Total costs in IT (EUR) / patient
F-Calprotectin	851,49	94,06	216,13	3848,2	160,9	114,0
CRP+ESR	657	35	325.61	5861.4	180.3	127.3
	Additional correctly diagnosed IBS	Additional correctly diagnosed IBD	Incremental costs in UK (£) / patient	Incremental costs in SW (SEK) / patient	Incremental costs in FR (EUR) / patient	Incremental costs in IT (EUR) / patient
	194,49	59,06	**-109,47**	**-2013,2**	**-19,44**	**-13,2**

Table 2: Summary results of F-calprotectin versus CRP+ESR in the European countries Sweden, France and Italy using the refined Markov model: F-calprotectin's sensitivity and specificity are (a) the same used in the NHS report and (b) calculated with a meta-analysis.

References:

1. NHS, CEP09041, February 2010.
2. Molodecky N.A., et al, Gastroenterology (2012), 142: 46-54.
3. Bellini M., et al, World Journal of Gastroenterology (2011), 17 (46): 5089-5096.
4. NICE clinical guideline 61, London (2008).
5. von Roon A.C., et al, Am J Gastroenterol (2007), 102: 803-813.
6. Tibble J.A., et al, Gastroenterology (2002), 123: 450-460.
7. Prof. Larsson A., Uppsala University (2012), unpublished data.
8. Limburg P.J., et al, Am J Gastroenterol (2000), 95: 2831-2837.
9. Schroder O., et al, Aliment Pharmacol Ther (2007), 26: 1035-1042.
10. Schoepfer A.M., et al, Dis Colon Rectum (2007), 50: 1697-1706.
11. Otten C.M., et al, Clin Chem Lab Med (2008), 46: 1275-1280.
12. Schoepfer A.M., et al, Inflamm Bowel Dis (2008), 14: 32-39.
13. Zafirova T., et al, "Utvärdering av en ny metod för analys av kalprotektin i feces på Phadia 250", Kemilaboratoriet, Jönköping. Poster presented at: Spring Meeting 2012 for Swedish Society of Clinical Chemistry
14. van Rheenen P., et al, BMJ (2010), Jul 15: c3369-c3380.

Analysis for diagnosis of celiac disease

Pedersen M
Department of Clinical Biochemistry, Nordsjælland Hospital, Denmark.

Objective: The genes that predispose to celiac disease (CD) are known as the HLA-DQ genes, and are found on the HLA-class II complex of DNA. More than 95% of CD patients share HLA-DQ2 genotype and most of the remainder have HLA-DQ8 genotype. We wished to evaluate whether the high negative predictive value of non-HLA-DQ2/DQ8 genotype could contribute to an efficient system for screening for celiac disease in the general population.

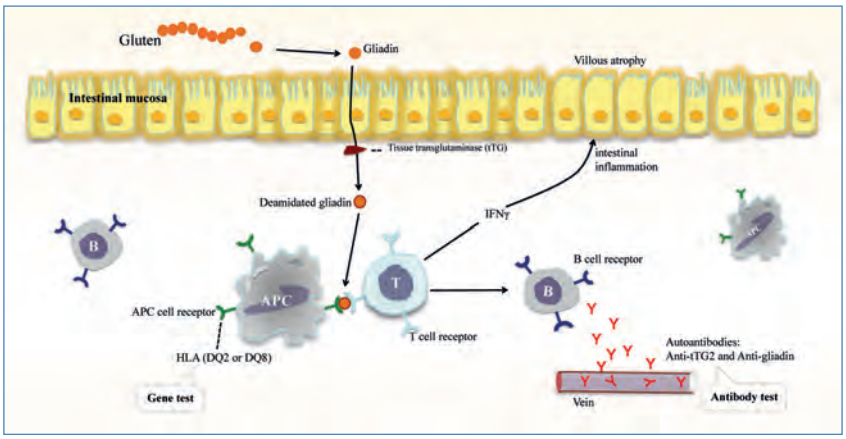

Table 1: Determination of HLA genotypes predicts, on the basis of high negative predictive value (99.9%) the probability of negative antibody tests.

Patients and Methods: 2444 Danish patients with suspected gluten intolerance were tested for HLA genotype, IgA antibodies to tissue transglutaminase (TG2) and IgG and IgA antibodies to deamidated gliadin peptides.

Conclusion:

- Determination of HLA genotypes predicts the probability of a negative antibody test.
- Positive antibody test indicates celiac disease, therefore HLA test for HLA-DQ2 and HLA-DQ8 is recommended as the first test.
- HLA positive patients should be analysed for the concentration of specific celiac serum antibodies to Deamidated Gliadin Peptides (DGP) and immunoglobulin A anti-tissue TransGlutaminase type 2 antibodies (TG2).
- High levels of these antibodies indicate celiac disease, and patients should be started on a gluten-free diet (GFD). If GFD results in clinical improvement and normalization of antibody levels, the diagnosis of celiac disease is confirmed.
- HLA negative patients ought to be investigated for other diseases.

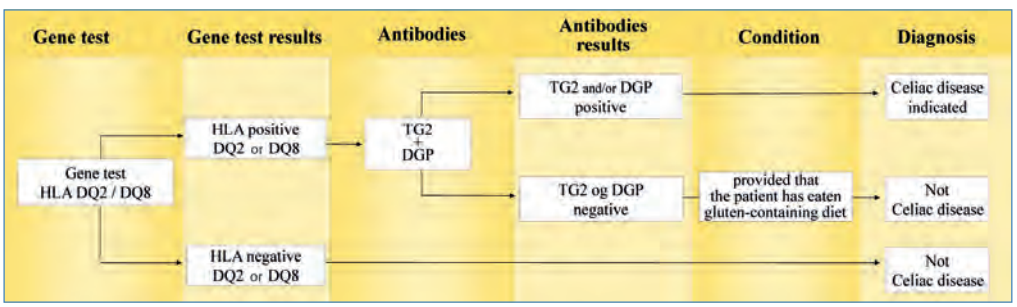

Table 2: Suggested testing schedule for celiac disease screening.

Population screening for celiac disease in Danish adults

Horwitz A[1], Skaaby T[1], Jørgensen T[1], Rumessen J[2], Linneberg A[1]
[1] Research Centre for Prevention and Health, Glostrup Hospital, Denmark, [2] Department of Internal Medicine Gentofte Hospital, Denmark.

Objective: We aimed to determine the prevalence of celiac disease by serologic screening followed by clinical evaluation of seropositive individuals.

Patients and Methods: 2,297 men and women aged 24-74 years living in Copenhagen, Denmark were screened for IgA antibodies to transglutaminase (tTG) using the EliA Celikey assay and for IgG and IgA antibodies to deamidated gliadin using the EliA deamidated gliadin anti-IgG and IgA assays respectively. Patients with positive serology were referred for gastroenterologic evaluation and were tested for HLA-DQ2/DQ8 genotype.

Results: A total of 2.4% were serological test positive (figure 2). Of these, 16 were HLA-DQ2/DQ8 negative. Seropositivity to gliadin (>10) was more common than seropositivity to tTG (>7), the latter also being more strongly associated with HLA-DQ2/DQ8 positivity.

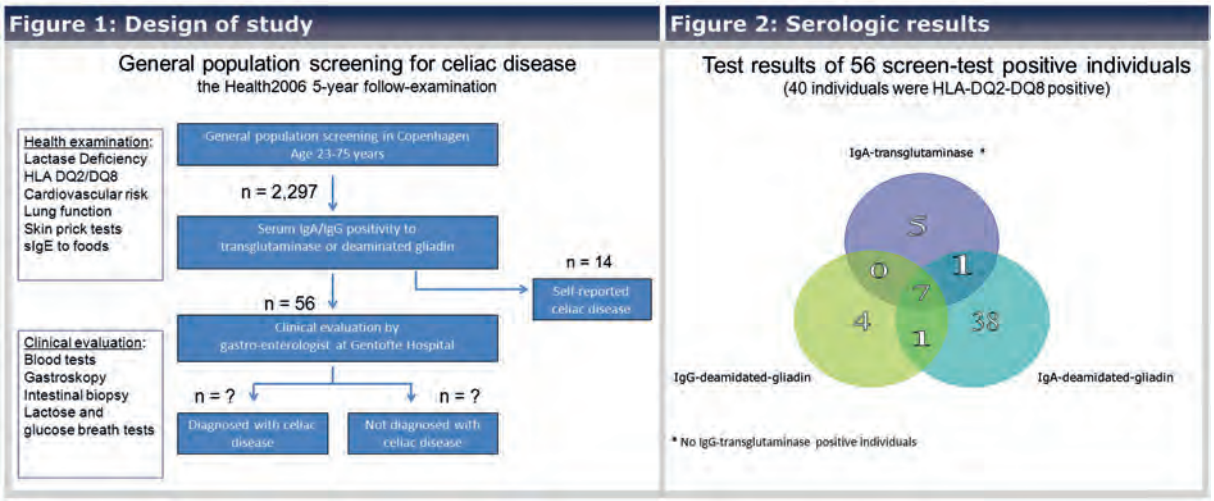

Figure 1: Design of study.

Figure 2: Serologic results.

Conclusion:

- In this adult general population, seropositivity to TTGs (n=13) or gliadin (n=51) was seen in 2.4%.
- Clinical evaluations of seropositive individuals are on-going and results will provide data on efficacy and costs of possible screening programs in the whole population or high-risk groups.

Analysis for the diagnosis of lactose intolerance

Pedersen M
Department of Clinical Biochemistry, Nordsjællands Hospital, Denmark.

Objective: To suggest an algorithm to determine whether a patient's apparent lactose intolerance symptoms are caused by primary lactose intolerance or secondary lactose intolerance.

Gene Test: In European populations, primary lactose tolerance due to lactase persistence beyond infancy correlates with the single allele carrying the CT(-1390) variant genotype on the 2q21-22 chromosome. The genetic test for this mutation is simply carried out on leukocytes and will show the genotype as C/C, C/T or T/T. Only C/C is associated with lactose intolerance. However, lactose intolerance can also be the result of non-genetic causes (e.g., coeliac disease, inflammatory gastrointestinal disease, acute gastroenteritis or cytostatic treatment) or mutations in other alleles, particularly a variant at GT(-13915) which is common in non-European populations.

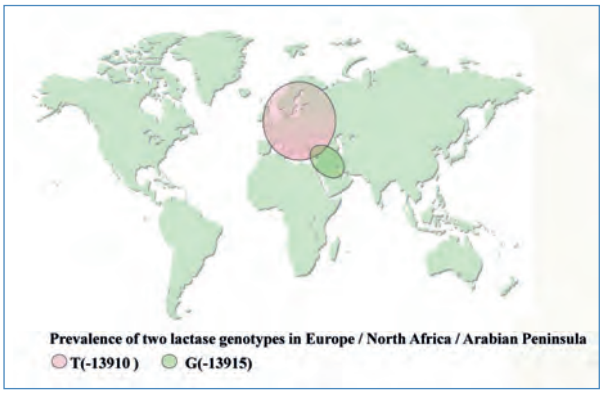

Figure 1: Prevalence of two lactase genotypes in Europe / North Africa / Arabian Peninsula.

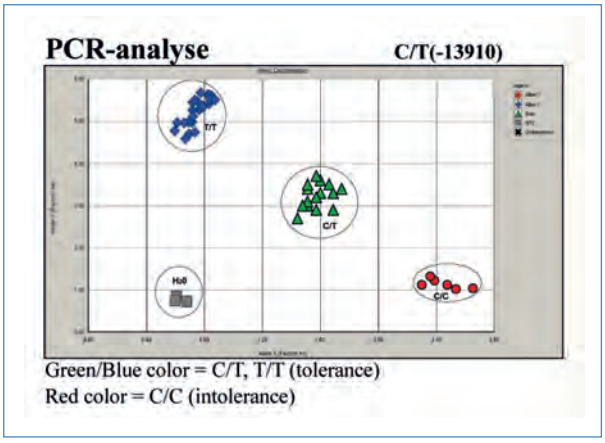

Figure 2: The genetic test for the mutation CT(-13910), is analysed on DNA purified from leucocytes. The results will show determination of the genotypes C/C, C/T or T/T. Only the genotype C/C is associated with lactose intolerance. The gene test will not show lactose intolerance.

Oral lactose tolerance test. The oral tolerance test should be applied for the diagnosis of secondary lactose intolerance and primary lactose intolerance with other genetic origins. The fasting patient drinks 0.4 L water containing 0.05 kg lactose within 5 min. Blood samples are taken after 15, 30, 45 and 60 min to detect changes in the glucose concentration.

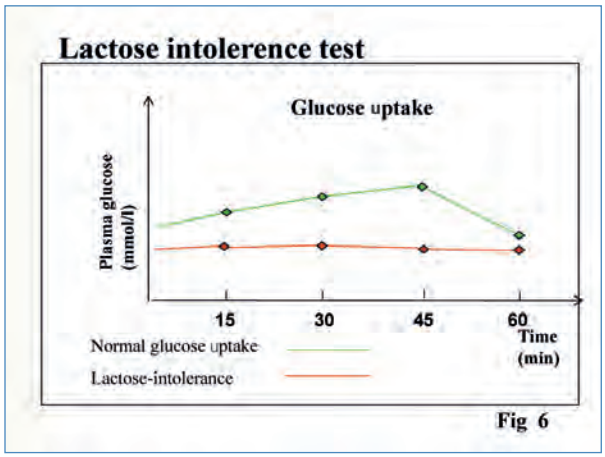

Figure 3: Lactose intolerance test. A flat glucose curve with maximum increment of less than 1.4 mmol/L combined with abdominal pain and/or diarrhea – strongly indicate lactose intolerance.

Conclusion:

The genetic test shows whether there is a genetic predisposition to lactose in patients, and is recommended as the first test in patients with symptoms of lactose intolerance.
The ethnic origin of the patient can be important in the diagnostic process because some populations with C/C (e.g., Africa and Saudi Arabia GT(-13915) have lactase persistence

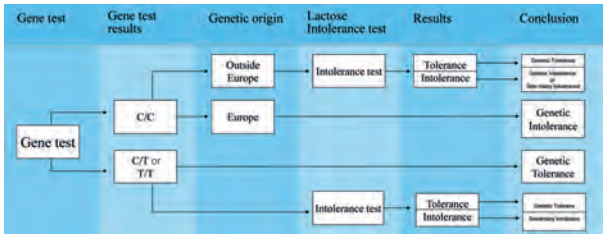

Figure 4: Suggested algorithm for determining the cause of lactose intolerance.

due to other genetic mutations. Secondary lactose intolerance caused by gastrointestinal disease with epithelial damage such as e.g., coeliac disease and chronic inflammatory diseases should be investigated by an oral lactose tolerance test.

EliA Calprotectin: Validation of the first fully automated fecal calprotectin test for the diagnosis of inflammatory bowel diseases

Mummert M, Löbke C, Stumpf T
Phadia GmbH, Thermo Fisher Scientific, Freiburg, Germany.

Objective: We evaluated the clinical performance of the new fully automated EliA Calprotectin assay (Phadia AB, part of Thermo Fisher Scientific) regarding the differentiation of inflammatory bowel diseases (IBD) such as Crohn's disease and ulcerative colitis from irritable bowel syndrome (IBS).

Patients and Methods: Stool samples from 132 patients with IBD and 59 patients with IBS and other functional bowel diseases (BD) have been evaluated for fecal calprotectin using EliA Calprotectin on the Phadia 250 instrument and two assays from other suppliers. All assays used a cut-off of 50 mg/kg.

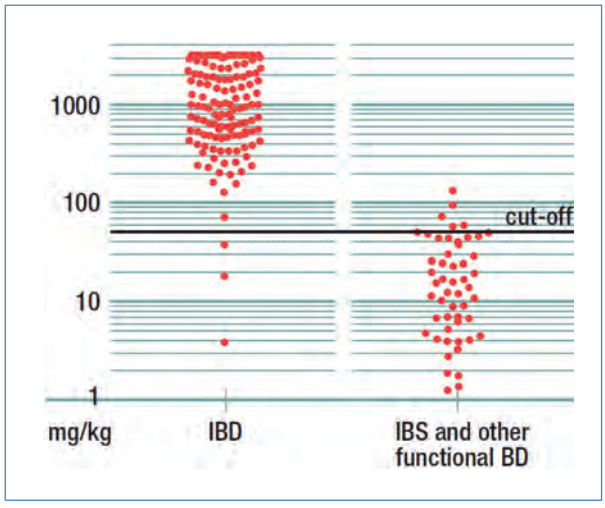

Figure 1: Performance of EliA Calprotectin in 191 clinically defined patients; internal study.

	EliA	Supplier 1	Supplier 2
Sensitivity	97.7 %	96.7 %	99.2 %
Specificity	89.8 %	89.8 %	76.3 %
PPV	0.96	0.96	0.90
NPV	0.95	0.93	0.98
LR+	9.58	9.48	4.19
LR-	0.03	0.04	0.01

Table 1: Performance data of EliA Calprotectin and tests from two other suppliers.

Results: EliA Calprotectin showed a very good agreement with patient diagnosis (figure 1). The test shows an excellent clinical performance which is comparable to supplier 1 and better than supplier 2 (table 1).

Conclusion: The study showed that EliA Calprotectin is able to differentiate clearly between inflammatory bowel diseases (IBD), irritable bowel syndrome (IBS) and other functional bowel disorders (BD).
The outstanding performance of EliA Calprotectin is underlined by the high sensitivity and the high specificity of the test. Most important, the predictive values and the likelihood ratios give excellent values assuring high clinical usefulness of the test in routine practice.

ImmunoDiagnostics Journal No. 7.2013

- The EliA method has the best discriminative power between patients with and without inflammatory bowel diseases.
- The EliA Calprotectin method showed good precision.
- Children younger than six months had the highest values of fecal calprotectin.
- The EliA method showed a higher clinical concordance than the competitor ELISA.
- Device extraction varies strongly which may partly explain the differences in calprotectin results obtained with the two devices.
- The measurement of fecal calprotectin reduces the number of unnecessary endoscopies via a lower false positive rate (compared to CRP and ESR).

thermoscientific.com/phadia

Legal Manufacturer: Phadia AB, Uppsala, Sweden

Thermo Fisher Scientific – Phadia GmbH, Munzinger Str. 7, D-79111 Freiburg, Germany, Tel: +49 761 47-805-0, autoimmunity@phadia.com

Head office Sweden +46 18 16 50 00
Austria +43 1 270 20 20
Belgium +32 2 749 55 15
Brazil +55 11 33 45 50 50
China +86 25 89 60 57 00
Czech Republic +420 220 51 87 43
Denmark +45 70 23 33 06
Finland +358 9 3291 0110
France +33 161 37 34 30
Germany +49 761 47 805 0
India +91 11 4610 7555/56
Italy +39 026 416 34 11
Japan +81 3 53 65 83 32
Korea +82 2 20 27 54 00
Norway +47 216 732 80
Portugal +351 214 23 53 50
South Africa +27 11 793 5337
Spain +34 93 57 658 00
Sweden +46 18 16 60 60
Switzerland +41 433 43 40 50
Taiwan +886 225 16 09 25
The Netherlands +31 306 02 37 00
United Kingdom / Ireland +44 19 08 76 91 10
USA +1 800 346 4364
Other countries +46 18 16 50 00

Thermo SCIENTIFIC
Part of Thermo Fisher Scientific

Order No. 52-5506-06 Freiburg 07/2013