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Agreement Between Home-Based Measurement of Stool Calprotectin and ELISA Results for Monitoring Inflammatory Bowel Disease Activity

Anke Heida,[*] Mariska Knol,[*] Anneke Muller Kobold,[‡] Josette Bootsman,[*] Gerard Dijkstra,[§] and Patrick F. van Rheenen[*]

[*]Department of Paediatric Gastroenterology, Hepatology and Nutrition, University of Groningen, University Medical Centre Groningen, Groningen, the Netherlands; [‡]Department of Laboratory Medicine, University of Groningen, University Medical Center Groningen, Groningen, the Netherlands; [§]Department of Gastroenterology, Hepatology and Nutrition, University of Groningen, University Medical Center Groningen, Groningen, the Netherlands

BACKGROUND & AIMS: **An increasing number of physicians use repeated measurements of stool calprotectin to monitor intestinal inflammation in patients with inflammatory bowel diseases (IBDs). A lateral flow-based rapid test allows patients to measure their own stool calprotectin values at home. The test comes with a software application (IB*Doc*; Bühlmann Laboratories AG, Schönenbuch, Switzerland) that turns a smartphone camera into a results reader. We compared results from this method with those from the hospital-based reader (Quantum Blue; Bühlmann Laboratories AG) and enzyme-linked immunosorbent assay (ELISA) analysis.**

METHODS: **In a single-center comparison study, we asked 101 participants (10 years of age or older) in the Netherlands to perform the IB*Doc* measurement on stool samples collected at home, from June 2015 to October 2016. Participants then sent the residual extraction fluid and a fresh specimen from the same bowel movement to our pediatric and adult IBD center at the University Medical Center Groningen, where the level of calprotectin was measured by the Quantum Blue reader and ELISA analysis, respectively. The primary outcome was the agreement of results between IB*Doc* and the Quantum Blue and ELISA analyses, determined by Bland-Altman plot analysis.**

RESULTS: **We received 152 IB*Doc* results, 138 samples of residual extraction fluid for Quantum Blue analysis, and 170 fresh stool samples for ELISA analysis. Spearman's rank correlation coefficient was 0.94 for results obtained by IBDoc vs Quantum Blue and 0.85 for results obtained by IBDoc vs ELISA. At the low range of calprotectin level (<500 μg/g), 91% of IB*Doc*–Quantum Blue results were within the predefined limits of agreement (±100 μg/g), and 71% of IB*Doc*–ELISA results were in agreement. At the high range of calprotectin level (≥500 μg/g), 81% of IB*Doc*–Quantum Blue results were within the predefined limits of agreement (±200 μg/g) and 64% of IB*Doc*–ELISA results were in agreement.**

CONCLUSIONS: **Measurements of fecal levels of calprotectin made with home-based lateral flow method were in agreement with measurements made by Quantum Blue and ELISA, as long as concentrations were <500 μg/g. For patients with concentrations of fecal calprotectin above this level, findings from IB*Doc* should be confirmed by another method. (Netherlands Trial Registration Number: NTR5133).**

Keywords: Biomarker; Telemedicine; Point-of-care test; Monitoring IBD Activity.

Crohn's disease and ulcerative colitis are progressive inflammatory bowel diseases (IBDs) that may result in irreversible bowel damage. The ultimate goal of treating IBD patients is to achieve symptom control and stop disease progression to change the natural course of the disease. The desired treatment target is mucosal healing and fecal calprotectin (FC) levels correlate well with this target.[1–4] Asymptomatic patients whose FC

Abbreviations used in this paper: **CI, confidence interval; ELISA, enzyme-linked immunosorbent assay; FC, fecal calprotectin; IBD, inflammatory bowel disease.**

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levels drift away from the target range have an increased risk to develop a disease flare in the next 2–3 months, while repeated FC levels in the normal range suggest sustained remission.[5]

For 8 years we have been following children with IBDs by periodically measuring calprotectin levels in their sent-in stool samples with an enzyme-linked immunosorbent assay (ELISA). Although there is little agreement among IBD experts about the optimal cutoff points for calprotectin, in children we considered levels below 250 μg/g as indicative for disease remission (green),[6] levels above 500 μg/g as indicative for disease flare (red), and levels between 250 and 500 μg/g provided little guidance and required short-term retesting (orange). Both physicians and patients found repeated testing of calprotectin and the traffic light color coding helpful to guide therapy, but ELISA testing is time consuming and requires a high level of expertise to perform.[7] Point-of-care calprotectin tests, including the Quantum Blue lateral flow immunoassay (Bühlmann Laboratories AG, Schönenbuch, Switzerland), are less time consuming, but patients are still forced to send or bring a stool sample to the hospital.[8] Bühlmann Laboratories AG recently developed a lateral flow–based calprotectin test and a software application (IB*Doc*) that turns an ordinary smartphone camera into a reader for quantitative measurements at home. The software application enables patients to perform a measurement and receive the result without delay, provided that there is an Internet connection available. We aimed to compare this new method with the hospital-based lateral flow reader Quantum Blue and the established ELISA method to see whether these tests agreed sufficiently for the new to replace the old, or to use the 3 interchangeably.

Methods

This study was a single-center prospective method comparison study, performed at the pediatric and adult IBD center at the University Medical Center Groningen (Groningen, the Netherlands).

Participants

Eligible participants were 10 years or older with good knowledge of the Dutch language who had a smartphone that was validated to run the IB*Doc* application (Supplementary Table 1) and did not have an ileostomy. We contacted candidate participants by telephone before their next planned visit to the outpatient clinic. When patients were interested in participation, we explained the procedure during the same telephone conversation. We then sent a study package including a pictorial algorithm of the procedural steps, including instructions for sample extraction and measurement, to the patient's home.

Stool Collection and Sampling

Participants defecated onto a stool collection sheet (included in the IB*Doc* package) held above the toilet water and collected 2 samples from the same bowel movement. The first sample was collected with the classical screw top container with spatula for ELISA measurement at the hospital laboratory. The second sample was taken with a CALEX valve extraction device (Bühlmann Laboratories AG) (Figure 1).

After performing the home test, both the CALEX valve extraction device and the screw-top container were sent in a resealable biomaterial envelope to the Department of Laboratory Medicine of the University Medical Center Groningen.

Calprotectin Measurements

The level of calprotectin was measured 3 times (Figure 2). First, patients used the IB*Doc* application on their smartphone to read the FC level in the CALEX extraction device, using a lateral flow technique. To do so they had to place the camera above the cassette and a picture was automatically taken. The image was then analyzed and the quantitative calprotectin result was directly shown on the screen (an instruction video of the step-by-step procedure can be found at www.ibdoc.net). At the same time the research team received a notification that an IB*Doc* measurement was performed with a direct link to this result on a secured web portal. Second, one experienced laboratory technician in the University Medical Center Groningen, who was blinded for IB*Doc* results, used the Quantum Blue Extended Reader (Bühlmann Laboratories AG) to read the FC level in the send in CALEX extraction device, also with a lateral flow technique.[9] Third, the fresh stool sample was extracted in the hospital laboratory and the FC level was measured with the ELISA technique. The fresh samples were manually weighted and stored at −20°C until analysis. The stool samples were then thawed for calprotectin measurements with the fCAL ELISA (Bühlmann Laboratories AG) on a Dynex DS2 Automated ELISA system (Alpha Labs, Easleigh, UK). The IB*Doc* and Quantum Blue tests covered a measurable range of calprotectin from 30 to 1000 μg/g, and the ELISA tests covered a range of 40 μg/g and above. For the analyses we therefore registered calprotectin values below 40 μg/g as 40 μg/g and values above 1000 μg/g as 1000 μg/g.

Outcome Measures

The primary outcome was the agreement of results between IB*Doc* and the clinically accepted ELISA method, using Bland-Altman plot analysis. First, we reasoned that disagreement in the lower range of the test (ie, between 40 and 500 μg/g) could lead more easily to misinterpretation of disease activity than disagreement in the higher range (>500 μg/g). We therefore used predefined acceptable

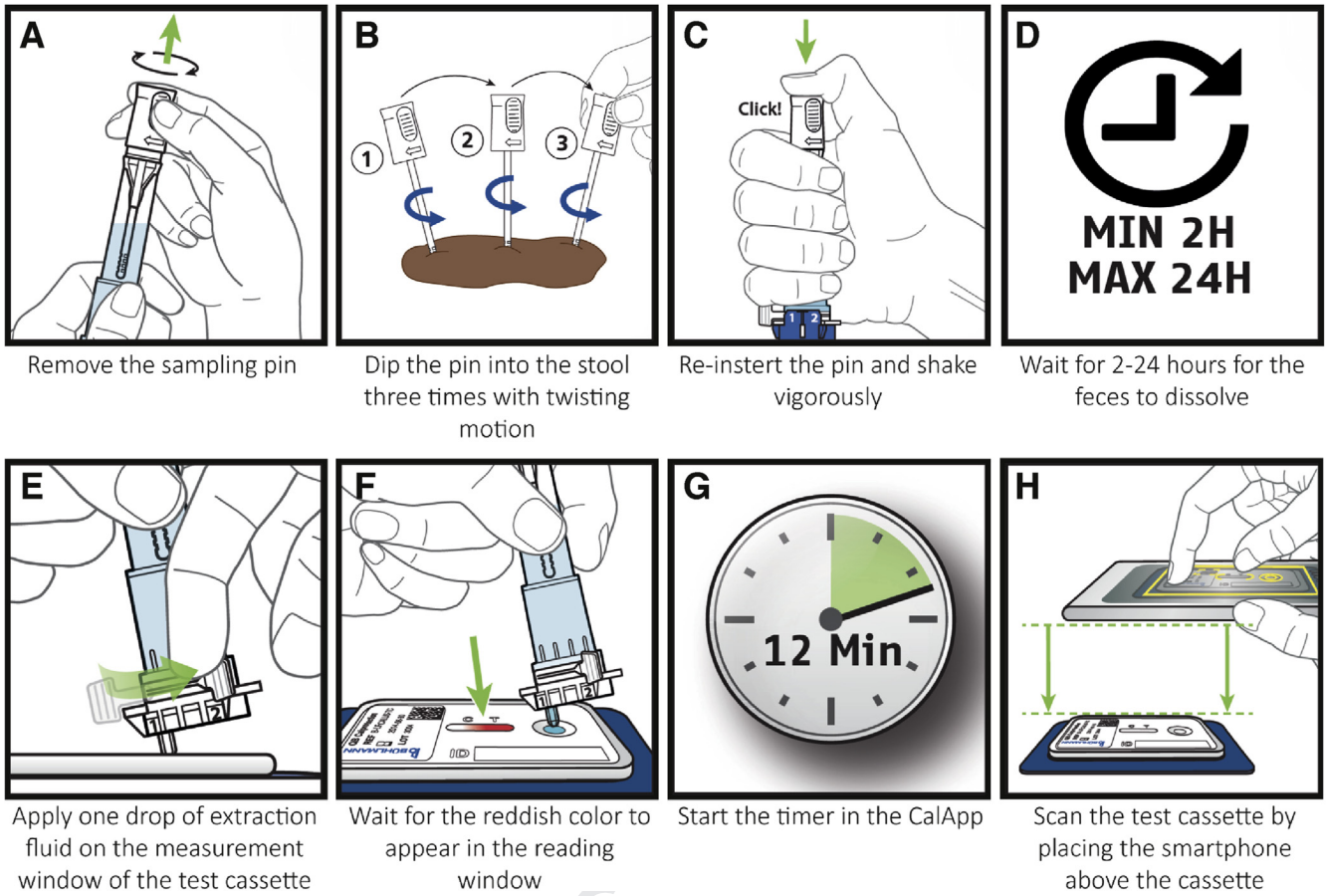

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Figure 1. IB*Doc* sampling and measurement. Adapted with permission from Bühlmann Laboratories AG.[23]

limits of difference, which were arbitrary set at ±100 μg/g for the lower range, and ±200 μg/g for the higher range. Second, we assessed concordance of ELISA and IB*Doc* readings in each of the 3 FC ranges used in our clinical practice (ie, <250 μg/g, 250–500 μg/g, and >500 μg/g). Next to this, as there is currently no consensus among IBD experts about the range of FC associated with mucosal healing, we also report concordance of ELISA and IB*Doc* readings for other frequently used dichotomous cutoffs (namely 50, 150, 200, 250, and 300 μg/g). Other outcome measures included agreement between patient-performed IB*Doc* measurements and hospital-based Quantum Blue measurements of the same extract, and an evaluation of the usability of the IB*Doc* method by patients.

Quality Measurement of Scanning Methods

Prior to the study, we verified the quality of the scanning methods of both the IB*Doc* application on an iPhone 4S and the Quantum Blue Extended reader, as described in the Supplementary Methods and Results and Supplementary Table 2.

Sample Size Calculation

We aimed to include at least 100 paired samples in the lower range of the test (ie, between 40 and 500 μg/g). This sample size was based on the recommendation of Bland.[10]

Statistical Analysis

Data were recorded electronically by using SPSS version 23.0 for Windows (IBM Corporation, Armonk, NY). Agreement between IB*Doc* and ELISA results, and between IB*Doc* and Quantum Blue results was compared with a Bland-Altman plot.[11] The Bland-Altman plot assigns the average of the old and new method on the x axis, and the difference between both on the y axis. Furthermore, we calculated the Passing-Bablok regression coefficient and Spearman rank correlations coefficient. Concordance of IB*Doc* and ELISA readings in each of the 3 FC ranges used in our clinical practice were presented in a scatterplot. Graphs were constructed with GraphPad Prism, version 5.04 for Windows (GraphPad Software, San Diego, CA). A *P* value below .05 was significant.

Ethical Considerations

This study was performed according to the Declaration of Helsinki. The Medical Ethical Committee of the University Medical Center in Groningen decided that a study measuring markers in voluntary stool samples did

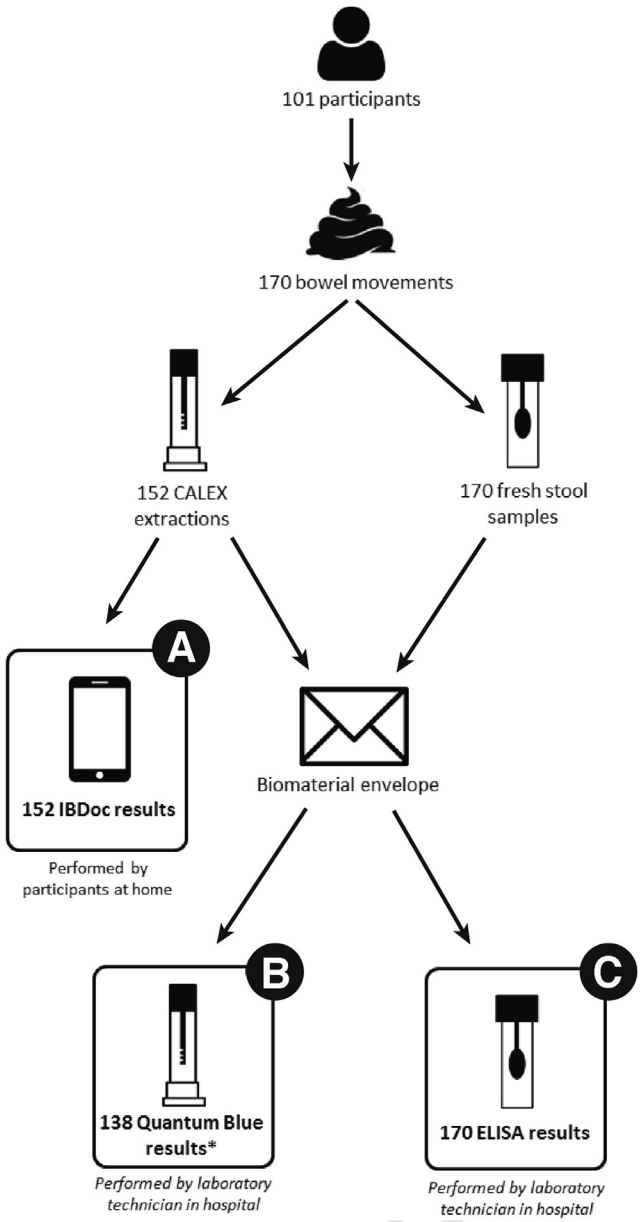

Figure 2. Study flow indicating 3 calprotectin measurements from the same bowel movement. ELISA, enzyme-linked immunosorbent assay.

not require approval according to the Dutch Medical Research Involving Human Subjects Act. All adult participants, legal guardians from pediatric participants, and children 12 years of age and older gave informed consent to use data generated by routine medical care. The data were collected and recorded by the investigators in such a manner that subjects could not be identified, directly or through identifiers linked to the subjects. This study was conducted in compliance with the Clinical Trial Agreement, the study protocol, designated Standard Operating Procedures and the international standard for clinical studies of medical devices (ISO 14155: 2011 Clinical investigation of medical devices for human subjects – Good Clinical Practice). All authors had access to the study data and reviewed and approved the final manuscript.

Results

Between June 2015 and October 2016, 306 random patients with IBD were approached by telephone, of which 211 were willing to participate. Sixty of them did not have access to a validated smartphone and were excluded from participation. The remaining 151 patients received a study package. In the end 101 patients actively participated and sampled 170 bowel movements (Figure 2). Median age of the participants was 24 (range, 10–59) years (Table 1). A total of 152 IB*Doc* results were transmitted to the secured web portal. Eighteen attempts to measure and transmit an IB*Doc* measurement failed for various reasons, including application dysfunction, slow adjustment of focus when scanning the test cassette, or being too much in a hurry to await the test result. The hospital laboratory received 138 CALEX valve extraction devices and 170 screw-top containers. Median transport time was 2 (range, 0–7) days, and 82% of samples arrived in the hospital laboratory within 72 hours after collection (Supplementary Figure 1).

*IB*Doc *vs ELISA*

We compared IB*Doc* and ELISA measurements in 152 paired samples. In Figure 3*A* we present a Bland-Altman plot with 124 measurements situated in the lower calprotectin range ($\leq$500 μg/g) and 28 measurements in the higher calprotectin range (>500 μg/g). We found 81% (100 of 124) and 64% (18 of 28) of IB*Doc* measurements were within predefined limits of agreement in respectively the lower and higher calprotectin range. The mean difference (IB*Doc* minus ELISA) was –1.7 μg/g in the lower range and –52 μg/g in the higher range.

Passing-Bablock regression analysis showed a slope of 0.80 (95% confidence interval (CI), 0.72–0.88) with an

Table 1. Patient Demographics

	n	Stool samples	Median calprotectin (IQR) (μg/g)[a]
Age			
- Children (<18 y of age)	19	58	40 (40–198)
- Adults (≥18 y of age)	82	94	195 (69–588)
Disease activity during stool sampling[b]			
- Symptomatic disease	Not applicable	50	445 (199–1010)
- Asymptomatic disease	Not applicable	84	47 (40–150)
- Unknown	Not applicable	18	420 (89–713)

IQR, interquartile range.
[a]Measured with enzyme-linked immunosorbent assay.
[b]Disease activity, as assessed by the Physician's Global Assessment, was reported when the interval between stool sampling and face-to-face encounter with the doctor was shorter than 1 month.

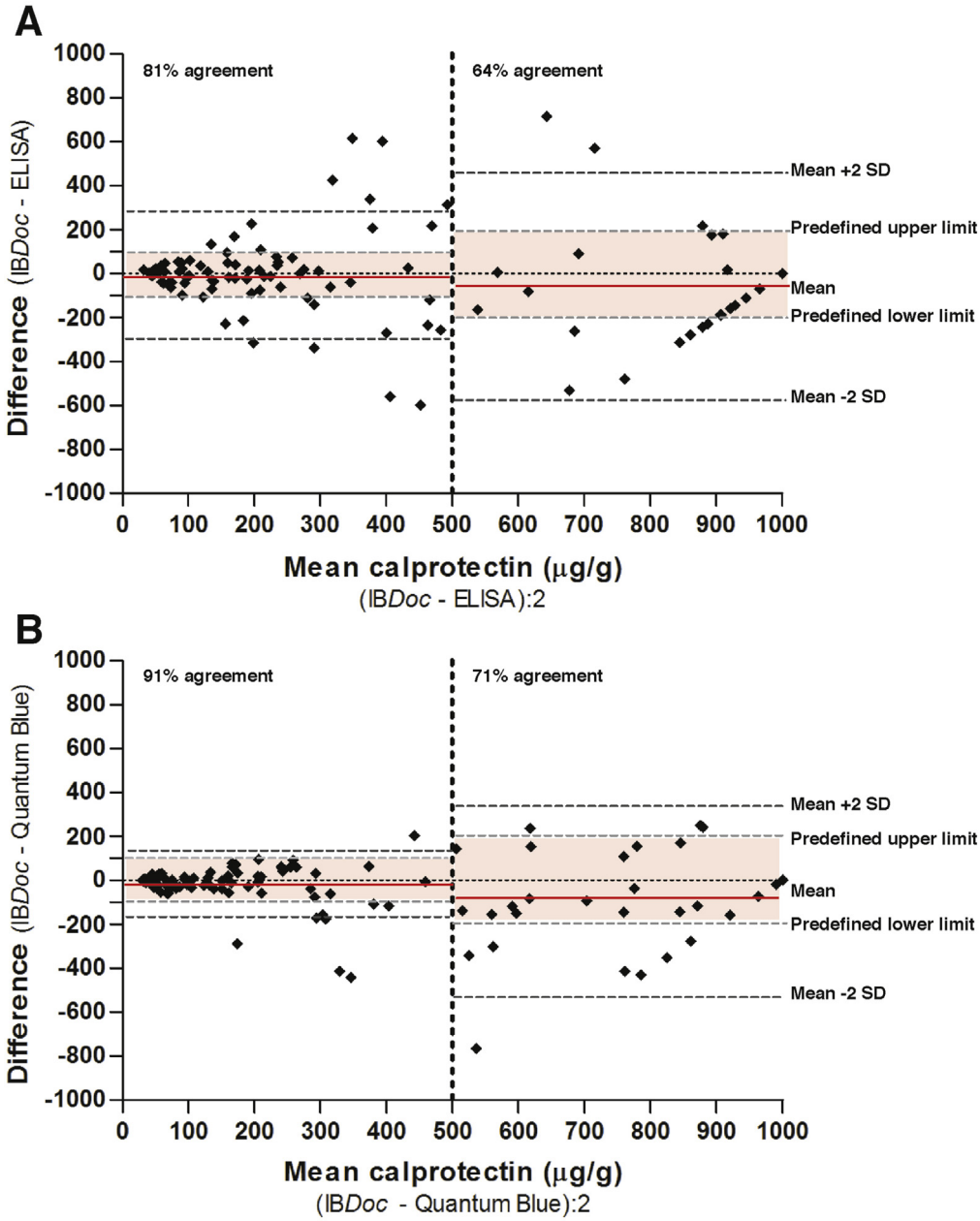

Figure 3. Bland-Altman plot showing difference against mean. (*A*) IB*Doc* and (enzyme-linked immunosorbent assay (ELISA) measurements. (*B*) IB*Doc* and Quantum Blue measurements. The pink zone corresponds with our predefined limits of agreement, which were arbitrary set at ±100 µg/g for the lower calprotectin range, and ±200 µg/g for the higher range. The outer lines correspond with the 95% limits of agreement (SD, 1.96).

intercept of 45.2 (95% CI, 10.9–79.6) and R^2 of 0.72. Spearman's rank correlation coefficient was 0.85 ($P < .001$).

The concordance between IB*Doc* and ELISA readings in each of the 3 FC ranges used in our clinical practice is presented in Figure 4. A total of 108 of 152 test pairs (71%) were concordant. Discordant test pairs leading to overt misinterpretation of disease activity (ie, calprotectin >500 µg/g with one method and <250 µg/g with the other) were observed in 6 of 152 stool samples (4%). Two of 6 discordant test pairs, depicted in the right lower corner of Figure 4, were caused by 1 participant who did not observe the advised incubation time. As a consequence, detachment of fecal material from the sampling grooves was incomplete. Stool consistency, transport time, age of participant (adult or child), and type of smartphone were not clearly related to discordant results (data not shown). The concordance between IB*Doc* and ELISA reading for other frequently used dichotomous cutoff values is presented in Supplementary Table 3.

*IB*Doc *vs Quantum Blue*

We compared patient-performed IB*Doc* smartphone readings and Quantum Blue readings by laboratory staff in 138 pairs of the same extraction fluid and show the corresponding Bland-Altman plot in Figure 3*B*. In the lower calprotectin range 95 of 104 (91%) IB*Doc* readings were within predefined limits of agreement, and in the higher ranges 24 of 34 IB*Doc* results (71%). The mean

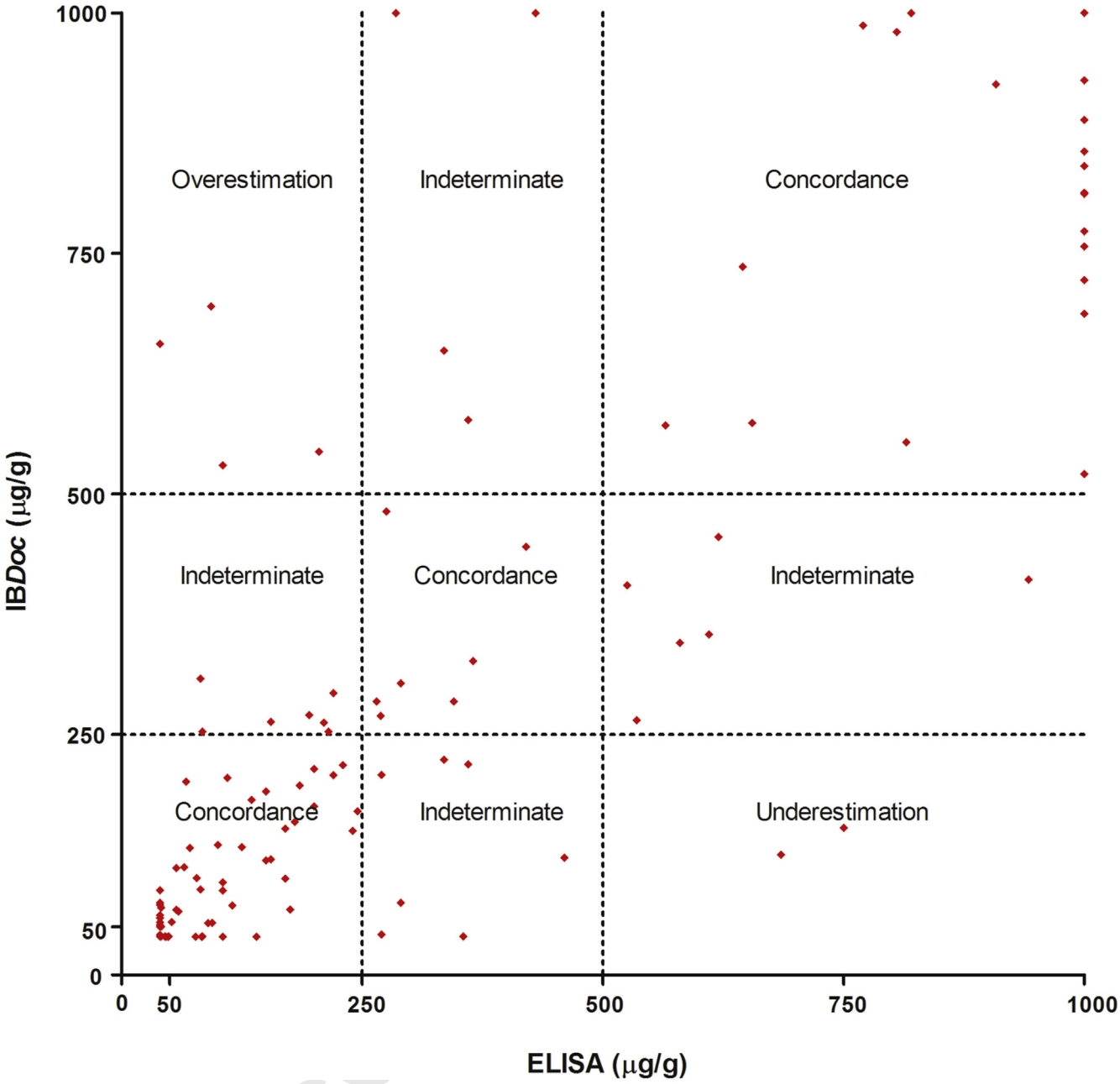

Figure 4. Scatterplot showing calprotectin readings with IB*Doc* against enzyme-linked immunosorbent assay (ELISA) method. Concordance is defined as calprotectin levels in the same category (<250, 250–500, >500 µg/g) for both IB*Doc* and ELISA.

difference (IB*Doc* minus Quantum Blue) was –16 ug/g in the lower range and –84 ug/g in the higher range. Passing-Bablock regression analysis showed a slope of 0.85 (95% CI, 0.79–0.91) with an intercept of 12 (95% CI, 16–40) and R^2 of 0.84. Spearman's rank correlation coefficient was 0.94 ($P < .001$).

Self-Reported Usability

Sixty-three participants returned the questionnaire about the usability of the home test (response rate, 62%). A total of 87% of the respondents were of the opinion that the test was not difficult to perform. Holding the smartphone in the right position to scan the test cassette was perceived as the most difficult step in the home test, and 97% of the respondents were interested in using the home test in the future.

Discussion

Summary of Main Findings

The results show that the majority of calprotectin measurements performed at home with a lateral flow immunoassay and smartphone reader agreed sufficiently with the ELISA-based quantification of calprotectin in the

hospital laboratory, provided that calprotectin levels are below 500 μg/g. In the higher calprotectin range a substantial proportion of pairs exceeded the predefined limits of agreement (±200 μg/g). Furthermore, we showed that the smartphone reader used by patients at home performed as good as the point-of-care Quantum Blue reader in the hospital.

Additional benefits of the home test include a reduction of the burden on hospital laboratory resources and a more patient-friendly sampling technique (with a pin instead of a spatula).[12]

Comparisons With Existing Literature

To the best of our knowledge, this is the first study that compared the performance of the FC home test with both a point-of care test (Quantum Blue) using the same extraction fluid and the ELISA test.

In 2010 a Danish research team first described the use of a lateral flow device that could be read by a tabletop scanner connected to a computer with special software (CALPRO Inc, Oslo, Norway).[13] In the same paper it was shown that the lateral flow device could also be analyzed by taking a picture with a mobile phone and sending it to a server for evaluation. Both methods showed acceptable agreement compared with ELISA, but in this study the tests were carried out by an experienced laboratory technician who used the same extracted sample for all 3 methods. The same group recently reported the results of a study comparing long distance reading of a lateral flow technique from a different manufacturer (CalproSmart, Calpro AS) with ELISA.[14] They found a significant but lower Spearman rank correlation compared with our IB*Doc* results ($r = 0.67$ vs $r = 0.85$). The reader used in this study covered a measurable range of calprotectin from 30 to 600 μg/g, and as a consequence of this smaller range the maximum difference that could be measured between the lateral flow test and ELISA was 570 μg/g, compared with 960 μg/g in our study. Major disadvantage in the Danish study included the use of a single type of smartphone for standardization purposes, whereas we included measurements from 16 different types of validated smartphones.

Limitations of the Study

There are some limitations in our study that need to be addressed. First, the study participants were patients with a suitable smartphone and interest in home testing, rather than random IBD patients. We might have included a sample of patients with higher socioeconomic status and better education than others. Second, we observed a median delay of 2 days between IB*Doc* home testing and arrival of stool in our hospital, which could have influenced the agreement between the results. A recent study on the stability of calprotectin in fresh stool showed no significant difference in concentrations between samples kept at room temperature for 1–3 days, but between 3 and 7 days a mean decrease of 28% was found.[15] In our study 82% of fresh stool samples arrived at the hospital laboratory within 3 days, and none more than 7 days after collection. With the introduction of home extraction and reading potential degradation of calprotectin in send in stool samples will no longer be an issue of importance.

Implications for Clinical Practice

The evidence base for repeated FC testing in asymptomatic patients aimed at early recognition of disease exacerbation is accumulating.[5] Simultaneously, the number of telemonitoring initiatives for IBD care is rising.[16–19] Calprotectin home monitoring with a smartphone fits perfectly in the current spirit of the times and is another important step toward patient-centered care.[20] Whether FC home testing is cost effective should be evaluated in future studies. The actual price for IB*Doc* tests is approximately €30, compared with approximately €41 per ELISA test (which includes labor and equipment costs).

When repeated calprotectin home testing is implemented it is important to realize that the total variation between successive measurements can be influenced by biological variation (fluctuations within same subject), preanalytical variation (differences in collection technique, transport, storage, and handling of stool), and analytical variation (differences in precision of assay). In our study, taking too little time for the extraction process explained 2 of 6 discordant test pairs. Technical competence is not only an important element of training for hospital based laboratory technicians, but also for patients who wish to use a point-of-care calprotectin test at home. We therefore recommend to train interested patients in a skills lab until proficiency criteria have been met.[21]

Conclusions

We found sufficient agreement between the home-used lateral flow test and the hospital-based ELISA test in the lower ranges of calprotectin to use this new test for telemonitoring of patients with asymptomatic IBD. In line with recent literature about FC monitoring, we suggest that confirmation of elevated IB*Doc* readings is done before therapy adjustment is considered.[1,22]

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Reprint requests
Address requests for reprints to: Patrick F. van Rheenen, MD, PhD, University of Groningen, University Medical Centre Groningen, Department of Paediatric Gastroenterology, Hepatology and Nutrition, Internal Code CA 31, PO Box 30001, 9700 RB Groningen, the Netherlands. e-mail: p.f.van.rheenen@umcg.nl.

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Conflicts of interest
The authors declare no conflicts.

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Supplementary Material

Supplementary Table 1. ■■■

Validated smartphone types:	Number of readings in this study:
iPhone:	
- 4S	12 (8%)
- 5	27 (18%)
- 5c	4 (3%)
- 5s	17 (11%)
- 6	17 (11%)
- 6 plus	7 (5%)
- 6s	2 (1%)
- iPod touch 5th generation	0
HTC:	
- One	0
HUAWEI:	
- P8 lite	1 (1%)
SAMSUNG:	
- GALAXY S3	14 (9%)
- GALAXY S4	16 (11%)
- GALAXY S5	16 (11%)
- GALAXY S5 mini	2 (1%)
- GALAXY S6	7 (5%)
- GALAXY A3	0
- GALAXY A5	0
- GALAXY CORE PRIME LTE	0
SONY	
- Xperia Z3 compact	1 (1%)
- Xperia Z3 compact	1 (1%)
LG	
- G4	0
- G3	0

Supplementary Methods and Results: Quality assessment

Method: We prepared three homogenized stool pools representing a low, middle and high range calprotectin level (respectively 105μg/g, 186μg/g, and 507μg/g). We then prepared low, middle and high range CALEX extraction fluids. Thereafter, we performed 10 readings of the same test cassette at one level, readings of 10 test cassettes loaded with one extract (from the 3 pools), and readings of 10 test cassettes loaded with 10 different extracts (from the 3 pools).

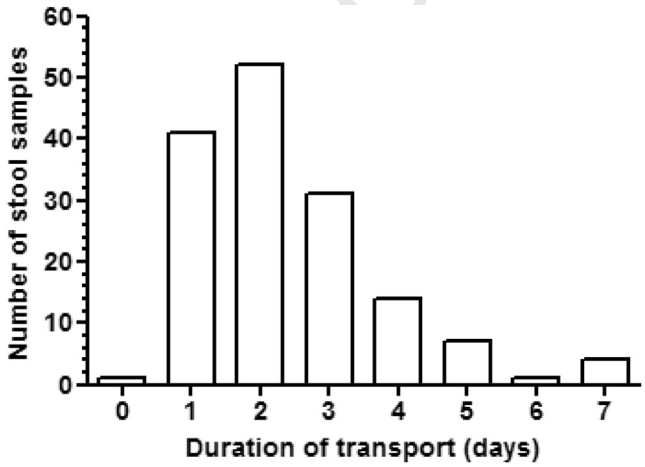
Results: Sample reproducibility results of both IB*Doc* and Quantum Blue are presented in the table below:

Supplementary Table 2. Coefficients of Variation in IB*Doc* Smartphone Readings and Quantum Blue Readings

	IB*Doc*	Quantum Blue
Variation in 10 readings of the same test cassette	4%	6%
Variation in readings of 10 test cassettes loaded with one extract		
Low-range calprotectin level (±105 μg/g)	11%	4%
Middle-range calprotectin level (±186 μg/g)	16%	11%
High-range calprotectin level (±507 μg/g)	10%	16%
Variation in readings of 10 test cassettes loaded with 10 different extracts		
Low-range calprotectin level (±105 μg/g)	17%	23%
Middle-range calprotectin level (±186 μg/g)	18%	19%
High-range calprotectin level (±507 μg/g)	25%	10%

Supplementary Table 3. Concordance Between IB*Doc* and ELISA Calprotectin Result for Frequently Used Dichotomous Cutoff Values

	Concordant + (IB*Doc* ↑; ELISA ↑)	Concordant - (IB*Doc* ↓; ELISA ↓)	Discordant (IB*Doc* ↓; ELISA ↑)	Discordant (IB*Doc* ↑; ELISA ↓)
Cutoff 50 μg/g	90 (59%)	45 (30%)	8 (5%)	9 (6%)
Cutoff 100 μg/g	69 (45%)	63 (41%)	10 (7%)	10 (7%)
Cutoff 150 μg/g	61 (40%)	74 (49%)	9 (6%)	8 (5%)
Cutoff 200 μg/g	50 (33%)	84 (55%)	8 (5%)	10 (7%)
Cutoff 250 μg/g	41 (27%)	91 (60%)	9 (6%)	11 (7%)
Cutoff 300 μg/g	34 (22%)	102 (67%)	8 (5%)	8 (5%)



Supplementary Figure 1. Overview of transport time.