IB*Doc*® Calprotectin Kit

Instructions for Use

Patients and Lay Users

LF-IBDOC8

8 tests

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TABLE OF CONTENTS

INTENDED USE

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BÜHLMANN IB*Doc*® is an *in vitro* diagnostic immunoassay for the quantitative determination of fecal calprotectin in human stool. The results of the assay are analyzed by a downloadable smartphone application. IB*Doc*® is intended as an aid to the assessment of inflammation of the intestinal mucosa for inflammatory bowel disease (e.g. Crohn's disease and ulcerative colitis) monitoring. IB*Doc*® is an assay developed for self-testing / home use by trained patients ages 12 and above that are under the care of a healthcare practitioner. The test may also be used in a near-patient or laboratory setting.

Disclaimer: The screenshots in this instruction for use are based on the iOS version of the IB*Doc*® app. The Android version might differ in layout but not in functionality.

PRINCIPLE OF THE ASSAY

IB*Doc*® is a home test to measure calprotectin in stool samples for patients ages 12 and above. Calprotectin is a protein released by neutrophilic immune cells, which are a hallmark of acute inflammation. Determination of fecal calprotectin levels helps to detect gastrointestinal (GI) inflammation and monitor inflammatory bowel disease (IBD). Low levels of calprotectin are an indication to your healthcare practitioner that you are in a state of IBD disease remission. Your treatment can be continued without any additional endoscopic, radiologic or other investigations. High calprotectin levels can act as a red flag signal for a possible GI tract inflammation. This will prompt further clinical and laboratory evaluation by your healthcare practitioner.

To determine calprotectin levels you will use the CALEX® Valve stool preparation device to collect a precise amount of stool sample. Inside the CALEX® Valve, calprotectin present in the stool sample is transferred to the extraction solution. The extract is then applied to the test cassette. Calprotectin in the sample is bound by anti-calprotectin antibodies linked to red gold particles. The red calprotectin-antibody-gold particles flow with the extract through the test cassette, are caught on and color the Test Line. Antibody-gold particles, not bound to calprotectin will color the Control Line. The Test and Control Lines are measured by a smartphone application (IB*Doc*® app). The result is calculated by IB*Doc*® app and sent to a secure server for the healthcare practitioner to review. The test has a measuring range of 30-1000 µg calprotectin / g stool and a linear range up to 700 µg/g.

MATERIALS AND ACCESSORIES PROVIDED IN ONE IB*Doc*® TEST SET (B-IBDOC):

Before running the test, please ensure that all components are in the set.

Quantity	Components
1	CALEX® Valve filled with extraction solution (5 mL)
1	Test Cassette
2	Stool Collection Papers
1	Quick Guide

THE COMPONENTS MUST BE STORED IN THE REFRIGERATOR (2-8 °C).

MATERIALS AND ACCESSORIES REQUIRED, BUT NOT PROVIDED:

- iOS or Android smartphone validated by BÜHLMANN for the use with IB*Doc*®.
- You can find a complete list of validated smartphones on **www.ibdoc.net**.
- An internet connection on your smartphone (see also important information).
- The smartphone application „IB*Doc*® ": Available from the Apple App Store or Google Play Store. Please note the IB*Doc*® application is having regular updates and might enforce them to make sure you are using the latest available version.
- Examination gloves, if necessary available from your doctor.

SUMMARY OF SAFETY AND PERFORMANCE (SSP):

A *Summary of Safety and Performance (SSP)* for BÜHLMANN IB*Doc*® can be received upon request from: support@ibdoc.net

IMPORTANT INFORMATION

- **Disease management should be performed together with your healthcare pracitioner (HCP). Do not adapt your treatment without consultation.**
- Contact your HCP if:
  - you feel that the IB*Doc*® result does not reflect your current health status.
  - the result is not displayed properly (see picture T, page 17 and chapter result interpretation, page 21).
  - you have any questions about IB*Doc*®.
- Please read the instruction for use carefully before starting the test procedure. Pages 8 to 10 contain an overview of all set components. The step by step prodecure walkthrough starts on page 11.
- Make sure that you are properly trained by your HCP before performing the test.
- Take your time to perform the test at your home and make sure you are not distracted.
- Keep your smartphone away from water to avoid damage.
- Additional costs for your internet connection may arise depending on your carrier.
- The test must be performed at room temperature (18 - 28°C) and ambient humidity (16-75% relative humidity).
- You can perform the IB*Doc*® test under different lighting conditions, but you must prevent direct sunlight, strong sideward-light or casting a shadow onto the test casette while reading the test cassette in test procedure - step 6.
- Your smartphone must have at least 20 % of battery charge left or connected into a power source.
- The CALEX® Valve and the test cassette must not be used after the expiry date printed on the labels. The test cassette is stable at room temperature for 4 hours after opening the pouch.
- The CALEX® Valve and the test cassette must not be reused.
- None of the components are poisonous.
- The extraction buffer in the CALEX® Valve contains components classified in accordance with the Regulation (EC) No. 1272/2008: 2-methyl-4-isothiazolin-3-one hydrochloride (conc. ≥ 0.0015 %), thus the extraction buffer may cause allergic skin reactions (H317).
- Avoid contact of extraction buffer with the skin, eyes, or mucous membranes. If contact does occur, immediately wash with generous amounts of water; otherwise, irritation can occur.
- For hygienic reasons, dispose of test components as soon as they are used and wash your hands.
- Patient specimens and the used kit components are potentially infectious and should be disposed of according to local state and federal regulations.
- A link to set your password will be sent to your IB*Doc*® email (username), which you provided to your HCP. If you fail to receive this message then check your spam folder.
- If the blue protection cap (figure 3, page 8) of the CALEX® Valve seems loose or has fallen off when opening the package, place it back on the outlet.
- If either the pouch holding the test cassette is damaged or the CALEX® Valve is leaking after opening the original packaging, do not use the test set.

IMPORTANT ADVICE FOR CORRECT TEST HANDLING

- **Many stool samples require up to 2 hours to fully detach** from the grooves (step 4, p. 14). It may be easiest to prepare and extract your stool sample (as decribed in test procedure - step 3 and 4) in the morning and continue with the next steps in the evening. Do not leave the processing of the extract for more than 24 hours. In this way, the stool sample has enough time to completely detach from the grooves and you are not under any time pressure for the rest of the test procedure.
- If you have problems to collect the stool sample i.e. the stool does not stick to the grooves of the CALEX® Valve, perform the test on another day. Use a new IB*Doc*® set.

CALEX® Valve

Figure 1:
CALEX® Valve

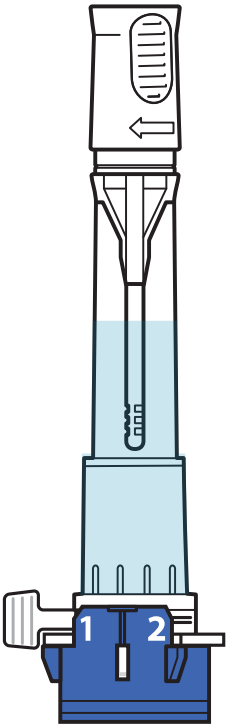


Figure 2:
Sampling pin

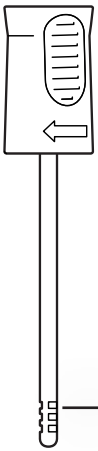


Figure 3:
CALEX® Valve body

Figure 4: Test cassette

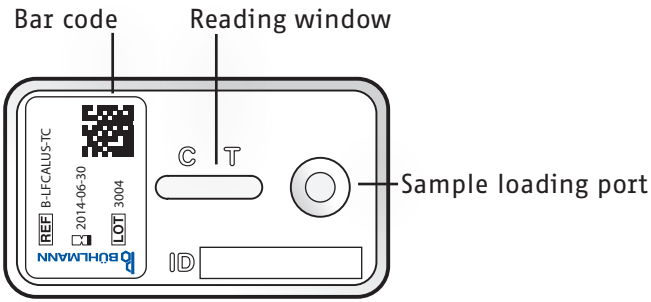


Figure 5a: IB*Doc*® app navigation menu

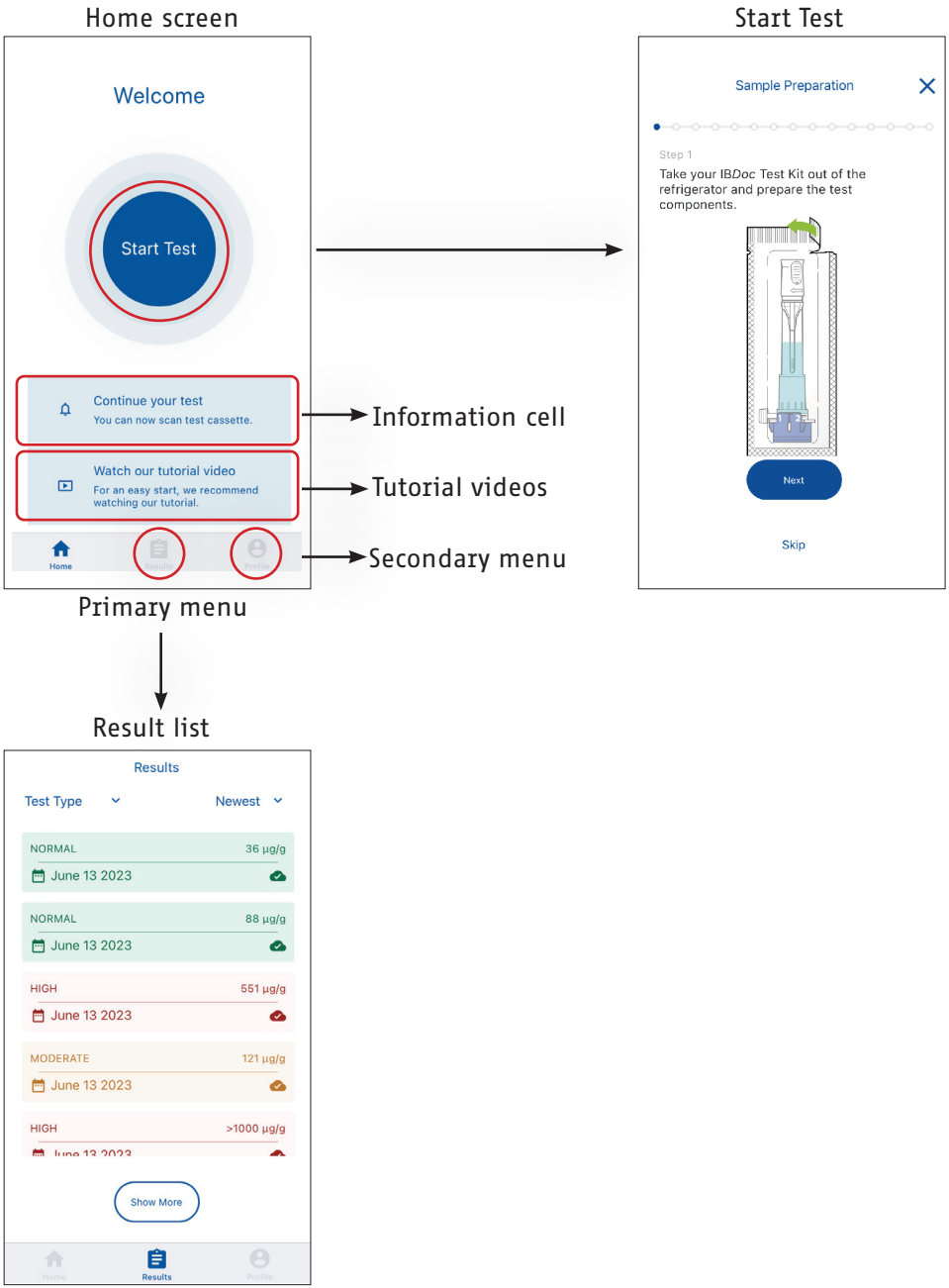
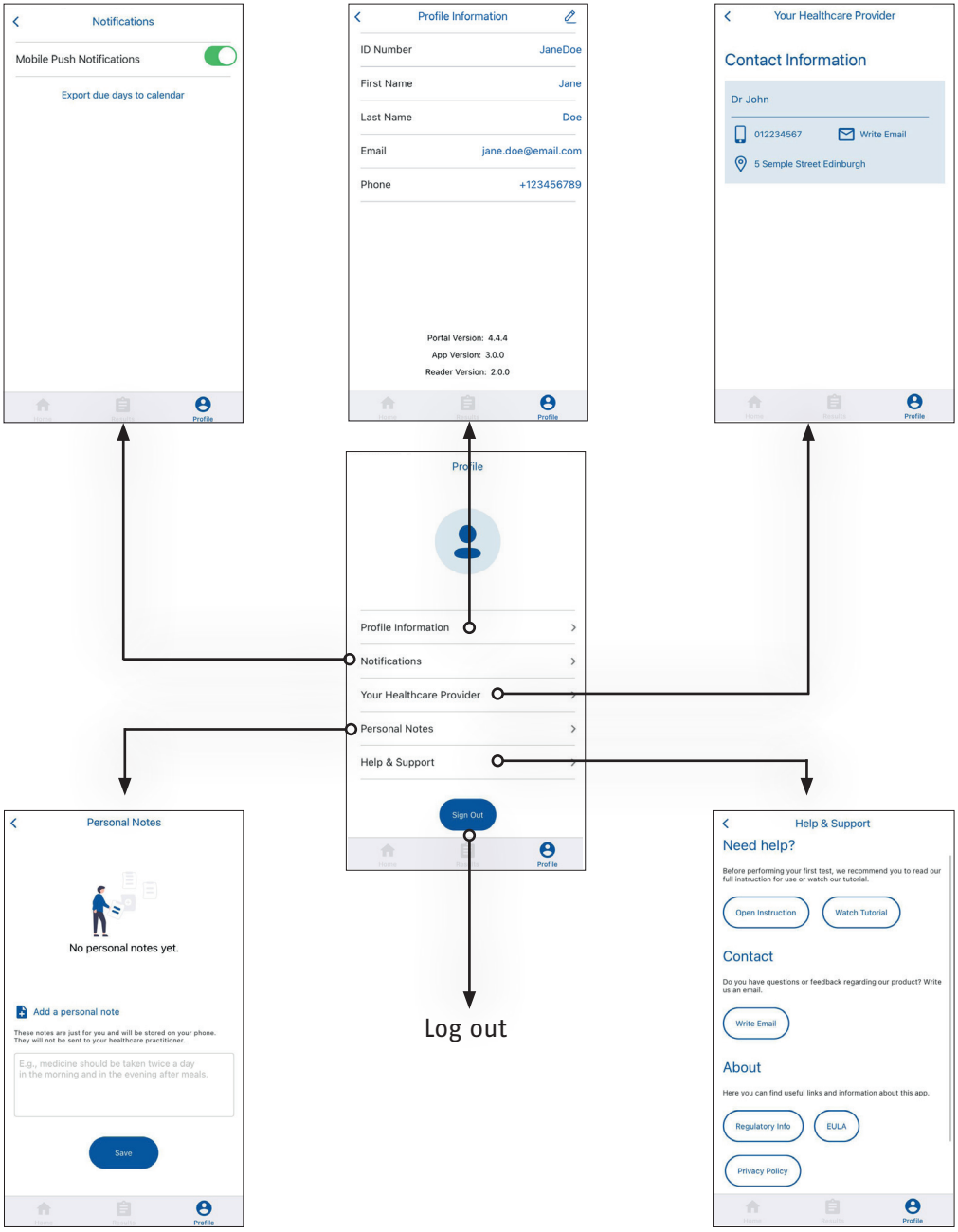


Figure 5b: IB*Doc*® app profile menu



STEP 1: INSTALLATION OF THE APP AND LOGIN

1.1 Search for „IB*Doc*® “ on the Apple App store or Google Play store.

1.2 Download and install the IB*Doc*® app onto your smartphone (A).

Note: Please verify that you have an iOS or Android smartphone validated by BÜHLMANN. You can find a complete list of validated smartphones on www.ibdoc.net. Login will be blocked on a smartphone that is not validated.

1.3 Make sure that you have a stable internet connection.

Note: Please be aware that you need an internet connection to log into IB*Doc*® app at first startup.

1.4 Tap on the IB*Doc*® icon and start the login process.

Note: At the first start up you will have to agree that the IB*Doc*® app is allowed to send you push notifications. A push notification will send you a reminder one day before the next test is due.

An onboarding screen explaining the IB*Doc*® system is shown at first start-up.

You will have to agree that the IB*Doc*® app is allowed to use the camera.

1.5 Enter your IB*Doc*® account email address (username) and password (B).

Note: If you forgot your password you can reset it by tapping on the „Forgot Password“ button (B). Once you entered your IB*Doc*® account email address (username), a link to reset your password will be sent to your email address.

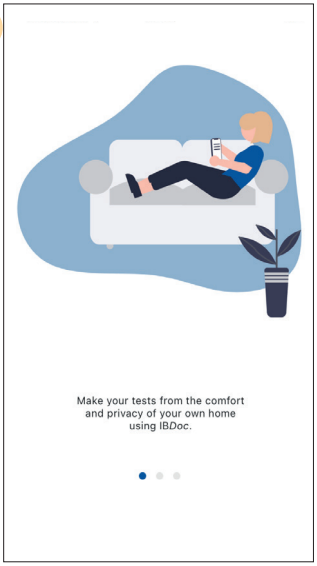
1.6 Read and accept the End User Licence Agreement (EULA) and the Privacy Policy.

Note: Due to data protection and security reasons your session expires after 7 days and you will need to log in again.

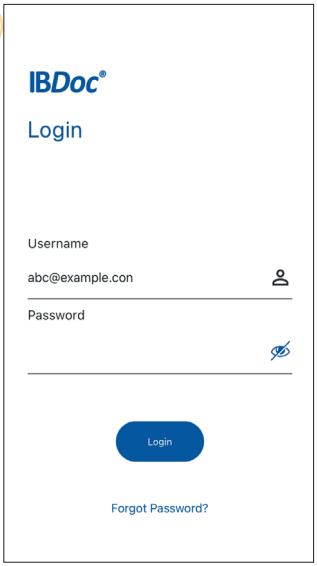
1.7 In case you are requested to update the IB*Doc*® app, search for „IBDoc®“ on the Apple App store or Google Play store and click on "Update".

Note: To uninstall the app, long press on the app icon on the home screen and click on "Uninstall" or "Remove App".

A



B



STEP 2: PREPARATIONS FOR THE TEST PROCEDURE

2.1 After login you see the home screen (fig. 5a, p. 9). To start your test, tab on the "Start Test" button (C).

Note: Two information cells are displayed on the Home Page (fig. 5a, p. 9). The first one gives you information about your tests (date of the next test due, stage of the current test, etc). The second one will allow you to consult the tutorial video.

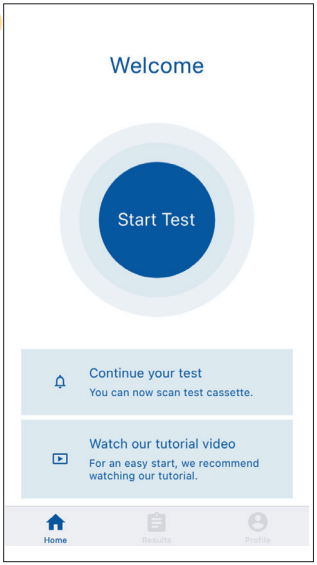
2.2 You can freely navigate between the tutorial screens and displayed text information (D). If you are an experienced user you may skip these instructions (by tapping the "skip" button) and start with the test procedure right away (see step 3).

2.3 Now you have completed the smartphone preparations. Put it aside but keep it in reach for later steps. Start with the test procedure (step 3).

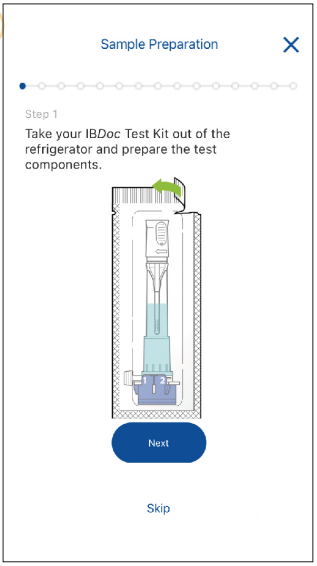
Take the IB*Doc*® test set out of the fridge and keep the single components in a dry and shaded place until needed in the test procedure.

Note: Keep the test cassette and CALEX® Valve in the pouches until you need them in the following steps.

C



D



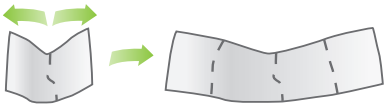
STEP 3: STOOL COLLECTION

The subsequent steps 3 and 4 must be performed continuously without interruption

3.1 Please empty your bladder first if necessary, since urine can affect the test.

3.2 Unfold the stool collection paper by holding the open ends and gently pulling outwards (E).

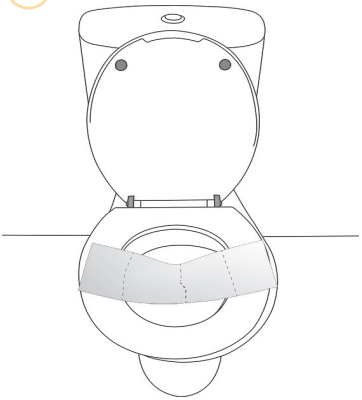
E



3.3 Place the stool collection paper on top of the toilet seat near the back (F).

Caution: The paper must not touch the toilet water.

F



3.4 Make sure that the stool collection paper is correctly fixed on the toilet seat.

3.5 Make sure that your stool sample is caught on the collection paper (G).

Note: In case your stool collection fails the first time, you have a second stool collection paper provided with the test set.

If needed put your examination gloves on and proceed to step 4 of the test procedure.

G

STEP 4: STOOL PREPARATION / EXTRACTION

4.1 Remove the CALEX® Valve from the packaging.

4.2 Hold the CALEX® Valve with the white cap at the top and remove the white sampling pin by simultaneously turning clockwise and pulling it upwards (H).

4.3 Dip the dosing tip with the grooves into the stool, and twist before removing. Repeat the procedure at 3 to 5 different positions of the stool sample in order to fill the grooves of the dosing tip (fig. 2, p. 8) completely (I).

Caution: Make sure that all grooves are completely filled with stool. It does not matter if the dosing tip of the sampling pin is fully covered with stool, since excess stool will be stripped off at the next step.

4.4 Place the sampling pin back into the funnel of the CALEX® Valve body (fig. 3, p. 8) and push it into the final locking position. You feel and hear a "click" (J).

Note: After the stool sample is collected, you can flush the remaining stool down the toilet together with the stool collection paper.

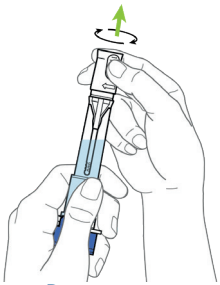
4.5 Shake the CALEX® Valve vigorously for 10 seconds (K) and let it stand for 2 hours on the blue protection cap.

Note: Since many stool samples require up to 2 hours to fully detach from the grooves you have to wait at least 2 hours before proceeding with the next step. Continue with the procedure at a convenient time point within the next 24 hours.

There is a 2 to 24 hours timer at this step in the app tutorial. If started, the timer will send you a notification when 2 hours are up or if 1 hour is left of the 24 hours.

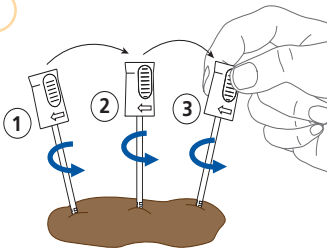
Keep the CALEX® Valve in a dry and shaded place during the waiting period.

H

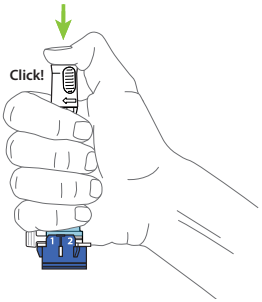


Caution: Do not remove the blue protection cap!

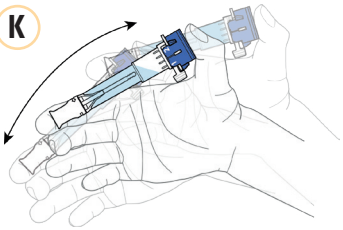
I



J



K



STEP 5: LOADING THE TEST CASSETTE

The subsequent steps 5 to 7 must be performed continuously without interruption.

Caution: Make sure you have your smartphone ready and that you are logged into the IB*Doc*® app.

5.1 Unpack the test cassette and place it on an even surface.

5.2 Shake CALEX® Valve again vigorously for 10 seconds. Holding the CALEX® Valve upright, flick the bottom of the CALEX® Valve to remove any airbubbles trapped in the outlet (L).

Caution: To get accurate test results it is important that the grooves of the dosing tip are free before the next step. If there is any residual stool left in the dosing tip, repeat step 5.2 before continuing.

5.3 Remove the protection cap (M, 1) and place the outlet of the CALEX® Valve onto the circular sample loading port (fig. 4, p. 8) of the test cassette (M, 2). Turn the throttle (fig. 3, p. 8) from position 1 to position 2 counterclockwise (N) and make sure that the outlet remains in close contact with the sample loading port.

5.4 As the liquid is loaded (0, 1) a reddish color appears in the reading window. Allow the reddish color to reach the middle of the reading window of the test cassette (0, 2 arrow). This will take 20 to 30 seconds.

5.5 Start the timer in the IB*Doc*® app immediately (0, 3).

Caution: Open the throttle of the CALEX® Valve only once. The test is designed to work with the first drop released.

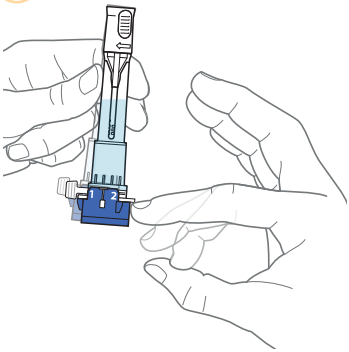
5.6 Remove the CALEX® Valve from the sample loading port of the test cassette and put the protection cap back on.

Note: The CALEX® Valve must be used only once.

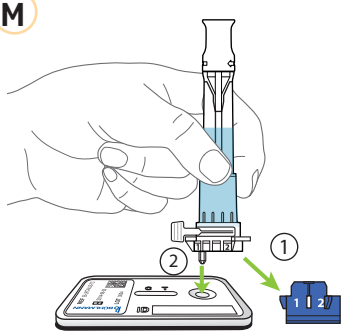
5.7 Leave the test cassette for 12 minutes until the timer starts to "beep". Then proceed immediately to step 6 of the test procedure.

Note: Make sure that your phone is not on mute, so you can hear the timer ring.

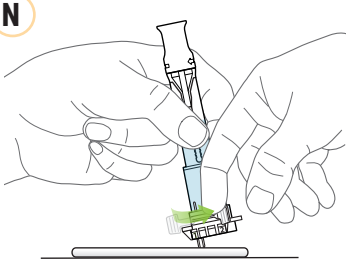
L



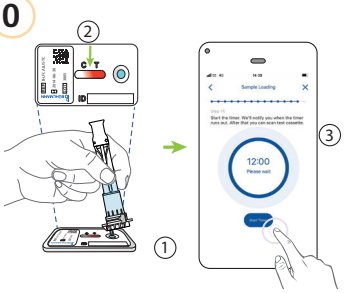
M



N



O



STEP 6: READING THE TEST CASSETTE

Note: Make sure that the test cassette is positioned on a plain and uniform surface (P). Do not place test cassettes on furniture edges, patterned or dark backgrounds as this may interfere with the image capture by your smartphone.

6.1 Once the timer has run out, proceed immediately to take an image of the test cassette by pressing the „Scan“ button.

Caution: A time out message will appear 90 seconds after pressing the “Scan“ button. The camera view will close and the IB*Doc*® app will return to the start screen.

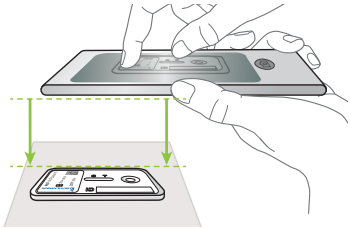
6.2 Align the frame in the camera view with the edges of the test cassette (R). Touch the screen to focus, if necessary.

Note: Hold your smartphone horizontally to the test cassette and not at an angle (Q).

Move smartphone slowly up and down to get the frame in proper alignment with the test cassette. If the smartphone is in the correct position, the frame will turn from red (R) to green (S). If the color does not change, touch the screen to refocus on the image. Once the position is indicated as correct (green), please hold your smartphone at the same position until the reading is complete. Please make sure that you scan the test cassette in under 1 minute.

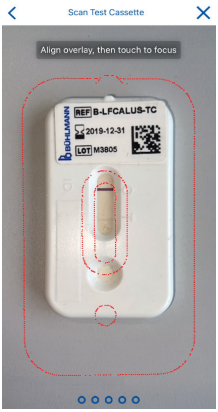
6.3 Once the IB*Doc*® app has found good images to analyse, five dots gradually appear and the screen displays „Analysing test“.

P



Q

R



S

STEP 7: ADDING NOTES AND SAVING TEST RESULT

7.1 As soon as the test cassette has been read, the IB*Doc*® app takes you to the result view (T).

Note: If you do not have an internet connection while performing the test, the result may appear as „Pending“ in a blue box until an internet connection is established at the next login. Your test result is not lost in any case.

7.2 By tapping into the „Notes“ box you can leave a comment for yourself or for your healthcare professional.

Note: Only full stops and commas are allowed as punctuation marks.

7.3 Once you have typed your note, tap the „Submit“ button (T) to save the test result.

Note: Test results are sent automatically to the IB*Doc*® Portal and your HCP is notified via email. If the smartphone does not have an internet connection at the time the test cassette is measured, the test result is automatically sent the next time an internet connection is established.

You can review your test result history at any time by accessing the test result list or the mobile portal (fig. 5a, p. 9).

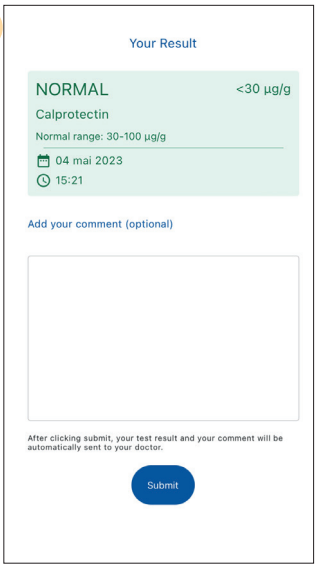
7.4 After the test result is saved, dispose of the CALEX® Valve and the test cassette (U).

7.5 You have reached the end of the test procedure. The IB*Doc*® app will return to the home screen.

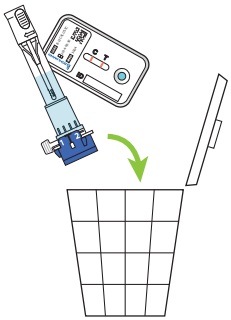
Caution: Do not read the same test cassette again.

Note: Uploaded results will be displayed by a cloud symbol with a check mark. Results that are not yet uploaded to the IB*Doc*® Portal will be displayed with an arrow in the cloud symbol. You can perform a manual upload by manually pulling down the page to refresh the result list (fig. 5a, p. 9).

T

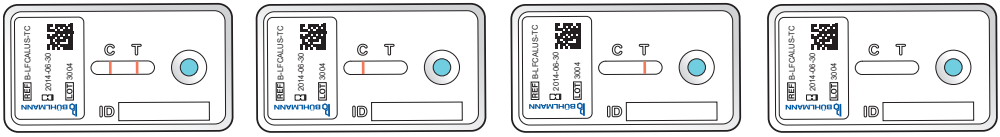


U



QUALITY CONTROL FEATURES

Figure 6: Test results



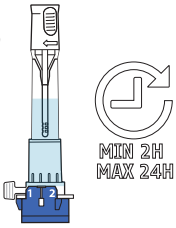
VALID	VALID	INVALID	INVALID
Control Line (C) and Test Line (T) are visible.	Control Line (C) is visible. The calprotectin concentration is below the detection limit and the Test Line (T) is not visible.	Control Line (C) is not visible.	Control Line (C) is not visible.

For a valid result, the Control Line (C) has to be visible. If the signal intensity of the Control Line (C) is below a threshold after 12 minutes of incubation time, the test result is also invalid and the test has to be repeated using another test cassette. The IB*Doc*® app determines the validity of the test cassette automatically.

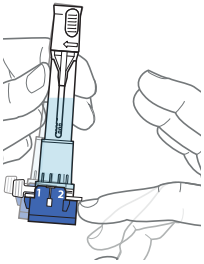
CRITICAL STEPS IN THE TEST PROCEDURE

To ensure optimal IB*Doc*® test performance always remember:

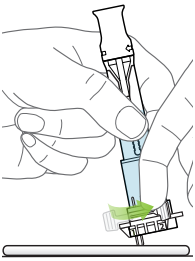
Stool collection (step 4.3) Make sure all the grooves of the sampling pin are completely filled with stool. Do not worry about excess stool. This will be removed by the CALEX® Valve.



Before you release the extract on the test cassette (step 5.3), allow the CALEX® Valve to stand for 2-24 hours at room temperature.



Before you release the extract on the test cassette (step 5.3), flick the bottom of the CALEX® Valve to release any air bubbles trapped in the outlet.



During extract release onto the test cassette (step 5.4), keep the outlet of the CALEX® Valve in contact with the sample loading port until the reddish color reaches the middle of the test cassette.

THE NEXT SECTION OF THE INSTRUCTION FOR USE DESCRIBES THE RESULT INTERPRETATION AND PERFORMANCE EVALUATION OF THE IB*Doc*® AND IS IN PARTICULAR INTENDED FOR HEALTHCARE PRACTITIONERS.

LIMITATIONS AND CONTRAINDICATIONS

- Multiple fecal calprotectin measurements performed at up to 4 weeks intervals have been suggested to have best diagnostic accuracy in predicting clinical relapse in patients [14, 15].
- It is advised that IBD patient monitoring with IB*Doc*® be established during disease remission. This will allow optimal determination of rising calprotectin levels that may indicate disease relapse.
- Fecal calprotectin levels determined by IB*Doc*® are intended as an aid to IBD monitoring and should be interpreted in combination with other clinical and laboratory findings.
- Fecal calprotectin results should be considered an adjunctive target for treatment [13].
- IB*Doc*® testing should be performed by users ages 12 and above only.
- Fecal calprotectin levels in newborns and young children can be significantly increased [16, 17].
- It is recommended that fecal calprotectin testing in patients below the age of 18 should be performed under parental supervision.
- Patients that are on or have been on continuous NSAID medication (i.e., Aspirin®, Ibuprofen, Aleve®, Excedrin®) may have elevated fecal calprotectin values and specimens from these patients should not be tested or used as part of the diagnostic interpretation.
- Only validated smartphone models can be used with the IB*Doc*® (more information available on www.ibdoc.net).
- An additional control of the test strip image for any abnormalities is recommended when assessing IB*Doc*® test results. Please refer to fig. 6 p. 18.

RESULT INTERPRETATION

The following IB*Doc*® result categories reflect condensed knowledge of published cut-offs and, specifically, clinical performance studies for BÜHLMANN fCAL tests (please refer to section: summary of clinical literature). Thresholds can be classified as a color code or as a value:

- Normal: Fecal calprotectin levels below 100 µg/g can reliably indicate patients, with low risk of clinical relapse, in endoscopic remission. Invasive endoscopic procedures can be avoided in these patients [1-7,9-13].
- Moderate: Fecal calprotectin levels between 100-300 µg/g may indicate the necessity of tighter control in the following period to assess disease development tendencies.
- High: Fecal calprotectin levels above 300 µg/g should be repeated and, if raised levels are confirmed, prompt further investigative procedures [1-7,9-13].

The above IB*Doc*® result categories are default settings and may be adjusted. It is advised that healthcare practitioners verify the default thresholds by determining the patient's baseline calprotectin level during disease remission.

A false negative result for a patient with endoscopic inflammation, that is a calprotectin result in a green category that should appear red, is highly unlikely. However it is important that the patient remains under the care of a healthcare practitioner and reports any clinical symptoms to prevent delay of appropriate clinical decisions and treatment, should a false negative result occur.

Studies have shown that high calprotectin levels, above 300 µg/g, will not always indicate development of a clinical relapse. High calprotectin levels should be treated as a red flag signal and repeated. Confirmation of raised levels should prompt further investigative procedures.

Correlation of calprotectin levels and the inflammatory state of patients' intestinal mucosa, according to endoscopic evaluations, were determined in three independent studies using BÜHLMANN fCAL tests.

	Study 1 (Spain) [Ref. 1]	Study 2 (Spain) [Ref. 2]	Study 3 (Australia, New Zealand) [Ref. 3]	Study 4 (UK) [Ref. 4]
Patient number and demographics	89 (CD[1]) Ages 32-58 44 % male	123 (UC[2]) Ages: 18-85 66.4 % male	99 (CD[1] after resection) Ages: 29-47[3] 46.5 % male	26 (UC[2]) Ages: 32-49[3] 44 % male
Calprotectin level chosen as the decision point	272 µg/g	280 µg/g	100 µg/g	187 µg/g
% of patients with values below the decision point in endoscopic remission[4]	98 %	86 %	91 %	100 %
% of patients with values above decision point with endoscopic relapse[5]	76 %	80.3 %	53 %	72 %

Table 1: Correlation of calprotectin levels with IBD disease activity determined by endoscopic evaluations. Results for studies 1 and 2 were obtained with BÜHLMANN lateral flow assays (Quantum Blue® fCAL and Quantum Blue® fCAL high range). Results in study 3 were obtained with the BÜHLMANN fCAL® ELISA. Results in study 4 were obtained with IB*Doc*®.

[1] CD = Crohn`s disease patients,
[2] UC = Ulcerative Colitis patients,
[3] Interquartile range (IQR),
[4] Negative predictive values (true negative / (true negative + false negative)),
[5] Positive predictive values (true positive / (true positive + false positive)).

The diagnostic value of calprotectin in predicting clinical remission and relapse, according to patients symptoms, clinical activity indices, unplanned need for therapy escalation, hospitalization or emergency was determined in three studies using BÜHLMANN fCAL tests.

	Study 5 (UK) [Ref. 5]	Study 6 (Spain) [Ref. 6]	Study 7 (Spain) [Ref. 7]
Number of patients in a study	92 (CD[1]) 38 % male	30 (CD[1]) adalimumab therapy Ages: 24-64 43.3 % male	33 (CD[1]) 20 (UC[2]) infliximab therapy Ages: 18-68 47.2 % male
Follow-up time after calprotectin measurement	12 months	4 months	12 months
Calprotectin level chosen as decision point	240 µg/g	204 µg/g	160 µg/g
% of patients with values below decision point in clinical remission[3]	96.8 %	100 %	96.1 %
% of patients with values above decision point in clinical relapse[4]	27.6 %	75 %	68.7 %

Table 2: Results for study 5 were obtained with the BÜHLMANN fCAL® ELISA. Results for studies 6 and 7 were obtained with BÜHLMANN lateral flow assays (Quantum Blue® fCAL and Quantum Blue® fCAL high range).

[1] CD = Crohn`s disease patients
[2] UC = Ulcerative Colitis patients
[3] Negative predictive values (true negative / (true negative + false negative))
[4] Positive predictive values (true positive / (true positive + false positive)).

USER PERFORMANCE EVALUATION

Sixty-one (61) patients diagnosed with ulcerative colitis (n=29) or Crohn's disease (n=32), according to classical criteria (66% female, mean age (± SD): 36 years (±11.8)) were enrolled at three study sites and provided a single stool specimen.[8]

IB*Doc*® test results were generated by the patients with their own smartphones as well as by health-care providers (HCP) at the clinical sites using two different smartphone models, running the Android and iOS version of the IB*Doc*® app software, respectively. IB*Doc*® results (patients' and HCPs' results) were compared to reference calprotectin values from the same sample. To establish reference values multiple measurements of three independent CALEX® Cap stool preparations were performed with the BÜHLMANN fCAL® ELISA in a laboratory.

Fifty-one (51) patients had both patients' IB*Doc*® results and laboratory ELISA reference measurements available for comparison. None of the 51 patients obtained a false-positive result (red → green) or false-negative (green → red) result. The total within-result category agreement achieved by patients was 88% (see table 3). All 61 CALEX® Valve extracts prepared by the patients and measured with an ELISA could be compared with the HCPs' IB*Doc*® measurement. A total agreement of 89% was obtained by HCPs. (To optimize test performance, please refer to the critical steps in the test procedure section).

IB*Doc*® Result Patient

ELISA Reference	Normal ≤100	Moderate 101-299	High ≥300	TA/Total
Normal ≤100	**19**	2	0	19/21
Moderate 101-299	3	**8**	1	8/12
High ≥300	0	0	**18**	18/18
TA/Total	19/22	8/10	18/19	**45/51**

Total Target Agreement **88%**

Table 3: Agreement of results obtained by patients using IB*Doc*® with laboratory reference measurements (BÜHLMANN fCAL® ELISA) from the same stool sample. TA= Target Agreement

Out of the 61 patients performing the IB*Doc*®, 97% (59 of 61) positively responded that they had understood the instruction in the app. Seventy-nine (79) percent (48 of 61) of patients agreed that the IB*Doc*® was easy to use. Eighty-five (85) percent (52 of 61) of patients agreed that they were willing use the home kit in the future.

METHOD COMPARISON

The method comparison study was performed according to the CLSI guideline EP09-A3. At least one-hundred and forty-five (145) clinical samples were measured, according to the instructions for use, with the IB*Doc*® Calprotectin Kit on two smartphones with different operating systems, one Android and one iOS based, and with the BÜHLMANN fCAL® turbo assay. Measurements were performed over eight (8) days using three (3) IB*Doc*® Calprotectin Kit lots (table 4).

Description	Read out	N	Bias at clinical decision point (95%CI)	
			100 µg/g	300 µg/g
IB*Doc*® Calprotectin Kit **vs.** BÜHLMANN fCAL® turbo	iOS	148	-10.8% (-13.6%, -2.7%)	-12.2% (-16.0%, -6.7%)
	Android	145	8.3% (0.6%, 14.5%)	7.1% (-2.5%, 15.5%)

Table 4: Bias of IB*Doc*® measurements at clinical decision points when compared to reference calprotectin values obtained with the BÜHLMANN fCAL® turbo test. IB*Doc*® measurements were performed with three test cassette lot and two smartphone models. (n= number of samples, CI: Confidence interval)

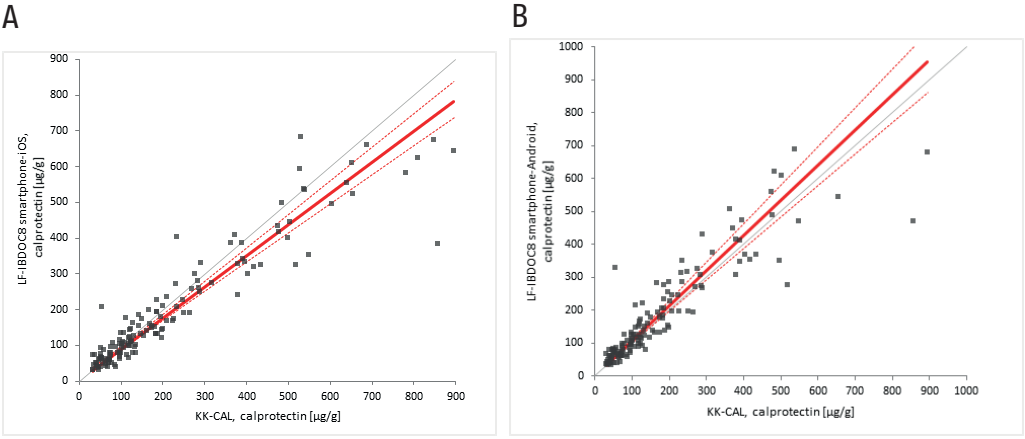


Figure 7: Scatter plot of IB*Doc*® with smartphone-iOS (A) or smartphone-Android (B) and BÜHLMANN fCAL® turbo (KK-CAL) measurements with Passing-Bablok regression analysis. The Passing-Bablok linear fit is depicted as a red solid line. 95% confidence intervals are indicated by red dotted lines.

RECOVERY

Eight (8) stool specimen extracts from clinical left-over stool samples were spiked with 60.2 µg/g and 120.4 µg/g calprotectin from calibrator material. „Baseline" samples were spiked with corresponding amount of extraction buffer. "Baseline" and „baseline + spike" samples were measured in thirteen (13) replicates using one IB*Doc*® Calprotectin Kit lot and two smartphones with different operating systems, one Android and one iOS based. The results fullfilled the acceptance criterion of 70-130% recovery (table 5).

	Sample No.	Level 1	Level 2		Level 3	Level 4	Level 5		Level 6
smartphone A - iOS	**Sample target value [µg/g]**	<50	50-80		80-110	110-150	150-325		>325
	Baseline [µg/g]	46.9	58.6	61.9	81.7	123.3	151.7	237.1	543.0
	Spike value [µg/g]	60.2	60.2	60.2	60.2	60.2	120.4	120.4	120.4
	Expected (baseline+spike) [µg/g]	107.1	118.8	122.1	141.9	183.5	272.1	357.5	663.4
	Observed (baseline+spike) [µg/g]	114.0	130.5	129.4	149.4	192.8	302.4	375.0	611.9
	% Total recovery	106.5	109.8	106.1	105.3	105.1	111.1	104.9	92.2

	Sample No.	Level 1	Level 2		Level 3	Level 4	Level 5		Level 6
smartphone B - Android	**Sample target value [µg/g]**	<50	50-80		80-110	110-150	150-325		>325
	Baseline [µg/g]	57.5	66.9	65.6	86.5	134.8	176.6	271.7	634.6
	Spike value [µg/g]	60.2	60.2	60.2	60.2	60.2	120.4	120.4	120.4
	Expected (baseline+spike) [µg/g]	117.7	127.1	125.8	146.7	195.0	297.0	392.1	755.0
	Observed (baseline+spike) [µg/g]	122.8	142.9	149.4	159.9	217.4	346.9	434.9	721.0
	% Total recovery	104.4	112.5	118.8	109.0	111.5	116.8	110.9	95.5

Table 5: IB*Doc*® recovery results obtained with one test cassette lot, analyzed with smartphones A and B, running the iOS/ the Android version of the IB*Doc*® app respectively.

PRECISION

Within-laboratory precision: ≤30% CV

Within-laboratory precision of the IB*Doc*® Calprotectin Kit was established according to the CLSI guideline EP05-A3. Measurements were performed using two smartphone models with different operating systems, one Android and one iOS based, on one (1) test cassette lot and six (6) extracted stool samples with calprotectin concentrations covering the measuring range of the assay and clinical decision points.
Testing was done over twenty (20) nonconsecutive days, in two (2) runs with two (2) replicates per run/sample. Acceptance criteria for repeatability and within-laboratory precision (≤30% CV and ≤35% CV for samples with calprotectin concentrations ≤300 µg/g and >300 µg/g, respectively) were fulfilled for all samples (table 6).

	Within-Laboratory Precision (smartphone A - iOS)				Within-Laboratory Precision (smartphone B - Android)			
	N	Mean [µg/g]	SD [µg/g]	%CV	N	Mean [µg/g]	SD [µg/g]	%CV
1	79	39.2	11.8	30.0	77	47.5	9.1	19.1
2	80	76.9	19.9	25.9	80	82.6	17.1	20.8
3	80	129	33.8	26.1	77	136	23.0	17.0
4	79	218	52.9	24.3	80	227	47.8	21.0
5	80	353	81.1	23.0	80	388	77.0	19.8
6	80	528	125.4	23.7	80	579	142.8	24.6

Table 6: Within-laboratory precision data of IB*Doc*® results analyzed with smartphones A and B, running the iOS/ the Android version of the IB*Doc*® app respectively.

PRECISION

Lot-to-Lot Reproduciblity: ≤30% CV

Reproducibility of IB*Doc*® Calprotectin Kit was established according to the CLSI guideline EP05-A3. Measurements were performed using two smartphone models with different operating systems, one Android and one iOS based, using three (3) different test cassette lots and six (6) extracted stool samples with calprotectin concentrations covering the measuring range of the assay and clinical decision points. Testing was done over five (5) days, in one (1) run with five (5) replicates per run/sample. Acceptance criteria for lot-to-lot reproducibility (≤30% CV and ≤35% CV for samples with calprotectin concentrations ≤300 µg/g and >300 µg/g, respectively) were fulfilled for all samples (table 7).

	Reproducibility (smartphone A - iOS)				Reproducibility (smartphone B - Android)			
	N	Mean [µg/g]	SD [µg/g]	%CV	N	Mean [µg/g]	SD [µg/g]	%CV
1	74	44.6	11.0	24.7	75	55.3	12.5	22.5
2	75	89.3	22.3	24.9	75	95.4	23.4	24.5
3	75	154	37.1	24.1	75	162	35.1	21.6
4	75	228	48.0	21.0	74	253	50.8	20.1
5	75	383	85.9	22.4	75	430	87.0	20.2
6	75	577	135	23.5	75	655	151	23.0

Table 7: Reproducibility data of IB*Doc*® results analyzed with smartphones A and B, running the iOS/ the Android version of the IB*Doc*® app respectively.

STOOL PREPARATION / EXTRACTION REPRODUCIBILITY

Between-extraction precision: 0.0% - 14.5% CV
Extraction reproducibility: 15.6% - 29.3% CV

The stool preparation / extraction reproducibility study was established according to the CLSI guideline EP05-A3. Eight (8) clinical stool specimens, selected to reflect different stool consistencies: solid, semi-solid and liquid, with calprotectin concentrations covering the measuring range of the test and clinical decision points, were extracted two times with each of three CALEX® Cap lots by two operators on two days. Each stool extract was tested in three (3) replicates using one (1) reagent lot of the IB*Doc*® Calprotectin Kit and two different smartphone models, one iOS and one Android based.

Sample	N	Mean Conc. [µg/g]	Within-extraction (within-run)		Between-extraction		Between-day		Between-lot		Between-operator		Total	
			SD [µg/g]	CV [%]	SD [µg/g]	CV [%]	SD [µg/g]	CV [%]	SD [µg/g]	CV [%]	SD [µg/g]	CV [%]	SD [µg/g]	CV [%]
1	72	43.7	8.0	18.4	0.0	0.0	5.6	12.9	0.0	0.0	0.0	0.0	9.8	22.5
2	72	54.5	9.1	16.7	7.8	14.4	2.9	5.3	0.0	0.0	5.2	9.5	13.4	24.6
3	72	77.7	11.3	14.6	9.2	11.8	0.0	0.0	4.1	5.3	0.0	0.0	15.1	19.5
4	72	158.5	30.7	19.3	0.0	0.0	14.0	8.8	0.0	0.0	5.3	3.3	34.1	21.5
5	72	180.8	25.5	14.1	26.3	14.5	0.0	0.0	11.0	6.1	16.3	9.0	41.6	23.0
6	72	277.5	52.1	18.8	31.1	11.2	0.0	0.0	36.0	13.0	40.4	14.6	81.3	29.3
7	72	587.2	104.4	17.8	44.2	7.5	26.3	4.5	15.6	2.6	0.0	0.0	117.5	20.0
8	72	619.8	100.6	16.2	52.9	8.5	54.6	8.8	0.0	0.0	0.0	0.0	126.1	20.3

Table 8: Within-extraction, between-extraction, between-day, between-lot, between-operator and total results from extraction reproducibility study with IB*Doc*® Calprotectin Kit and iOS based smartphone as readout.

Sample	N	Mean Conc. [µg/g]	Within-extraction (within-run)		Between-extraction		Between-day		Between-lot		Between-operator		Total	
			SD [µg/g]	CV [%]	SD [µg/g]	CV [%]	SD [µg/g]	CV [%]	SD [µg/g]	CV [%]	SD [µg/g]	CV [%]	SD [µg/g]	CV [%]
1	72	52.3	7.1	13.5	0.0	0.0	5.4	10.3	0.0	0.0	0.0	0.0	8.9	17.0
2	72	64.6	10.3	16.0	8.7	13.4	6.4	10.0	0.0	0.0	0.0	0.0	14.9	23.1
3	71	90.6	15.0	16.5	9.9	10.9	5.6	6.2	4.9	5.4	7.0	7.7	20.6	22.8
4	72	183.0	33.1	18.1	6.7	3.6	11.3	6.2	0.0	0.0	7.7	4.2	36.4	19.9
5	72	214.7	28.1	13.1	31.1	14.5	0.0	0.0	7.5	3.5	23.7	11.0	48.7	22.7
6	72	330.1	56.8	17.2	35.2	10.7	24.0	7.3	39.7	12.0	23.7	7.2	84.8	25.7
7	72	679.5	83.9	12.4	51.4	7.6	38.6	5.7	0.0	0.0	8.8	1.3	106.1	15.6
8	72	720.6	87.8	12.2	42.9	6.0	61.6	8.6	0.0	0.0	0.0	0.0	115.5	16.0

Table 9: Within-extraction, between-extraction, between-day, between-lot, between-operator and total results from extraction reproducibility study with IB*Doc*® Calprotectin Kit and Android based smartphone as readout.

SENSITIVITY

LIMIT OF BLANK (LOB)
LIMIT OF DETECTION (LOD)
LIMIT OF QUANTITATION (LOQ)

The LoD was established according to the CLSI guideline EP17-A2 using the classical approach, parametric analysis and a LoB <20 µg/g, determined using the non-parametric analysis.

The LoQ was established according tot the CLSI guideline EP17-A2, based on 90 determinations and a precision goal of 30% CV.

All IB*Doc*® results obtained in the sensitivity study were analyzed using two different smartphone models, one running the Android and one the iOS versions of the the IB*Doc*® app, respectively. The results of LoB, LoD and LoQ studies for IB*Doc*® are summarized in table 10.

Smartphone	Lot	Limit of Blank (LoB)	Limit of Detection (LoD)	Limit of Quantitation (LoQ)
Android	1	**6.0 µg/g**	12.5 µg/g	19.5 µg/g
Android	2	5.0 µg/g	**13.2 µg/g**	22.9 µg/g
iOS	1	0.0 µg/g	7.8 µg/g	17.0 µg/g
iOS	2	0.0 µg/g	8.9 µg/g	**23.4 µg/g**

Table 10: Limit of Blank, Limit of Detection and Limit of Quantitation values obtained with two different smartphone models, one iOS and one Android based and two test cassette lots.

LINEARITY

Linear range: 30 - 700 µg/g

The linear range of the IB*Doc*® Calprotectin Kit was determined according to the CLSI guideline EP06-Ed2. For fecal calprotectin the method has been demonstrated to be linear from 30 - 700 µg/g, within an allowable deviation of ±20% / ±15 µg/g in this interval.

Smartphone	Lot	Levels	N	Measuring Interval [µg/g]	Linear regression parameters		
					Intercept	Slope	R^{2*}
iOS	1	1-11	109	16.5 - 1080.3	-11.9	1.11	0.996
	2	1-13	128	21.2 - 997.6	-4.7	0.99	0.975
Android	1	1-8	79	26.0 - 812.3	-4.8	1.24	0.980
	2	1-11	108	25.9 - 817.1	-3.0	1.13	0.995

Table 11: Summary of linearity analyses of test results for two smartphone models, running the iOS/ the Android version of the IB*Doc*® app respectively and two reagent lots. * based on mean results from each level.

HIGH DOSE HOOK EFFECT

Samples with calprotectin concentrations of up to 11.2 x 10^3 µg/g can be measured without limiting the measuring range of the IB*Doc*® Calprotectin Kit. The high dose hook effect study was performed on two independent reagent lots and the concentrations were established based on measurements performed with the BÜHLMANN fCAL® turbo assay.

INTERFERING SUBSTANCES

The susceptibility of the IB*Doc*® Calprotectin Kit to oral pharmaceuticals, nutritional supplements, haemoglobin as well as entheropathological microorganisms was assessed according to CLSI guideline EP07-A3. Bias in results exceeding 30% was considered interference. No interference was detected with listed substances, in table 12, up to the indicated concentrations. No interference was detected with entheropathological microorganisms, listed in table 13, up to the indicated amounts of colony forming units (CFU) per mL of stool specimen extract.

Trade name (Substance)	**Target concentration (mg/50 mg stool)**
Duofer Fol (Iron (II) sulfate)	0.11
Prednisone (Prednisone)	0.31
Imurek (Azathioprine)	0.19
Salofalk (Mesalamine (5-aminosalicylic acid))	5.21
Agopton (Lansoprazole)	0.18
Asacol (Mesalamine (5-aminosalicylic acid))	2.50
Vancocin (Vancomycin)	2.00
Bactrim (Sulfamethoxazole + Trimethoprim)	1.7 + 0.35
Ciproxine (Ciprofloxacin)	1.25
Food supplement	**Target concentration (mg/ 50 mg stool)**
Vitamin E (DL-α Tocopherol Acetate)	0.30
Berocca (Multivitamin)	1.06
Hemoglobin	**Target concentration (mg/50 mg stool)**
Human hemoglobin	1.25

Table 12: Substances and their target concentrations that were tested and show no interference in the IB*Doc*® assay.

Microorganism	**Concentration (CFU/ mL)**
Escherichia coli	2.9×10^7
Salmonella enterica subsp. enterica	8.2×10^7
Klebsiella pneumoniae subsp. pneumonia	4.5×10^7
Citrobacter freundii	5.5×10^7
Shigella flexneri	5.0×10^7
Yersinia enterocolitica subsp. enterocolitica	5.3×10^6

Table 13: Microorganisms that were tested and show no interference in the IB*Doc*® assay.

BIBLIOGRAPHY

1. Lobatón T, López-García A, Rodríguez-Moranta F, et al. A new rapid test for fecal calprotectin predicts endoscopic remission and postoperative recurrence in Crohn's disease. J Crohns Colitis. 2013, 7(12):e641-51.
2. Lobatón Ortega T, Rodríguez-Moranta F, Lopez A, et al. A new rapid quantitative test for fecal calprotectin predicts endoscopic activity in ulcerative colitis. Inflamm Bowel Dis. 2013, 19(5):1034-42.
3. Wright EK, Kamm MA, De Cruz P, et al., Measurement of fecal calprotectin improves monitoring and detection of recurrence of Crohn's disease after surgery. Gastroenterology. 2015, 148(5):938-947.
4. Walsh A, Kormilitzin A, Hinds C, et al. Defining Faecal Calprotectin Thresholds as a Surrogate for Endoscopic and Histological Disease Activity in Ulcerative Colitis-a Prospective Analysis. J Crohns Colitis. 2019 Mar 30;13(4):424-430.
5. Naismith GD, Smith LA, Barry SJ et al. A prospective evaluation of the predictive value of faecal calprotectin in quiescent Crohn's disease. J Crohns Colitis. 2014, 8(9):1022-9.
6. Ferreiro-Iglesias R, Barreiro-de Acosta M, Lorenzo-Gonzalez A et al. Usefulness of a rapid faecal calprotectin test to predict relapse in Crohn's disease patients on maintenance treatment with adalimumab. Scand J Gastroenterol. 2015, 23:1-6.
7. Ferreiro-Iglesias R1, Barreiro-de Acosta M, Otero Santiago M, Lorenzo Gonzalez A, Alonso de la Peña C, Benitez Estevez AJ, Dominguez-Muñoz JE. Fecal calprotectin as Predictor of Relapse in Patients With Inflammatory Bowel Disease Under Maintenance Infliximab Therapy. J Clin Gastroenterol. 2015, 50(2):147-51.
8 . Moore AC, Huang VW, Bourdages R, et al. IBDoc Canadian User Performance Evaluation. Inflamm Bowel Dis. 2019 May 4;25(6):1107-1114.
9. Guardiola J., Lobatón T, Rodríguez-Alonso L, et al. Fecal Level of calprotectin Identifies Histologic Inflammation in Patients with Ulcerative Colitis In Clinical And Endoscopic Remission. Clinical Gastroenterology and Hepatology 2014, 12(11):1865-70.
10. Lasson A, Öhman L, Stotzer PO et al. Pharmacological intervention based on fecal calprotectin levels in patients with ulcerative colitis at high risk of a relapse: A prospective, randomized, controlled study. United European Gastroenterol J. 2015 ;3(1):72-9.
11. Lin JF, Chen JM, Zuo JH et al. Meta-analysis: fecal calprotectin for assessment of inflammatory bowel disease activity. Inflamm Bowel Dis. 2014 Aug;20(8):1407-15.
12. Bressler B, Panaccione R, Fedorak RN, et al. Clinicians' guide to the use of fecal calprotectin to identify and monitor disease activity in inflammatory bowel disease. Can J Gastroenterol Hepatol. 2015, 29(7):369-72.
13. Peyrin-BL, Sandborn W, Sands BE et al. Selecting Therapeutic Targets in Inflammatory Bowel Disease (STRIDE): Determining Therapeutic Goals for Treat-to-Target. Am J Gastroenterol. 2015, 110:1324-38
14. Molander P, Färkkiläb M, Ristimäkic A, et al. Does Fecal calprotectin Predict Short-Term Relapse After Stopping Tnfalpha-Blocking Agents In Inflammatory Bowel Disease Patients In Deep Remission? Journal of Crohn's and Colitis, 2015, 33–40.
15. De Vos M, Dewit O, D'Haens G et al. Consecutive fecal calprotectin measurements to predict relapse in patients with ulcerative colitis receiving infliximab maintenance therapy. Inflamm Bowel Dis. 2013, 9:2111-2117.
16. Fagerberg UL, Lööf L, Myrdal U et al. Colorectal inflammation is well predicted by fecal calprotectin in children with gastrointestinal symptoms. J Pediatr Gastroenterol Nutr. 2005, 40(4):450-5.
17. Kapel N, Campeotto F, Kalach N et al. Faecal calprotectin in term and preterm neonates. J Pediatr Gastroenterol Nutr. 2010 51(5):542-7.

CHANGELOG/ SHIPPING DAMAGE

CHANGELOG

Date	Version	Change
2024-05-27	V7.0	- Adjustment of the linear range to 700µg/g - Update to chapter *Materials and Accessories required, but not provided* - New chapter *Summary of safety and performance* - Update to chapter *Important information* - New figure 5b: IB*Doc*® app profile menu - Additional information in the assay procedure step 1, 2 and 5 - Revision of chapter *Limitations and contraindications* - Revision of chapter *Result interpretation*: - Update to chapter *Summary of clinical literature* - Revision of *Performance characteristics* - Update to chapter *Symbols* including symbols for near-patient testing and for self-testing - Addition of Health Canada Licence number

SHIPPING DAMAGE (INFORMATION INTENDED FOR HCP ONLY)

Please notify your distributor, if this product was received damaged.

INCIDENT REPORTING IN EU MEMBER STATES

INCIDENT REPORTING IN EU MEMBER STATES

If any serious incident in relation to this device has occurred, please report without delay to the manufacturer and competent authority of your Member State.

SYMBOLS

Symbol	Explanation
	Use by
	Consult Instruction for Use
	Temperature limitation
	Batch code
	Order Code
	Manufacturer
	Number of Tests
	Do not re-use
	Translations
	For self-testing
	For near-patient testing

Health Canada Licence: 98903, Device class: 3

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