QDx Instacheck™ Calprotectin

INTENDED USE

QDx Instacheck™ Calprotectin is a fluorescence Immunoassay (FIA) for quantitative determination of Calprotectin (MRP8/14; S100A8/S100A9) in human feces. It is useful as an aid in management and monitoring of the reflex gastrointestinal inflammation caused by several pathologies (inflammatory bowel disease, colorectal cancer and some enteropathies).
For *in vitro* diagnostic use only.

INTRODUCTION

Calprotectin is a cytosolic protein present in neutrophils, whose concentration increases in the stool by Inflammatory Bowel Disease (IBD), specifically Crohn's disease and Ulcerative Colitis. The stability of Calprotectin to degradation keeps it stable in stools for up to seven days at room temperature and much longer periods at -20 °C. Calprotectin inhibits zinc-dependent enzyme systems, as a result, kills microbes and induces apoptosis in normal and cancer cells. In the presence of calcium, calprotectin is significantly resistant to proteolytic degradation and so is stable in stools keeps at room temperature for seven days. The fecal concentration of Calprotectin correlates with the histologic and endoscopic patterns of the intestinal inflammation in IBD patients.

PRINCIPLE

The test uses a sandwich immunodetection method; the detector antibody binds to antigen in the sample, forming antigen-antibody complexes, and migrate onto the nitrocellulose matrix to be captured by the other immobilized-antibody on the test strip.
The more antigen in sample, the more antigen-antibody complex is formed, which lead to a stronger intensity of the fluorescence signal from detector antibodies, which is processed by the instrument for QDx Instacheck™ tests to show the calprotectin concentration in the sample.

COMPONENTS AND REAGENTS

QDx Instacheck™ Calprotectin consists of 'Cartridges', 'Extraction Buffer Tubes', 'ID chip' and 'Instruction for Use'

- The cartridge contains a test strip, the membrane which has anti human calprotectin at the test line, with rabbit IgG at the control line.
- Each cartridge is individually sealed in an aluminum foil pouch containing a desiccant. 25 sealed cartridges are packed in a box which also contains an ID chip.
- The extraction buffer contains bovine serum albumin (BSA), detergent and sodium azide as a preservative in HEPES buffer.
- The extraction buffer is pre-dispensed in an extraction tube. 25 extraction buffer tubes are packaged in the cartridge box.

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use only.
- Follow the instructions and procedures described in this 'Instruction for use'.
- Use only fresh samples and avoid direct sunlight.
- There should be no contamination with urine or water in samples.
- Samples should not be taken during menstruation, hemorrhoids or when using rectal medications.
- Lot numbers of all the test components (cartridge, ID chip and extraction buffer) must match each other.
- Do not interchange the test components between different lots or use the test components after the expiration date, either of which might yield misleading of test result(s).
- Do not reuse cartridges or extraction buffer tubes. An extraction buffer tube should be used for processing of one sample only. A cartridge should be used for testing one sample only.
- The cartridge should remain sealed in its original pouch before use. Do not use the cartridge, if is damaged or already opened.
- For shipping, samples must be packed in accordance with the local regulations.
- Allow the cartridge, extraction buffer and sample to be at room temperature for approximately 30 minutes.
- The instrument for QDx Instacheck™ tests may generate slight vibration during use.
- Used extraction buffer tubes, pipette tips and cartridges should be handled carefully and discarded by an appropriate method in accordance with relevant local regulations.
- An exposure to larger quantities of sodium azide may cause certain health issues like convulsions, low blood pressure and heart rate, loss of consciousness, lung injury and respiratory failure.

- **QDx Instacheck™ Calprotectin** will provide accurate and reliable results subject to the bellow conditions.
- **QDx Instacheck™ Calprotectin** should be used only in conjunction with instrument for QDx Instacheck™ test.

STORAGE AND STABILITY

Storage condition		
Component	Storage Temperature	Shelf life
Cartridge	4 - 30 °C.	20 months
extraction buffer	4 - 30 °C.	20 months

- After the cartridge pouch is opened, the test should be performed immediately.

LIMITATIONS OF THE TEST SYSTEM

- The test may yield false positive result(s) due to the cross-reactions and/or non-specific adhesion of certain sample components to the capture/detector antibodies.
- The test may yield false negative result(s) due to the non-responsiveness of the antigen to the antibodies which is most common if the epitope is masked by some unknown components, so therefore not being able to be detected or captured by the antibodies. The instability or degradation of the antigen with time and/or temperature may also cause false negative result as it makes antigen unrecognizable by the antibodies.
- Other factors may interfere with the test and cause erroneous results, such as technical/procedural errors, degradation of the test components/reagents or presence of interfering substances in the test samples.
- Any clinical diagnosis based on the test result must be supported by a comprehensive judgment of the concerned physician including clinical symptoms and other relevant test results.

MATERIALS SUPPLIED

REF IFPC-36

Components of **QDx Instacheck™ Calprotectin**

- **Cartridge Box:**
  - Cartridges 25
  - Extraction buffer tube 25
  - ID Chip 1

- Instruction for Use 1

MATERIALS REQUIRED BUT SUPPLIED ON DEMAND

Following items can be purchased separately from **QDx Instacheck™ Calprotectin**.

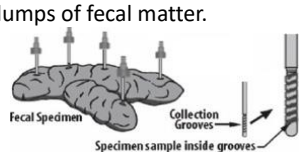
Please contact our sales division for more information.

- **QDx Instacheck™ II** REF FPRR021
- **QDx Instacheck™ reader** REF FR203
- **Printer** REF FPRR007
- **Boditech Calprotectin Control** REF CFPO-211

SAMPLE COLLECTION AND PROCESSING

The sample type for QDx Instacheck™ Calprotectin is human feces.

- Invert an extraction buffer tube and loosen the cap which is attached a sampling stick (yellow color).
- Introduce the sampling stick into the fecal sample six times at different sites. In order to get sampling even in the spirals of the stick and to ensure appropriate specimen to buffer ratio, try to avoid obtaining clumps of fecal matter.



- Return the stick to the extraction buffer tube. Tighten the cap thoroughly and shake the tube vigorously so as to disperse the specimen throughout the extraction buffer in the tube
- If not to be used immediately after addition of fecal sample, extraction buffer tube should be refrigerated but must be analyzed using the test cartridge within 7 days.
- The collected specimen should be tested as soon as possible but may be held up to 24 hours at 2-8 °C prior to testing. If testing will be delayed more than this time frame, samples should be frozen at -20 °C.
- Samples stored frozen at -20 °C for 6 months showed no performance difference.
- Repeated freezing and thawing can result in the change of test values.

TEST SETUP

- Check the components of **QDx Instacheck™ Calprotectin**: Sealed Cartridge, Extraction Buffers tubes and ID Chip.
- Ensure that the lot number of the cartridge matches with that of the ID chip as well as that on the Extraction Buffer.
- Keep the sealed cartridge (if stored in refrigerator) and the extraction buffer at room temperature for at least 30 minutes just prior to the test. Place the cartridge on a clean, dust-free and flat surface.
- Turn on the instrument for QDx Instacheck™ tests.
  (Please refer to the 'Instrument for QDx Instacheck™ test Operation Manual' for complete information and operating instructions.)

TEST PROCEDURE

1) Collect sample according to the sample collection method using a sampling stick in the 'sample collection and processing'.
2) Break off the black tip on the outside of the black cap.
3) Discard 3 drops of reagent onto the paper towel before applying to the cartridge.
4) Hold the vial upside down and transfer 3 drops of the sample mixture and load it into the sample well on the cartridge.
5) Leave the sample-loaded cartridge at room temperature for 10 minutes.
   ⚠ *Scan the sample-loaded cartridge immediately when the incubation time is over. If not, it will cause inexact test result.*
6) To scan the sample-loaded cartridge, insert it into the cartridge holder of the instrument for QDx Instacheck™ tests. Ensure proper orientation of the cartridge before pushing it all the way inside the cartridge holder. An arrow has been marked on the cartridge especially for this purpose.
7) Tap 'Start' button on the instrument for QDx Instacheck™ tests to start the scanning process.
8) Instrument for QDx Instacheck™ tests will start scanning the sample-loaded cartridge immediately.
9) Read the test result on the display screen of the instrument for QDx Instacheck™ tests.

INTERPRETATION OF TEST RESULT

- Instrument for QDx Instacheck™ tests calculates the test result automatically and displays hemoglobin concentration of the test sample in terms of mg/kg.
- The cut-off (reference value): 50 mg/kg
- The Reference value:

Value	Interpretation
<50 mg/kg	Negative
50 – 100 mg/kg	Borderline area, to be repeated (within 4-6 weeks)
>100 mg/kg	Positive

- In case of a positive result (above 50 mg/kg), consult a physician to discuss the test result. The physician may decide further course of action.
- Working range: 10-1000 mg/kg

QUALITY CONTROL

- Quality control tests are a part of the good testing practice to confirm the expected results and validity of the assay and should be performed at regular intervals.
- The control tests should be performed immediately after opening a new test lot to ensure the test performance is not altered.
- Quality control tests should also be performed whenever there is any question concerning the validity of the test results.
- Control standards are not provided with QDx Instacheck™ Calprotectin. For more information regarding obtaining the control standards, contact the technical section at Diasys Diagnostics India Private Limited.
  (Please refer to the instruction for use of control material.)

PERFORMANCE CHARACTERISTICS

- **Analytical sensitivity**

Limit of Blank (LoB)	2.91mg/kg
Limit of Detection (LoD)	4.35 mg /kg
Limit of Quantification(LoQ)	10 mg /kg

- **Analytical specificity**
  - **Cross-reactivity**

There was no significant cross-reactivity from materials listed below with the **QDx Instacheck™ Calprotectin test** measurements.

Cross-reactivity materials	Concentration
Helicobacter pylori	1.2 x 10^8 CFU/mL
Campylobacter jejuni	1.2 x 10^8 CFU/mL
Candida albicans	1.2 x 10^8 CFU/mL
Enterobacter cloacae	1.2 x 10^8 CFU/mL
Escherichia coli	1.2 x 10^8 CFU/mL
Pseudomonas aeruginosa	1.2 x 10^8 CFU/mL

- Interference

  There was no significant Interference from these materials with the **QDx Instacheck™ Calprotectin** test measurements.

Interference materials	Concentration
Human hemoglobin	2,000 µg/mL
Transferrin	4,000 mg/mL
Prednisolone	8.31 µmol/L
Ciprofloxacin	30.2 µmol/L
Stearic acid	0.4 mmol/L
Palmitic acid	6 mmol/L
Metronidazole	701 µmol/L
Vancomycin	69 µmol/L

■ **Precision**

- Between lot

  One person tested three different lots of **QDx Instacheck™ Calprotectin,** five times at each concentration of the control standard.

- Between person

  Three different persons tested one lot of **QDx Instacheck™ ™ Calprotectin**, five times at each concentration of the control standard.

- Between day

  One person tested **QDx Instacheck™ Calprotectin** for five days, five times at each concentration of the control standard.

- Between site

  One person tested one lot of **QDx Instacheck™ Calprotectin** at three different sites, five times at each concentration of the control standard.

Calprotectin. (mg/kg)	Between-lot		Between-person	
	AVG	CV(%)	AVG	CV(%)
10	9.7	10.04	9.86	10.9
50	48.5	5.69	50.16	6.56
500	495.63	3.66	493.26	3.37
Calprotectin. (mg/kg)	Between-day		Between-site	
	AVG	CV(%)	AVG	CV(%)
10	9.78	5.57	9.95	9.02
50	49.52	1.87	50.22	6.27
500	498.33	3.44	501.59	3.1

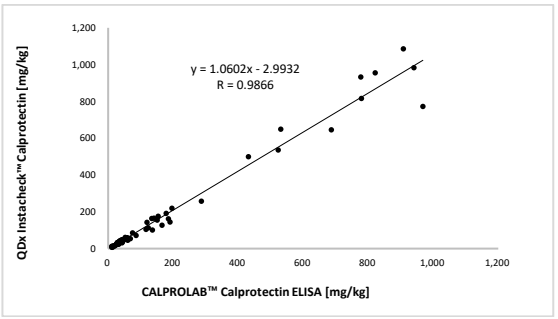
■ **Accuracy**

The accuracy was confirmed by 3 different lots testing ten times each different concentration.

Calprotectin. (mg/kg)	Lot 1	Lot 2	Lot 3	AVG	Recovery (%)
10	10.47	9.46	9.70	9.88	99%
50	49.73	50.49	51.04	50.42	101%
500	493.96	500.90	506.58	500.48	100%

■ **Comparability**

Calprotectin concentrations of 60 clinical samples were quantified independently with **QDx Instacheck™ Calprotectin** and CALPROLAB™ Calprotectin ELISA (Calpro AS, Norway) as per prescribed test procedures. Test results were compared and their comparability was investigated with linear regression and coefficient of correlation (R). Linear regression and coefficient of correlation between the two tests were Y= 1.0602x -2.9932 and R = 0.9866 respectively.



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Note: Please refer to the table below to identify various symbols

Σ	Sufficient for <n> tests
	Read instruction for use
	Use by Date
LOT	Batch code
REF	Catalog number
	Caution
	Manufacturer
EC REP	Authorized representative of the European Community
IVD	In vitro diagnostic medical device
	Temperature limit
	Do not reuse
CE	This product fulfills the requirements of the Directive 98/79/EC on in vitro diagnostic medical devices

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