QDx Instacheck™ Calprotectin

INTENDED USE

QDx Instacheck™ Calprotectin is a fluorescence immunoassay (FIA) for the 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 antibodies in buffer bind to antigens in the sample, forming antigen-antibody complexes, and migrate onto nitrocellulose matrix to be captured by the other immobilized streptavidin on a test strip.

More antigens in the sample will form more antigen-antibody complexes which lead to stronger fluorescence signal by detector antibodies, which is processed by the instrument for QDx Instacheck™ tests to show calprotectin concentration in the sample.

COMPONENTS AND REAGENTS

QDx Instacheck™ Calprotectin consists of 'cartridges' and 'extraction buffer tubes'.

- The cartridge contains the membrane called a test strip, which has anti-calprotectin at the test line, and rabbit IgG at the control line. All cartridges are individually sealed in an aluminum foil pouch containing a desiccant, and they are further packaged in a box.
- The extraction buffer contains sodium azide in HEPES as a preservative. It is pre-dispensed in extraction buffer tubes. The extraction buffer tubes are packed in a box.

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use only.
- Follow the instructions and procedures described in this 'Instructions for use'.
- Use only fresh samples and avoid direct sunlight.
- Samples should not be taken during menstruation, hemorrhoids or when using rectal medications.
- There should be no contamination with urine or water in samples.
- Lot numbers of all the test components (cartridge, extraction buffer tube and ID chip) must match with 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 incorrect test result(s).
- Do not reuse cartridges or extraction buffer tubes. A cartridge should be used for testing one sample only. An extraction buffer tube should be used for processing of one sample only.
- The cartridge should remain sealed in its original pouch until just before use. Do not use cartridge, if pouch is damaged or has already been opened.
- For shipping, samples must be packed in accordance with local regulations.
- If test components and/or sample are stored in refrigerator, then allow cartridge, extraction buffer tube and sample to be at room temperature for approximately 30 minutes before use.
- The instrument for QDx Instacheck™ tests may generate slight vibration during use.
- Used cartridges, extraction buffer tubes and pipette tips should be handled carefully and discarded by an appropriate method in accordance with relevant local regulations.
- The extraction buffer tube contains sodium azide (NaN_3), and it may cause certain health issues like convulsions, low blood pressure, low heart rate, loss of consciousness, lung injury and respiratory failure. Avoid contact with skin, eyes, and clothing. In case of contact, rinse immediately with running water.
- **QDx Instacheck™ Calprotectin** will provide accurate and reliable results subject to the below conditions.
- **QDx Instacheck™ Calprotectin** should be used only in conjunction with the instrument for QDx Instacheck™ tests.

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 antigens to the antibodies which is the 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 antigens with time and/or temperature may also cause false negative result as it makes the antigens 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 in conjunction with clinical symptoms and other relevant test results.

STORAGE AND STABILITY

Storage condition			
Component	Storage Temperature	Shelf life	Note
Cartridge	2- 30 °C	20 months	Disposable
Extraction buffer tube	2 - 30 °C	20 months	Disposable

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

MATERIALS SUPPLIED

REF **IFPC-36**

Components of **QDx Instacheck™ Calprotectin**

- Cartridge Box:
  - Cartridge 25
  - Extraction buffer tube 25
  - ID chip 1
  - Instruction for use 1

MATERIALS REQUIRED BUT SUPPLIED ON DEMAND

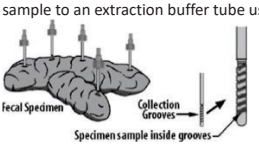
Following items can be purchased separately from **QDx Instacheck™ Calprotectin**. Please contact our sales division for more information.

- **QDx Instacheck™-II** REF FPRR039

SAMPLE COLLECTION AND PROCESSING

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

- Collect the sample feces in a clean and dry container.
- Invert an extraction buffer tube and loosen the cap where the sampling stick (yellow color) is attached.
- Poke the sampling stick into the fecal sample about 5 to 6 times at different sites. Whilst collecting the sample with the sampling stick, make sure to exclude large solid lumps.
  (In case the fecal matter is in liquid form, transfer 10 µL of the sample to an extraction buffer tube using a pipette.)



- Return the stick to the extraction buffer tube. Tighten the cap thoroughly and shake the tube vigorously around 10 times so as to disperse the specimen throughout the extraction buffer in the tube.
- Sample (feces) storage periods are as below:
  - Sample (feces) stored at room temperature showed no performance difference for 4 hours.
  - Sample (feces) stored at refrigerator (2~8°C) showed no performance difference for 72 hours.
  - Sample (feces) stored at freezer (-20°C) showed no performance difference for 8 weeks.
- The sample mixture storage periods in extraction buffer tube are as below:
  - The sample mixture in an extraction buffer tube stored at room temperature showed no performance difference for 7 days.
  - The sample mixture in an extraction buffer tube stored at refrigerator (2~8°C) showed no performance difference for 10 days.
- However, it is recommended to use the sample mixture in the extraction buffer on the same day after sampling.
- The storage period may vary depending on the condition and type of feces.
- As a repeated freeze-thaw cycle may affect the test result, do not refreeze previously frozen sample.

TEST SETUP

- Check the contents of **QDx Instacheck™ Calprotectin**: Sealed cartridges, extraction buffer tubes, an ID chip and an instructions for use.
- Ensure that the lot number of the cartridge matches that of the extraction buffer tube as well as an ID chip.
- If the sealed cartridge and the extraction buffer tube have been stored in a refrigerator, place them on a clean and flat surface at room temperature for at least 30 minutes before testing.
- Turn on the instrument for QDx Instacheck™ tests.
- Insert the ID chip into the 'ID chip port'.

※ **Please refer to the Instrument for QDx Instacheck™ tests operation manual for complete information and operating instructions.**

TEST PROCEDURE

<Multi test mode>

1. Collect sample using a sampling stick according to the sample collection method described in the 'sample collection and processing'.
2. Shake the assembled extraction buffer tube about 10 to 15 times.
3. Break off the black tip on the outside of the black cap.
4. Discard 5 drops of reagent onto the paper towel before applying to the cartridge.
5. Hold the tube upside down and apply 3 drops of the sample mixture and load it into the sample well on the cartridge.
6. 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 inaccurate test result.*

7. 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 is marked on the cartridge especially for this purpose.
8. Tap the 'Start' button on the instrument for QDx Instacheck™ tests.
9. The instrument for QDx Instacheck™ tests will start scanning the sample-loaded cartridge immediately.
10. Read the test result on the display screen of the instrument for QDx Instacheck™ tests.

<Single test mode>

1. The test procedure is same with 'Multi test mode 1)-5)'.
2. Insert the sample-loaded cartridge into the 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 is marked on the cartridge especially for this purpose.
3. Tap the 'Start' button on the instrument for QDx Instacheck™ tests.
4. The cartridge goes inside the instrument for QDx Instacheck™ tests and will automatically start scanning the sample-loaded cartridge after 10 minutes.
5. Read the test result on the display screen of the instrument for QDx Instacheck™ tests.

INTERPRETATION OF TEST RESULT

- The instrument for **QDx Instacheck™** tests calculates the test result automatically and displays calprotectin concentration of the test sample in terms of mg/kg.
- Cut-off: 50 mg/kg
- 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

- Working range: 10 – 1,000 mg/kg

PERFORMANCE CHARACTERISTICS

- **Analytical sensitivity**
  - Limit of Blank (LoB) 2.475 mg/kg
  - Limit of Detection (LoD) 4.76 mg/kg
  - Limit of Quantitation (LoQ) 10 mg/kg

- **Analytical specificity**
  - Cross reactivity

    Biomolecules listed in the following table were added to the test sample(s) at concentrations much higher than their normal physiological levels in the feces. **QDx Instacheck™ Calprotectin** test results did not show any significant cross-reactivity with these biomolecules.

Cross-reactants	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

    Interferents listed in the following table were added to the test sample at the concentration mentioned below. **QDx Instacheck™ Calprotectin** test results did not show any significant interference with these materials.

Interferents	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
DMF	2%
DMSO	2%

- **Precision**

  3 Lots of **QDx Instacheck™ Calprotectin** were tested for 30 days (10 days per 1 Lot at 1 site by one operator). Each standard material was tested 2 times per day. For each test, each material was duplicated.
  - Repeatability (within-run precision)

    Repeatability of **QDx Instacheck™ Calprotectin** was evaluated with results of 1 Lot.
  - Total precision (within-laboratory)

    Total precision of **QDx Instacheck™ Calprotectin** was evaluated with results of 1 Lot.
  - Lot to lot precision

    Lot to lot precision of **QDx Instacheck™ Calprotectin** was evaluated with results of 3 Lots.
  - Between-person

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

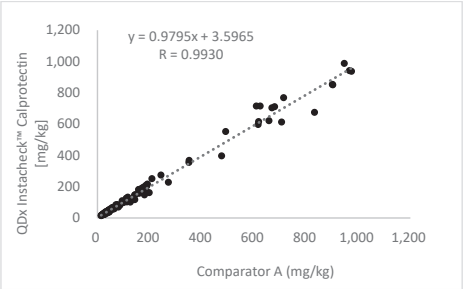
    One person tested one lot of **QDx Instacheck™ Calprotectin** at three different sites, ten times at each concentration of the control standard.

Calprotectin [mg/kg]	Repeatability		Total precision		Lot to lot precision	
	AVG [mg/kg]	CV (%)	AVG [mg/kg]	CV (%)	AVG [mg/kg]	CV (%)
25	23.77	4.4	23.70	4.2	24.90	5.6
50	49.25	4.6	48.94	5.0	50.46	6.1
250	245.83	3.8	246.33	3.5	248.93	4.5

Calprotectin [mg/kg]	Between-person		Between-site	
	AVG [mg/kg]	CV (%)	AVG [mg/kg]	CV (%)
25	25.19	6.9	24.75	7.1
50	50.66	5.2	50.54	5.4
250	248.27	5.2	249.03	5.4

- **Comparability**

  Calprotectin concentrations of 100 clinical samples were quantified independently with **QDx Instacheck™ Calprotectin** (QDx Instacheck™-II) and comparator A as per prescribed test procedures. Test results were compared, and their comparability was investigated with linear regression and correlation coefficient (R). The regression equation and correlation coefficient are as below.



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

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

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