Manual

IDK® TurbiCAL®

For the quantitative in vitro determination of calprotectin in stool

Valid from 2021-10-04

REF **TU1021.1**
TU1021.2
TU1021.8

Σ 100/200/800

+2 °C +8 °C

IVD

CE

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1. INTENDED USE

***IDK*® TurbiCAL®** assay is a latex turbidimetric assay for the quantitative detection of calprotectin in human stool samples.

***IDK*® TurbiCAL®** is intended to exclusively differentiate IBD patients with inflammation from IBD patients without inflammation and from irritable bowel syndrome (IBS).

This assay has a simple performance and multiple applications. This product is optimised for several automated analyser. For professional *in vitro* diagnostic use only.

2. INTRODUCTION

Calprotectin is a complex of the calcium binding proteins S100A8 and S100A9 and a well-established biomarker for the differentiation between organic and functional intestinal diseases. In addition, calprotectin has been established as a valuable parameter in inflammatory bowel disease (IBD) for primary diagnosis as well as for monitoring of disease activity.

It is often difficult to distinguish between patients suffering from irritable bowel syndrome (IBS) and those with inflammatory bowel diseases (IBD). Symptoms of both diseases are comparable and final diagnosis requires a multitude of elaborate, even invasive, examinations. The use of calprotectin as a reliable and non-invasive marker in primary care allows for a quick and reliable differentiation between patients who need further invasive procedures and patients with functional disease for whom a conservative treatment is sufficient.

Furthermore, calprotectin is a reliable biomarker to indicate relapse in advance in patients suffering of IBD. The determination in stool correlates very well with histological and endoscopic findings of the disease activity in Crohn's Disease and Ulcerative Colitis. Calprotectin provides a measure to objectively assess the response to the treatment and enables physicians to monitor patients that are in clinical remission in order to check for disease activity allowing early intervention in case of a treatment relapse of IBD.

3. MATERIAL SUPPLIED

Label	Kit components	Quantity for cat. no.		
		TU1021.1	TU1021.2	TU1021.8
Reagent 1	Reagent 1, ready-to-use	1x 27 ml	2 x 27 ml	8 x 27 ml
Reagent 2	Reagent 2, ready-to-use	1 x 5 ml	1 x 8 ml	4 x 8 ml
CAL 0–5	Calibrators 0–5, ready-to-use	6 x 1 ml	6 x 1 ml	6 x 1 ml
CTRL 1	Control 1, ready-to-use	2 x 1 ml	2 x 1 ml	2 x 1 ml
CTRL 2	Control 2, ready-to-use	2 x 1 ml	2 x 1 ml	2 x 1 ml

The supplied reagents are in neutral tubes that are not compatible with the used analyzer. Therefore, it is necessary to fill the corresponding reagents into compatible reagent vessels.

4. MATERIAL REQUIRED BUT NOT SUPPLIED

- Sample collection container
- Centrifuge, 3 000 *g*
- Vortex
- Calibrated precision pipettors (adjustable volume) and 10–1 000 µl single-use tips
- Disposable gloves and laboratory equipment
- Automated analyser (list of compatible analysers see annex information)
- Reagent vessels that are approved for the used analyser
- *IDK*® **TurbiTUBE**® for sample collection (cat. no.: TU0091.A)

5. STORAGE AND PREPARATION OF REAGENTS

- All test reagents are ready-to-use. Test reagents are stable until the expiry date (see label) when stored at 2–8 °C. Do not freeze any component of the kit. Keep away from direct sunlight.
- Always consider that the reagent containers must be properly closed to avoid any contamination.

6. STORAGE AND PREPARATION OF SAMPLES

Sample storage and stability

Collect a sufficient amount of human stool in a clean and dry container (no preservatives or transport media is necessary).

The raw stool samples can be stored at 2–8 °C for 7 days. If not immediately tested, stool samples can be frozen and stored at -20 °C for a maximum of 6 months. In this case, the sample must be totally thawed, and brought to room temperature (15–30 °C) before testing.

Stool samples must be homogenised as thoroughly as possible prior to preparation.

Stool extract is stable at 2–8 °C for 7 days.

Extraction of the stool samples

Stool Sample Application System (***IDK*® TurbiTUBE®**) (cat. no.: TU0091.A)

Stool sample tube – Instructions for use

Please note that the dilution factor of the final stool suspension depends on the amount of stool sample used and the volume of the buffer.

***IDK*® TurbiCAL®** with 1.5 ml sample extraction buffer:

Applied amount of stool:	15 mg
Buffer Volume:	1.5 ml
Dilution Factor:	1:100

Please follow the instructions for the preparation of stool samples using the ***IDK*® TurbiTUBE®** as follows:

a) The raw stool sample has to be thawed and brought to room temperature (15–30 °C). For particularly heterogeneous samples we recommend a mechanical homogenisation using an applicator, inoculation loop or similar device.

b) Unscrew the tube (yellow part of cap) to open. Insert the yellow dipstick into the sample. The lower part of the dipstick has notches which need to be covered completely with stool after inserting it into the sample. Place dipstick back into the tube. When putting the stick back into the tube, excess material will be stripped off, leaving 15 mg of sample to be diluted. Screw tightly to close the tube.

Hint: In case of liquid stool samples we recommend to pipette 15 µl of liquid sample into the tube.

c) Vortex the tube well until no stool sample remains in the notches. Important: Please make sure that you have a maximally homogenous suspension after shaking. Especially with more solid samples, soaking the sample in the tube with sample extraction buffer for ~ 10 minutes improves the result.

d) Samples should be centrifuged for ~ 10 minutes at 3 000 *g* to obtain a sediment-free sample. Floating material such as grain shells can be neglected.

e) Carefully unscrew the complete cap of the tube including the blue ring plus the dipstick. Discard cap and dipstick. Make sure that the sediment will not be dispersed again.

7. ASSAY PROCEDURE

Principle of the test

***IDK*® TurbiCAL®** is a latex turbidimetric assay for the quantitative detection of calprotectin in human stool samples.

***IDK*® TurbiCAL®** assay is based on agglutination reactions. These involve *in vitro* aggregation of microscopic latex particles. This aggregation consists in the specific reaction between antigen and antibodies. Antigens contained in the sample bind with the antibodies' anti-antigen coated on polystyrene latex particles. The sample is mixed with a suspension containing antibodies against the antigen bound to latex particles. If the antigen is present in the sample, it will react with the antibodies and form an aggregate. If no antigen is present in the sample, the mixture will maintain its clear appearance.

Test Procedure

Preparation of the calibration curve

The calibrator liquid vials of ***IDK*® TurbiCAL®** (all calibrators) are ready to use. For calibration use only ***IDK*® TurbiCAL®** calibrator vials. The calibrators containing recombinant calprotectin at different concentrations. The exact concentrations are indicated on the label of each vial.

Calibration of the system at least once a month is highly recommended. The system must be recalibrated when the reagent lot is changed or when the controls are out of the assigned range (see the quality control protocol provided with the kit).

Calibration and control vials

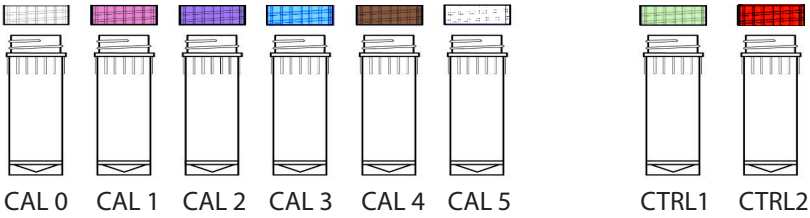

Fig 1: Prepared calibrators (5 points and blank) and controls for performing the calibration curve.

Label	Concentration	Lid Colour
CAL 0	0 µg/g	transparent
CAL 1	50 µg/g	pink
CAL 2	100 µg/g	purple
CAL 3	250 µg/g	blue
CAL 4	750 µg/g	brown
CAL 5	1 500 µg/g	white
CTRL 1	Control 1*	green
CTRL 2	Control 2*	red

* The batch-dependent concentration is given by the quality control protocol.

8. QUALITY CONTROL

For quality control use only ***IDK*® TurbiCAL®** Control 1 (CTRL 1) and ***IDK*® TurbiCAL®** Control 2 (CTRL 2). These are two ready-to-use liquid controls containing different concentrations of recombinant calprotectin. The respective concentration for each vial is given by the quality control protocol.

The use of control materials at two different concentrations is recommended in order to verify test precision.

Controls should be assayed every day before running extracted patient samples to validate the calibration curve.

If the obtained results are out of the tolerance range, the equipment, the reagents and the technique must be reviewed.

Since the sample dilution is already considered in the calibration curve, the dilution factor is 1.

In case **another dilution factor** has been used, multiply the obtained result by the dilution factor used.

Reference Range

We recommend each laboratory to establish its own reference concentration range.

Reference range in stool samples

Calprotectin concentrations lower than 50 µg of calprotectin/g of stool are considered as normal values and do not indicate to an inflammation of gastro-intestinal tract.

Calprotectin concentrations between 50 and 200 µg calprotectin/g of stool are considered as an abnormal presence being indicative of mild inflammation of gastro-intestinal tract, therefore monitoring and follow-up of the patient is recommended.

Calprotectin concentration values higher than 200 µg of calprotectin/g of stool are indicative of a severe inflammation of gastrointestinal tract.

9. PERFORMANCE CHARACTERISTICS

Analytical sensitivity

Limit of Detection (LoD): **See application note.**

Prozone

Studies have been carried out to a concentration of 25 mg of calprotectin/g of stool and no false negative results have been observed. Studies using higher concentrations have not been carried out.

Data obtained by the analyser Biolis 24i (Tokyo Boeki)

Clinical sensitivity and specificity

An evaluation was performed comparing a turbidimetric assay (***IDK*® TurbiCAL®**) and another commercial immunoassay (**Calprest®**, Eurospital). The results were as follows:

	Sensitivity	Specificity
***IDK*® TurbiCAL®** vs **Calprest®** (Eurospital)	**94 %**	**> 99 %**

The results showed a high sensitivity and specificity to detect calprotectin using ***IDK*® TurbiCAL®**.

Interferences

An evaluation was performed to determine the interferences of ***IDK*® TurbiCAL®** with other substances. No interferences were found against tested substances: BSA, Hemin, Ascorbic acid.

Cross reactivity

An evaluation was performed to determine the cross reactivity against other faecal markers occasionally present in faeces, such as: bovine and pig haemoglobin, bovine transferrin, bovine lactoferrin and human haemoglobin transferrin and lactoferrin. No cross reactivity was found.

10. LIMITATIONS

***IDK*® TurbiCAL®** should only be used in human stool samples. The use of other samples, such as blood, serum, plasma, urine, cerebrospinal fluid, oral fluid, synovial fluid or empyema fluid, has not been established. ***IDK*® TurbiCAL®** must be used with properly extracted faecal samples.

The results obtained determine the concentration of calprotectin in faecal samples. A positive result should be contrasted with additional diagnostic procedures. Colonoscopy and a biopsy are the most adequate methods to detect and quantify the inflammation in the intestine.

If symptoms persist further diagnostic procedures should be carried out. Negative results do not exclude the presence of inflammation in the intestine. Some diseases as celiac diseases, microscopic colitis and polys can involve mononuclear inflammation.

Stool samples from patients taking non-steroidal anti-inflammatory drugs (NSAID) could show positive results.

Neonatal faecal calprotectin levels have been reported higher than those in normal children with a mean of 167 µg/g (range 22–860 µg/g).

11. PRECAUTIONS

- All reagents in the kit package are for in vitro diagnostic use only.
- A trained person in the turbidimetric technique and the use of the auto-analyser is required. Manual application of the test kit is not possible.
- The analyser should be ready before performing any assay.
- Read and follow the instructions for use provided with the kit.
- If the result exceeds the measurement range, use the sample diluent to dilute the sample and repeat the assay again.
- Reagents should not be used beyond the expiration date stated on the kit label.
- Reagents should not be used if the primary packaging is damaged or opened.
- Do not interchange different lot numbers of any kit component within the same assay.
- Only **new** empty reagent vessels are strictly recommended, because washed or recycled/reused reagent vessels may influence the test result.

12. DISPOSAL

- Liquid test components, pipets tips, tubes etc. are to be treated as ordinary laboratory waste, unless otherwise stated. The solutions should be discarded in a proper container after testing following local regulations.
- All the specimens should be considered potentially hazardous and handled in the same manner as an infectious agent. Other potentially infectious materials (e.g. sample collection container) must be disposed in accordance with official regulations.

13. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- This assay was produced and distributed according to the IVD guidelines of 98/79/EC.
- Follow Good Laboratory Practices, wear protective clothing, use disposal gloves, eye protection and mask. Do not eat, drink or smoke in the working area.

- In the event of warranty claims, notifications of defects or serious incidents, notify Immundiagnostik AG immediately, but at the latest within 14 days after the problem occurring. Please send the product together with a written complaint (including e-mail, address and telephone number) to Immundiagnostik AG.
- If stored properly (2–8 °C), Immundiagnostik AG guarantees, after the first opening, no loss of activity of the kit components for the following three months and at least 20 opening-closing cycles.
- Any variation of the test procedure, which is not coordinated with the producer, may influence the results of the test. Immundiagnostik AG can therefore not be held responsible for any damage resulting from incorrect use.
- *IDK*® **TurbiCAL**® is a trademark of Immundiagnostik AG.

14. REFERENCES

1. Matsuura et al. (2018), Fecal calprotectin reflects endoscopic activity in patients with small-bowel Crohn's disease according to double-balloon endoscopy findings. Nagoya J Med Sci 80(2):257-266
2. Walker et al. (2018), Faecal calprotectin effectively excludes inflammatory bowel disease in 789 symptomatic young adults with/without alarm symptoms: a prospective UK primary care cohort study. Aliment Pharmacol Ther 1-14
3. Chen et al. (2012), Fecal Calprotectin as a Correlative Marker in Clinical Severity of Infectious Diarrhea and Usefulness in Evaluating Bacterial or Viral Pathogens in Children. JPNG 55:541-547

15. ANNEX - COMPATIBLE TURBIDIMETRIC ANALYSERS

Abbott	Alinity
Abbott	Architect c1000/c4000/c8000
Awareness	Chemwell-T
Beckman Coulter	AU680
Biobase	BK-Mini
Biosystems	A15
DiaSys	Respons 910
Erba	XL-180 & XL-200
Mindray	BS200E

Roche	Cobas c111/ /c311/ c501/ c702
Siemens	ADVIA 1800/ 2400
Siemens	ATELLICA
Tecom	TC220
Tokyo Boeki	Biolis 24i/ Biolis 30i/ Biolis 50i
Ortho Clinical Technologies	Vitros 5600

Applications notes for the use of our turbidimetric products on various instruments can be obtained on request from our technical support.

Used symbols:

 Temperature limitation

 Catalogue number

 In Vitro Diagnostic Medical Device

 To be used with

 Manufacturer

 Contains sufficient for <n> tests

 Lot number

 Use by

 Attention

 Consult instructions for use

 Consult specification data sheet

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