BÜHLMANN fPELA® turbo

Pancreatic elastase turbidimetric assay
for professional use

Reagent Kit

B-KPELA-RSET
Version A2

For *In Vitro* Diagnostic Use

Rx Only

Manufacturer

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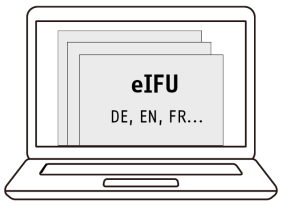

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INTENDED USE

The BÜHLMANN fPELA® turbo is an *in vitro* diagnostic test for the quantitative determination of pancreatic elastase in human fecal extracts. The results can be used as an aid to determination of exocrine pancreatic insufficiency in patients suffering from conditions such as chronic pancreatitis and cystic fibrosis in conjunction with other laboratory and clinical findings. The BÜHLMANN fPELA® turbo assay is intended to be run on clinical chemistry analyzers. For laboratory use only.

PRINCIPLE OF THE ASSAY

The BÜHLMANN fPELA® turbo test is a particle enhanced turbidimetric immunoassay (PETIA) and allows automated quantification of pancreatic elastase in fecal extracts on clinical chemistry analyzers. Fecal samples are extracted with extraction buffer using the CALEX® Cap extraction device and applied at a final dilution of 1:500. The extracts are incubated with reaction buffer and mixed with polystyrene nanoparticles coated with pancreatic elastase-specific antibodies (immunoparticles). Pancreatic elastase available in the sample mediates immunoparticle agglutination. Sample turbidity, measured by light absorbance, increases with pancreatic elastase-immunoparticle complex formation and is proportional to pancreatic elastase concentration. The detected light absorbance allows quantification of pancreatic elastase concentration via interpolation on an established calibration curve.

REAGENTS SUPPLIED

Reagents	Quantity	Code	Preparation
Reaction Buffer (R1) MES buffered saline	1 vial 27.0 mL	B-KPELA-R1	Ready to use
Immunoparticles (R2) Polystyrene beads coated with rabbit antibodies against human pancreatic elastase	1 vial 5.1 mL	B-KPELA-R2	Ready to use

Table 1: Reagents supplied

REAGENT STORAGE AND STABILITY

Unopened reagents
Store at 2-8°C. Do not use kit past expiration date printed on the labels.
On-board stability[1]
Store for up to 3 months (91 days) at 5-12°C

Table 2: Storage and stability of reagents

Do not freeze reagents!

[1] On-board stability was established on the Roche cobas® 6000 c501 instrument

MATERIALS REQUIRED BUT NOT PROVIDED

Reagents	Quantity	Code
BÜHLMANN fPELA® turbo Calibrator Kit Calibrators 1-6 for establishment of six-point calibration curve	1 x 6 vials 1 mL/vial	B-KPELA-CASET
BÜHLMANN fPELA® turbo Control Kit Controls low and high	3 x 2 vials 1 mL/vial	B-KPELA-CONSET
CALEX® Cap device Extraction device filled with extraction buffer	50 tubes 200 tubes 500 tubes	B-CALEX-C50 B-CALEX-C200 B-CALEX-C500

Table 3: Materials required but not provided

WARNINGS AND PRECAUTIONS

- This test is for *in vitro* diagnostic use only.
- This kit contains components classified in accordance with the Regulation (EC) No. 1272/2008: 2-methyl-2-isothiazolin-3-one (conc. ≥0.0015%), thus the reagents may cause allergic skin reactions (H317).
- Avoid contact of reagents with the skin, eyes or mucous membranes. If contact does occur, immediately wash with generous amounts of water; otherwise, irritation can occur.
- It is recommended that the test be handled by qualified personnel, in accordance with Good Laboratory Practice (GLP).
- The immunoparticles contain potentially infectious substances of animal origin and should be handled with caution. Disposal of any discarded materials should be in accordance with local requirements.
- R1 contains MES (2-(*N*-morpholino)ethanesulfonic acid) that can be irritating to eye and skin. Handle with due caution.
- R2 contains polystyrene nanoparticles.
- Unused solution should be disposed according to local state and federal regulations.

Technical Precaution

- Please equilibrate reagents, controls, calibrators and samples as described in the application note.

- Evaporation of calibrators and controls on the analyzer could lead to incorrect results. Run the assay immediately after loading the analyzer.
- Do not mix reagents R1 and R2 of different reagent lots or switch caps between reagents.
- Reagent R2, once frozen, cannot be used anymore.
- The assay is designed for fecal extract samples prepared using the BÜHLMANN CALEX® Cap device.
- Ensure that samples have no bubbles prior to running the test.
- Sample carry over depends on the clinical chemistry analyzer. For more information refer to the analyzer specific application note.

SPECIMEN COLLECTION AND STORAGE

For the extraction procedure, less than 1 g of native stool specimen is required. Collect stool specimen into plain tubes.

Important: The specimen must be collected without any chemical or biological additives.

Specimen transport and storage

Stool specimens should be processed by the laboratory within 6 days of collection. The stool specimens may be shipped and stored at ambient temperature (up to 28°C) or refrigerated. For longer storage freeze the specimens at -20°C. More than 2 freeze-thaw cycles are not recommended.

STOOL SAMPLE EXTRACTION AND EXTRACT STORAGE

Liquid stool samples cannot be measured using the BÜHLMANN fPELA® turbo assay (see section “Limitations” below).

Follow the instruction for use provided with the CALEX® Cap device kit. Fecal sample extracts prepared using the CALEX® Cap device will have a final dilution of 1:500 and are ready to use.

Important: Let the stool extract stand for at least one hour before proceeding with centrifugation and subsequent measurement.

Important: Centrifuge the CALEX® Cap device for 10 minutes at 1000-3000 x g prior to running the BÜHLMANN fPELA® turbo procedure.

Pancreatic elastase extracts obtained with the CALEX® Cap device are stable at room temperature (≤ 28°C) for 8 days; at 2-8°C for 12 days and at -20°C for at least 24 months.

CALEX® Cap extracts can be frozen and stored within the CALEX® Cap device. Extracts can be subject to five freeze-thaw cycles. Prior to

measurement, allow frozen extracts to equilibrate to room temperature, vortex thoroughly for 10 seconds and centrifuge according to the instruction for use of the assay.

ASSAY PROCEDURE

Application notes / assay installation

Assay procedures for the BÜHLMANN fPELA® turbo are established on several clinical chemistry analyzers. Validated application notes describing installation and analysis on specific instruments are available from BÜHLMANN upon request. Corresponding instrument manuals must be considered for instrument setup, maintenance, operation and precautions.

Reagent preparation

The reagents supplied are ready to use. Mix gently before loading onto the instrument. The reagent bottles may fit directly into the instrument, unless otherwise stated in the application note.

Establishment of the calibration curve

The BÜHLMANN fPELA® turbo Calibrator Kit is used to establish a six-point calibration curve according to the instrument manual. Calibrator values are lot-specific. A new calibration must be performed for each new calibrator and reagent lot. Otherwise, periodic calibrations should be performed every one to two months according to the instrument specific application notes. Refer to the QC-data sheet provided with the BÜHLMANN fPELA® turbo Calibrator Kit for assigned calibrator values. Contact BÜHLMANN support if calibration cannot be performed without error.

QC controls

The BÜHLMANN fPELA® turbo Control Kit should be assayed each day before running patient fecal sample extracts to validate the calibration curve. The controls have assigned value ranges indicated on the QC-data sheet supplied with each lot of the BÜHLMANN fPELA® turbo Control Kit. The control measurements must be within the indicated value ranges to obtain valid results for patient fecal sample extracts.
If the control values are not valid, repeat measurement with fresh controls. If control values remain invalid, recalibrate the assay. If valid control values cannot be reproduced, after performing the steps described above, contact BÜHLMANN support.

Patient fecal sample extract measurement

Once a calibration curve is established and validated with the controls, patient fecal extracts may be measured. Perform patient fecal extract measurement according to the application note and instrument manual.

Results

Results are calculated automatically on the clinical chemistry analyzer and presented in µg/g unless otherwise stated in the corresponding analyzer-specific application notes.

STANDARDIZATION

The BÜHLMANN fPELA® turbo is standardized against an internal reference material.

LIMITATIONS

- High water content of the stool sample (liquid stool samples) leads to an underestimation of the fecal pancreatic elastase concentration. Stool sample collection on a different day is recommended.
- Test results should be interpreted in conjunction with information available from clinical assessment of the patient and other diagnostic procedures.
- The risk of false negative diagnosis is higher for patients with mild and moderate insufficiency compared to patients with severe insufficiency (ref. 1). The utility of fecal pancreatic elastase testing is limited in patients with diabetes (ref. 2, 3).
- Interference studies indicate a positive bias of 20% in samples containing pancreatin (Creon®) corresponding to a daily dose of 757.5 kU/day, assuming a daily stool weight of 150 g. This is moderately below the maximum recommended dose of 800 kU/day.
- Interference studies indicate a negative bias of 20% in samples containing lansoprazole corresponding to a daily dose of 229.1 mg/day, assuming a daily stool weight of 150 g and a lansoprazole excretion factor of 64%. This is above the maximum recommended dose of 180 mg/day.
- Interference studies indicate a negative bias of 20% in samples containing esomeprazole corresponding to a daily dose of 266.7 mg/day. This is above the maximum recommended dose of 160 mg/day.
- Interference studies indicate a negative bias of 20% in samples containing omeprazole corresponding to a daily dose of 285.0 mg/day. This is above the maximum recommended dose of 120 mg/day.

RESULT INTERPRETATION

Fecal Elastase Concentration	Indication
200 to >500 µg/g	Normal pancreatic function
<200 µg/g	Pancreatic insufficiency

Table 4: Result interpretation (ref. 1)

EXPECTED VALUES

One hundred and twenty eight (128) stool samples from self-declared healthy volunteers were measured using the BÜHLMANN fPELA® turbo. The following results were obtained:

Fecal Elastase Concentration	Number of healthy subjects
200 to >500 µg/g	114
<200 µg/g	14

Table 5: Expected values

PERFORMANCE CHARACTERISTICS

The presented performance characteristics have been established on a Roche cobas® 6000 c501 instrument, unless otherwise indicated. Refer to analyzer-specific application notes for the performance characteristics on other clinical chemistry analyzers.

Method comparison: BÜHLMANN fPELA® turbo vs ScheBo® Pancreatic Elastase 1™

The method comparison study was performed according to the CLSI guideline EP09-A3. One hundred and eight (108) samples were measured using 3 lots of BÜHLMANN fPELA® turbo over 4 days. Mean reference values, with elastase concentrations falling within the range of 21.0-444.9 µg/g, were established with 2 lots (2 replicates per lot) of the ScheBo® Pancreatic Elastase 1™ test. Bias was determined using Passing-Bablok linear regression and Bland-Altman analysis.

Bland-Altman Analysis			Passing-Bablok Regression Analysis			
Mean bias (95% CI)	Lower LoA (95% CI)	Upper LoA (95% CI)	Slope (95% CI)	Intercept (95% CI)	Bias at 200 µg/g (95% CI)	r
3.6% (-4.8%, 12.1%)	-83.0% (-97.4%, -68.5%)	90.2% (75.8%, 104.7%)	0.90 (0.80, 1.04)	13.7 (-3.0, 27.2)	-2.7% (-17.2%, 14.0%)	0.847

Reproducibility: 1.9-6.2% CV

Reproducibility was established according to the CLSI guideline EP05-A3 using a 3 instruments/lots x 5 days x 5 replicates study design and an acceptance criterion of 15% CV. Testing was performed on Roche c501, Beckman Coulter AU480, and Mindray BS380 instruments. Six (6) stool specimen extracts were assayed.

ID	Mean [µg/g]	n	Within-run		Between-day		Between-lot/ instrument		Total	
			SD [µg/g]	%CV	SD [µg/g]	%CV	SD [µg/g]	%CV	SD [µg/g]	%CV
S1	78.8	75	1.6	2.1%	0.4	0.5%	1.1	1.4%	2.0	2.5%
S2	188.7	75	1.3	0.7%	0.7	0.3%	3.4	1.8%	3.7	1.9%
S3	284.3	75	1.6	0.6%	2.1	0.8%	10.2	3.6%	10.5	3.7%
S4	400.5	75	2.2	0.5%	1.7	0.4%	11.2	2.8%	11.6	2.9%
S5	912.3	75	21.6	2.4%	0.0	0.0%	52.7	5.8%	56.9	6.2%
S6	1998.6	75	23.8	1.2%	9.5	0.5%	67.4	3.4%	72.1	3.6%

Repeatability: 0.6-1.9% CV

Within-laboratory precision: 0.9-2.2% CV

Repeatability and within-laboratory precision were established according to the CLSI guideline EP05-A3 using the standardized 20 days x 2 runs x 2 replicates study design and an acceptance criterion of 10% CV. Six (6) stool specimen extracts were assayed.

ID	Mean [µg/g]	n	Within-run		Between-day		Between-lot/ instrument		Total	
			SD [µg/g]	%CV	SD [µg/g]	%CV	SD [µg/g]	%CV	SD [µg/g]	%CV
S1	77.0	80	1.5	1.9%	0.8	1.1%	0.0	0.0%	1.7	2.2%
S2	188.9	80	1.2	0.7%	1.3	0.7%	1.8	1.0%	2.6	1.4%
S3	273.0	80	2.1	0.8%	2.1	0.8%	1.8	0.7%	3.5	1.3%
S4	404.1	80	2.4	0.6%	1.4	0.4%	2.3	0.6%	3.6	0.9%
S5	946.6	80	9.1	1.0%	7.5	0.8%	7.9	0.8%	14.2	1.5%
S6	2046.0	80	11.8	0.6%	6.9	0.3%	21.5	1.1%	25.5	1.2%

Extraction reproducibility – CALEX® Cap: 6.9%-13.8% CV

The extraction reproducibility was established according to the CLSI guideline EP05-A3 using a 2 days x 2 operators x 3 CALEX® Cap lots x 3 extractions x 2 replicates study design. Six (6) clinical stool specimens were assayed.

ID	Mean [µg/g]	n	Within-extraction		Between-extraction		Total precision	
			SD [µg/g]	%CV	SD [µg/g]	%CV	SD [µg/g]	%CV
10236	54.7	72	1.1	2.1%	2.9	5.3%	4.4	8.1%
10202	97.9	72	0.8	0.8%	4.5	4.6%	10.7	10.9%
10265	230.8	72	2.0	0.9%	13.4	5.8%	16.0	6.9%
10192	396.6	72	2.9	0.7%	34.7	8.8%	44.2	11.1%
10177	930.9	72	13.8	1.5%	98.0	10.5%	128.8	13.8%
10185	2126.5	72	13.9	0.7%	189.1	8.9%	204.3	9.6%

Accuracy/ Recovery: 99.8-107.7%

Six (6) stool specimen extracts from clinical samples with elastase levels ranging from 15.8 - 355.7 µg/g were spiked with 55 µg/g elastase in calibrator material. Spiking was performed at 10% of the specimen extract volume. “Baseline” samples were spiked with the corresponding amount of extraction buffer. “Baseline” and “baseline + spike” samples were measured in four (4) replicates.

Sample carry-over

The sample carry over was established according to the CLSI guideline EP10-A2. No statistically significant carry over with the BÜHLMANN fPELA® turbo test on Roche cobas® 6000 c501 instrument was detected.

Limit of Blank (LoB): 0.8 µg/g

The LoB was established according to the CLSI guideline EP17-A2 using the classical approach and non-parametric analysis.

Limit of Detection (LoD): 2.3 µg/g

The LoD was established according to the CLSI guideline EP17-A2 using the classical approach and parametric analysis.

Limit of Quantitation (LoQ): 5.7 µg/g

The LoQ was established according to the CLSI guideline EP17-A2, based on 60 determinations and a precision goal of 20% CV.

Linearity range: 3.4 to 5024.2 µg/g

The linear range of the BÜHLMANN fPELA® turbo was determined according to the CLSI guideline EP06-A. Samples with a concentration of over 500 µg/g were diluted automatically 1:10 by the analyzer. A maximum deviation from linearity of 10% or 10 µg/g, for samples below 100 µg/g, was allowed.

High Dose Hook Effect

Samples with concentrations of up to 17'231 µg/g can be measured without limiting the measuring range of the assay. Including a prozone check extends the testing range up to 18'891 µg/g.

Cross-reactivity

Cross-reactivity of the BÜHLMANN fPELA® turbo assay with molecules similar to human elastase was assessed according to the CLSI guideline EP07-A2. Bias in results exceeding 10% was considered interference.

No cross-reactivity was detected with the following molecules: porcine chymotrypsin or porcine elastase at concentrations of 1 µg/mL in stool sample extract.

Interfering substances

The susceptibility of the BÜHLMANN fPELA® turbo assay to oral pharmaceuticals, nutritional supplements as well as hemoglobin was assessed according to the CLSI guideline EP07-A2. Bias in results exceeding 10% was considered interference.

No interference was detected with the following substances [Concentration in mg [2] / 50 mg stool]: Prednisone (1.5), acetylcysteine (Fluimucil®) (1.8); lumacaftor/ivacaftor (Symdeko®) (0.8), metformin (3.0), glimepiride (Amaryl®) (6.0 µg), ciprofloxacin (Ciproxin®) (1.5), ibuprofen (2.4), multivitamin (Berocca®) (15 µg), pantoprazole (Nycomed®) (0.16), hemoglobin (12.5).

Interference was detected for the following substances: pancreatin (Creon®) (800 U), lansoprazole (Agopton®) (0.18), esomeprazole (Esomeprazol-Mepha®) (0.16), and omeprazole (Omeprazol-Mepha®) (0.12). Polynomial fitting of bias versus interferent concentration indicated that bias of 20% is exceeded for concentrations above 252.5 U for pancreatin, 0.0475 mg for lansoprazole, 0.0875 mg for esomeprazole and 0.095 mg for omeprazole.

[2] Unless otherwise indicated

REFERENCES

1. Vanga et al., 2018; Diagnostic Performance of Measurement of Fecal Elastase-1 in Detection of Exocrine Pancreatic Insufficiency – Systematic Review and Meta-analysis
2. Hahn et al., 2008, Low fecal elastase 1 levels do not indicate exocrine pancreatic insufficiency in type-1 diabetes mellitus
3. Creutzfeldt et al., 2005, Follow-Up of Exocrine Pancreatic Function in Type-1 Diabetes mellitus.

CHANGELOG

Date	Version	Change
2022-07-20	A2	Update to chapter "*warnings and precautions*", "*limitations*", "*method comparison*" and "*interfering substances*" Update to pancreatic elastase extracts stability at -20°C Revision of chapter "*symbols*" Inclusion of notified body number to CE-mark – conformity assessment procedure according to IVDR 2017/746

INCIDENT REPORTING IN EU MEMBER SATES

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.

SHIPPING DAMAGE

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

SYMBOLS

BÜHLMANN use symbols and signs listed and described in ISO 15223-1. In addition the following symbols and signs are used:

EN: electronic instruction for use available in different languages at:/ **BG**: електронни инструкции за употреба на различни езици на адрес:/ **CS**: elektronický návod k použití dostupný v různých jazycích na adrese:/ **DA**: elektronisk brugsanvisning på forskellige sprog på:/ **DE**: elektronische Gebrauchsanweisung in verschiedenen Sprachen verfügbar unter:/ **EL**: ηλεκτρονικές οδηγίες χρήσης διαθέσιμες σε διάφορες γλώσσες στη διεύθυνση:/ **ES**: instrucciones de uso electrónicas disponibles en diferentes idiomas en:/ **ET**: elektrooniline kasutusjuhend, mis on saadaval erinevates keeltes aadressil:/ **FR**: un mode d'emploi électronique disponible en différentes langues à l'adresse:/ **HU**: különböző nyelveken elérhető elektronikus használati utasítás a következő címen:/ **IT**: istruzioni elettroniche per l'uso disponibili in diverse lingue su:/ **LT**: elektroninės naudojimo instrukcijos įvairiomis kalbomis:/ **LV**: dažādās valodās pieejama elektroniska lietošanas instrukcija:/ **NO**: elektronisk instruksjon for bruk tilgjengelig på forskjellige språk på:/ **PL**: elektroniczna instrukcja obsługi dostępna w różnych językach na stronie:/ **PT**: instrução electrónica para utilização disponível em diferentes línguas em:/ **RO**: instrucțiuni electronice de utilizare disponibile în diferite limbi la adresa:/ **SK**: elektronický návod na použitie dostupný v rôznych jazykoch na:/ **SL**: elektronska navodila za uporabo so na voljo v različnih jezikih na:/ **SR**: elektronsko uputstvo za upotrebu dostupno na različitim jezicima na:/ **SV**: elektronisk bruksanvisning på olika språk på följande adress:

http://www.buhlmannlabs.ch/support/downloads/

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