CLINICAL AND LABORATORY ASPECTS OF ^{13}C-BREATH TEST EVALUATED BY NDIRS INFRARED SPECTROMETRY

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INTRODUCTION

Modern, **non-invasive methods analysing $^{13}C/^{12}C$ ratio** are available for the dynamic tests of gastrointestinal functions.

This study summarizes pre- and post-analytical aspects of the **^{13}C-breath-test** (BT) evaluated by means of **isotope selective nondispersive infrared spectrometry** (NDIRS) - Isomax 4000 (Isodiagnostika). Moreover, the sources of inaccuracy in test results are identified: (a) uncertain baseline ^{13}C abundance, (b) inaccuracy of the spectrometer, and (c) uncertainty in CO_2 production, which also burden cummulative BT where IR/IRMS measuring instruments are used.

Regarding (a), an estimate is presented that is closer to reality than the commonly used **PDB standard**. To address (b), the **accuracy of measurements** is assessed by a statistical analysis and by measuring IRMS calibrated samples every four months. After 14 cycles of checking, the calculated mean bias of the Isomax 4000 equals 5.23 %. Concerning (c), two published estimates of CO_2 production are used and compared: a BSA-based (Body Surface Area) estimate, and a BMR-based (Basal Metabolic Rate) estimate.

To measure **gastrointestinal functions**, 500 BT have been performed since 2002: 53 tests with **^{13}C-xylose**, 161 with **^{13}C-mixed triglyceride**, and 286 with **^{13}C-urea**. These include 215 cumulative (6 hours) ^{13}C breath tests, especially exocrine pancreatic tests with ^{13}C-mixed triglyceride. The cut-off value for these pancreatic tests was calculated as the mean value of the recovery levels - 2SD in a group of 45 subjects without chronic pancreatitis. It is observed that the BMR-based calculation has led to greater ^{13}C recovery values than the BSA approach. The cause of this discrepancy is explained, and a corrected, more accurate approach is proposed.

ISOMAX 4000 INSTRUMENT

POCT (POINT OF CARE TESTING)
2 CHANNEL ANALYSER
FOR ^{13}C - BREATH TESTS

NDIRS MEASUREMENT
(NON-DISPERSIVE INFRARED SPECTROSCOPY)

2000 ml ALUMINIUM BAGS
FOR BREATH AIR SAMPLING
ENABLING REPEATED MEASUREMENTS AND KINETIC EVALUATIONS

UNCERTAINTY OF $^{13}CO_2$

The uncertainty in the **results of $^{13}CO_2$** breath tests has three sources:

- uncertain **baseline ^{13}C abundance** (a)
- inaccuracy of the **spectrometer** (b)
- uncertainty in **CO_2 production** (c)

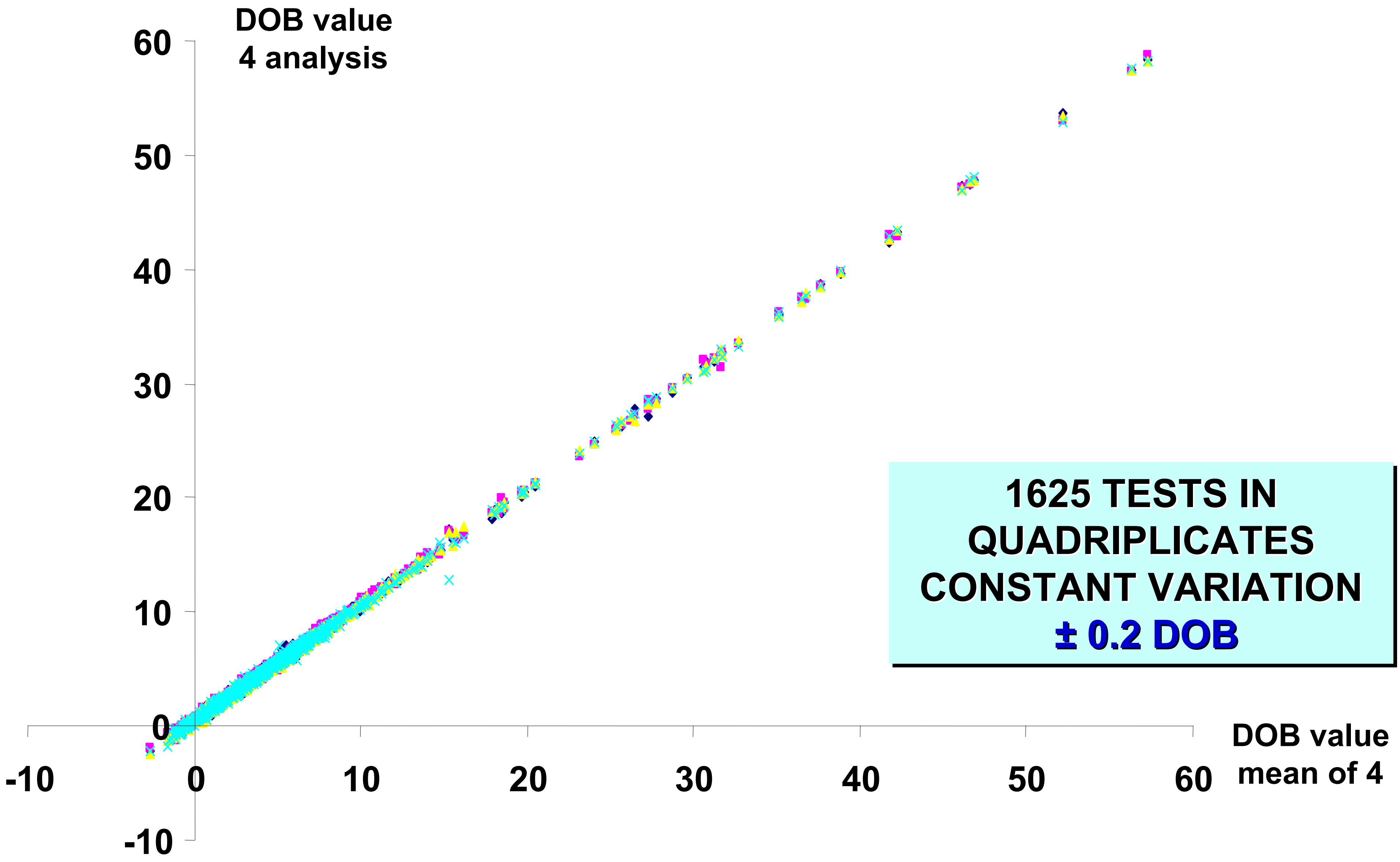
Regarding (a), it is fairly justified to consider the uncertainty is very limited. Its impact is almost negligible if compared with (b) and (c). Although the basal ^{13}C abundance is estimated on the basis of general facts, the bounds are rather tight. It means that the accuracy gained in a cummulative breath test using IRMS, where the reference basal ^{13}C is known, would not be significant.

The **accuracy of the spectrometer** comprises two aspects:

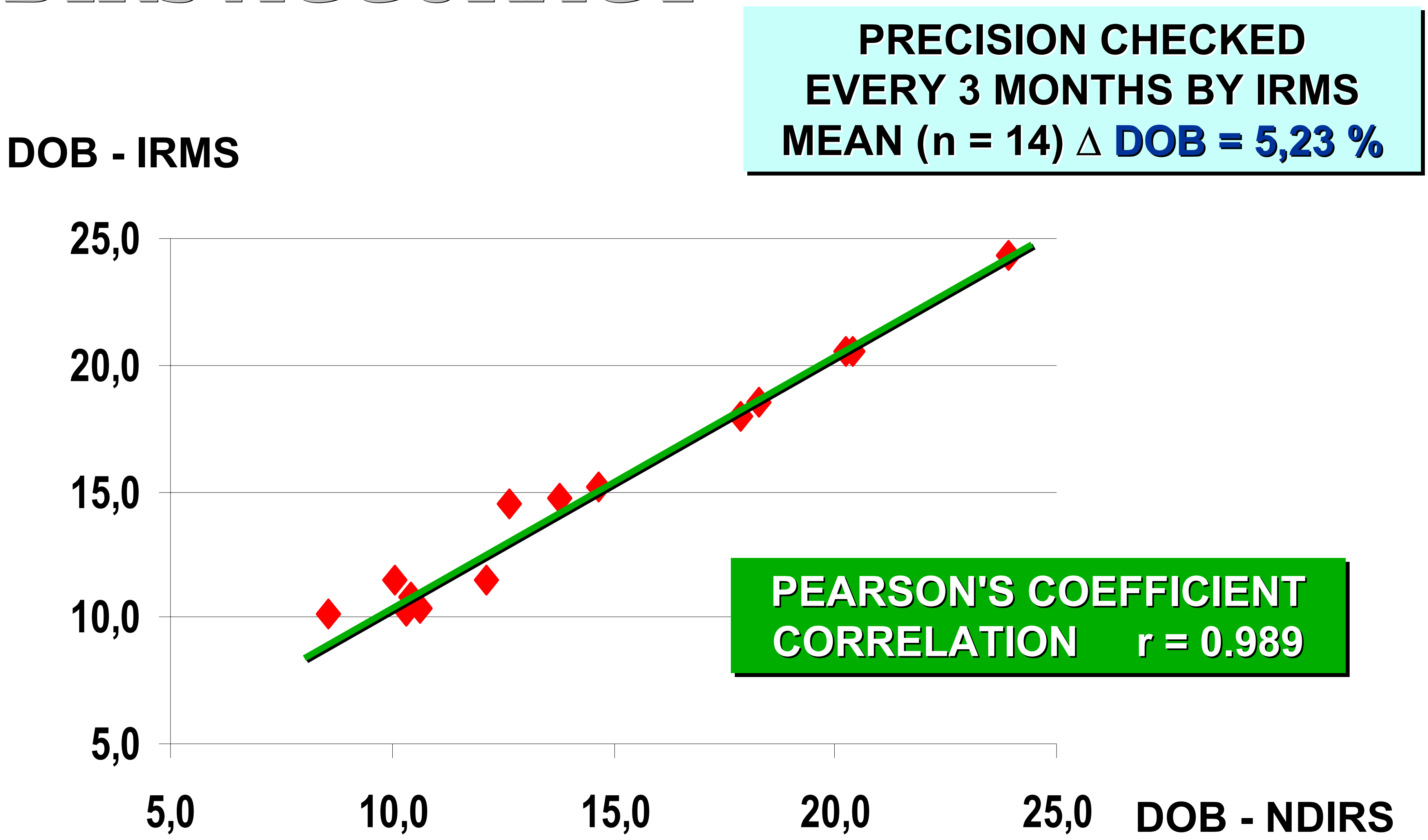
- the accuracy of repetitive measurements
- the absolute accuracy, the relation to a true value.

The breath-test analysis also reveals that a more serious danger for the credibility of $^{13}CO_2$ breath tests could be the **uncertainty in the amount of CO_2 exhaled by the patient.** We consider the uncertainty in CO_2 production the most important source of uncertainty in breath tests results. The IRMS-based cumulative breath tests also suffer from this sort of uncertainty.

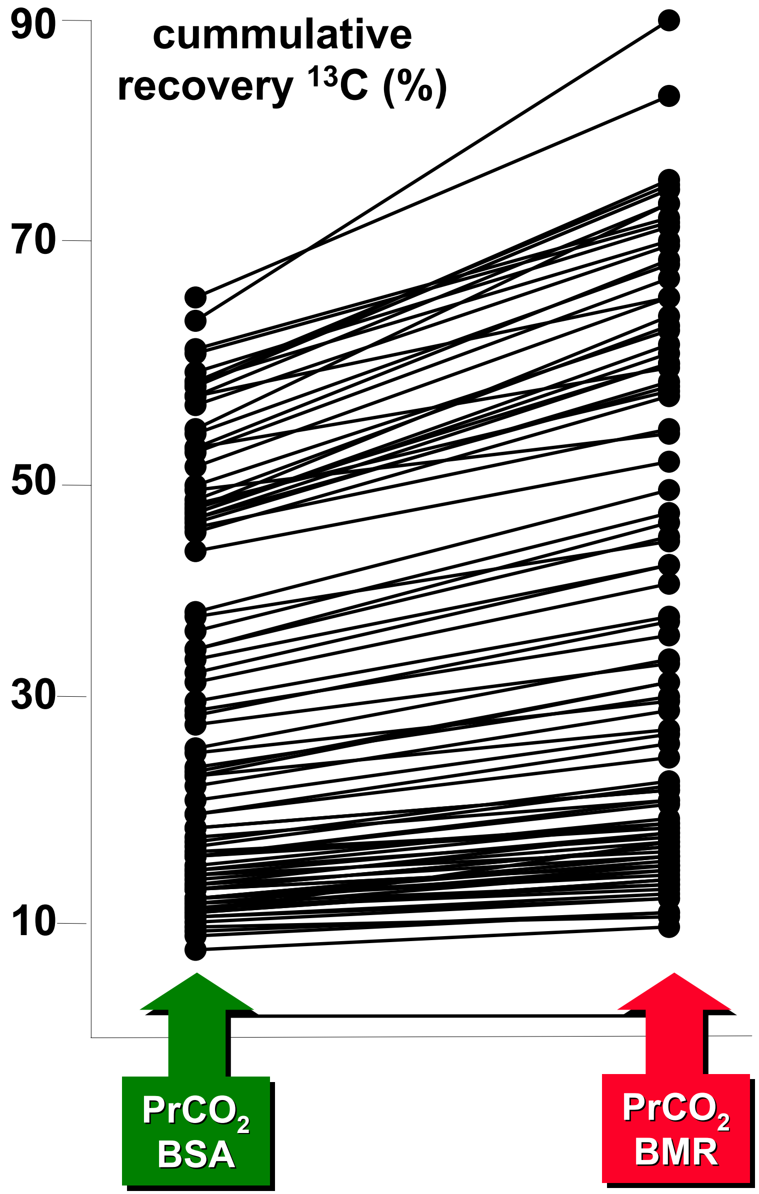
NDIRS ACCURACY



NDIRS ACCURACY



^{13}C RECOVERY CALCULATION



PrCO$_2$BSA = BSA * 300 mmol/h
BSA = W^0,5378 * H^0,3963 * 0,024265
body surface (weight, height) only

PrCO$_2$BMR = BMR * 2,49 mol/d
BMR = αW + βH + γ
α, β, γ = constants for age, sex
body surface (weight, height)
age, sex corrected

215 FUNCTIONAL TESTS
AVERAGE DIFFERENCES = 26.2 %
RANGE = 7.8 - 79.4 %

^{13}C-MTG TEST

TEST PROCEDURE

TWO SAMPLE BAGS AFTER OVERNIGHT FASTING
PANCREATIC SUBSTITUTION THERAPY 3DAY EXCLUDED
STIMULATION MEAL
4 CRISP SLICES, MAIZE WITH FIBRES
(WITHOUT CHOLESTEROL, GLUTEN-FREE)
2 x 10g RAMA (VEGETABLE FAT WITHOUT MILK PROTEINS)
TEST SUBSTANCE ADMINISTRATION - 250mg ^{13}C-MTG
STIRRED INTO VEGETABLE FAT
HOURLY BREATH-BAG SAMPLING (1 - 6 hr)

TEST ANALYTICS

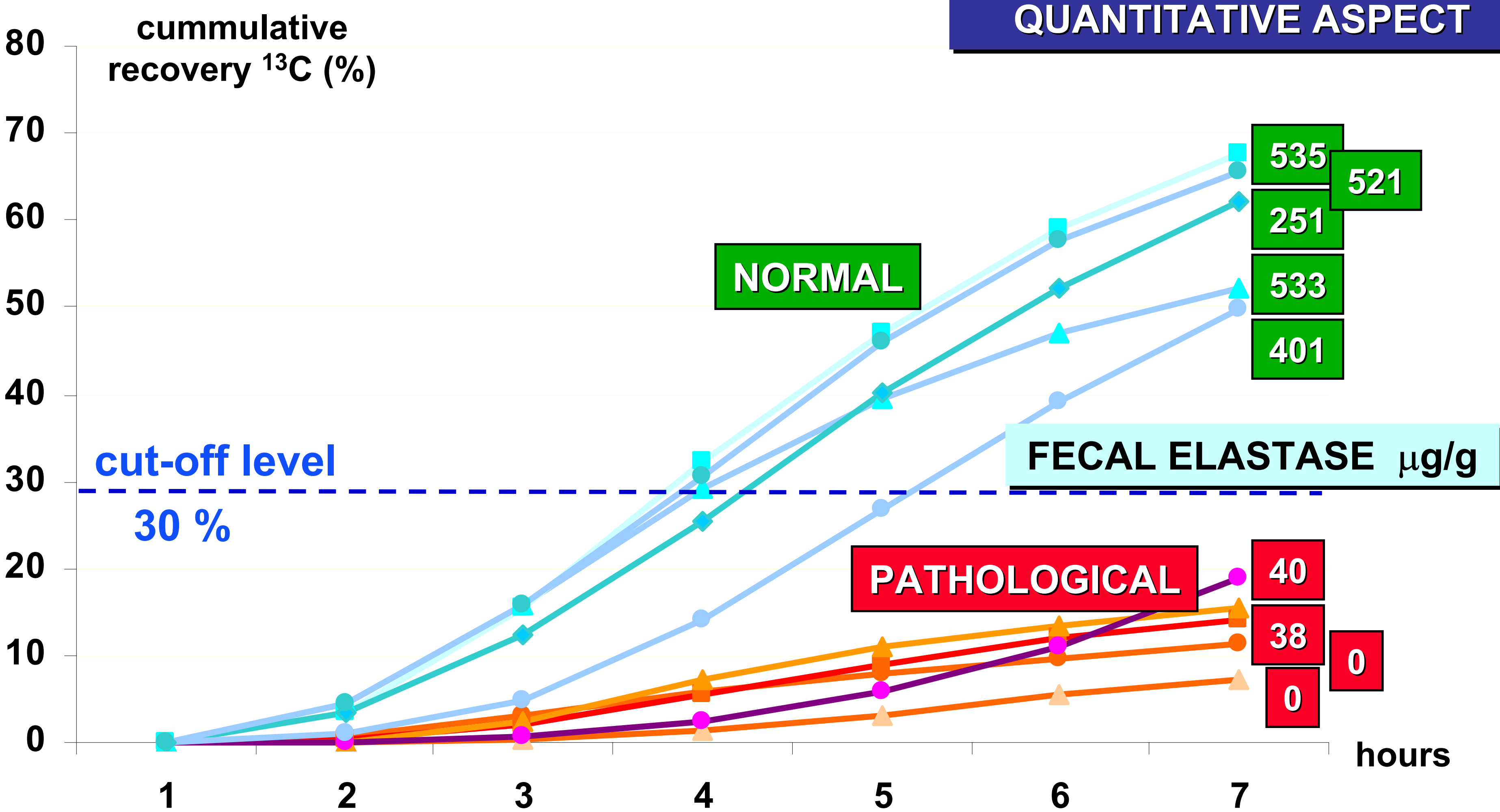
DOB MEASUREMENT OF EACH SAMPLE $^{13}CO_2 : {}^{12}CO_2$ (in ‰)
T_x SAMPLE AGAINST T_0 (DOB = Delta Over Baseline)

EVALUATION OF PANCREATIC INSUFFICIENCY

BSA CALCULATED (BASED ON WEIGHT, HEIGHT)
BMR AND CO_2 PRODUCTION CALCULATED (MS Excel)
CUMMULATIVE RECOVERY FOR 6 HOURS CALCULATED

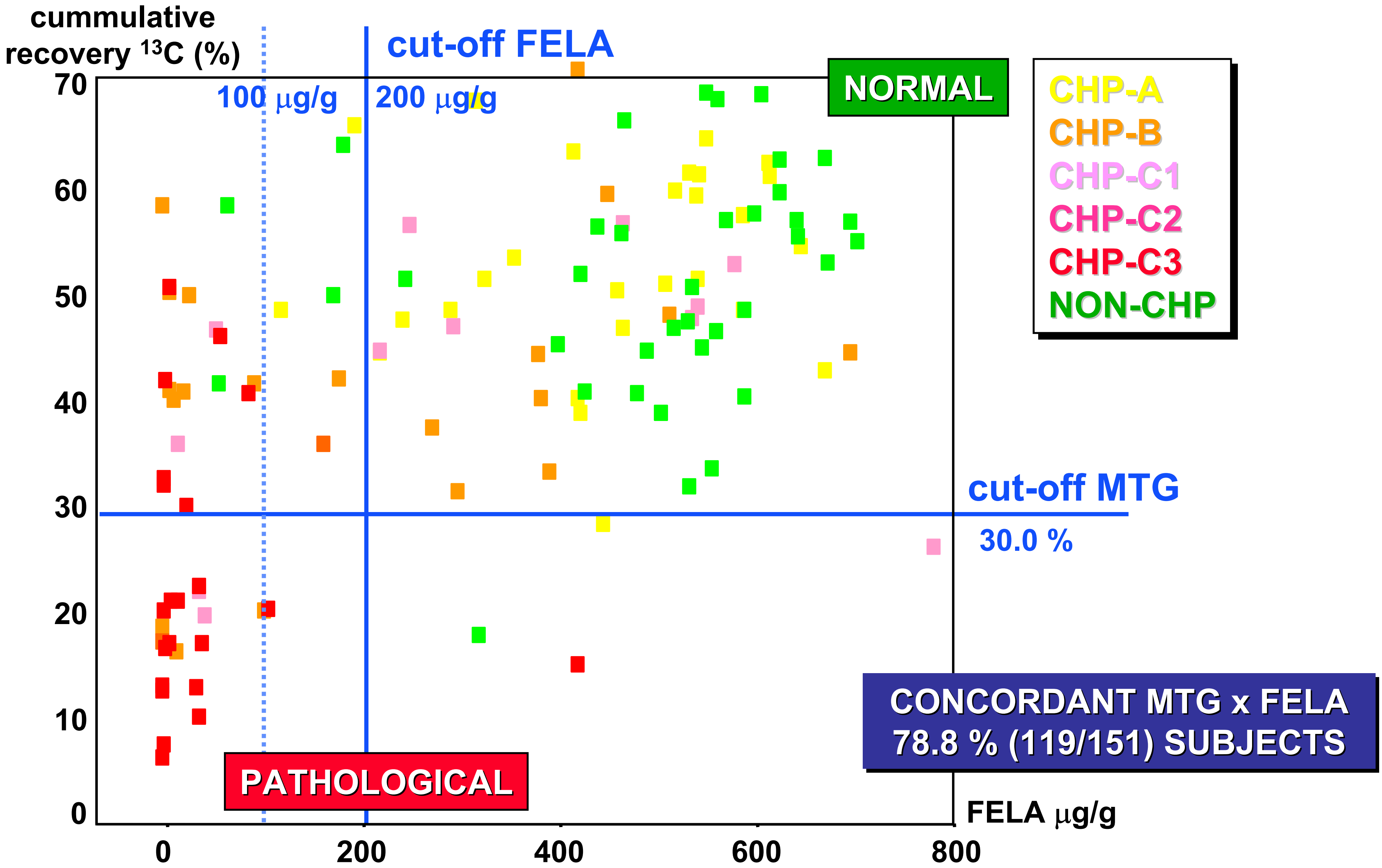
^{13}C-MTG TEST

6 HR CUMMULATIVE RECOVERY of ^{13}C MARKER QUANTITATIVE ASPECT



CUMMULATIVE RECOVERY of $^{13}CO_2$ in % DURING 6 HOURS AFTER 250mg MTG, 10 CASES (5 WITHOUT PANCREATIC INSUFFICIENCY) AND FECAL ELASTASE-1 LEVEL BY ELISA

^{13}C-MTG - FELA in CHP



^{13}C-UREA TEST

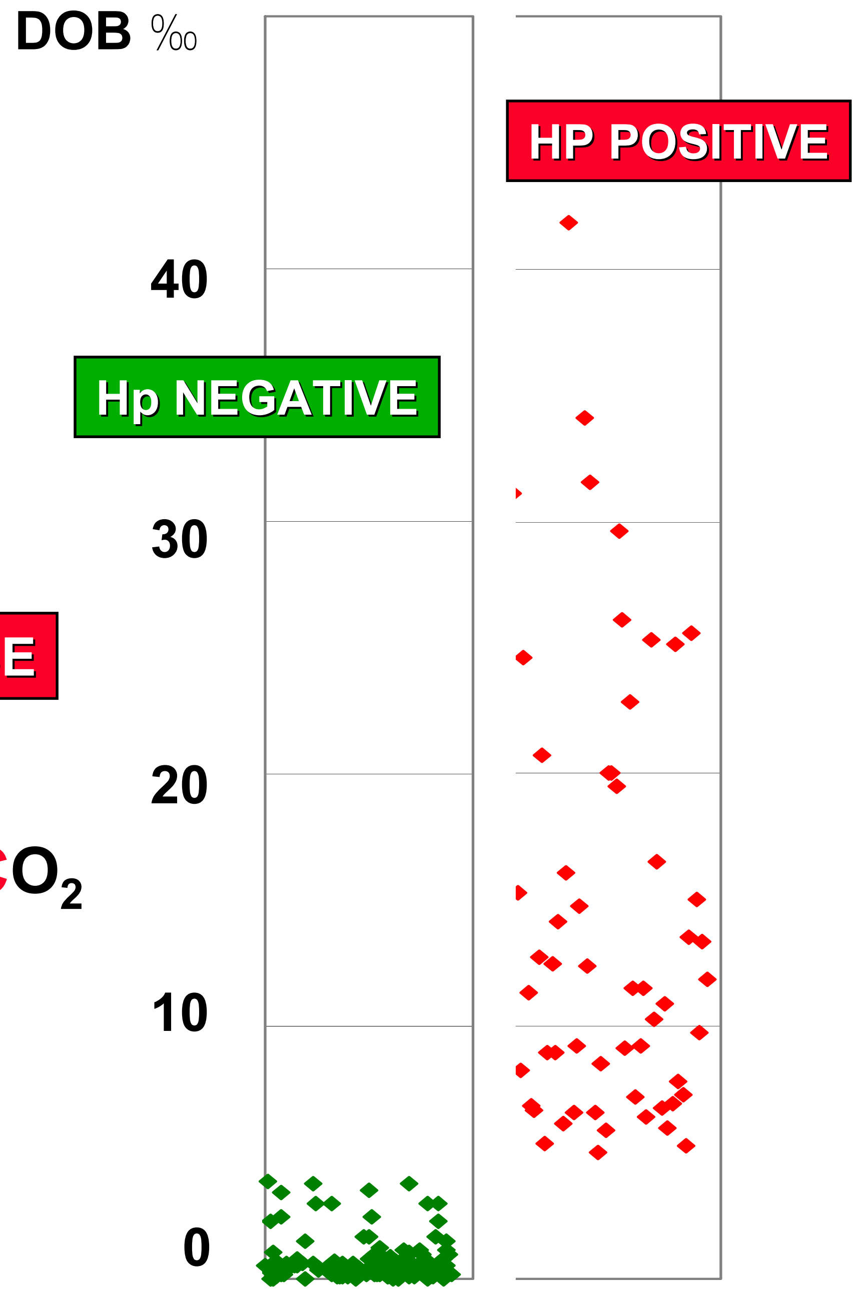
END-POINT TESTS
DOB VALUES of ^{13}C MARKER
CUTT-OFF DEFINED TEST

SUBSTRATE: ^{13}C-UREA
DOSAGE: 75 mg
TEST TIME: 30 minutes

Hp - UREASE

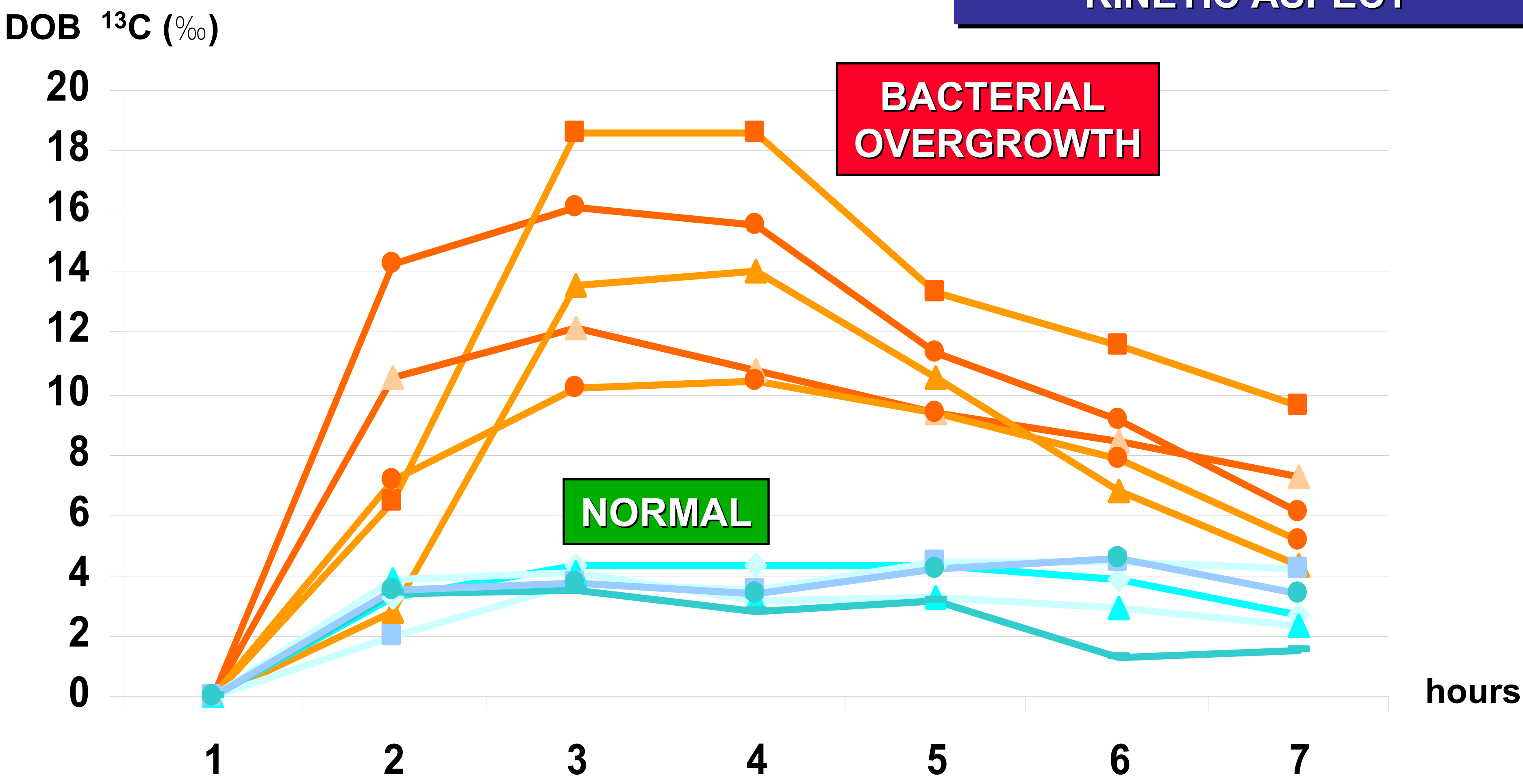
$$NH_2\text{-}^{13}CO\text{-}NH_2 + H_2O = 2\ NH_3 + {}^{13}CO_2$$

NUMBER OF UBT TESTS:	284
NEGATIVE RESULTS:	222
POSITIVE RESULTS:	56
GRAY ZONE (DOB 4 - 5):	6



^{13}C-XYLOSE TEST

6 HR KINETIC TESTS
DOB VALUES of ^{13}C MARKER
KINETIC ASPECT



DOB VALUES of $^{13}CO_2$ in % DURING 6 HOURS
AFTER 100mg XYLOSE, 10 CASES - 5 WITH BACTERIAL OVERGROWTH

CONCLUSIONS

Isomax 4000 analyser for ^{13}C-breath test could be used for range of functional tests in gastroenterology diagnostics.

Intraassay variability of measured DOB calculated on 1625 quadriplicates of breath samples in range 0 - 60 DOB is 0.189 DOB value, precission of measurement was checked every 3 months using calibration samples with IRMS reference value. We found the mean difference NDIRS x IRMS to be 5.2%.

The uncertainty in the results of $^{13}CO_2$ breath tests has three sources: uncertain baseline ^{13}C abundance, inaccuracy of the spectrometer and uncertainty in CO_2 production. We consider the uncertainty in CO_2 production the most important source of uncertainty in breath tests results.

^{13}C-mixed triglyceride test (MTG) for exocrine pancreatic function was performed in 161 patients suspected of chronic pancreatitis using 250mg of Glyceryl-1,3-dioctadecanoate-2-octanoate-1-^{13}C. Cummulative recovery < 30 was interpreted as pancreatic insufficiency. When compared with fecal elastase-1 level we found 78.8% consensual results.

^{13}C-xylose test (XBT) for bacterial overgrowth was performed in 53 patients suspected of small bowell bacterial overgrowth with 100mg of ^{13}C-xylose. Those with the peak higher than 6.0 DOB were checked by surgery and results confirmed by a small bowel content cultivation.