Please write clearly using CAPITAL LETTERS.
All fields are mandatory.

For internal purposes
LCD/LCB

PrenaTest®

WM-3051-EN-003

Enter barcode number

## Responsible doctor

*Country code

Practice/hospital

Title/first name/last name

Street/number

CC*

Postcode/city

Telephone/fax

ID (if known)

Laboratory sending the samples/Distributor

## Patient information

First name/last name

Date of birth (DD/MM/YYYY)

# Order to perform the PrenaTest® to exclude or detect a 22q11.2 microdeletion (associated with DiGeorge syndrome) and velo-cardio-facial syndrome (Shprintzen syndrome)

This examination is particularly useful in cases of abnormalities on ultrasound, for example during organ screening, which may correlate with a DiGeorge or velo-cardio-facial syndrome (Shprintzen syndrome):

**Reasons for the genetic examination**

- ☐ Congenital heart defect (as the most important prenatal characteristic of a 22q11.2 microdeletion in ultrasound examinations)
- ☐ Detection of an aberrant subclavian artery
- ☐ Growth retardation
- ☐ Increased nuchal translucency
- ☐ Kidney malformation
- ☐ Cleft lip and palate
- ☐ Small thymus
- ☐ Other medical reasons

This examination can **be selected only in combination with the PrenaTest® option 3,** however, not with PrenaTest® option 1 or 2.
The analysis is free of charge for your patient.
**The cffDNA level has to be at least 13% (see reverse).**

☐ **In combination with the initial order for PrenaTest® option 3**
Result report normally in 4 – 6 business days* (in comination with the results for the trisomies 21, 18, 13 and the gonosomal aneuploidies).
Please send this completed and signed order form together with the initial order and the blood samples in the return box.

* Business days are Mon.-Fri. (not including Saturday, Sunday and public holidays). Prices are including VAT and transport

## Patient consent

The responsible doctor or the laboratory sending the samples confirm by signing that the patient
a) has received explanations and human genetic counseling in accordance with national legislation and has consented to the genetic examination,
b) has agreed to have her test result sent to the laboratory sending the samples for forwarding to the responsible doctor,
c) has agreed to the storage and processing of her personal data by the laboratory sending the samples to LifeCodexx AG.

| Place | Date (DD/MM/YYYY) | Signature/Stamp of the responsible doctor | Signature/Stamp of the distributor/ laboratory sending the samples |
|---|---|---|---|
| | | ✗ | ✗ |

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Chief Executive Officer: Dr. Michael Lutz | Freiburg im Breisgau District Court, Commercial registry 701989 | Registered office: Konstanz | VAT ID no. DE 258862614

# Information for performing the PrenaTest® to exclude or detect a 22q11.2 microdeletion, associated with DiGeorge syndrome and velo-cardio-facial syndrome (Shprintzen syndrome)

## 22q11.2 microdeletion [1]

DiGeorge and velo-cardio-facial syndrome are caused by a microdeletion on the long arm of chromosome 22 in position 11. This 22q11.2 microdeletion is one of the most common chromosomal abnormalities which occurs in one out of approx. every 6000 newborns. This occurs spontaneously in more than 90% of those affected and 6% to 28%[2] of those affected may also have inherited it from a parent. The clinical abnormalities, particularly heart defects, may have different manifestations, depending on the severity of the disease or may not be present at all. Knowledge about DiGeorge or velo-cardio-facial syndrome may be of importance in the prenatal as well as in the neonatal period.

## Test accuracy of the PrenaTest® for the determination of the 22q11.2 microdeletion

Data from synthetic pooled DNA specimens as well as several specimens from pregnant women whose unborn child had a 22q11.2 microdeletion were investigated. In all cases, a 22q11.2 microdeletion was correctly detected. The validation process was performed in three phases:

**Phase 1** Four synthesized specimens, each of which had a different cffDNA level (16%, 8%, 4% and 2%) and which had a 22q11.2 microdeletion, were analyzed several times. The 22q11.2 microdeletion of the two specimens with a cffDNA level of 8% and 16% was correctly determined, while the results of the specimens with a lower cffDNA level were not significant.

**Phase 2** Four specimens from pregnant women whose unborn children had a 22q11.2 microdeletion were retrospectively tested and the 22q11.2 microdeletion was correctly detected in each case. In the case of the fourth specimen, a cardiac defect (DORV; double outlet right ventricle) was confirmed by ultrasound. The DiGeorge syndrome was confirmed after birth.

**Phase 3** In a final internal blinded study, specimens from phase 2 with a 22q11.2 microdeletion as well as euploidic specimens were investigated. All specimens were correctly classified. Due to the low number of cases, a concrete test sensitivity and specificity cannot currently be derived.

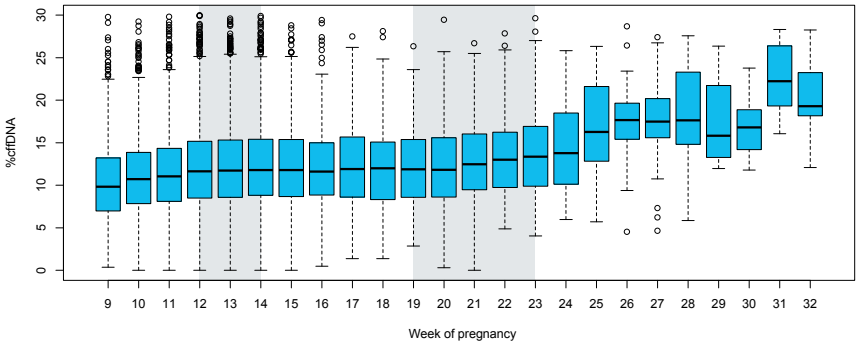
| Correctly classified specimens 16/16 | Total detection rate 2/2 | False-positive rate 0/14 |
|---|---|---|

## Limits of the test method for the determination of the 22q11.2 Microdeletion

### 1. cffDNA level

The detection or exclusion of a 22q11.2 microdeletion is currently only possible for specimens which have a cffDNA level of at least 13%, since in the scope of the validation, the cffDNA level of the patient specimens investigated was between 13% and 27%. Please note: According to Fig. 2, the cffDNA level is highly individual and only increases starting in the 20th week of pregnancy. Therefore it is possible that due to a cffDNA level that is too low, no test result can be achieved. A new blood sample is not requested provided the cffDNA level is sufficient for determining fetal trisomies 13, 18, 21 as well as gonosomal aneuploidies.

**Fig. 1: Correlation between cffDNA level and week of pregnancy based on data from LifeCodexx AG from routine clinical practice in Germany (8/2012 – 12/2015)**




| Week of pregnancy 12 – 14 | Week of pregnancy 19 – 23 | |
|---|---|---|
| 39% | 45% | Samples with cffDNA ≥ 13% |
| 61% | 55% | Samples with cffDNA < 13% |

### 2. Mother is the carrier of the microdeletion

It is possible that the mother is the carrier of the 22q11.2 microdeletion but not the unborn child. This can lead to discordant (false-positive) test results.

### 3. Size of the microdeletion

In more than 85% of affected persons, the deletion includes a region measuring approx. 2.5 megabases in the 22q11.2 region of chromosome 22. This region is investigated with the PrenaTest®. A small percentage of affected persons has an even smaller deletion or point mutation in the affected region which cannot be detected with the PrenaTest®. This can lead to discordant (false-negative) test results.

### 4. Microduplication in the investigated gene region

In the investigated gene region of chromosome 22, microduplications can also occur. These are not determined with the PrenaTest®.

**Literature**

1 OMIM# of the disease: 188400 (DGS), 192430 (VCFS)

2 Sandrin-Garcia P, Macedo C, Martelli LR, et al. Recurrent 22q11.2 deletion in a sibship suggestive of parental germline mosaicism in velocardiofacial syndrome. Clin Genet. 2002;61(5):380–383

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