PrenaTest®

PrenaTest® I Details to the result reports

Examination method and analysis result

The PrenaTest® for the determination of the examined chromosomal aneuploidies is based on molecular genetic methods such as qPCR and next generation sequencing (NGS) using the CE-marked software PrenaTest® DAP.plus.[1-4] The threshold values of the analysis scores which are used to differentiate a positive from a negative test result differ for the chromosomal aneuploidies due to biological and analytical factors. For the determination of gonosomal aneuploidy, additional evaluation criteria are used and thus the analysis score alone is not significant.

Validity

The results should always be considered in the context of other clinical criteria. Conspicuous results have a high predictive value. PrenaTest® is an advanced, non-invasive test method with a high accuracy, which is currently classified as 'not fully diagnostic'. International and national recommendations and opinions have to be taken into account.[5-7]

Diagnostic value for singleton pregnancies

Performance evaluation of the NGS-based PrenaTest® in studies of 2012/2013
The diagnostic accuracy of the PrenaTest® has been validated by clinical studies in which LifeCodexx AG was substantially involved. The European validation study (EVS) comprised the analysis and the reporting of 468 blood samples.2 Within the scope of a further study with samples from the partner company Sequenom Inc., USA, (Sequenom Collective Study, SCS) we analysed and reported an additional 340 samples. In summary, the performance qualification comprises the analysis of 808 samples including 75 trisomy 21 cases (EVS 41, SCS 34), 14 trisomy 18 cases (EVS 8, SCS 6) and 8 trisomy 13 cases (EVS 5, SCS 3). 806 out of 808 samples have been classified correctly (99.8%). Within the EVS, one result was false-negative for trisomy 21 and one result was false-positive for trisomy 18 (with an aberration of chromosome 10). This translates into a detection rate for trisomy 21 of 98.7% with a false-positive rate of 0%. The numbers of trisomy 13 and 18 cases tested are insufficient to draw conclusions on its sensitivity and specificity for these trisomies.

Table 1: Number of cases and overall detection rates of fetal trisomies 13, 18 and 21 within the study collectives

	EVS 2012	SCS 2013	Overall
Correctly classified samples	466/468 (99,6%)	340/340 (100%)	806/808 (99,8%)
Trisomy 13	5/5 (100%)	3/3 (100%)	8/8 (100%)
Trisomy 18	8/8 (100%)	6/6 (100%)	14/14 (100%)
Trisomy 21	40/41 (97,6%)	34/34 (100%)	74/75 (98,7%)
Overall detection rate	**53/54 (98,1%)**	**43/43 (100%)**	**96/97 (99,0%)**
False-positive rate	**1/414 (0,2%)**	**0/297 (0%)**	**1/711 (0,1%)**

Table 2: PrenaTest® sensitivity & specificity for the detection of fetal trisomy 21 for singleton pregnancies (mosaics as well as structural aberrations are not included).

	EVS 2012	SCS 2013	Overall
Sensitivity (lower unilateral 95% confidence intervall)	97,6% (88,9%)	100% (91,6%)	**98,7%** **(93,8%)**
Specificity (lower unilateral 95% confidence intervall)	100% (99,3%)	100% (99,0%)	**100%** **(99,6%)**

Diagnostic value for twin pregnancies

Within the scope of the performance qualification for the NGS-based PrenaTest® for multiple pregnancies 60 twin as well as 2 triplet pregnancies have been investigated (comprising 16 samples from our partner company Sequenom Inc., USA). Among the twin pregnancies there were 6 trisomy 21 cases, which have been confirmed by karyotyping (1monochorionic, concordant; 5 dichorionic, discordant). Using the PrenaTest® all 6 cases have been determined correctly. The remaining samples exhibited inconspicuous results. No fetal trisomy 13 or 18 occurred within the scope of the performance qualification, therefore no conclusions on the PrenaTest® accuracy for trisomies 13 and 18 in multiple pregnancies can be drawn. There were too few triplet samples for a performance qualification and a statement on test accuracy for triplets.

Table 3: Results of the performance review of the PrenaTest® for the determination of fetal trisomies 13, 18 and 21 for multiple pregnancies (all positive and one part of the negative results (SQNM study) were tested using karyotyping)

	EVS 2012	SQNM study 2013	Overall
Correctly classified samples	46/46*	16/16	62/62*
Trisomy 21	2/2	4/4	6/6
Trisomy 13/18	0	0	0
Detection rate	**2/2**	**4/4**	**6/6**
* including 2 triplet pregnancies			

Diagnostic value for gonosomal aneuploidies

The NGS-based PrenaTest® for gonosomal aneuploidy (Turner, Triple X, Klinefelter and XYY syndrome) was tested on a total of 434 specimens from single pregnancies. During this testing, 11 out of 12 affected fetuses, that is 92 %, were correctly determined. Moreover, five discordant, „false positive“ results were obtained. At present, based on the low number of cases examined, LifeCodexx AG will not separately report any sensitivities and specificities for gonosomal aneuploidy.

Table 4: Results of the performance review of the PrenaTest® for the determination of gonosomal aneuploidy for singleton pregnancies

	EVS 2012	SQNM study 2013	Overall
Correctly classified samples	377/383* (98,4%)	51/51 (100%)	428/434* (98,6%)
Turner syndrome	7/8 (87,5%)	3/3 (100%)	10/11 (90,9%)
Triple X syndrome**	0	0	0
Klinefelter syndrome**	0	0	0
XYY syndrome	1/1 (100%)	0	1/1 (100%)
Overall detection rate	**8/9 (88,9%)**	**3/3 (100%)**	**11/12 (91,7%)**
False-positive rate	**5/374 (1,3%)**	**0/48 (0%)**	**5/422 (1,2%)**

* Three samples with normal, male karyotype were classified as false-positive for Klinefelter syndrome. In two of the samples the cffDNA content was below 5%. The third sample showed an abnormal chromosome X value, which could be a maternal triple X and which was not determined by conventional karyotyping in the study. The reason for the false-positive results of the two other samples classified for Turner syndrome could be a maternal mosaic or a low cffDNA content.
** The Triple X and Klinefelter syndrome were examined independently of this performance qualification in the context of research projects and successfully determined.

General limitations of the genetic testing method

1. In general, no statements regarding structural chromosomal changes, mosaics or polyploidy can be made with the PrenaTest®. Also, chromosomal disorders in which other chromosomes than 13, 18, 21, X and Y are affected, as well as other genetic diseases, are not the subject of the present examination.

2. The examined fetal DNA is primarily derived from the cytotrophoblast and is released by apoptosis and necrosis of trophoblast cells of the placenta. As such, it is only possible to achieve a level of diagnostic certainty close to that attained via direct chorionic villus sampling. Consequently, mosaics or fetoplacental discrepancies in trisomies 21, 18 or 13 resp. gonosomal aneuploidy are not recognisable. In the event of a fetoplacental discrepancy, this can also mean that the PrenaTest® result is not representative for the unborn child.

3. Undisclosed *vanished twins* can contribute a sufficient proportion to the total cffDNA fraction to cause a positive PrenaTest® result being not representative for the continuing singleton pregnancy.

4. An existing maternal mosaic can lead to a conspicuous PrenaTest® result which may not be representative for the unborn child. These mosaics primarily affect gonosomal aneuploidy. For example, cases of pregnancies in women affected with Turner syndrome were based on mosaic findings (45, X/46, XX) in the women.

5. An existing maternal gonosomal aneuploidy as the Triple X syndrome can lead to a conspicuous PrenaTest® result which may not representative for the unborn child. This means, for example, that a positive PrenaTest® result with a reference to a chromosome disorder 47, XXY does not necessarily represent a fetal chromosomal abnormality. Rather, it should be examined whether and how the pregnant woman has a triple X syndrome.

Fetal gender determination

For PrenaTest® and PrenaTest® Plus gender will also be determined using next generation sequencing. Both methods for gender determination have not been validated in a clinical study. A –male– result is reported, if a Y chromosomal marker is detected by QuantYfeX® and a sufficient number of Y chromosomal sequences are detected by next generation sequencing. In the case of a twin pregnancy this means that at least one of the fetuses is male. A –female– result is reported, if no Y chromosomal marker is detected and only a small number of Y chromosomal sequences are deteced by next generation sequencing. In very rare cases the fetal gender cannot be determined clearly by QuantYfeX® or the results of QuantYfeX® and of next generation sequencing deviate from each other. Then, for PrenaTest® and PrenaTest® Plus, we will determine the gender on the basis of the quantity of Y chromosomal sequences detected by next generation sequencing of the blood sample.

Please note:

LifeCodexx AG work is state of the art in terms of science and technology. LifeCodexx notes that a validity of 100% in the use of the PrenaTest® at the practice cannot be expected. There are risks that can never be fully excluded, no matter how meticulous the genetic analysis is. Nevertheless, all possible measures and safety precautions are undertaken to avoid these risks and other errors

Literature

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