EndoPredict®

EndoPredict® Report Generator CE

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EndoPredict® Report Generator Version V3.1

User Manual

EndoPredict® Report Generator is a product for *in-vitro*-diagnostics.

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Contents

Translated from the German language

1. Abbreviations and Definitions

A	Specimen A, first specimen on the PCR plate
B	Specimen B, second specimen on the PCR plate
Ct	Cycle Threshold: PCR cycle at which the fluorescence threshold is reached (normalised, interpolated)
Delta Ct values	Relative expression level of a gene normalised to the mean of the three reference genes (CALM2, OAZ1 and RPL37A)
EP	EndoPredict®
EPclin	Predictive score that is calculated from the EndoPredict® score, the tumour size and the number of positive lymph nodes
EPRG	EndoPredict® Report Generator
EV-SSL	Extended validation secure sockets layer; cryptographic technique ensuring an authentic and intercept-secure internet connection
kPCR	Kinetic PCR; synonymous with qPCR
LCL	Lower Control Limit
NEG	Negative control
PCR	Polymerase Chain Reaction
PDF file	File with the extension `.pdf`, comprising the EP report
POS	Positive control
qPCR	Quantitative PCR
SS III RT/Platinum Taq Mix	SuperScript® III Platinum® One-Step Quantitative RT-PCR System Custom
Text file	File with the extension `.txt`, comprising the Ct values and generated using the VERSANT software
UCL	Upper Control Limit
VERSANT software	Device software of the VERSANT® kPCR Molecular System by Siemens Healthcare Diagnostics

In the text below, the following notation is used:

italics	References
bold	Texts in the graphic user interface (menus, buttons, tabs, ...)
bold italic	Keyboard shortcuts
`Courier`	Keywords in files or file names
«Quotes»	Quotations from the EP Report

2. Operation

The web application «EndoPredict® Report Generator» (EPRG) calculates the EndoPredict® Score (EP) from measurements of the expression of various genes. From this information, plus details on tumour size and number of positive lymph nodes, the EPclin score is determined. The calculation is performed according to the publication

Filipits et al: A new molecular predictor of distant recurrence in ER-positive, HER2 negative breast cancer adds independent information to conventional clinical risk factors. Clinical Cancer Research, 2011.

In addition, EPRG offers analysis of various quality parameters of the underlying values and additional controls. As a result of these calculations, a report in the form of a PDF file is created, which can be saved and printed out by the user.

EndoPredict® Report Generator is free and available without authentication from the internet. The server addresses are `www1.endopredict.com` and `www2.endopredict.com`. Data transfer to the server and back is encrypted so that any patient data cannot be read by third parties.

EndoPredict® Report Generator and the generated EndoPredict® reports are designed for trained staff of diagnostic laboratories. The generated reports are also intended for physicians involved in the care of breast cancer patients.

3. Statement of Intended Use

The EndoPredict® Report Generator is an in vitro diagnostics product for determining the risk of distant metastases in patients with oestrogen-receptor-positive, HER2-negative primary breast cancer under exclusively adjuvant endocrine therapy. For the intended purpose, the EndoPredict® Report Generator may only be applied to RNA samples extracted from formalin-fixed, paraffin-embedded breast cancer tissue, and it may be used only in combination with Sividon's Tissue Preparation Reagents or the VERSANT® Tissue Preparation Reagents, with the VERSANT® kPCR AD module and the EndoPredict® TAQO / SuperScript® III Platinum® One-Step Quantitative RT-PCR System Custom and the EndoPredict® Kit.

The EndoPredict® Report Generator is designed for use by experts trained in the assay techniques required for performance of the test and in the application of the system. The results of the EndoPredict® test may be used only in the proper context, together with other established methods and clinical / pathological factors for the prognosis and stratification of patients with breast cancer.

4. Application of the EndoPredict® Report Generator

4.1. Providing the measurement data

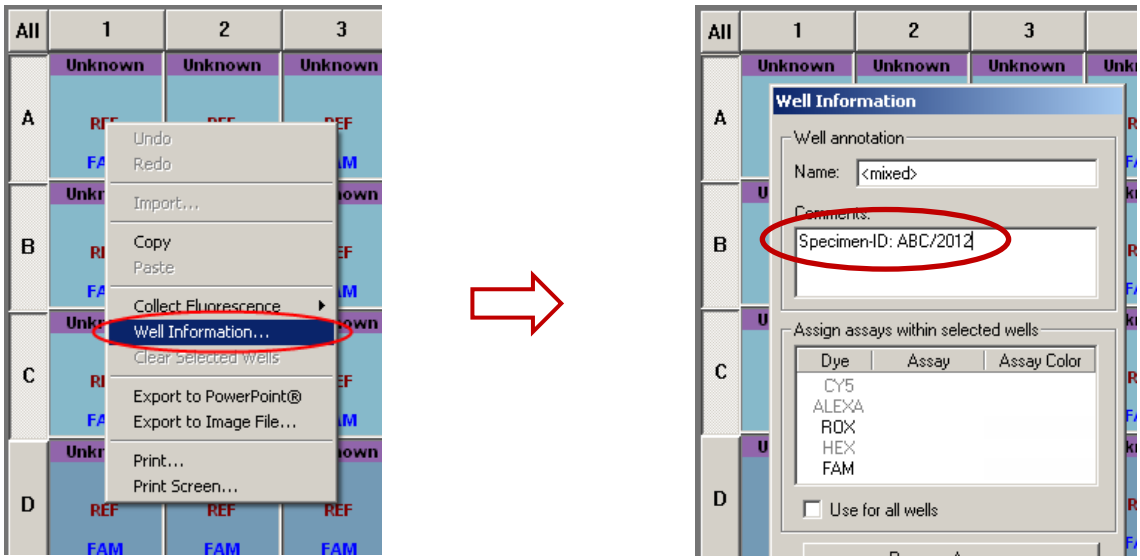
EPRG generates a report from the qPCR data, which must be exported beforehand using the software of the VERSANT qPCR system used. For details, see the

«IFU_Endopredict»[1] instruction manual; a quick starter for the export of measurement data follows here:

The EndoPredict® test was performed on a VERSANT™ kPCR device by Siemens with the associated VERSANT kPCR software version 1.1 (VERSANT software) and the «EndoPredict CE» default settings. EPRG requires the measured Ct values and also checks the plate layout. This information is already defined in the VERSANT software and then exported to the text file. The defaults define the conditions of the qPCR run and the plate layout, the temperature profile, the settings for «Analysis Term Settings» and the format of the text report. All test settings essential for EndoPredict® are stored in the respective default (see instruction manual «IFU_Versant_Defaults»[2]).

At the end of the PCR, you should visually inspect all qPCR curves in the VERSANT software. The text report is then exported as a text file **(File → Export Text Report → Export Text Report To Text File).**

You can identify the specimen(s) for example by reference to the specimen receipt number of the individual tumour patient already in the VERSANT software and also store the identification there: this is then included in the text file after export as well and will appear in the EndoPredict® Report (see section 5.1). To this purpose, select all the wells of the respective specimen in **Plate Setup** (A1 to C12 for specimen A and F1 to H12 for specimen B), open the context menu with the right mouse button and select **Well Information.** Then enter the text[3] «`Specimen-ID:`» in the dialogue **Well Information** into the text box **Comments**, followed by the required identification.



For full control of the data, it is also mandatory to name the EndoPredict® plate and qREF batch (positive control) used. In analogy to the input of the specimen-ID, these data can be stored in the VERSANT software already at this point or defined later in the user interface of the EPRG. If they have already been stored in the VERSANT software, the information is automatically copied into the input fields of the user interface upon upload of the text file. For entering the plate number and the qREF batch prefer-

[1] Available from Sividon Diagnostics, for contact data see page 2
[2] Available from Sividon Diagnostics, for contact data see page 2
[3] For compatibility with version 2.0, alternatively «`PatID:`» may be entered.

ably the wells of the controls (D1 to E12) should be selected. Open the context menu with the right mouse button and select **Well Information.** The plate number and the qREF batch each have to be entered on a separate line of the text box **Comments**, so after entering the first number use the ***Enter*** key to terminate the line. If the plate number or the qREF batch share a line with other information, the data entered cannot be assigned correctly by EPRG.

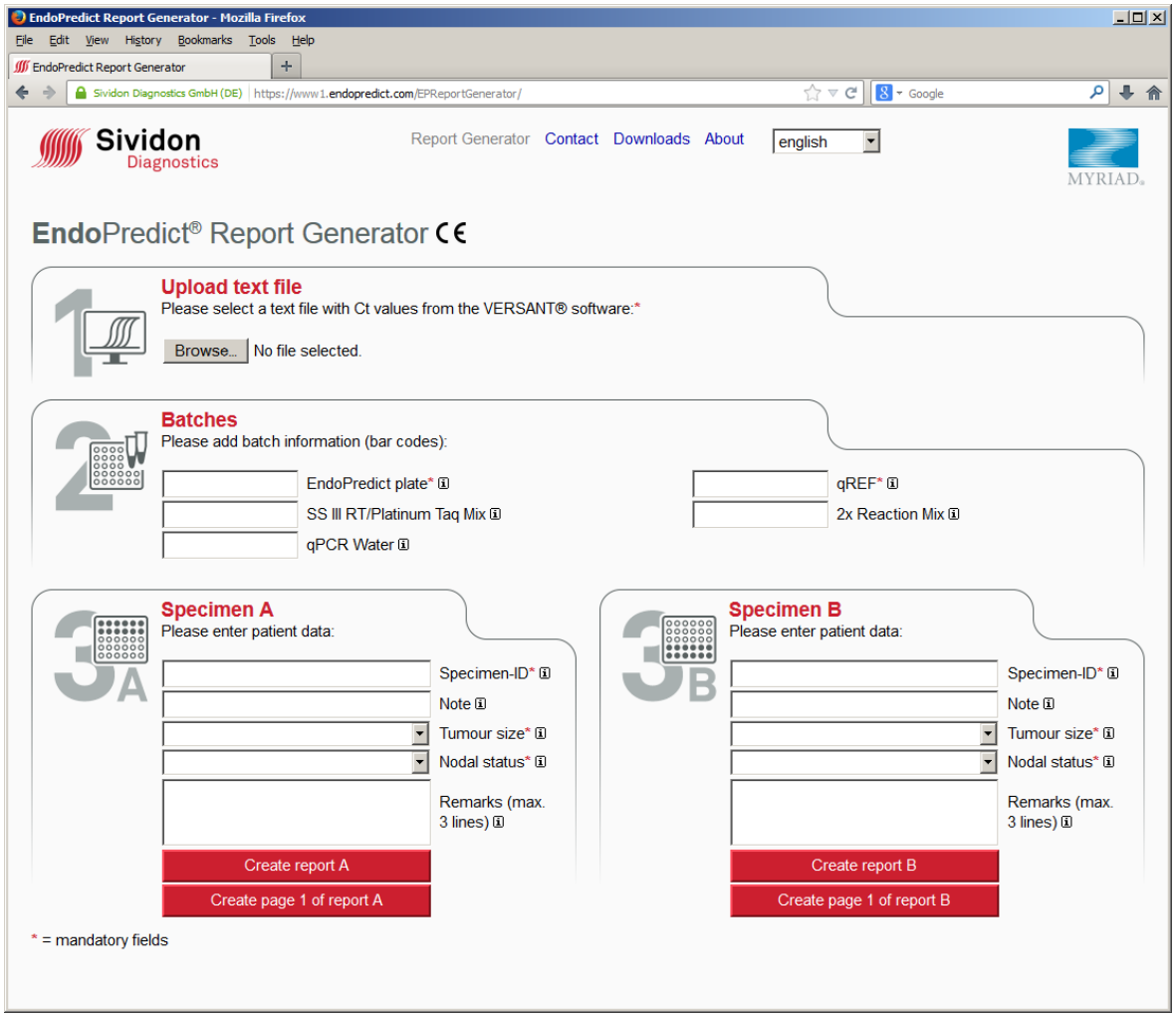
As an alternative to using the keyboard, you can use a bar code scanner to enter the plate number and the qREF batch. The bar code scanner should be used only on the device-specific PC, as its factory default setting is «U.S. keyboard». On the device-specific PC, all numeric and alphanumeric characters are read correctly.

4.2. Starting the web application

Start a browser and enter the address (URL) of one of the following alternatives:

`www1.endopredict.com` OR `www2.endopredict.com`

This takes you to the following website:

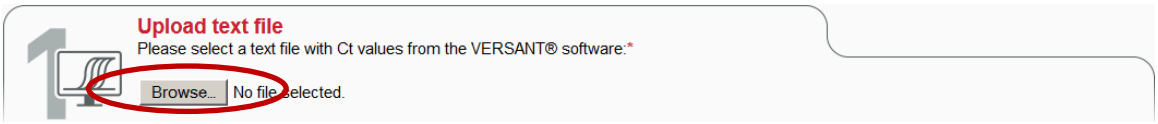


For secure data transmission, the website uses an Extended Validation SSL certificate (EV-SSL). This security certificate confirms the identity of the organisation to the browser. When you visit the website, the padlock icon next to the site URL in the address bar identifies the secure connection. Depending on your browser, the name of the organisation responsible for the content of the website is displayed next to the symbol, and/or parts of the address bar may additionally be displayed in green. Clicking the padlock icon displays further information regarding the identity of the website.

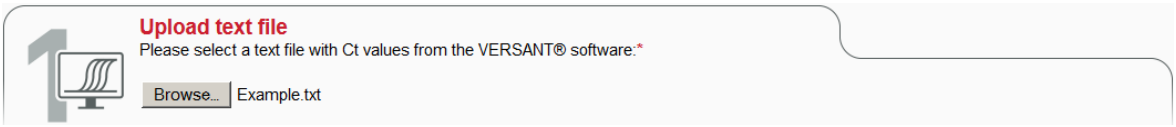
EPRG was developed for the following browsers: Chrome 33, Firefox 27, Internet Explorer 9 and 11, and Safari 5.

4.3. Uploading a text file

The first step on the main page of EPRG is to select a text file from the file system of your computer. In the box **Upload text file** click the **Browse** button.



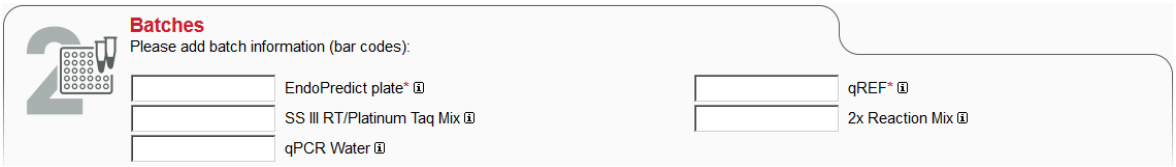
A file selection dialogue opens: Navigate to the desired folder and select your text file. The selected file is then displayed:



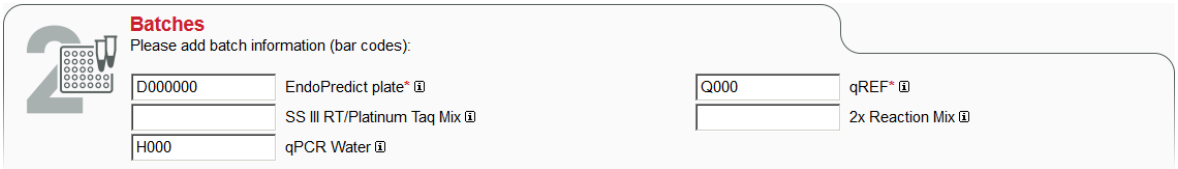
The screen may look different for different browsers.

4.4. Entering batch-relevant information

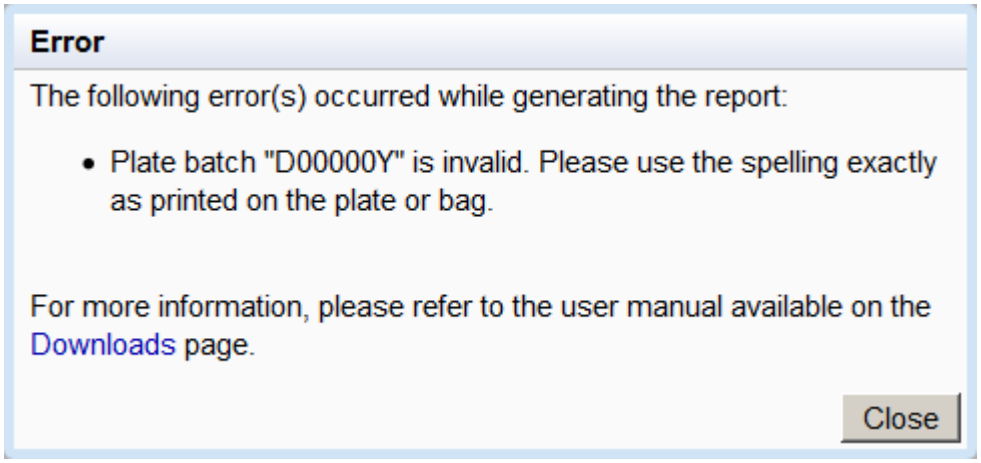
In the next step you have the option of entering the batch numbers of all reagents used for the EP test, so that all the test-related information can later be summarised on page 3 of the report. If this information has already been registered in the VERSANT software (see section 4.1), it will appear automatically in the input fields.

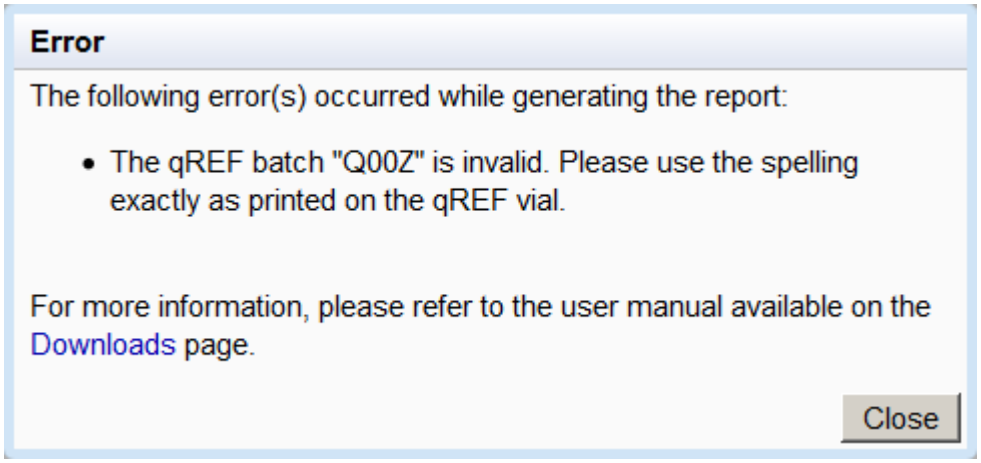


It is mandatory to enter the serial number of the EndoPredict® plate and the qREF batch used; optionally, all other test-relevant reagent batches, such as the batches of the SS III RT / Platinum Taq mix, of the 2× Reaction Mix and of the qPCR water, may be entered. The serial number of the EndoPredict® plate is printed on the label of the aluminium composite bag and also on a narrow label on the right side of the EndoPredict® plate. The batch number of the qREF used is found on the label of the corresponding screw-cap tube.



The combinations of letters and numbers can be manually entered via the keyboard or read using a bar code scanner. The bar code scanner should be used only on the device-specific PC, as its factory default setting is «U.S. keyboard". On the device-specific PC, all numeric and alphanumeric characters are read correctly. However, if a PC with a different language or country keyboard is used, alphanumeric characters may be not correct. If the bar code scanner cannot be used with the device-specific PC, you must always pay attention to correct reproduction or correct the characters read if necessary. Otherwise you will get error messages like the following:

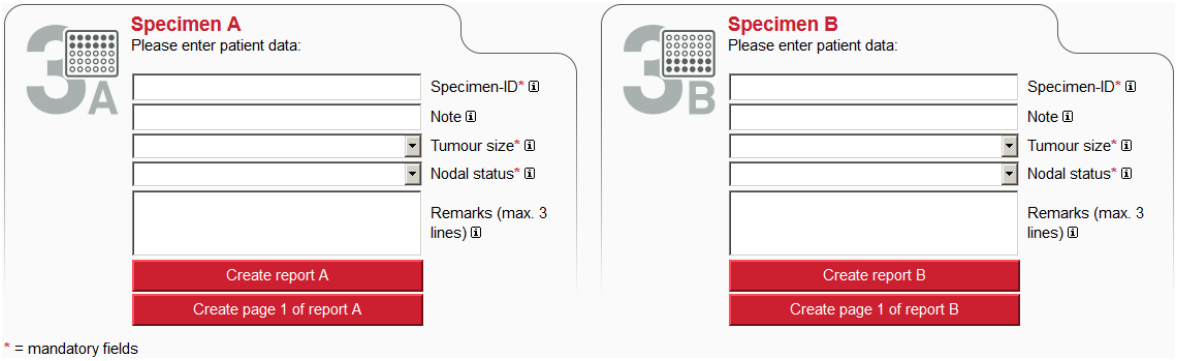




EPRG draws on databases by means of which it checks the expiration dates of the registered reagents and from which it determines the control limits valid for the batch (applies only to the qREF batch and the EndoPredict® plate). The results of these checks are shown in the report (see section 5.11).

4.5. Entering patient information

EPRG can handle one or two specimens on one PCR plate: These are referred to as Specimen A and Specimen B, respectively. For each specimen there is an area for further entries.



The **Specimen-ID** is used to identify the specimen and is shown on each page of the report. This input is mandatory. Please note that the space reserved for the specimen ID in the report is limited. To protect privacy please do not enter the name or the birth date of the patient.

In addition to the specimen-ID, you can enter more text into the **Note** field. The note appears in the report under the specimen-ID, input is optional.

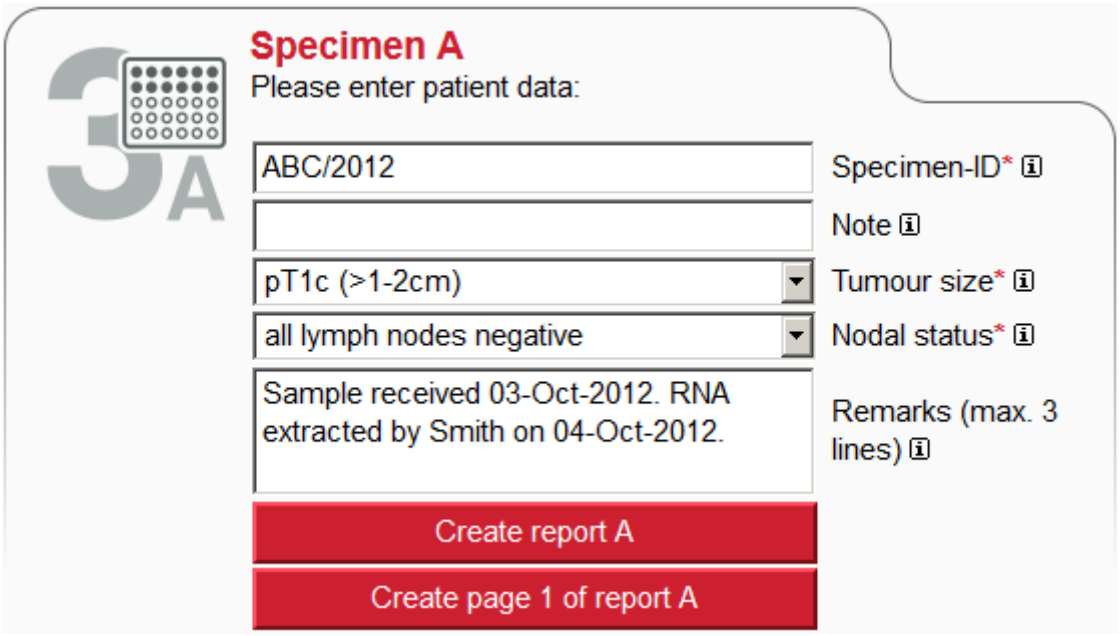
The tumour size and nodal status are selection boxes that must be filled with information from the respective drop-down list. If you have information about the two clinical parameters (i.e. select anything other than **unknown),** in the report the EPclin score is calculated and displayed graphically. Please note the exact definition of the categories: tumour size is based on the TNM classification, where pT1a (up to 0.5 cm) and pT1b (> 0.5 cm to 1 cm) are combined but distinguished from pT1c (> 1 cm to 2 cm). The nodal status has its own categories which should not be confused with the TNM classification! Micro metastases are evaluated as **1 to 3 positive lymph nodes** (according to the TNM classification).

If you select **unknown** for tumour size and / or nodal status, in the report an additional page will be generated that contains a table with all 16 possible combinations of tumour size and nodal status and the respective associated individual EPclin results.

Tumour size and nodal status have no influence on the EP score.

You can also enter additional free text in the **Comments** field, which will be printed on p. 3 or 4 of the report. Please note that the space in the report is limited to three lines which are broken automatically at the right edge of the report. If your entry should not fit completely into the report to be printed, at the end the note «<rest truncated>» will be added.

For the further sections of this manual, by way of example we consider the following entries:



4.6. Creating Reports

By clicking the button **Create report A** or **Create report B**, you generate the report for Specimen A or B, respectively. The report is a PDF file, which you can now view (i.e. open) directly or save.

By clicking the button **Create page 1 of report A** or **Create page 1 of report B** you have the option of creating only the first page of the report, e.g. to communicate the test result to the commissioning colleague. In case **unknown** is selected for tumour size and / or nodal status, the above mentioned additional page containing the table with all the 16 possible combinations of tumour size and nodal status will also be contained in the report.

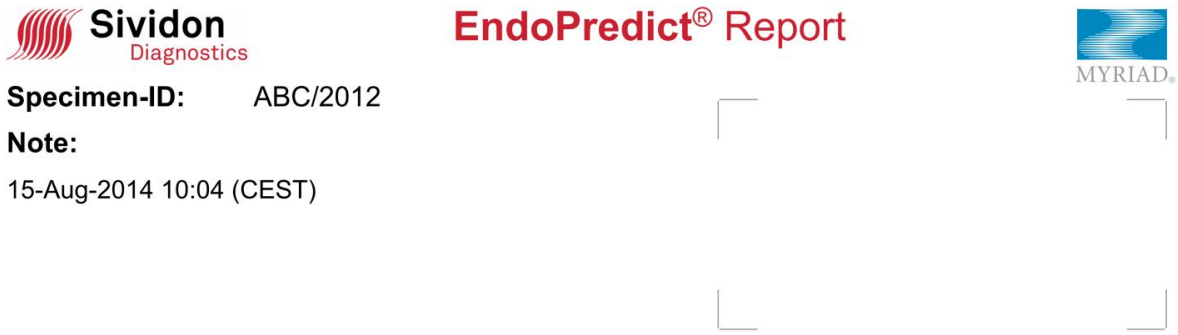
The PDF file is saved under the same file name as the text file, with the suffix `_A` or `_B` added to the filename. It is recommended to follow this default so that at a later date it will be easier to track which text file any particular report is based upon. Please note that the directory which the browser offers for saving the report will generally not be the one where the text file is stored!

You can newly create the report at any time. With identical input (including the text file) you will get a report with identical contents, with the following exceptions: date and time in the report header and possibly the results of the verification of the expiration dates of the EndoPredict® plate, the qREF, the SS III RT/Platinum Taq Mix, the 2x Reaction Mix and the qPCR water.

5. The EndoPredict® Report

The EndoPredict® report usually comprises three pages[4]. The first page comprises the patient information and the results (EP and EPclin). The following pages primarily contain information based on the control of the qPCR plate, on page 2 in the form of tables and on page 3 in the form of text messages. If tumour size and / or nodal status have been specified as unknown, an additional page is inserted after page 1 which comprises a table that contains the EPclin score and the 10-year metastasis risk for all 16 possible combinations of tumour size and nodal status. If the clinical parameters become subsequently available, the individual EPclin score and the corresponding risk of recurrence can be determined from the table. However, it is also possible to upload the text file again and enter the clinical information before creating the report.

5.1. Page header of the report



At the very top of the first page of the report there is a header which bears the company logo of the manufacturer on the left side and that of the distributor on the right side. Below it there is the specimen-ID, which comes either from the Well Comment entered in the text file or from the entry in the EPRG user interface. In case of conflict between the specimen-ID stored in the text file and the information entered into the user interface, the latter is used; however, in a note on page 3 the discrepancy is pointed out. Under specimen-ID is the note, which behaves identically to the specimen-ID. Underneath that, the time at which the report was created is listed. To the right of this, on the first page of the report, there is some space reserved for the stamp of the executive laboratory.

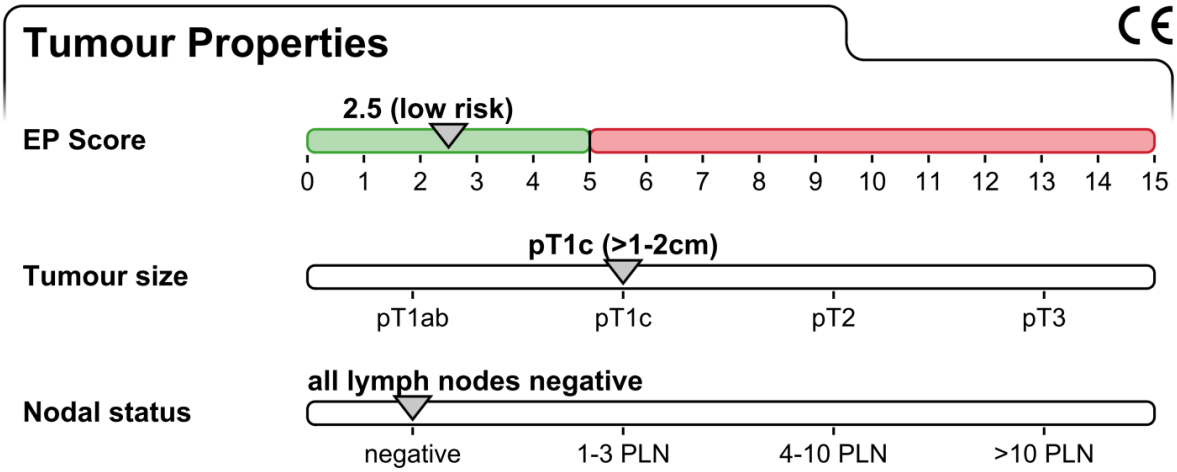
Starting from page 2, in the header on the right, the page number and the total number of pages are indicated:

[4]The page numbering described in the following assumes a report with tumour size and nodal status known. Otherwise (see section 5.5) a page 2 will be inserted and all other pages shifted accordingly.

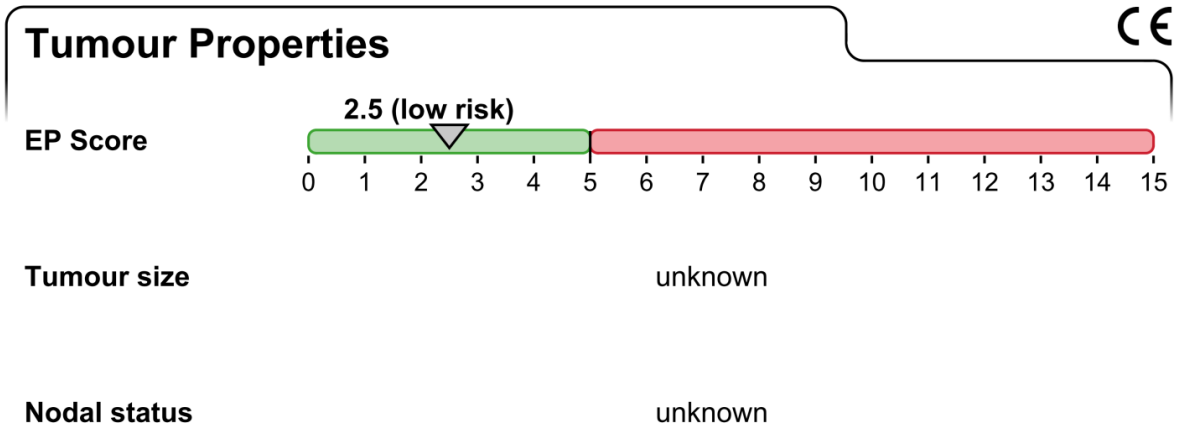
5.2. The tumour properties

Under the header, on page 1 the tumour properties are shown, beginning with the EP score, which is represented numerically and graphically. According to *Filipits et al., 2011* it is always between 0 and 15, where EP scores of less than 5 are designated as «low risk» and EP scores of 5 or more as «high risk». The risk class is listed verbally in parentheses after the determined EP score. In certain cases, the EP score cannot be calculated from the measured data: if this is the case, at this point an error message is printed; in addition a respective message (see sections 5.13 and 6) is shown on the last report page describing the problem in more detail.

«Tumour size» and «Nodal status» are displayed numerically and graphically according to the entries in the browser (Section 4.5).



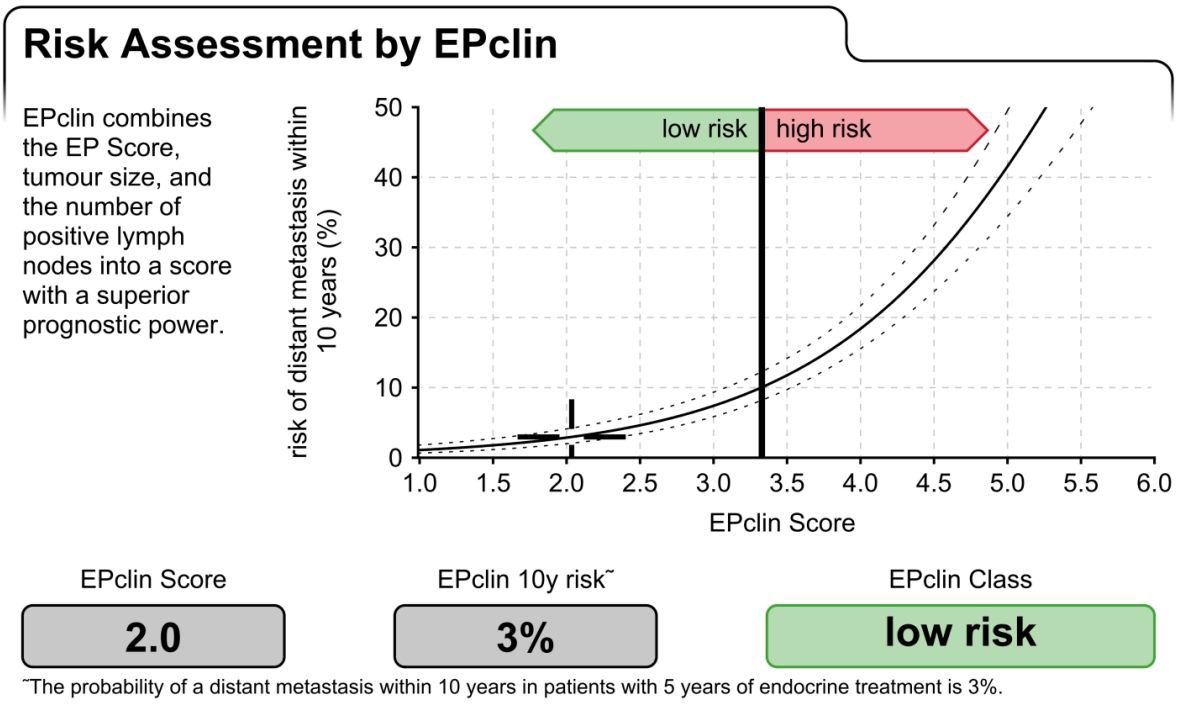
If entered as **unknown,** this is also indicated here accordingly:



5.3. The risk as assessed by EPclin

If you have entered information on tumour size and nodal status, the EPclin score according to *Filipits et al., 2011* is calculated and shown on page 1 of the report. From the EPclin score, the probability of metastasis formation within 10 years is estimated according to *Filipits et al., 2011*, and both values are presented graphically. The curves show the general relationship which was calculated on the basis of a model, where the dashed curves form the 95 % confidence interval. The crosshairs indicate the EPclin results for the individual patient of this report.

EPclin scores of less than 3.3 (probability of metastasis < 10% within 10 years) are considered as «low risk», the others as «high risk».



5.4. Assessment of the Report

On page 1 below, the test result can be approved by signature of the pathologist (or not, usually on the basis of the quality information on the following report pages). In addition, there is room for handwritten comments.

Page 1 is completed by the reference to the literature underlying the EndoPredict® test and the calculation of the EP and EPclin scores.

Pathologists Approval

Controls were run as required
and test result is valid.

authorized signature

Based on: Filipits et al. (2011): A new molecular predictor of distant recurrence in ER-positive, HER2-negative breast cancer adds independent information to conventional clinical risk factors. Clinical Cancer Research 17: 6012-6020.

5.5. EPclin in case of unknown tumour size or nodal status

If you have selected **unknown** in the browser for tumour size and / or nodal status, the EPclin score cannot be calculated. Instead, on page 2 the report will include a table with all possible combinations of tumour size and nodal status, and the respective EPclin results.

Manual Risk Assessment by EPclin

EPclin combines the EP Score, tumour size, and the number of positive lymph nodes into a score with a superior prognostic power. Tumour size and/or the nodal status were unknown when generating this report. Once tumour size and nodal status become available the following table allows for manually calculating the EPclin score, the risk of a metastasis within 10 years, and the EPclin risk class. The table content is calculated from the EP Score for the tumour specimen described in this report.

Nodal status	Tumour size: pT1ab (<=1cm)	pT1c (>1-2cm)	pT2 (>2-5cm)	pT3 (>5cm)
all lymph nodes negative	1.7 2% low risk	2.0 3% low risk	2.4 4% low risk	2.7 6% low risk
1 to 3 positive lymph nodes	2.3 4% low risk	2.7 5% low risk	3.0 8% low risk	3.4 10% high risk
4 to 10 positive lymph nodes	3.0 7% low risk	3.31 10% low risk	3.7 14% high risk	4.0 19% high risk
more than 10 pos. lymph nodes	3.6 13% high risk	4.0 18% high risk	4.3 24% high risk	4.7 32% high risk

Top: EPclin score
Middle: probability of a distant metastasis within 10 years
Bottom: risk class (threshold = 10% for probability)
Red: high risk

In a paper form of this report please pick the tumour size (column) and the number of positive lymph nodes (row) and mark the intersecting cell corresponding to the correct tumour size and nodal status. Copy the EPclin score, the marked percent metastasis risk, and risk class into the result boxes below:

EPclin Score	EPclin 10y risk˜	EPclin Class
	%	risk

˜The "EPclinRisk10" is the probability of a distant metastasis within 10 years in patients with 5 years of endocrine treatment.

In addition to the EPclin score, the table also indicates the probability of metastasis formation within 10 years according to the data (see section 5.3). Probabilities of less than 10 % (equivalent to EPclin scores of less than 3.3) are marked «low risk»; probabilities of 10 % or more are described as «high risk» and highlighted in red.

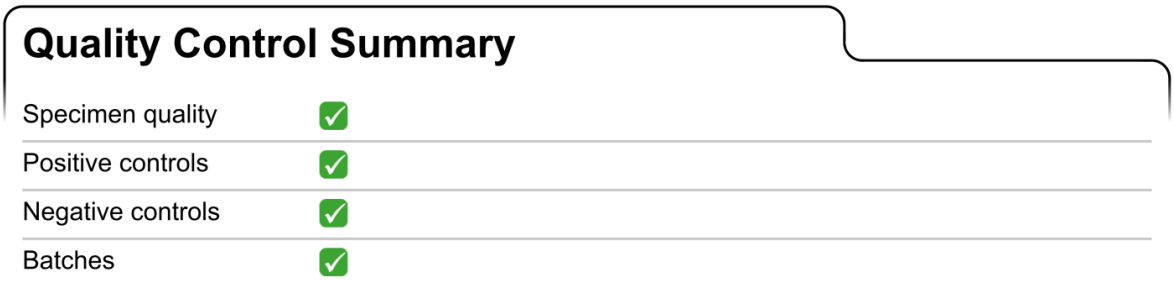
If you have printed the report and later determined tumour size and nodal status, you can read the EPclin score, the corresponding 10-year metastasis probability and the EPclin class from the table and enter them manually into the appropriate fields below the table.

5.6. Overview of software-side quality control

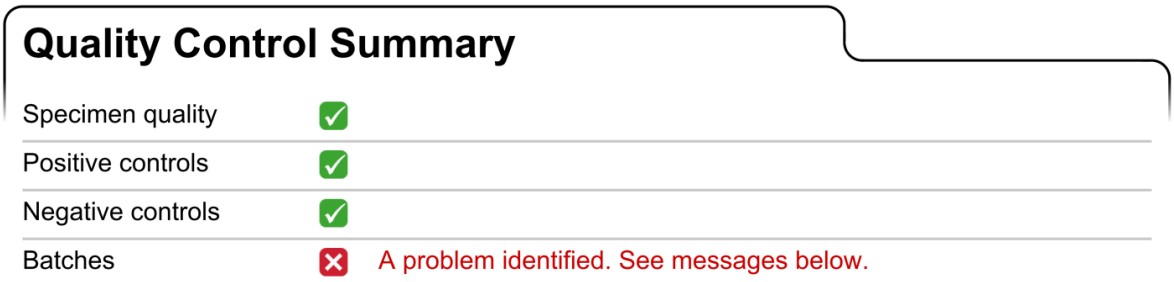
Pages 2 and 3 of the report essentially deal with the quality control of the PCR measurements. This is done through a series of quality checks in the form of simple rules. If any such quality check fails (i.e. if the rule is violated), it is highlighted in the report at various points: (i) in the summary (section 5.7), (ii) in one of the tables on page 2 or 3 in the form of a coloured marking (sections 5.8 to 5.11) and (iii) on page 3 in the form of a text message (section 5.13).

5.7. Quality control summary

Page 2 of the report begins with a summary of the results of the quality checks. The quality checks are divided into four categories: If all four show a green check mark, all checks have been passed.



If any quality check has failed, in that category a red cross appears in lieu of the green check mark. The red cross is followed by an appropriate message. On page 3 of the report, all the problems identified are listed separately in the section «Messages».



5.8. Ct values in the plate layout

In the middle of page 2 there is a table summarising the Ct values, showing the arrangement of the wells on the PCR plate. The main function of this table is to clearly present the immediate measurement results.

Since each report includes the results of only one patient, Ct values of another specimen that may be present on the same qPCR plate are not displayed: In the image below in rows F to H.

In addition to the Ct values, the table comprises the EndoPredict® plate layout that marks the gene and material («A» for specimen A, «B» for specimen B, «POS» for positive control and «NEG» for negative control) for each well. If a well is not described in the text file (see instruction manual «IFU_Endopredict»), the corresponding cell is labelled «<omitted>» and highlighted in yellow.

In each control well it is determined whether the Ct value is within the range permitted by the control limits: if so, the corresponding cell remains white, otherwise it turns red.

According to *Filipits et al., 2011,* the replicates of a gene are checked for outliers (rogue results) during the calculation of the EP scores. If an outlier is detected, it is excluded from the calculation of the EP and marked in light blue in the table. Individual outliers may occasionally occur due to the nature of the PCR as a method of analysis and do not constitute a relevant quality risk. They are the reason why the measurements are done in triplicate.

Ct Values

	1	2	3	4	5	6	7	8	9	10	11	12
A	AZGP1 A 21.07	BIRC5 A 32.23	OAZ1 A 25.51	DHCR7 A 28.99	IL6ST A 25.12	MGP A 21.03	CALM2 A 23.71	RBBP8 A 26.85	STC2 A 23.72	UBE2C A 29.71	RPL37A A 21.92	HBB A No Ct
B	AZGP1 A 21.21	BIRC5 A 32.13	OAZ1 A 25.65	DHCR7 A 28.76	IL6ST A 24.99	MGP A 20.85	CALM2 A 23.53	RBBP8 A 27.01	STC2 A 23.45	UBE2C A 29.62	RPL37A A 21.89	HBB A No Ct
C	AZGP1 A 21.29	BIRC5 A 32.22	OAZ1 A 25.37	DHCR7 A 28.86	IL6ST A 24.98	MGP A 20.69	CALM2 A 23.53	RBBP8 A 27.25	STC2 A 23.97	UBE2C A 29.94	RPL37A A 21.89	HBB A No Ct
D	HBB POS 26.72	BIRC5 POS 26.55	OAZ1 POS 23.34	DHCR7 POS 23.91	IL6ST POS 23.05	MGP POS 24.62	CALM2 POS 20.51	RBBP8 POS 25.39	STC2 POS 24.22	UBE2C POS 23.37	RPL37A PO. 18.82	AZGP1 POS 24.73
E	AZGP1 NEG No Ct	BIRC5 NEG No Ct	OAZ1 NEG No Ct	DHCR7 NEG No Ct	IL6ST NEG No Ct	MGP NEG No Ct	CALM2 NEG No Ct	RBBP8 NEG No Ct	STC2 NEG No Ct	UBE2C NEG No Ct	RPL37A NE. No Ct	HBB NEG No Ct
F	AZGP1 B	BIRC5 B	OAZ1 B	DHCR7 B	IL6ST B	MGP B	CALM2 B	RBBP8 B	STC2 B	UBE2C B	RPL37A B	HBB B
G	AZGP1 B	BIRC5 B	OAZ1 B	DHCR7 B	IL6ST B	MGP B	CALM2 B	RBBP8 B	STC2 B	UBE2C B	RPL37A B	HBB B
H	AZGP1 B	BIRC5 B	OAZ1 B	DHCR7 B	IL6ST B	MGP B	CALM2 B	RBBP8 B	STC2 B	UBE2C B	RPL37A B	HBB B

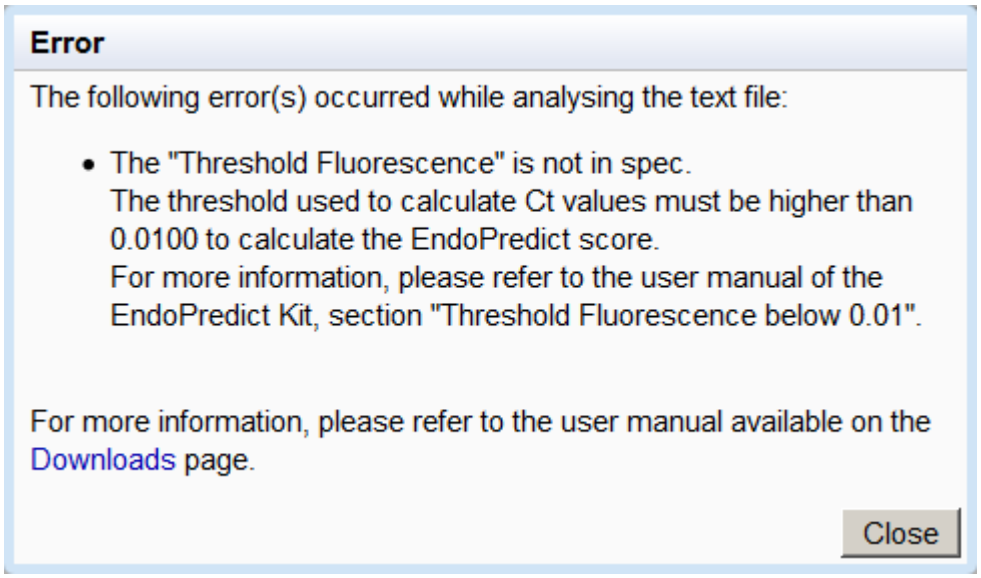
5.9. Controls

The following is a section on controls, beginning with the examination of the threshold value for determining the Ct values in the PCR software. In the user interface of the VERSANT software this is called **Threshold Fluorescence** and appears in the text file in the `Threshold (dRn)` column. It is determined automatically by the VERSANT software from the fluorescence curves and is listed on page 2 in the «Controls» section under the headers.

Controls

Threshold fluorescence: 0.0464

If it is too low, the Ct values of the controls are often not within the specifications, and it is recommended that you correct the appropriate settings in the VERSANT software to achieve an adequate **Threshold Fluorescence** (see the «IFU_Endopredict» instruction manual, section «Threshold Fluorescence below 0.01»). An EndoPredict® report is generated only when the **Threshold Fluorescence** is greater than 0.01, otherwise the following error message will be displayed in the browser.



Under **Threshold Fluorescence**, the controls of the Ct values are displayed in a table. For each of the twelve genes of the EP algorithm (eight informative genes in alphabetical order, three reference genes also in alphabetical order, and one DNA marker gene) there is one positive and one negative control. Moreover, there is one negative DNA control of the specimen (here referred to as «specimen HBB").

The Ct value controlled by application of quality rules is shown in the two columns «Ct». In the specimen HBB negative control this is the mean value of three replicates; for the averaging for «No Ct» a value of 40 is used.

If the controlled value is between LCL and UCL (for positive controls) or above the LCL (for negative controls), these controls are within the specifications, and the appropriate cell in the «Controls» table will remain white. Otherwise the control is outside of specifications and it is highlighted in red. If for any control the corresponding Ct value is absent, in the column «Ct» only an empty box with yellow background will be shown.

If controls are outside the specification limits, or if the corresponding Ct values are absent, the validity of the result is not guaranteed. The test should be repeated.

Controls

Threshold fluorescence: 0.0464

control	positive Ct	positive limits	negative Ct	negative limit
AZGP1	24.73	(24.19 - 27.03)	No Ct	(≥36.00)
BIRC5	26.55	(24.06 - 28.87)	No Ct	(≥36.00)
DHCR7	23.91	(22.94 - 25.58)	No Ct	(≥36.00)
IL6ST	23.05	(22.59 - 25.61)	No Ct	(≥36.00)
MGP	24.62	(23.67 - 27.05)	No Ct	(≥36.00)
RBBP8	25.39	(24.68 - 27.84)	No Ct	(≥36.00)
STC2	24.22	(23.52 - 26.83)	No Ct	(≥36.00)
UBE2C	23.37	(22.22 - 25.52)	No Ct	(≥36.00)
CALM2	20.51	(19.78 - 22.37)	No Ct	(≥34.00)
OAZ1	23.34	(21.26 - 24.73)	No Ct	(≥34.00)
RPL37A	18.82	(17.80 - 20.03)	No Ct	(≥34.00)
HBB	26.72	(26.26 - 31.43)	No Ct	(≥36.00)
specimen HBB			No Ct	(≥38.00)

5.10. Delta Ct values

At the bottom of page 2 of the report, on the right side there is the «Delta Ct» table. The Delta Ct value represents an intermediate result in the calculation of the EP score and is listed in order to understand more easily how the EP is calculated.

Delta Ct

gene	dCt
AZGP1	22.67
BIRC5	12.82
DHCR7	15.21
IL6ST	19.21
MGP	22.95
RBBP8	16.88
STC2	19.79
UBE2C	13.54

ø Ct values of reference genes	limit
23.67	(≤27.00)

The mean of the Ct values of the three reference genes below the table is a measure of the total RNA quantity of the specimen. This should not be too low, since otherwise the potentially informative genes cannot be measured with sufficient precision. Therefore, this value is subject to a quality check by EPRG: if it is above 27, the number is

highlighted in red. The test should be repeated with an increased amount of total RNA (see instruction manual «EndoPredict», chapter «Troubleshooting – Insufficient yield»).

5.11. Batches

On page 3 of the report, the serial and batch numbers used are listed, provided they have been entered. The serial or batch numbers are used to check the expiration date of the EndoPredict® plate, the qREF batch, the SS III RT/Platinum Taq Mix, the 2x Reaction Mix and the qPCR water batch used (if specified). If the check is positive, a green check mark is shown in the expiration date column, otherwise a red cross and an appropriate message will appear.

Batches

		Shelf life
EndoPredict plate	D000000	✓
qREF	Q000	✓
SS III RT/Platinum Taq Mix		
2x Reaction Mix		
qPCR Water	H000	✓

Software version: V3.1.1, server: Testserver, data base: Test-DB.

If data for the EndoPredict® plate or for the qREF, the SS III RT/Platinum Taq Mix, the 2x Reaction Mix or qPCR water batch have already been entered into the PCR software and are in conflict with the data entered in the browser, the data from the browser will be used, but in the «Messages» section the conflicting input will be shown. Moreover, in the quality control summary on page 2 under the heading «Batches» a red cross is displayed. This also applies when expired batches were used.

Batches

		Shelf life
EndoPredict plate	D000001	✗ expired on 30-Nov-2013
qREF	Q000	✓
SS III RT/Platinum Taq Mix		
2x Reaction Mix		
qPCR Water	H000	✓

Software version: V3.1.1, server: Testserver, data base: Test-DB.

5.12. Test Result

The test results from page 1 are repeated on page 3 of the report in abridged form too.

Test result

EP Score: 2.5	Tumour size: n/a	Nodal status: n/a
EPclin Score: n/a	EPclin 10y risk˜: n/a	EPclin Class: unknown

˜The probability of a distant metastasis within 10 years in patients with 5 years of endocrine treatment is n/a.

5.13. Messages

The problems and conflicts of all indexed quality controls highlighted graphically on pages 2 and 3 are summarised in text form at the end of the report. In addition to the quality-related messages (Sections 5.7 to 5.11), all entries from the `Well Comment` column of the text file are listed which could not be allocated to any input field in the browser (e.g. `Specimen-ID` entries, plate numbers, qREF batches, SS III RT/Platinum Taq Mix batches, 2x Reaction Mix batches or qPCR water batches, see sections 4.1, 4.4 and 4.5). The entries are listed broken down by the material of the wells (A, B, POS or NEG), where multiple identical names are grouped.

Messages

[No]* Message	*Test result is valid* yes	no
[L01] The shelf life of the plate has expired.	☐	☐

*You can find further information under the given [No.] in the software manual (see the section “Downloads” on the EP Report Generator website).

Technical errors occurred. Please verify the data collected as outlined in the user’s manual and check if the EndoPredict test needs to be repeated.

Each message has an identifier (in the present example «[L01]»), which allows you to look up detailed information about the quality control and possible countermeasures in section 6. The first letter of the identifier assigns the message to a category:

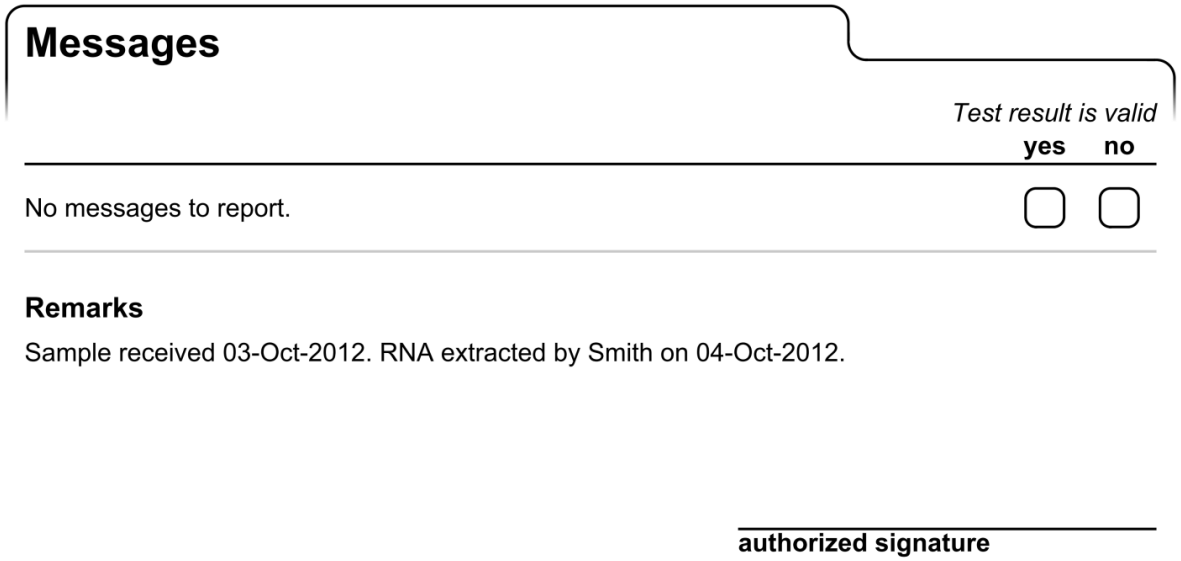
- L: Batch (previously lot), expired batch or conflicting batch information
- P: Positive control, abnormalities in the positive controls
- N: Negative control, abnormalities in the negative controls
- S: Specimen, abnormalities in specimen wells or in the calculation of the EP score

The identification code is followed by the text of the message. If several genes are affected by the same error / problem, this is summarised into a single message.

Here the pathologist responsible can select whether the indexed quality checks for the validity of the test results are relevant.

Any quality-related message results in an entry in the appropriate category in the summary on page 2 of the report (section 5.7). All quality-related red and yellow markings on pages 2 and 3 lead to corresponding messages.

Under the heading «Messages» there are also the remarks reproduced which have been entered into the browser (Section 4.5). A signature field concludes the report.



Messages

	Test result is valid yes	no
No messages to report.	☐	☐

Remarks

Sample received 03-Oct-2012. RNA extracted by Smith on 04-Oct-2012.

authorized signature

6. List of Messages

The following table lists all the possible entries under «Messages» on page 3 of a report.

Ident.	Message	Explanation, if necessary countermeasure
L01	The shelf life of the plate has expired.	The EndoPredict® plate used has exceeded its expiration date at the time of report generation. Please check if the expiration date had already been exceeded at the time of measurement, too. If so, repeat the test with a valid EndoPredict® plate.
L02	The shelf life of the qPCR water batch has expired.	The qPCR water aliquot used has exceeded the expiration date at the time of report generation. Please check if the expiration date had already been exceeded at the time of measurement, too. If so, repeat the test with a valid qPCR water batch.
L03	The shelf life of the qREF batch has expired.	The qREF batch used has exceeded its expiration date at the time of report generation. Please check if the expiration date had already been exceeded at the time of measurement, too. If so, repeat the test with a valid qREF batch.

Ident.	Message	Explanation, if necessary countermeasure
L04	The plate batch does not match the information from the text file (...).	The plate batch entered in the VERSANT software as **Well Comment** conflicts with the datum entered in the browser. As relevant information, the batch defined in the browser is used. Please make sure that the correct plate batch is used for the report. If the plate ID has been entered once manually and once via a bar code scanner which was not used on the device-specific PC, during reading of the ID with the bar code scanner some characters may be confused. Please correct any incorrect entries and create the report again.
L05	The qPCR water batch does not match the information from the text file (...).	The qPCR water batch entered in the VERSANT software as **Well Comment** conflicts with the datum entered in the browser. As relevant information, the batch defined in the browser is used. Please make sure that the correct qPCR water batch is used for the report. If the qPCR water batch has been entered once manually and once via a bar code scanner which was not used on the device-specific PC, during reading of the ID with the bar code scanner some characters may be confused. Please correct any incorrect entries and create the report again.
L06	The qREF batch does not match the information from the text file (...).	The qREF batch entered in the VERSANT software as **Well Comment** conflicts with the datum entered in the browser. As relevant information, the batch defined in the browser is used. Please make sure that the correct qREF batch is used for the report. If the qREF batch has been entered once manually and once via a bar code scanner which was not used on the device-specific PC, during reading of the ID with the bar code scanner some characters may be confused. Please correct any incorrect entries and create the report again.
L07	The shelf life of the SS III RT/Platinum Taq Mix batch has expired.	The SS III RT/Platinum Taq Mix batch used has exceeded its expiration date at the time of report generation. Please check if the expiration date had already been exceeded at the time of measurement, too. If so, repeat the test with a valid SS III RT/Platinum Taq Mix batch.

Ident.	Message	Explanation, if necessary countermeasure
L08	The shelf life of the 2x Reaction Mix batch has expired.	The 2x Reaction Mix batch used has exceeded its expiration date at the time of report generation. Please check if the expiration date had already been exceeded at the time of measurement, too. If so, repeat the test with a valid 2x Reaction Mix batch.
L09	The SS III RT/Platinum Taq Mix batch does not match the information from the text file (...).	The SS III RT/Platinum Taq Mix batch entered in the VERSANT software as **Well Comment** conflicts with the datum entered in the browser. As relevant information, the batch defined in the browser is used. Please make sure that the correct SS III RT/Platinum Taq Mix batch is used for the report. If the SS III RT/Platinum Taq Mix batch has been entered once manually and once via a bar code scanner which was not used on the device-specific PC, during reading of the ID with the bar code scanner some characters may be confused. Please correct any incorrect entries and create the report again.
L10	The 2x Reaction Mix batch does not match the information from the text file (...).	The 2x Reaction Mix batch entered in the VERSANT software as **Well Comment** conflicts with the datum entered in the browser. As relevant information, the batch defined in the browser is used. Please make sure that the correct 2x Reaction Mix batch is used for the report. If the 2x Reaction Mix batch has been entered once manually and once via a bar code scanner which was not used on the device-specific PC, during reading of the ID with the bar code scanner some characters may be confused. Please correct any incorrect entries and create the report again.
N01	The negative control of gene ... is positive.	The negative control of this gene is out of specification, i.e. the Ct value of the well is below the lower control limit. The test should be repeated.
N02	No negative control for gene	The negative control of this gene is absent. Please check in the VERSANT software whether the corresponding well really does not contain a Ct value. If so, the test should be repeated.
P01	The positive control of gene ... is outside the control limits.	The positive control of this gene is out of specification, i.e. the Ct value of the positive control of this gene is not between the lower and the upper control limit. The test should be repeated.

Ident.	Message	Explanation, if necessary countermeasure
P02	No positive control for gene	The positive control of this gene is absent. Please check in the VERSANT software as to whether the corresponding well really does not contain a Ct value. If so, the test should be repeated.
S01	All replicates of gene ... are missing. EP Score cannot be calculated.	The text file comprises no specimen measurement value for the specified gene. In the VERSANT software, all three wells of this gene were excluded from export for this specimen. Make sure that all wells are exported to the text file.
S02	All replicates of gene ... are outliers. EP Score cannot be calculated.	The Ct values of the specified gene are conspicuously far apart: Any of these can be a clearly incorrect value (outlier), so none of them is trustworthy. Repeat the test.
S03	The estimated confidence of the EP Score is not sufficient.	The confidence interval of the EP score is too wide (see *Filipits et al., 2011*); the EP score cannot be specified with sufficient precision. This is indicated by: high Ct values of the reference genes, high Ct values or «No Ct» for the informative genes, and/or a high number of outliers. Repeat the test with an increased quantity of RNA (see instruction manual «EndoPredict», section «Troubleshooting – Insufficient yield»).
S04	The majority of genes are not measured in triplicate: The precision of the EP Score is insufficient.	For six or more of the eleven genes (the HBB negative control of the specimen is not taken into account in the counting) of the EP algorithm the text files contains fewer than three replicates. The accuracy of the EP score is thereby jeopardized. Repeat the test and use three replicates for each of the eleven genes.
S05	The HBB-negative control of the specimen is positive. Repeating the DNA digestion may help.	In the text file the mean value of the HBB Ct values measured for the specimen («specimen HBB») falls short of the threshold value of 38. The specimen is thus contaminated with DNA, so a DNase redigest is necessary. Test the redigested specimen with EndoPredict® QC. If this is OK, repeat the test. If this issue fails to resolve the problem, refer to the instruction manual «EndoPredict» for more information in the «Troubleshooting» section.

Ident.	Message	Explanation, if necessary countermeasure
S06	The specimen-ID does not match the information from the text file (...).	The specimen-ID entered in the VERSANT software as **Well Comment** conflicts with the information provided via the browser. As relevant information, the specimen-ID defined in the browser is used. Please make sure that the correct specimen-ID is used for the report. If the specimen-ID has been entered once manually and once via a bar code scanner which was not used on the device-specific PC, during reading of the ID with the bar code scanner some characters may be confused. Please correct any incorrect entries and create the report again.
S07	The average of Ct values of reference genes is out of the specification validated by Sividon Diagnostics. Please increase the input RNA concentration.	The mean of the Ct values of the three reference genes is greater than 27. This means an insufficient quantity of RNA and thus a potentially imprecise EP score. Repeat the test with an increased quantity of RNA (see instruction manual «EndoPredict», chapter «Troubleshooting – Insufficient yield»).
S08	One or more replicates of a reference gene are «No Ct». Please increase the input RNA concentration.	At least one well of the three reference genes has the value «No Ct» (and is not an outlier). Thus, the mean value of the reference genes cannot be calculated. Repeat the test with an increased quantity of RNA (see instruction manual «EndoPredict», chapter «Troubleshooting – Insufficient yield»).
S09	All replicates of the HBB-negative control of the specimen are missing.	For the specimen there are no measured values for HBB, so no control for DNA contamination can be carried out. Check whether any curves have been unintentionally removed or forgotten upon export of the text file. If no data can be determined for HBB, the test must be repeated.
S10	The tumour size does not match the information from the text file (...).	The tumour size entered in the VERSANT software as **Well Comment** conflicts with the information provided via the browser. As relevant information, the tumour size defined in the browser is used. Please make sure that the correct tumour size is used for the report.
S11	The nodal status does not match the information from the text file (...).	The nodal status entered in the VERSANT software as **Well Comment** conflicts with the information provided via the browser. As relevant information, the nodal status defined in the browser is used. Please make sure that the correct nodal status is used for the report.

Ident.	Message	Explanation, if necessary countermeasure
S12	The remarks do not match those from the text file (...).	The remarks entered in the VERSANT software as **Well Comment** conflict with the information provided via the browser. As relevant information, the remarks defined in the browser are used. Please make sure that the correct remarks are used for the report.
S13	The note does not match the entry in the text file (...).	The note entered in the VERSANT software as **Well Comment** differs from the entry made in the browser. The note defined in the browser is used as relevant information. Please ensure that the correct note is used for the report.
	Well Comment˜ (A): (...)	Entry in the **Well Comment** in the PCR software (section 4.1) for a specimen well of specimen A
	Well Comment˜ (B): (...)	Entry in the **Well Comment** in the PCR software (section 4.1) for a specimen well of specimen B
	Well Comment˜ (NEG): (...)	Entry in the **Well Comment** in the PCR software (section 4.1) for a specimen well of a negative control
	Well Comment˜ (POS): (...)	Entry in the **Well Comment** in the PCR software (section 4.1) for a specimen well of a positive control

7. Released Versions

This section lists all released versions of EPRG and describes the major changes with respect to its predecessor.

Version	Changes
V3.0	First release with CE mark.
V3.1	Additional languages: Spanish, French, Italian, and Dutch. Report page 1 contains some space in the upper right corner for the pathologist's stamp or logo.