HER2DX® Software 6.1

Instructions For Use (IFU)

Document History		
Revision	**Date**	**Revision reason**
1.0	23rd Mar 2022	First release
2.0	29th Sep 2022	Added N2-3 in Nodul status classification in the section 5.3.2
3.0	21st Feb 2023	Change the web site
4.0	11th Apr 2023	Update recalculate
5.0	28th Sep 2023	Gramatical and clarity improvements. Product information of the v4.1 added.
6.0	14th Feb 2024	Incorporation of the RNA-SINT, improve of the Quality controls, multi-costumers and known issues.
7.0	26th Feb 2024	Minor update of product label screenshot due to hotfix 5.0.1.
8.0	12th Jun 2024	New security implementation (MFA, password complexity and expiry), reformulation of the quality controls into assay and sample quality, minor bug fixes.
9.0	29th Jul 2024	Remove the CE mark symbol and add "Exclusive use for performance studies". Selection of report language.

10.0	12nd Sept 2025	Added warning on the use of AJCC/UICC TNM classification in the Warnings and Precautions section. Referenced AJCC system in Clinical Variables section. Added statement indicating the HER2DX® Software is classified as non-hazardous.
11.0	29th Sept 2025	- Section 1.1, General description of the Device, has been updated with more detailed wording about gene signatures and clinical use. - Added the SSP (Summary of Safety and Performance) with reference to EUDAMED and the Reveal Genomics website. - A header was added to the document, including the product name, document version, and issue date. - Chapter 6.1 Requirements was updated and explicited with more detailed requirements regarding supported use environments and their specificities. - Section 9 (Performance Principles) updated and restructured. - Section 10 Limitations added (≥18 years, FFPE only, nCounter® data only, minimum RNA quality, not applicable to post-treatment samples, max. 12 samples/run). - Section 11 Performance characteristics expanded with analytical validation summary and detailed metrics.
12.0	26 March 2026	- Software version updated to 6.1 to reflect clarified wording of the Intended Purpose. - Include CE mark symbol (0344) and remove "Exclusive use for performance studies" statement. - Section 1.2: Intended purpose clarification. This change consists of a clarification of the Intended Purpose wording, without modification of device claims, algorithm, or clinical outputs, introducing the qualification of the device as a qualitative IVD MDSW and clarifying the target population, specimen type, and non-validated use conditions. - Section 1.4: Including pre-analytical acceptance criteria for tissue.

		- Section 3: Updated to include clarification on non-validated use (metastatic disease and HER2-negative tumors). - Section 6.2.1: Updated to include updated screenshots of the software landing page and product label (version 6.1). - Section 9.1 *Quality Controls* updated to clarify the origin, role and use of ERCC spike-in controls and RNA-SINT reference samples in the HER2DX workflow. - Section 10: Limitations were revised to incorporate defined pre-analytical acceptance criteria for tissue samples and to ensure full consistency with the overall documentation. - Section 11.2. Correction of the title. This section refers to Clinical performance. - References section: Addition of study references throughout the whole to improve traceability of the summarized data. - Multiple sections updated to remove non-applicable references.
13.0	5th May 2026	Section 9.2 updated to include clarification on the mathematical approach, specifying the use of pre-trained, fixed models and absence of adaptive learning or iterative processing at runtime. Section 9.2.1 updated to clarify that the penalized Cox proportional hazards model remains locked with fixed coefficients for all software versions.

HER2DX® Software 6.1

HER2DX® Software is a qualitative in vitro diagnostics (IVD) medical device software (MDSW) intended to help healthcare professionals to optimally select the best treatment option for adult patients with early-stage HER2+ breast cancer; the software integrates genomic data -quantitative gene expression- obtained from formalin-fixed paraffin-embedded (FFPE) tumor tissue samples collected prior to systemic treatment, and clinical data - tumor size and lymph nodal involvement (nodal status)-, to provide information on the long-term risk of relapse (HER2DX® relapse risk score), on the likelihood of achieving a pathologic complete response (pCR) following neoadjuvant therapy (HER2DX® pCR likelihood score), and *ERBB2* mRNA expression levels (HER2DX® *ERBB2* mRNA score). The HER2DX® Software, designed as a web application, is intended to be operated exclusively by authorized and trained healthcare professionals. The device has not been validated for use in metastatic disease, HER2-negative tumors, or post-treatment samples.

IVD

REF 00

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Warnings
For use by authorized and trained healthcare professionals.
Not indicated for breast cancer diagnostic purposes.

IFU Version 13.0, created 2026-05

CE 0344

Warning: Version Discrepancy

It is imperative to ensure that you are referring to the correct version of this Instructions for Use (IFU) document. Failure to use the most up-to-date version may result in inaccurate information being followed, potentially leading to misuse of the medical device and adverse outcomes for the patient. Always verify that you are using the latest version of the IFU before proceeding with any procedures or interventions. If you are unsure about the version or have any doubts, contact Support immediately for clarification.

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1. DEVICE DESCRIPTION AND SPECIFICATIONS

1.1. General description of the device

HER2DX® Software is an in vitro diagnostic (IVD) medical device software (MDSW) developed by REVEAL GENOMICS, S.L. to provide both prognostic (estimating the risk of recurrence) and predictive (estimating the likelihood of treatment response) information for patients with early-stage HER2-positive breast cancer. By analyzing gene expression and clinicopathological data, the software estimates the risk of disease recurrence and the likelihood of achieving a pathological complete response (pCR) to systemic therapy. This integrated information supports clinicians in tailoring the intensity of treatment according to individual risk and likelihood of treatment response, helping to avoid unnecessary toxicity and preserve quality of life in low-risk patients, while identifying those who may benefit from more intensive therapy. HER2DX® Software is intended for use by qualified healthcare professionals and does not replace clinical judgment. Final treatment decisions remain under the responsibility of the treating physician, based on the patient's full clinical context.

HER2DX® Software is a qualitative MDSW that generates three patient-specific scores by processing gene expression data and clinicopathological variables. The scores are computed through a fixed, pre-trained algorithm that integrates the expression levels of 27 genes, grouped into four biologically defined gene expression signatures, and combines them with two clinicopathological variables, tumor size (T) and nodal status (N). all of which are weighted according to their relative prognostic and predictive contribution. These scores are output as continuous values (1 to 99), and interpreted categorically based on predefined clinical thresholds[1]:

- **Gene signatures**: The 27 gene variables included in HER2DX® Software supervised learning algorithm are split into 4 gene expression signatures capturing various biological processes including immune infiltration, tumor cell proliferation, luminal differentiation, and the expression of the HER2 amplicon, giving rise to single scores. The 4 gene expression signatures are as follows:
    - The immune signature selected for HER2DX® Software was the 14-gene immunoglobulin (IGG) module (i.e., *CD27, CD79A, HLA-C, IGJ, IGKC, IGL, IGLV3-25, IL2RG, CXCL8, LAX1, NTN3, PIM2, POU2AF1 and TNFRSF17*), previously identified by unsupervised clustering of human breast tumors. The IGG signature has previously shown strong independent prognostic value in a large breast cancer dataset (N= 550), where patients did not receive adjuvant systemic therapy. [2]

  The other three gene signatures were identified from unsupervised clustering of the Short-HER HER2-positive dataset using data from 187-breast cancer-related genes. The genes selected were obtained from highly correlated gene clusters (correlation coefficient > 0.80);
    - The tumor cell proliferation signature includes 4 genes (i.e., *EXO1*, *ASPM*, *NEK2* and *KIF23*),

- The luminal differentiation signature includes 5 genes (i.e., *BCL2*, *DNAJC12*, *AGR3*, *AFF3* and *ESR1*), and
- The HER2 amplicon signature includes 4 genes located in the 17q11-12 chromosome (i.e., *ERBB2*, *GRB7*, *STARD3* and *TCAP*).

For each signature, the mean gene expression is calculated for each patient.

- **Clinical variables:**
  - Tumor size (T): size and/or extent of the main tumor. It is measured in cm being T1 (0-2 cm), T2 (2-5 cm), T3 (>5 cm), and T4 (tumor has broken through skin or attached to chest wall).
  - Nodal status (N):
    - Clinical Nodal status (cN): N0 (axillary and other nearby lymph nodes have no cancer), N1 (axillary lymph nodes have cancer, but can be moved around N2), axillary lymph nodes have cancer and are matted together or fixed to other structures (such as the chest wall) OR internal mammary nodes have cancer, but axillary lymph nodes don't appear to have cancer), N3 (infraclavicular (under the clavicle) nodes have cancer (axillary lymph nodes may or may not have cancer) OR internal mammary nodes and axillary lymph nodes have cancer OR supraclavicular (above the clavicle) nodes have cancer (axillary lymph nodes may or may not have cancer)).
    - Pathological Nodal status (pN): lymph node affectation being N0 (axillary and other nearby lymph nodes have no cancer or only have isolated tumor cells (individual cancer cells), when looked at under a microscope), N1 (micrometastases OR 1–3 axillary lymph nodes have cancer), N2 (4–9 axillary lymph nodes have cancer OR internal mammary nodes have cancer, but axillary lymph nodes don't have cancer) and N3 (10 or more axillary lymph nodes have cancer OR infraclavicular (under the clavicle) nodes have cancer OR internal mammary nodes have cancer plus 1 or more axillary lymph nodes have cancer OR 4 or more axillary lymph nodes have cancer plus internal mammary nodes have cancer or micrometastases found on sentinel node biopsy OR supraclavicular (above the clavicle) nodes have cancer).

Note: These definitions are based on the AJCC/UICC TNM classification system, which must be used for consistent and standardized staging.

- **Numerical scores**:
  - **HER2DX® Relapse risk score**: this score provides the patient's long-term risk of recurrence during follow-up at 10 years after (neo)adjuvant chemotherapy. Patients are classified as low risk (score from 1 to 49), or high risk (50 to 99) (Table 1).

- **HER2DX® pCR likelihood score**: this score provides the patient´s probability of achieving a pathological Complete Response (pCR) if treated with neoadjuvant therapy. Patients are classified as low, medium or high probability. It is categorized into low probability of pCR (score from 1 to 32), moderate probability of pCR (33 to 67), and high probability of pCR (68 to 99) (Table 1).
- **HER2DX® *ERBB2* mRNA score**: this score provides levels of *ERBB2* gene expression and this feature is linked to the tumor's responsiveness to anti-HER2 targeting in the absence of standard chemotherapy. Patients are classified as low (score from 1 to 32), medium (33 to 50), or high levels (51 to 99) (Table 1).

All three HER2DX® scores (the relapse risk score, pCR likelihood score, and *ERBB2* mRNA score) are prognostic and/or predictive in nature, expressed as percentiles (1 to 99) and categorized into low, medium (for pCR likelihood and *ERBB2* mRNA scores), or high levels.[1]

Table 1: HER2DX® Software score groups by score range

HER2DX Score	Score Range	Group
Relapse risk score	1-49 50-99	Low High
pCR likelihood score	1-32 33-67 68-99	Low Medium High
ERBB2 mRNA score	1 – 32 33-50 51-99	Low Medium High

The **HER2DX® Relapse risk score** is based on 3 gene expression-based signatures (immune, tumor cell proliferation, and luminal differentiation), together with tumor stage (T1 vs. T2-4) and nodal status (N0 vs. N1 vs. N2-3). The HER2DX® Relapse risk score is indicative of patient outcomes in HER2+ breast cancer. A statistically significant overexpression of genes within the immune and luminal signatures correlates with a good prognosis, whereas a significant overexpression of the proliferation signature, coupled with nodal status N1-3 and/or tumor status T2-4, is indicative of a poor prognosis.

The **HER2DX® pCR likelihood score** is based on 4 gene expression-based signatures (immune, tumor cell proliferation, HER2 amplicon, and tumor cell luminal differentiation), together with tumor size (T1 vs. T2-4) and nodal status (N0 vs. N1-3). Furthermore, the HER2DX® pCR likelihood score is associated with predictive responses to anti-HER2 therapies. Specifically, a significant overexpression of the immune, proliferation, and HER2 amplicon signature is associated with a complete pathological response (pCR), suggesting a positive response to treatment. In contrast, overexpression of the luminal signature, along with nodal status N1-3 and/or tumor status T2-4, suggests that the patient is likely a non-responder to anti-HER2 therapies.

The **HER2DX® *ERBB2* mRNA score** is based on the individual expression of the *ERBB2* gene.

Once the individual patient's three scores are calculated, they are extrapolated by referencing to databases that store the outcomes collected during the test's development phase. These databases serve as the foundation for the algorithm, which relies on them in the final step to compute the three numerical scores, presented as percentiles (ranging from 1 to 99) and categorized into low, medium (for pCR likelihood and *ERBB2* mRNA scores), or high levels.

The input to HER2DX® Software is the patient's genomic information and two clinicopathological variables (T and N). The patient's genomic information is obtained from a sample of the tumor that is sent to a laboratory for its genomic analysis. The samples can be collected by biopsy or surgical procedures, and they must not be previously exposed to any systemic therapies. In the laboratory, the RNA of the tumor sample is extracted and purified to perform a gene expression analysis of 27 set of genes and 5 housekeeping genes) using the nCounter® Analyses System (NanoString® Technologies, Seattle, USA).

The Summary of Safety and Performance (SSP) for HER2DX® is available in the website of REVEAL GENOMICS, S.L. (https://www.reveal-genomics.com/) and the European database on medical devices (EUDAMED) (https://ec.europe.eu/tools/eudamed). It can be accessed by searching the product using its Basic UDI-DI: 8437026625HER2DX94

1.2. Intended Purpose

HER2DX® Software is a qualitative in vitro diagnostics (IVD) medical device software (MDSW) intended to help healthcare professionals to optimally select the best treatment option for adult patients with early-stage HER2+ breast cancer; the software integrates genomic data -quantitative gene expression- obtained from formalin-fixed paraffin-embedded (FFPE) tumor tissue samples collected prior to systemic treatment, and clinical data - tumor size and lymph nodal involvement (nodal status)-, to provide information on the long-term risk of relapse (HER2DX® relapse risk score), on the likelihood of achieving a pathologic complete response (pCR) following neoadjuvant therapy (HER2DX® pCR likelihood score), and *ERBB2* mRNA expression levels (HER2DX® *ERBB2* mRNA score). The HER2DX® Software, designed as a web application, is intended to be operated exclusively by authorized and trained healthcare professionals. The device has not been validated for use in metastatic disease, HER2-negative tumors, or post-treatment samples.

1.3. Intended user

HER2DX® Software is intended to be used online operated by authorized and trained healthcare professional. In addition, HER2DX® Software must always be requested by a qualified healthcare professional. HER2DX® Software is not conclusive or prescriptive for use of any specific treatment or therapeutic product.

The information contained in the resulting report is intended to be interpreted by a licensed physician specialist (mainly medical oncologists but also gynecologists, surgeons, radiologists, among others specialist responsible for prescribing treatment for patients with breast cancer). The IVD MDSW HER2DX® Software cannot be considered in any case as a substitute for the physician's prescribing activity or for the required medical surveillance in any treatment to the patient. The healthcare professional has ultimate responsibility for all therapeutic decisions based on the patient diagnosis, medical history and family medical history, the indications of the medication, concomitant medications, other individual characteristics of the patient, and a comprehensive interpretation of the report. This *instructions for use* document is applicable only to this HER2DX® software online application, and for which data management is under the responsibility of Reveal Genomics S.L.

1.4. Type of specimen required

HER2DX® Software does not directly interact with any type of human specimen, however, its performance depends entirely on the quality of the input data, which are derived from formalin-fixed paraffin-embedded (FFPE) tumor tissue samples. These samples must be obtained from a biological specimen collected from the patient's primary tumor, either through core biopsy or surgical resection, and must be treatment-naïve (i.e., collected prior to systemic therapy).

The minimum requirements for specimen acceptance are:

- Tumor surface area ≥ 4 mm^2 and tumor cellularity ≥ 10%.
- RNA purity (OD260/280) between 1.7 and 2.5
- RNA concentration ≥12.5 ng/µL

The samples are processed using the nCounter® Analysis System (NanoString® Technologies), which generates standardized gene expression data of a set of defined breast cancer-related genes. that are uploaded to the software for analysis.

Other types of samples, such as frozen tissue or post-treatment tumor samples, have not been validated and are not recommended for use with HER2DX® Software.

The clinical variables used by the algorithm (tumor size and nodal status) must be obtained from the pathology report associated with the same specimen used for molecular analysis.

1.5. Testing population

Use of HER2DX® Software is indicated for patients with early-stage HER2+ breast cancer and who, according to their doctor's judgement, need to be treated. It is important to note that in all retrospective validation studies conducted, no exclusions were applied based on sex, hereditary cancer risk, menopausal status, or age (apart from the inclusion criterion of adults ≥18 years). Therefore, these characteristics cannot be excluded from the population in which the test may be applicable. Additionally, since the algorithm was developed and validated exclusively with treatment-naïve molecular profiles, the use of post-treatment samples may compromise its accuracy and reliability.

2. GLOSSARY

2.1. Abbreviations

All abbreviations used in this document are listed below.

Acronyms/Abbreviations	Meanings
AJCC	American Joint Committee on Cancer
ERBB2	erbB-2 Receptor Tyrosine Kinase 2
IFU	Instruction for Use
RNA	Ribonucleic Acid
IP	Internet Protocol
pCR	Pathological Complete Response
QC	Quality Control
IVD	In Vitro Diagnostic
EFS	Event-Free Survival
ERCC	External RNA Controls Consortium
RCC	Reporter Code Count
UICC	Union for International Cancer Control
TNM	Tumor, Node, Metastasis classification system

2.2. Definitions

The table below contains definitions specific to this document.

Terms	Definitions
RCC	Is a compressed data file and a source code and script file type that is mainly associated with C/C++

3. WARNINGS AND PRECAUTIONS

1. The HER2DX® Software is a stand-alone software in its own right which does not need to be used in combination with other device(s) to enable its use in accordance with its intended purpose. Results shall be taken in the context of other relevant clinico-pathological factors and standard practice of medicine.
2. The HER2DX® Software is not indicated for breast cancer diagnostic purposes.
3. The HER2DX® Software is only indicated for patients with early-stage HER2+ breast cancer (not validated in metastatic disease or HER2-negative tumors).
4. The HER2DX® Software is intended to be operated exclusively by authorized and trained healthcare professionals. Usage of the **HER2DX® Software** by an untrained healthcare professional or unauthorized user may lead to wrongfully handled or interpreted health information.
5. The accuracy of the HER2DX® scores depends on the correct classification of tumor size and nodal status. These clinical-pathological variables must be assessed following the latest edition of the AJCC/UICC TNM staging system.
6. If a patient's tumor size category is entered into the software incorrectly, the pCR likelihood score and relapse risk score classification may be adversely affected (e.g. shifted pCR likelihood score and relapse risk score and misclassification).
7. If a patient's nodal status is entered into the software incorrectly, the patient's test results may be reported incorrectly (e.g. shifted pCR likelihood score and relapse risk score and misclassification).
8. Always verify that the results of the report you select correspond to the right patient.
9. After using Software HER2DX®, always log out from your session to avoid access by a non-authorized user.
10. Users should be aware that any serious incidents related to the device must be promptly reported to both the manufacturer and the competent authority of the Member State in which the user and/or the patient is located.
11. Users should be aware that it is their responsibility to identify, analyze, evaluate and control any potential risks and hazards in relation to the access and usage of the HER2DX® Software. For potential hazards and its consequences, see chapter *7.9. Hazardous situations resulting from IT-network failure*.
12. Manual modification of RCC files is strictly forbidden. Any change performed by the end-user may lead to unexpected, incorrect, or inaccurate results, which will adversely affect the analysis and the report generated by the application.

4. TRAINING INFORMATION

The **HER2DX® Software** is intended to be run by authorized and trained healthcare professionals. Please contact Reveal Genomics for training information specific to running the HER2DX® software.

5. USER ROLES

The HER2DX® software application have four user roles as it follows:

1. Admin Role
2. Customer Admin Role
3. Research Role
4. User Role

1. Admin Role

The Administrator Role is responsible for the overall administration of the web application. This includes tasks such as:

a. The Admin role creates, deletes, and modifies user accounts.
b. The Admin role manages other users accounts.
c. The Admin role has the highest level of permissions in the user role hierarchy.
d. The Admin role can act as both a Regular User and a Researcher.

2. Customer Admin Role

a. This role creates, deletes and modifies user accounts that they lead.
b. This role sees all the logs registered for this customer.
c. This role can act as both a Regular User and a Researcher

3. Research Role – This role has a set of basic permissions configured by the Admin role. Its primary responsibility is to generate with the "Research" watermark.

a. Can supply the necessary documents for the application to generate a report based on the provided data.
b. Generates the report with the "Research" watermark.
c. By default, it does not have any administrative permissions assigned.

4. User Role – This role has a set of basic permissions which are set up by the Admin/Customer Admin role. The purpose of this role is to generate the report.

a. Can supply the necessary documents for the application to generate a report based on the provided data.
b. Can change its personal account such as password, email, phone number.

6. PROCEDURE

6.1. Requirements

The **HER2DX® Software** is a web-based application accessible via standard web browsers like Google Chrome or Mozilla Firefox through an internet connection. It can be used on PC or laptop devices without the need for additional on-site software or hardware.

Maintenance of the HER2DX® Software is the responsibility of the manufacturer, and only the support team have access to the infrastructure, services, and underlying software technology used for publishing the IVD MDSW.

This software is exclusively intended for operation by authorized and trained healthcare professionals. To perform the HER2DX® Software test, the patient's primary tumor size and nodal status information are required.

6.1.1. Intended Use Environment and Technical Requirements

The HER2DX® software is a cloud-based, web application that operates entirely through a supported internet browser. All computational logic and data processing are performed in the validated cloud environment; the user device is only required to access the application interface and display the generated results.

6.1.2. Supported Operating Systems

Validated operating system environments:

- Windows 10/Windows 11.

Other environments (e.g., macOS, Linux) may be technically compatible when using the supported browser versions below, as modern browsers provide cross-platform consistency.
However, these are not part of the validated configuration and use of macOS or Linux or other operating systems is at the user's sole responsibility.

Mobile and tablet platforms are not supported or validated.

6.1.3. Supported Browsers

- Google Chrome, version 119.0 or newer.
- Mozilla Firefox, version 119.0 or newer.

Browser versions must be kept up to date to ensure security and proper rendering of the application.

6.1.4. Minimum Hardware Requirements (User Device)

- CPU with frequency of 1.5 GHz or higher.
- 2 GB of RAM or more.
- 4.5 GB of available disk space.

The IVD MDSW is best viewed on screen size of 14" or bigger with a standard resolution 1,920 × 1,080 pixels or higher.

Internet connection sufficient for secure browser use (minimum 10 Mbps recommended). These requirements apply to the user's PC or laptop to ensure proper performance of the browser.

6.1.5. PDF Reader

HER2DX® Software result reports are generated in PDF format.
For opening and viewing the results, there is no external PDF reader required, as supported browsers (Google Chrome, Mozilla Firefox) include built-in PDF viewing capability.

An external PDF reader (e.g., Adobe Acrobat Reader version 2024.x.x or newer) is recommended if users wish to download reports for offline access, annotation or digital signature.

6.1.6. Cybersecurity and Supporting Infrastructure

Users must access the application from a secure, authorized workstation with standard IT protections (e.g., antivirus, firewall, controlled access).

Access is restricted to authorized users only; credentials must be handled securely.

The application is maintained in a hardened environment by the manufacturer; no user configuration is required.

The user should ensure local environment protections of their own IT systems according to their applicable local IT policies.

Users should report any suspected cybersecurity incident to Reveal Genomics using the contact details provided in this IFU.

6.1.7. Risks of Use Outside Intended Environment

Use of unvalidated operating systems (macOS/Linux), outdated browsers, mobile devices, or insecure networks may lead to degraded performance, inaccessibility, or unavailability of the application.

Clinical accuracy of results is not affected by the user device, as calculations occur in the validated cloud environment; however, unsupported configurations are outside the manufacturer's validated scope.

6.2. How to use the IVD MDSW HER2DX® Software

The users must access the IVD MDSW HER2DX® Software using private credentials (user and password). Thus, only authorized and trained healthcare professional users can access data managed and stored by HER2DX®. The username and password for authorized users in order to access the application will be provided by Reveal Genomics staff.

6.2.1. Access and Log-in

To access HER2DX® Software, go to the login page: https://her2dx.reveal-genomics.com. (Figure 1) and click on "Login".

- Enter your username or email address and password, and then click on the "Login" button. (Figure 2).
- Each user's personal password is confidential and should in no instance be shared with any study team members or third parties.
- Upon your first login, the application will prompt you to change your initial password with a new password. (Figure 3)
- In order to login, a multi-factor authentication is required. A verification code will be sent to your email address, which must then be entered into the designated field and then click on "Submit" button (Figure 4 + Figure 5)
- The application will prompt the Label of product & Instruction for Use (IFU) if there is any new version that has not been consulted yet (Figure 6 + Figure 7). Once accessed and accepted, the notifications will not appear on the next login.
- If the information inputted into the requested login fields is correct, you will be directed to the homepage (Figure 8 + Figure 9).

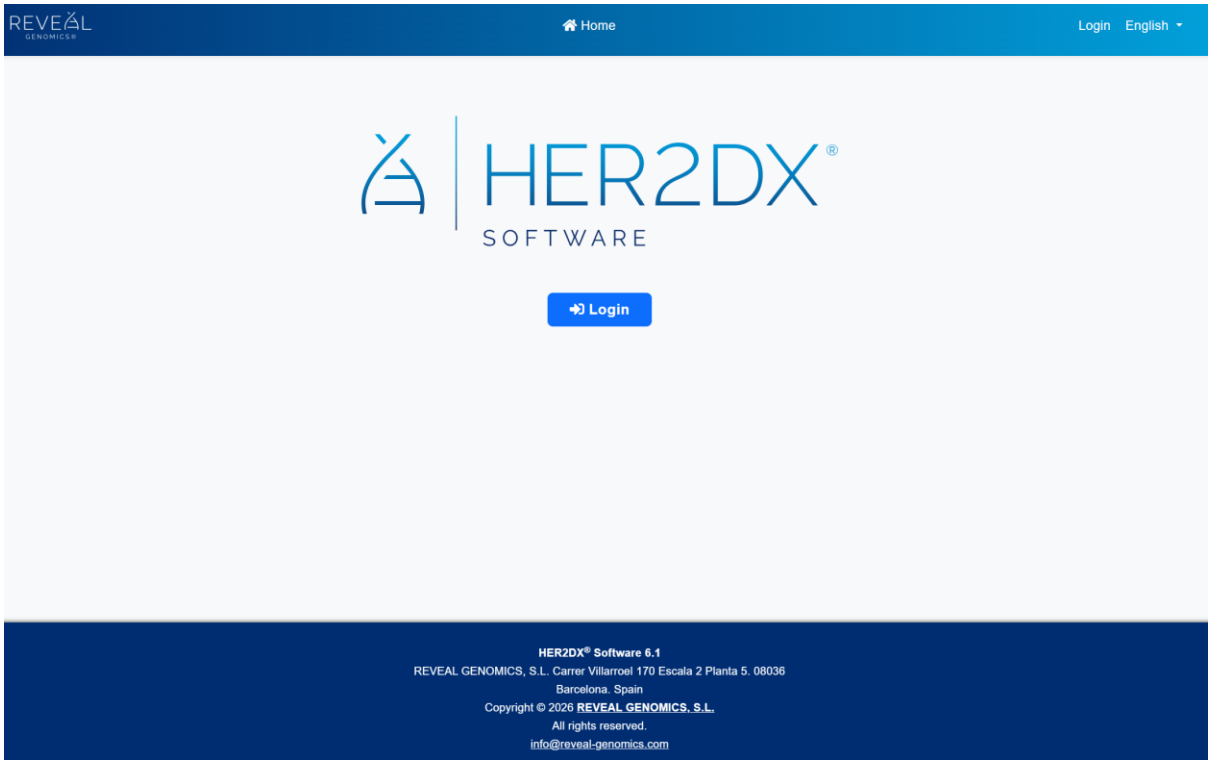


Figure 1 – Login Landing Page

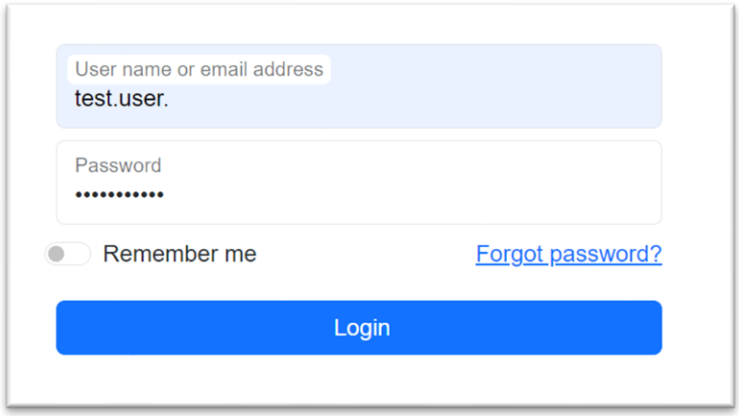


Figure 2 – Login

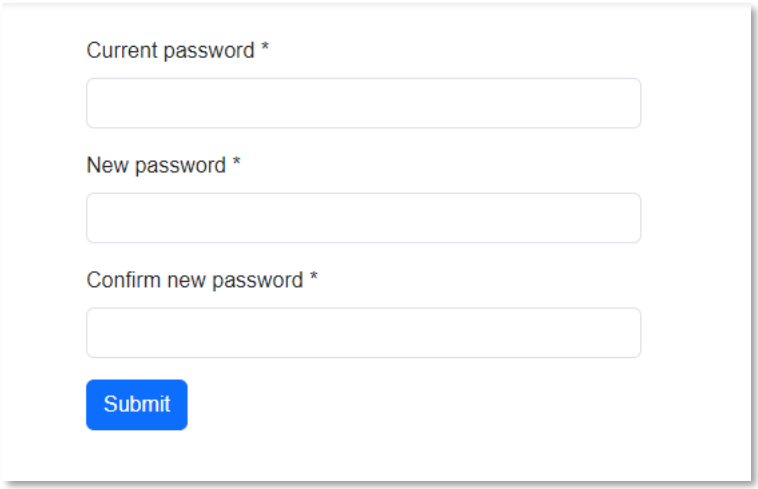


Figure 3 – First login- Password change

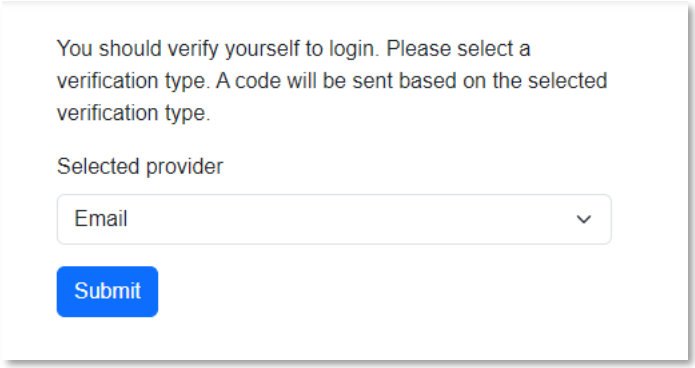


Figure 4 – Multi-factor authentication

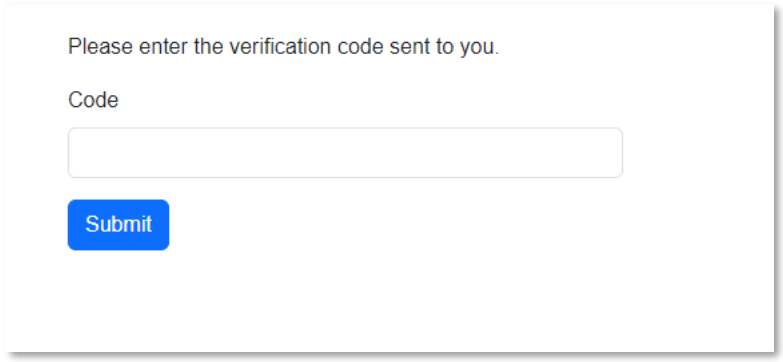


Figure 5 – Verification Code check

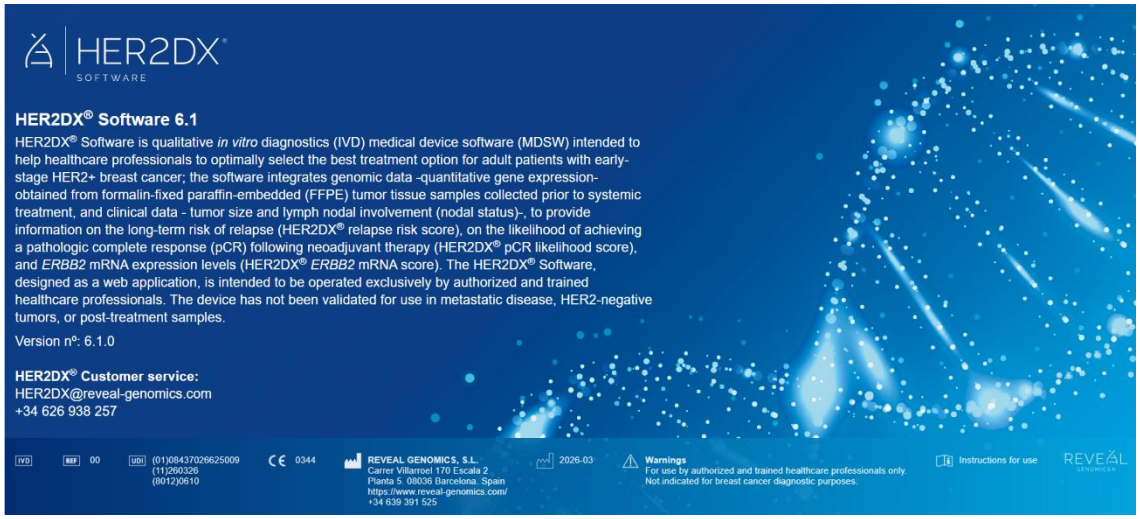


Figure 6 – Product Label notification

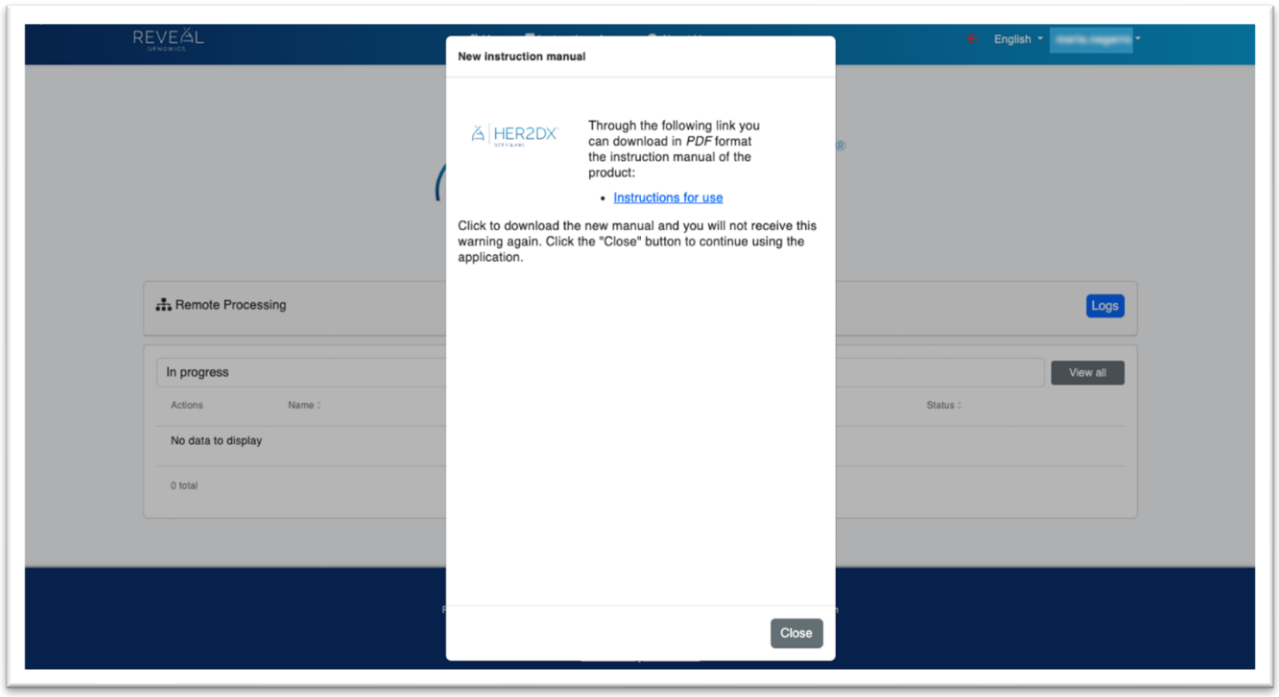


Figure 7 – New version of IFU pop-up notification

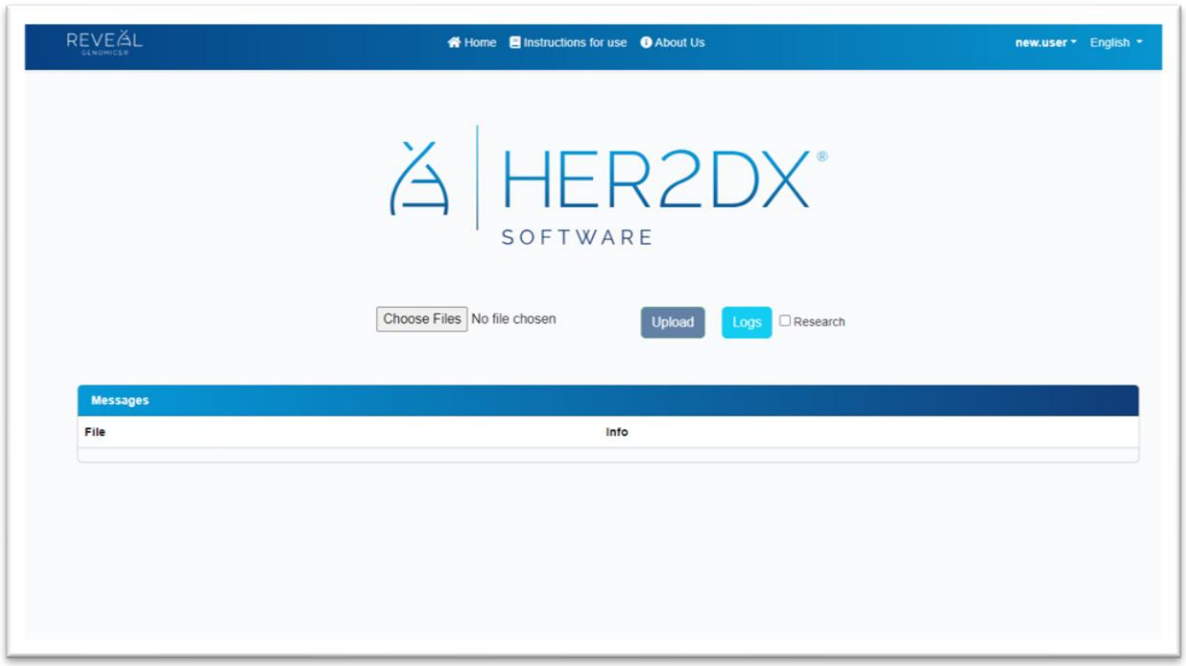


Figure 8 – Regular User Homepage

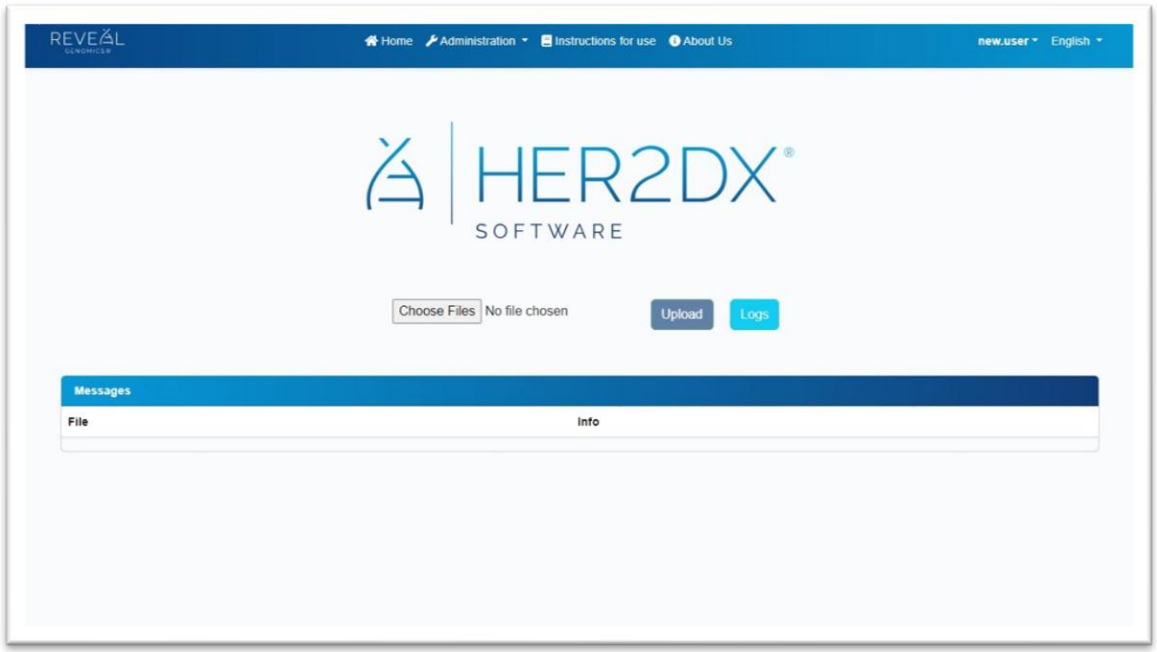


Figure 9 – Customer Admin Homepage

6.2.2. Forgotten Password and Change of Password

6.2.2.1. Forgot Password

- Click on the "Forgot Password?" button (Figure 10) on the login page,
- Enter the requested information and click on "Submit" (Figure 11).
- A notification will appear informing you that the email has been sent (Figure 12)
- You will receive the e-mail containing a link, in order to set a new password (Figure 13). Click on the link.
- You will be directed to HER2DX® Software application and you should input the "New password" and "Confirm new password" fields (Figure 14). *The password must contain at least 12 characters, at least 1 number, 1 capital letter and 1 special character.* Then click on "Submit" button (Figure 14).
- If the fields match, a notification will appear informing you that the password has been successfully reset and you will have the option to navigate on the Login page (Figure 15) in order to retry the Login.

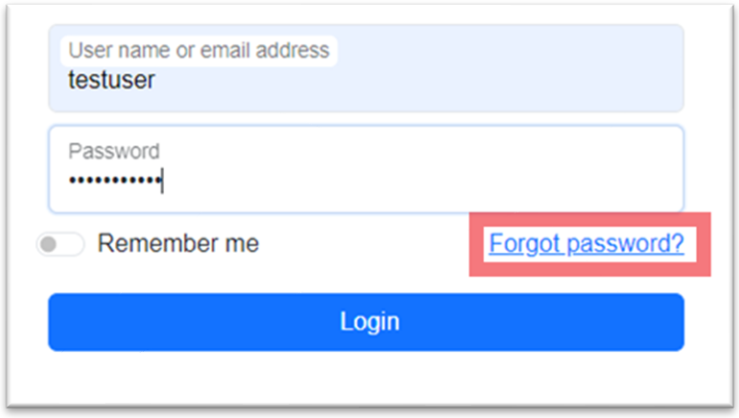


Figure 10 - Forgot password button

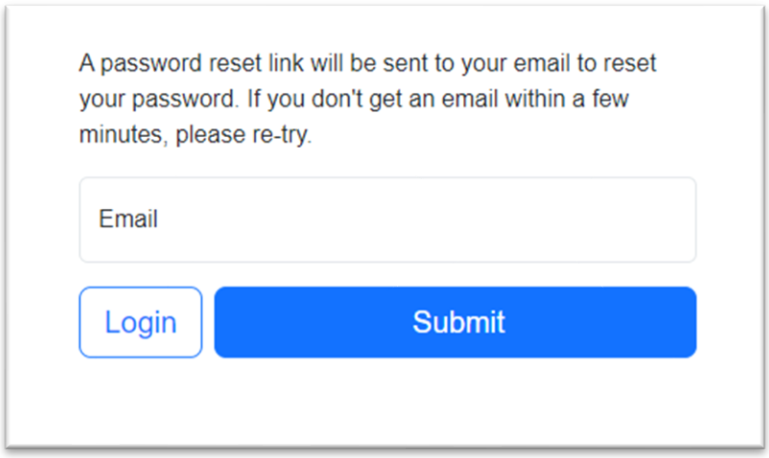


Figure 11 – Email address input for password recovery

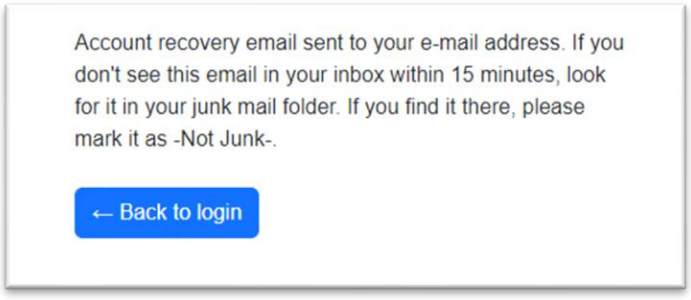


Figure 12 – Recovery email sent notification

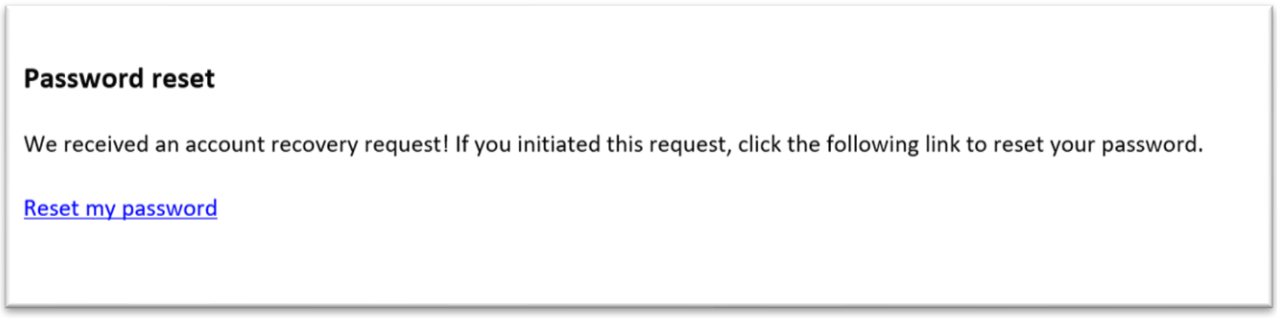


Figure 13 - Recovery email content + reset password link

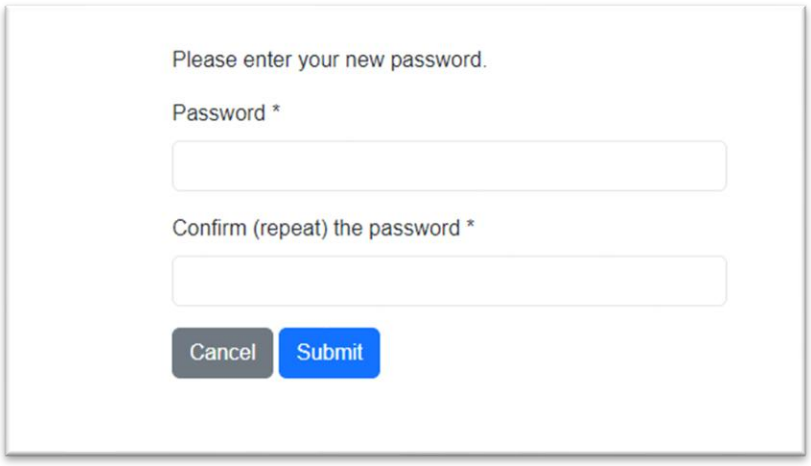


Figure 14 - Reset of the password

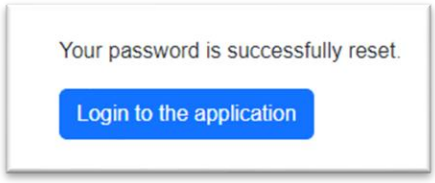


Figure 15 - Reset password notification

6.2.2.2. Change Password

- Enter "My account" section (Figure 16).
- Select "Change Password" option and input the required fields then click on "Save" button (Figure 17).
- If fields match and password is successfully changed, a notification will appear (Figure 18).

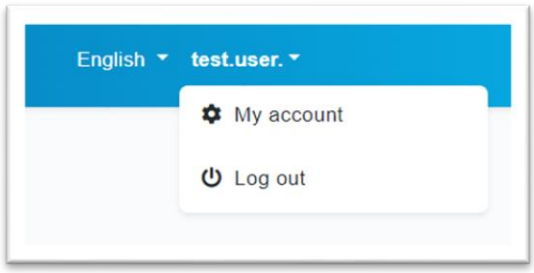


Figure 16 – My account

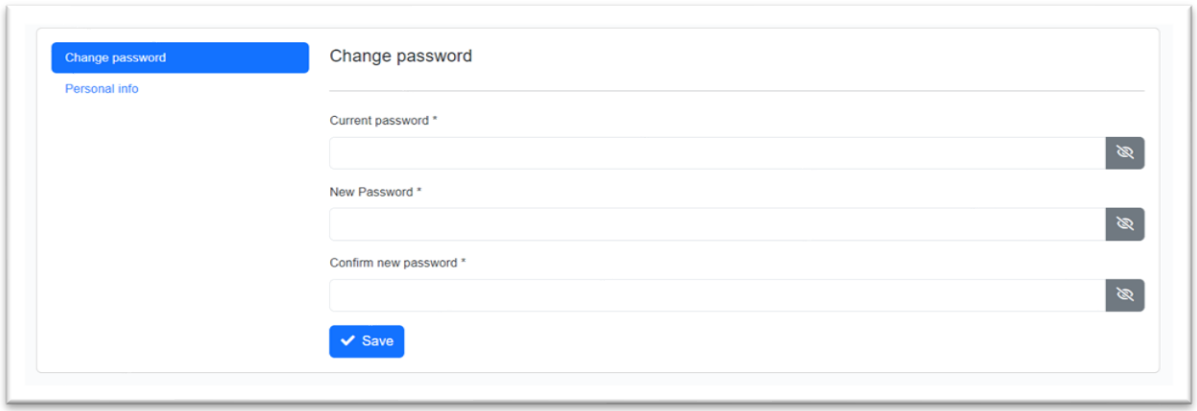


Figure 17 – Change password

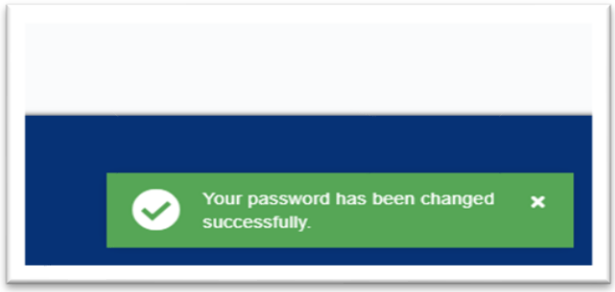


Figure 18 - Confirmation of changed password

6.2.3. Navigation and General Presentation of the Application

6.2.3.1. Customer Admin Navigation Menu

The application has a header (Figure 19) which allows users to access:

- **Home**: Refresh and access the main page.
- **Administration:** Access Identity Management functionality in order to visualize the Users assigned to the customer and their roles
- **Instructions for use**: Download the instructions for use.
- **About Us:** Label of the product.
- **Language:** Change language to English or Spanish.

- **Account Menu:** "Logout" or "My account", where you can change the password or check your personal settings.

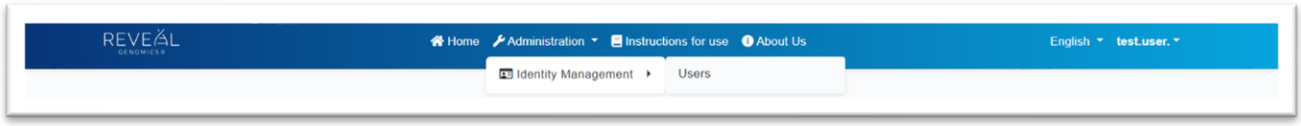


Figure 19 - Customer Admin user Navigation Menu

6.2.3.2. Regular User Navigation Menu

The application has a header, (Figure 20) which allows you to access:

- **Home**: To refresh and access the main page
- **Instructions for use**: To download the instructions for use
- **About Us**: Label of the product information
- **Language:** To change language to English or Spanish
- **Account Menu:** To "Log out" or access "My account", where you can change the password or check your personal settings.

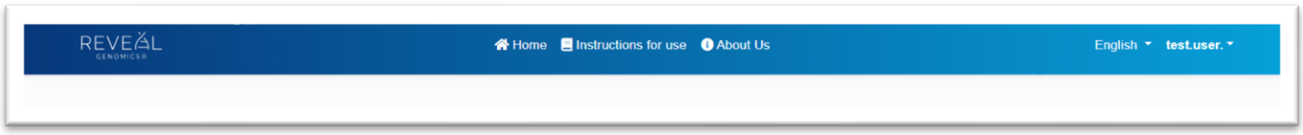


Figure 20 - Regular User Navigation Menu

6.3. Preparing The Files

6.3.1. How to name the SAMPLE ID and the RCC Files

The format of the **Sample ID** must meet the following points:

- Have a letter (e.g. E) along with a 2-digit number (year) followed by a dash line (e.g. E23-).
- After the dash line a 6 digits number has to be displayed (e.g. 001056)

The first letter of the Sample ID will be assigned by Reveal Genomics personnel.
E.g.: E23-001056

The format of the RCC file must meet the following format (**spaces are not allowed**):
20230118_HER2DX-M1-MAR_ E23-001056:

- 20230118 -> default by nCounter instrument
- HER2DX
- M1 or 2 -> In which instrument had run the samples
- Initials of the technician who have done the run-> e.g. Mar Alvarez Rodriguez: MAR
- SAMPLE ID: E23-001056

For the traceability of the RCC file name must contain the Sample ID.

You can run 1 sample or up to 10 samples at a time. It is necessary to put two RNA synthetic controls (reference controls) in each run and always the RCC FILE name of the Reference Control samples must contain **RNA-SINT** (in this exact format), e.g. RNA-SINT-01, RNA-SINT-02.

A
RCC_FILE
20230118_2023-01-18-HER2DX-M1-MAR_E22-027040_01.RCC
20230118_2023-01-18-HER2DX-M1-MAR_E22-027134_02.RCC
20230118_2023-01-18-HER2DX-M1-MAR_E23-001056_03.RCC
20230118_2023-01-18-HER2DX-M1-MAR_E23-000694_04.RCC
20230118_2023-01-18-HER2DX-M1-MAR_RNA-SINT-01_05.RCC
20230118_2023-01-18-HER2DX-M1-MAR_RNA-SINT-02_06.RCC

Figure 21 – How to name the RCC files

Note that the manual modification of the RCC files is strictly forbidden, in order to ensure the correct generation of the report analysis. Manual modification of the RCC file by the end-user will lead to unexpected, incorrect and inaccurate results which will affect the report file generated by the application.

6.3.2. Upload RCC files and Clinical Data

Depending on the user, you can either perform **manual processing** (Section 6.3.3) or **remote processing** (Section 6.3.4). Please follow the instructions below based on the user type you encounter.

6.3.2.1. Manual Processing

6.3.2.1.1. Create Clinical data file

In an excel file saved with the tab-delimited text files .txt format the following information must be filled in:

- **RCC_FILE*:** copy the name of the RCC file, has to be exactly the name that it's in the RCC FILE.
- **Sample ID***: It has to be the name of the sample that is on the RCC file.
- **Test ID***: Reveal ID, for example: HCDB-TR000X or C001-TR000X.
- **T***: T1, T2, T3, T4.
- **N***: N0, N1, N2, N3 and N2-3.
- **Language:** Select the language of the report: ES (Spanish), EN (English)

* All the cells must be completed.

A	B	C	D	E	F
RCC_FILE	Sample ID	Test ID	T	N	Language
20230118_2023-01-18-HER2DX-M1-MAR_E22-027040_01.RCC	E22-027040	HCDB-22-002	T2	N0	ES
20230118_2023-01-18-HER2DX-M1-MAR_E22-027134_02.RCC	E22-027134	HCDB-22-003	T3	N3	EN
20230118_2023-01-18-HER2DX-M1-MAR_E23-001056_03.RCC	E23-001056	C002-TR0053	T2	N1	ES
20230118_2023-01-18-HER2DX-M1-MAR_E23-000694_04.RCC	E23-000694	P012-TR0052	T2	N0	EN
20230118_2023-01-18-HER2DX-M1-MAR_RNA-SINT-01_05.RCC	REF1	REF1	NA	NA	NA
20230118_2023-01-18-HER2DX-M1-MAR_RNA-SINT-02_06.RCC	REF2	REF2	NA	NA	NA

Figure 22 – Example of format of the Clinical Data Her2DX.txt

The file will be saved with the following name ClinicalDataHER2DX_+ the date. Example: ClinicalDataHER2DX_11_01_2024.txt.

6.3.2.1.2. Upload of the RCC files and Clinical Data

On the Homepage there is a "Choose File" button, an "Upload" button and a "Logs" button. In order to upload the RCC files:

- Click on "Choose File" button (Figure 23) – a folder from the device will open (Figure 24)
- Select the RCC files from the samples + 2 RCC files of the RNA-SINT + ClinicalDataHER2DX.txt form the device (Figure 24).
- Click on "Open" (Figure 24).
- Check the total number of the selected files (Figure 25).
- Click on "Upload" button (Figure 23).
- A loading bar will appear on the top of the page as the files are uploading (Figure 26).
- Then the uploaded files will be displayed in a list on the Homepage (Figure 27) indicating whether the sample has been analyzed without any issues (OK) or with information about any potential errors.
- Then the web browser should automatically download the reports as PDF files on the device (Figure 28). If the browser configuration does not allow automatic download, you will be presented with a dialog for saving the reports.
- For multiple uploaded files you should expect multiple PDF reports (Figure 28).

Figure 23 - Manual upload of the RCC files

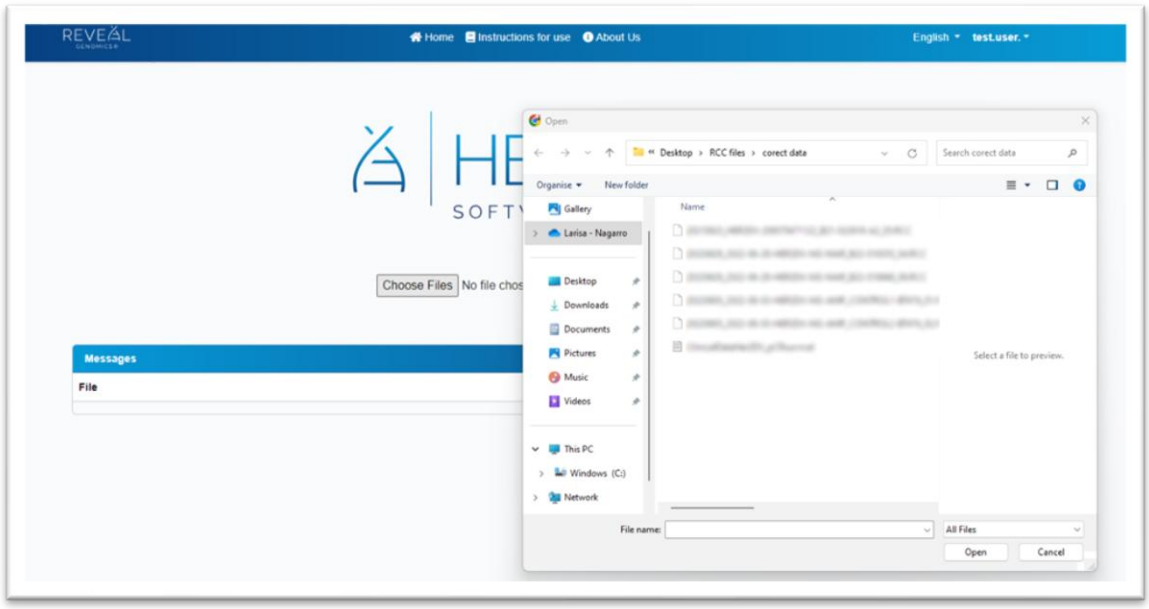


Figure 24 - Select the files from the device

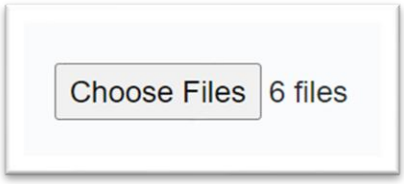


Figure 25- Total number of selected files

Figure 26 - Loading bar while files are uploading into the application.

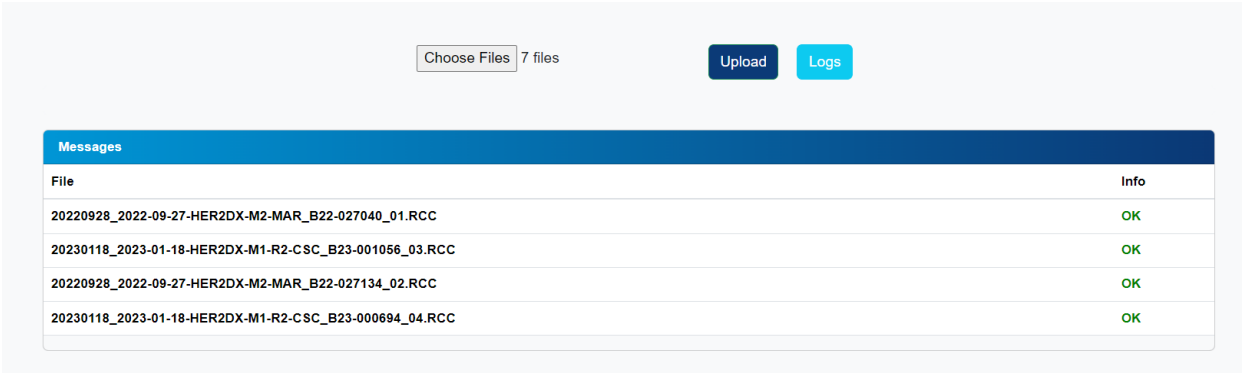


Figure 27 - Upload button and uploaded files displayed in a list

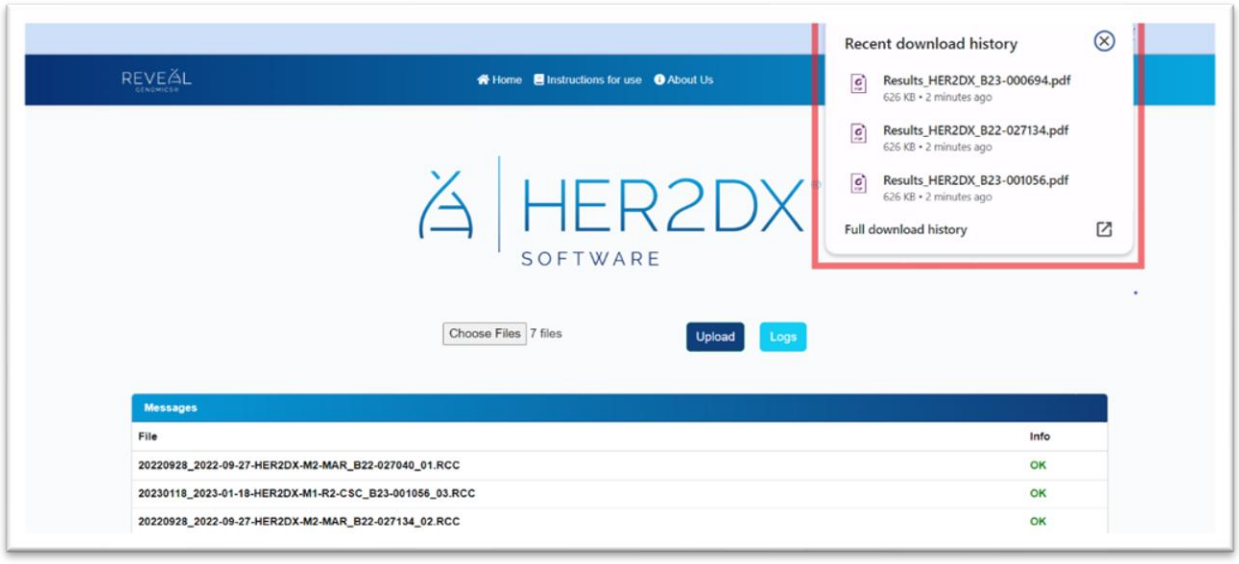


Figure 28 - Automatic download of files on device

6.3.2.2. Remote Processing

- This automatic upload process is achieved by sending the RCC files from the nCounter scanner to a specific customer email address that is previously provided by Reveal Genomics personnel.
- After the RCC files have been sent to the specific email, the Homepage will be displayed as in Figure 29.
- As for the files to appear on the Homepage you have to refresh the browser page, click on the "View All" button – (Figure 29). Then filter by date. After that, a list with the most recently uploaded set of RCC files is displayed. The status of the files will then appear as "Pending" (Figure 29).
- Click on "Review" button (Figure 29) in order to check information of the uploaded files.
- Ensure that T / N values are the correct ones (Figure 30).
- To delete the uploaded files, click on "Delete" button (Figure 30).
- In order to edit T / N values and the report language, click the "Edit" button located on the right side of each sample (Figure 31). If the report needs to be in Spanish, select ES (Spanish); by default, the report is generated in English (EN). Next, fill in the "Observation" field with the changes made and click "Save" in order to save the modifications (Figure 31). Note: The Medical History Number can only be added to the test ID field if requested by the ordering center. Please be careful when adding this information to ensure that the test ID code is not removed or modified.
- If changes are saved, a confirmation will appear (Figure 32).
- To calculate the files, click on "Calculate" button (Figure 30).
- After clicking on "Calculate" button, the application will validate the files and will proceed with the automatic download process of the PDF report into your device (Figure 33).

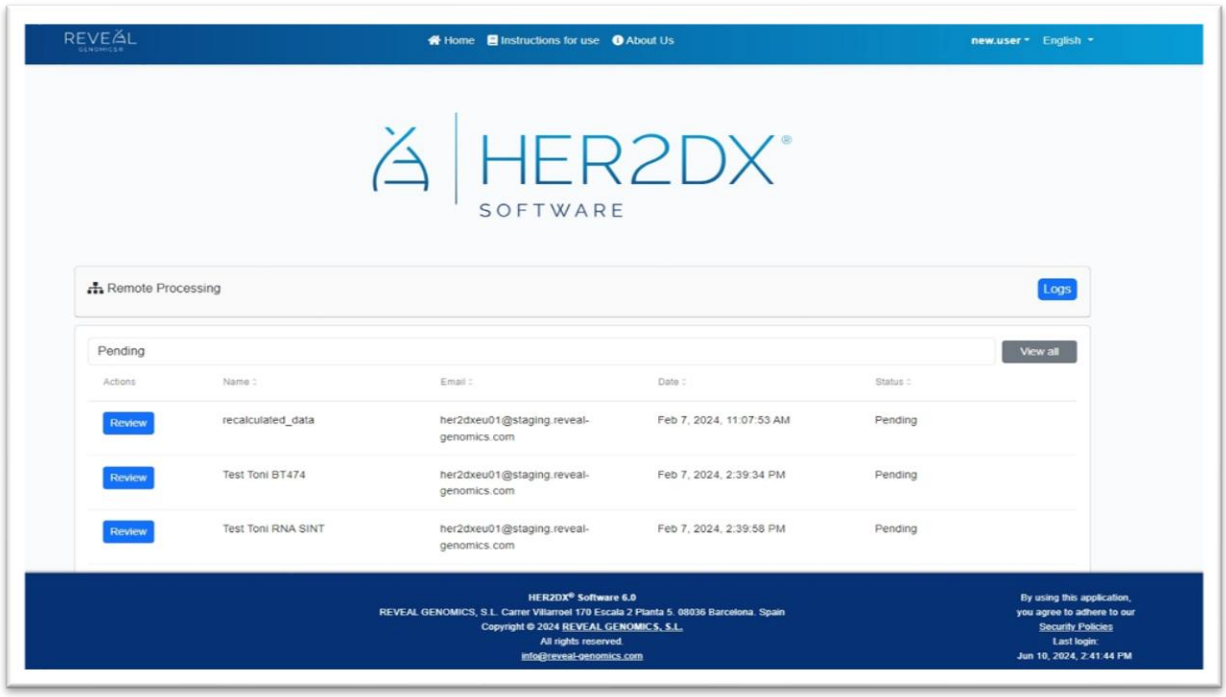


Figure 29 - Remote processing – Homepage after uploading the files

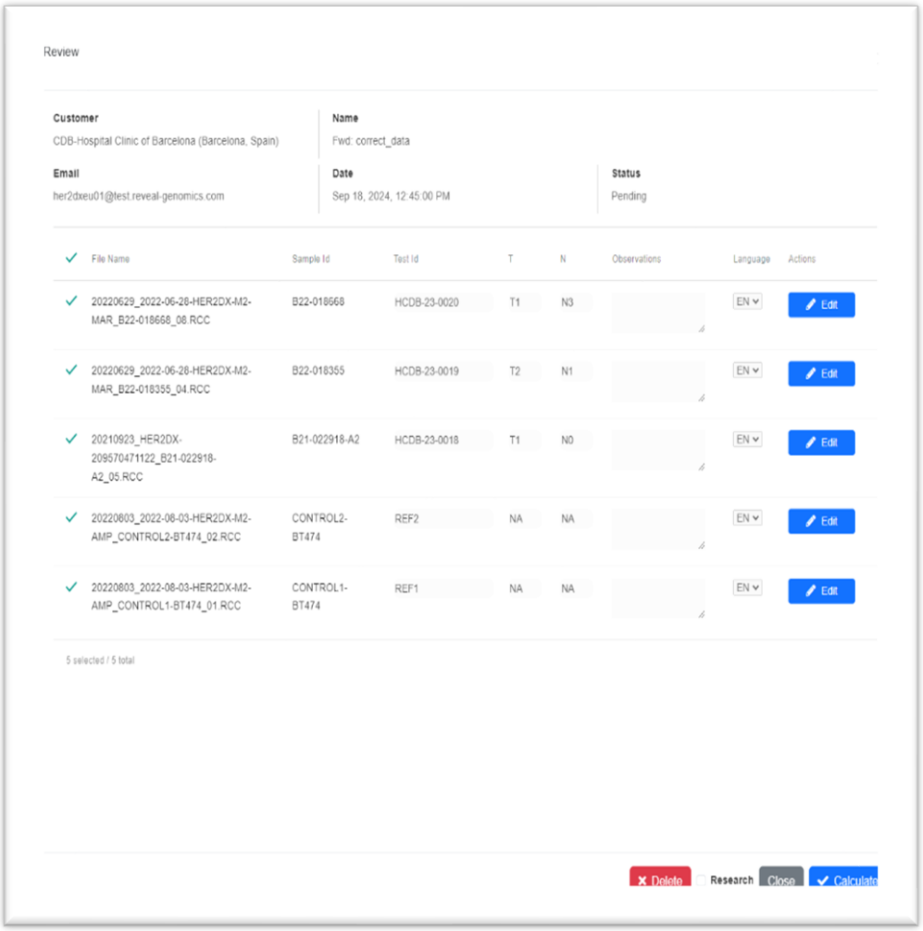


Figure 30 - Review of the uploaded files – Calculate / Close / Delete options

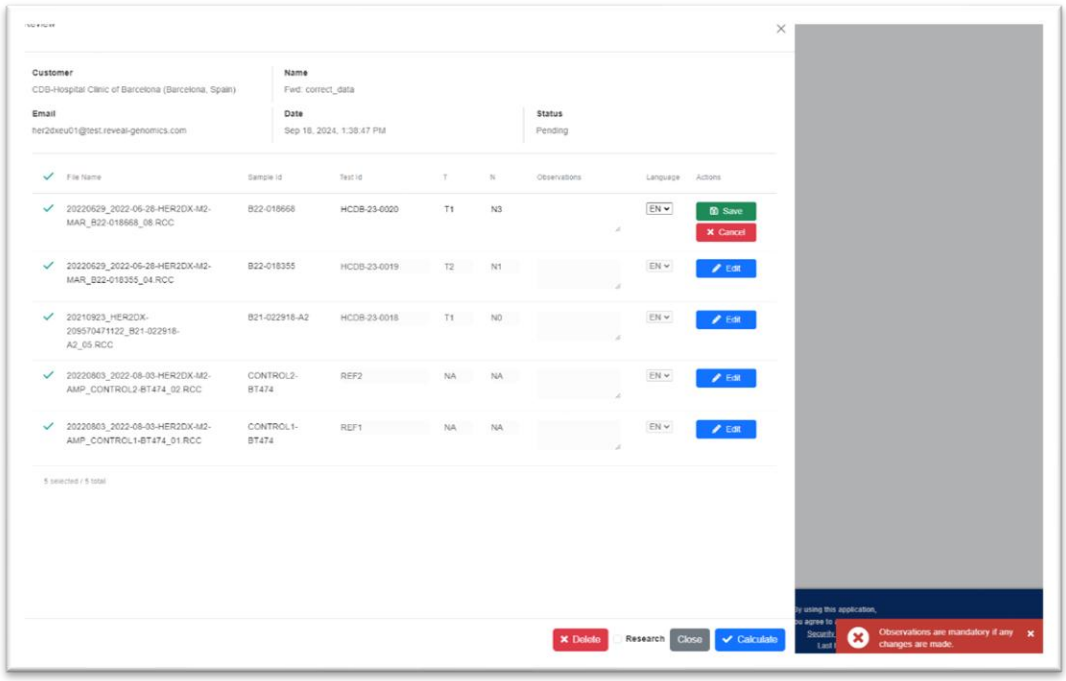


Figure 31 - Edit selected file and Save / Cancel the edit

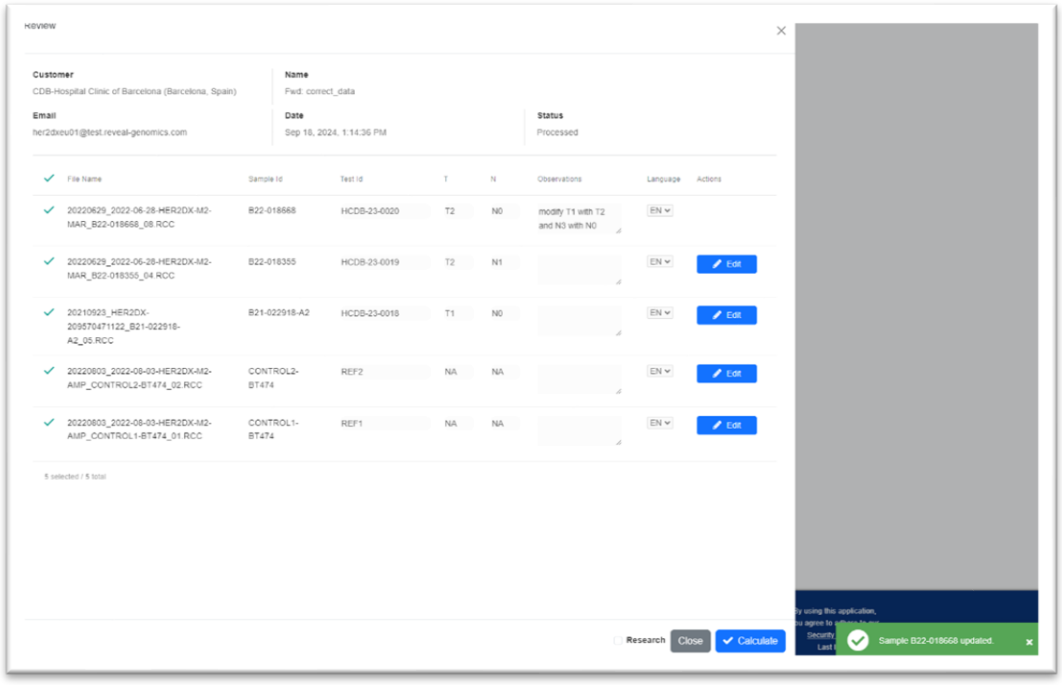


Figure 32 - Sample update notification

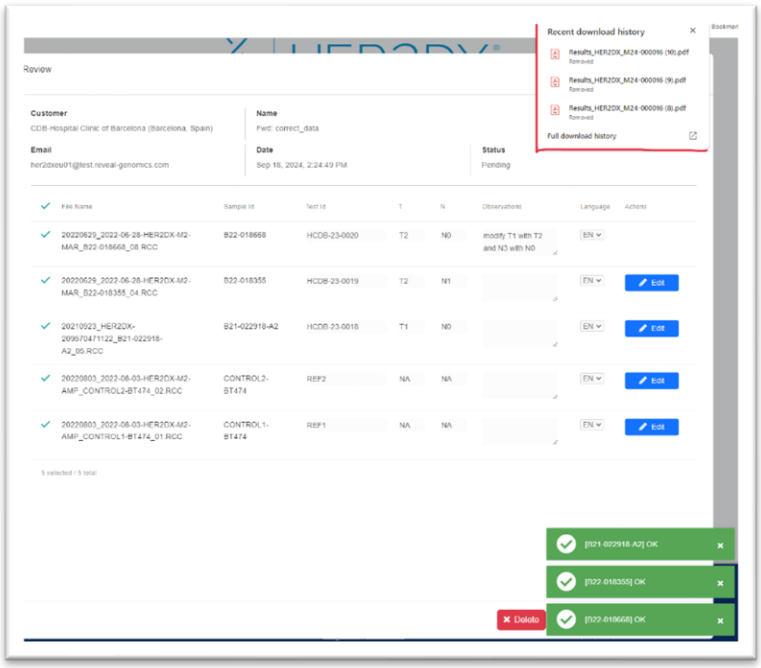


Figure 33 - Processed files and download to device

6.3.3. Logs

- To access the logs for each sample, click on the "Logs" button, displayed on both Manual or Remote processing Homepage (Figure 34/ Figure 35). Then click on "expand" icon (Figure 36). These Logs provide information on each score and quality control metrics.
- The Logs offer the possibility to visualize the PDF report or Recalculate the sample, as well to filter the information with the top listed filters: User, Sample, Run (Figure 36).

Figure 34 - Logs button on Manual Processing Homepage

Figure 35 - Logs button on Remote Processing Homepage

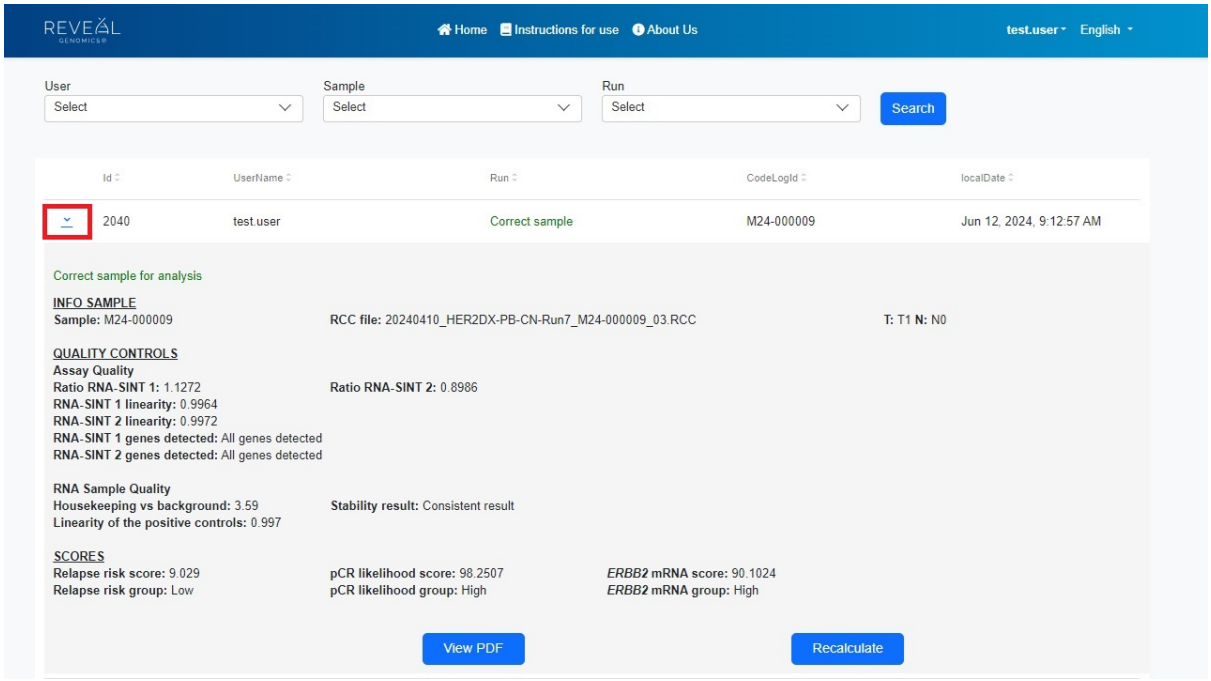


Figure 36 - Logs Page

6.3.4. Recalculations

In some cases, patients may be diagnosed with early-stage breast cancer (such as stage I or II) and show no evidence of cancer spread to the lymph nodes at the time of diagnosis (N0). However, during the surgical removal of the primary tumor, it may be discovered that the cancer has spread to nearby lymph nodes (N1).
For a file to be recalculated, it has to be previously processed (Calculated).

6.3.4.1. Recalculation of RCC file for Manual Processing:

- Before proceeding with the recalculation process, the T /N values will have to be updated into the Clinical Data File for the sample that needs to be recalculated (Figure 37) and also adding the 2 control reference (RNA-SINT) used in that run.
- Access Logs Page, then expand the sample that you want to recalculate, click on "Recalculate" button (Figure 38)
- A folder from the device will open allowing you to upload the updated Clinical Data file. After selecting the Clinical Data file, the user have to click on "Open" (Figure 39)
- The recalculation process will start, the sample will then appear on the Logs page as "Correct sample / Recalculated" in red color, and the application will download the recalculated report. (Figure 40)

	A	B	C	D	E
1	RCC_FILE	Sample ID	Test ID	T	N
2	20230118_2023-01-18-HER2DX-M1-MAR_E23-000694_04.RCC	E23-000694	HCDB-23-0002	T1	N2
3	20230118_2023-01-18-HER2DX-M1-MAR_RNA-SINT-01_05.RCC	REF1	REF1	NA	NA
4	20230118_2023-01-18-HER2DX-M1-MAR_RNA-SINT-02_06.RCC	REF2	REF2	NA	NA

Figure 37 - Lymph node status update into the Clinical Data file

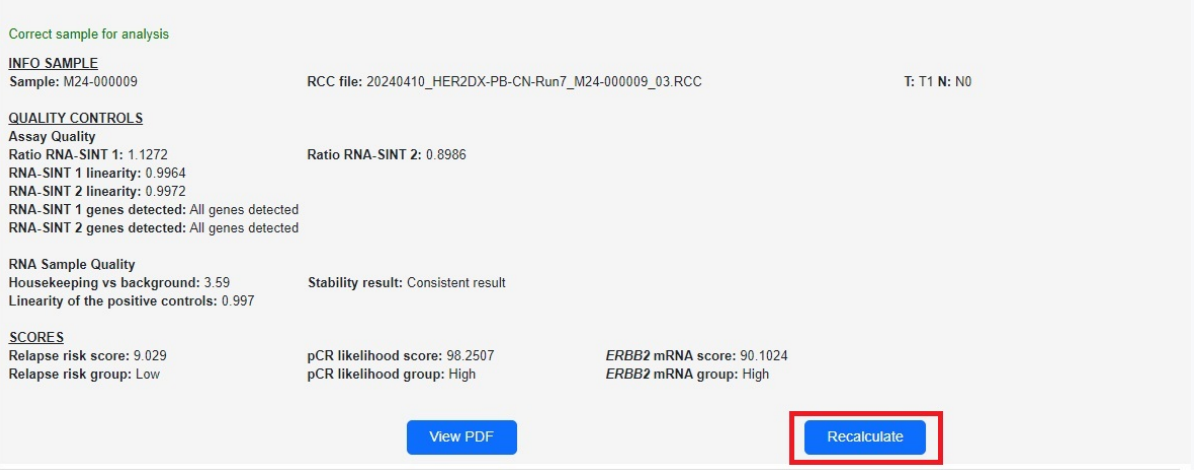


Figure 38 – Logs Page with "Recalculate" button

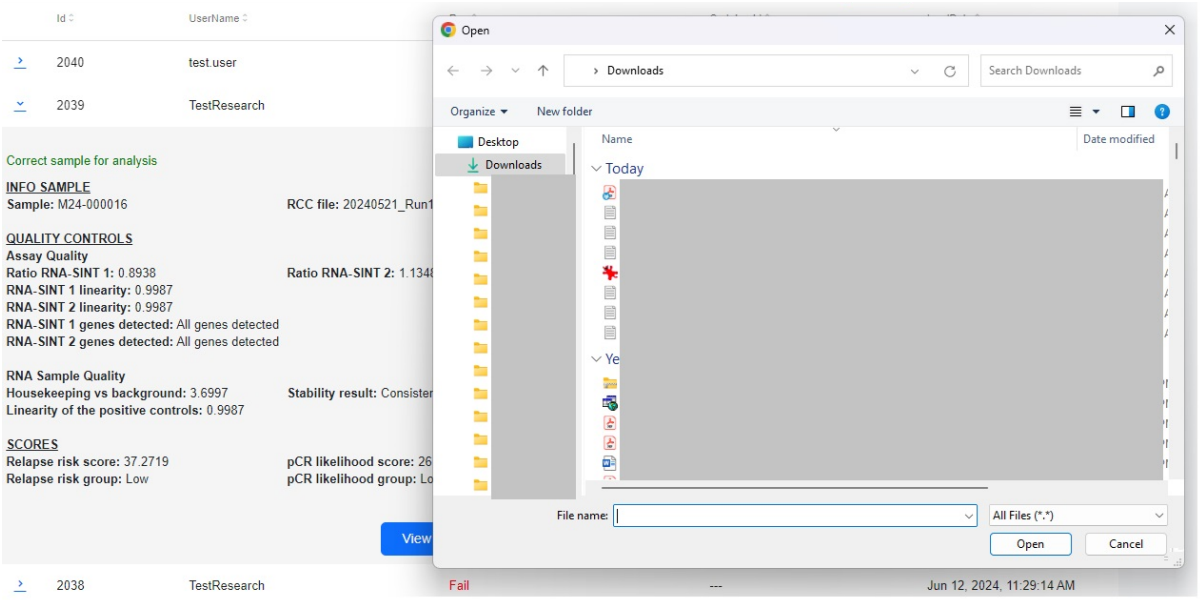


Figure 39 - Upload updated Clinical Data file from device

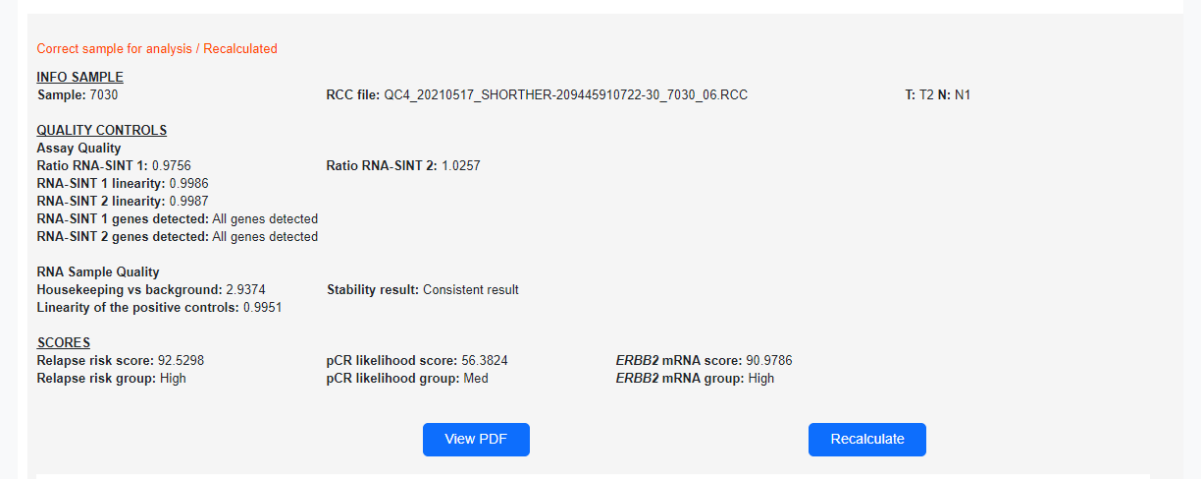


Figure 40 - Change of sample status + Recalculated report

6.3.4.2. Recalculation of RCC files for Remote Processing

- Access Logs Page, then expand the sample that you want to recalculate, click on "Recalculate" button (Figure 41).
- A new tab will open, asking you to input the updated T / N values. (Figure 42).
- The recalculation process will start, the sample will then appear on the Logs page as "Correct sample / Recalculated", and the application will download the recalculated report.

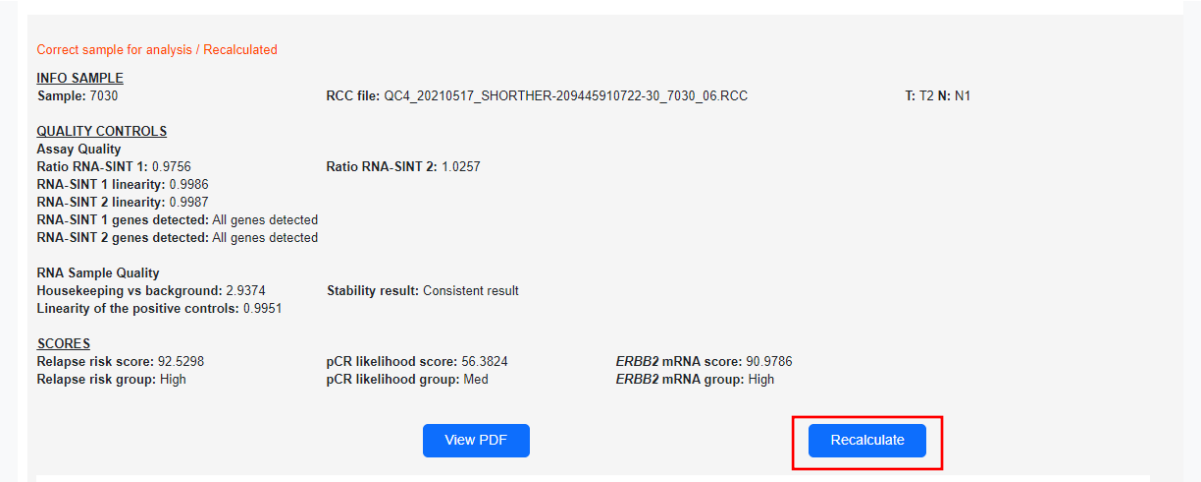


Figure 41 - Logs page with Recalculate button

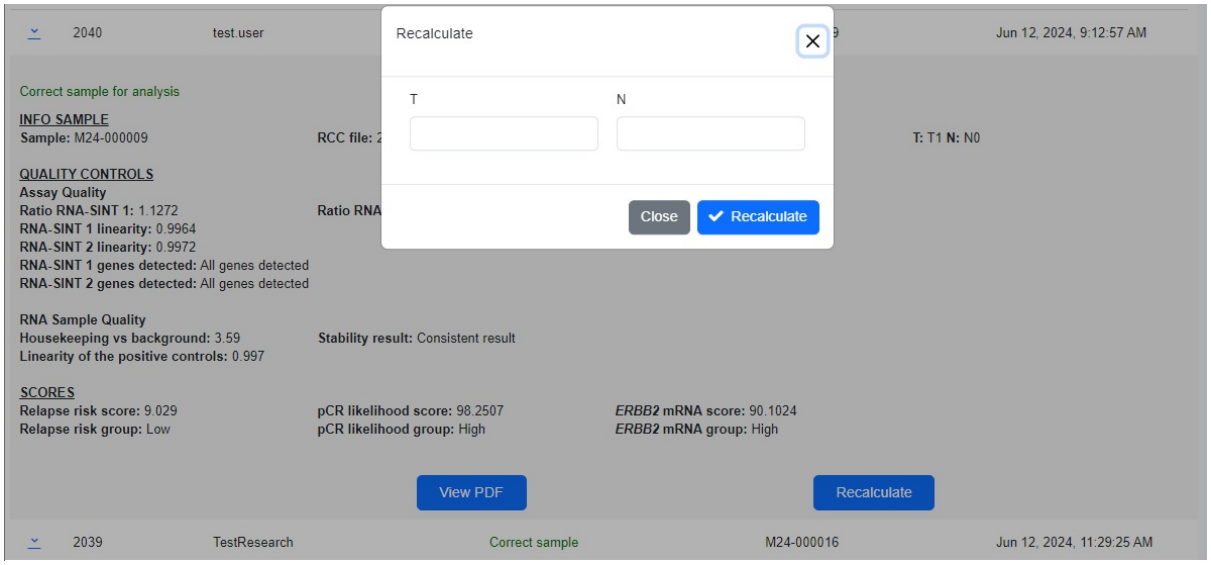


Figure 42 – Input of updated T / N values for a Remote processed file

6.3.5. Input Data Validations

These are some of the error messages that could appear:

Figure 43 – Closely resemble another sample / Duplicated sample

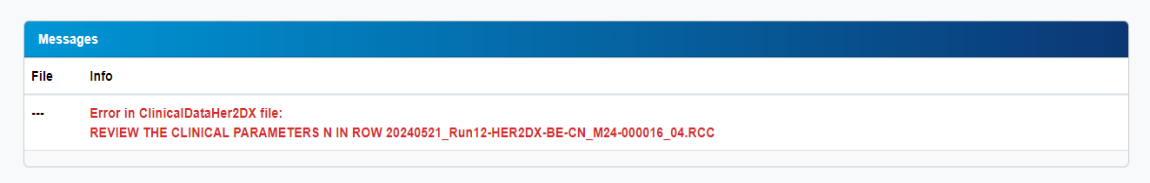


Figure 44 – Missing / Wrong N value

Figure 45 – Missing / Wrong RCC file

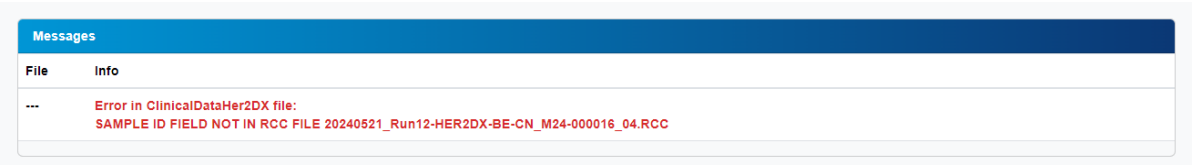


Figure 46 - Missing / Wrong Sample ID

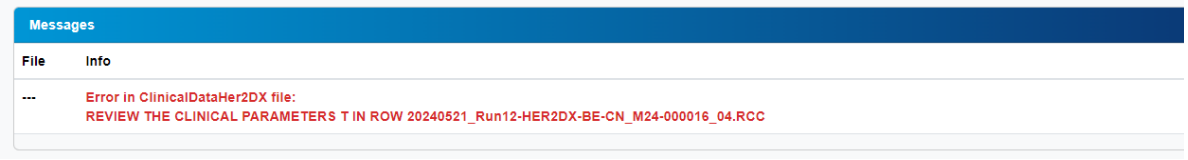


Figure 47 – Missing / Wrong T value

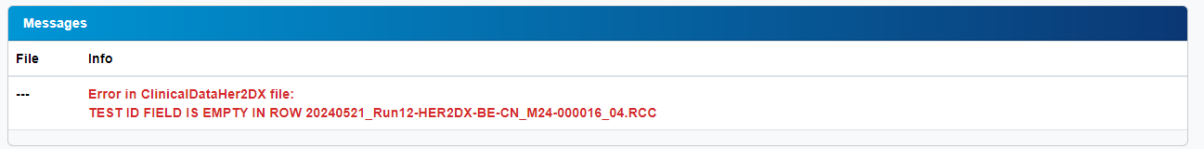


Figure 48 – Missing Test ID

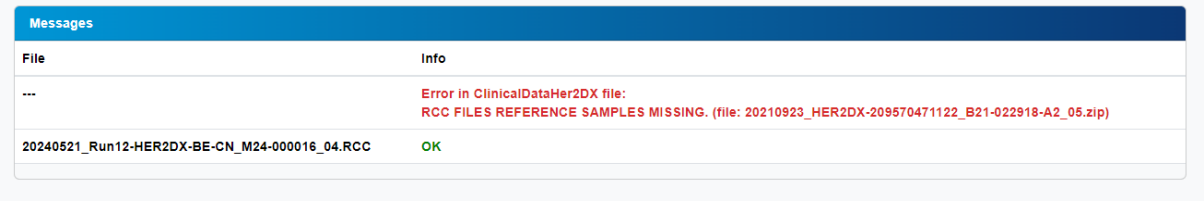


Figure 49 – Non RCC file – RNA

6.3.6. Log Out Of Your Account

In order to Log out of the application move the cursor to the username and click on "Logout" button on the top right corner of the page (Figure 48).
If "Log out" button is clicked, you will be directed to Login page.
Note: If the application is left 15 minutes unattended, the session will expire, and the user needs to log in again.

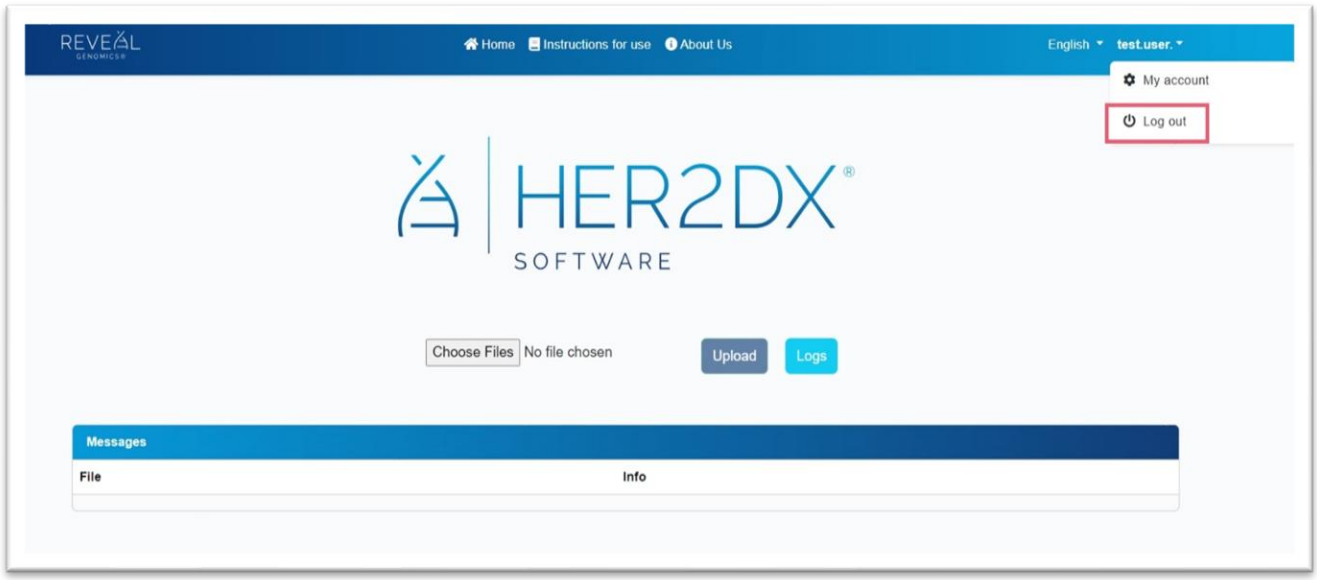


Figure 50 Log out button

7. TROUBLESHOOTING

7.1. Known issues and workarounds:

a. "Remember me" function on the Login page does not retain the Username.
Solution: use the browser's built-in User/Password management function.

b. On screen sizes smaller than 14 inch, the Filtering fields of the Logs section might render and show possible values in a truncated drop-down list.
Solution1: re-size the browser window.
Solution2: re-size the displayed information by using the CTRL + mouse scroll button or the browser's Zoom functionality.

c. Closing the browser tab might not terminate the user session. If a browser tab is re-opened and the user navigates to the HER2DX® Software main page, the last logged-in user is automatically authorized and authenticated.
Solution: close the browser completely or manually log out from the application before closing the browser tab. As general best practice, always lock the screen whenever the computer is left unattended.

7.2. Login issues:

a. Unable to login – Ensure that you are entering the correct username and password. Check for any typos in the login credentials

b. Incorrect username or password – If you've forgotten your password, use the "Forgot Password" link to reset it.

c. Account locked due to multiple failed attempts – If your account is locked, wait for the specified duration or contact support for assistance.

7.3. User role issues:

a. Incorrect access or missing features – User might not be assigned to the correct role – If you are experiencing access denied errors, contact your administrator for proper permissions.

7.4. File upload issues:

a. File is not processed successfully – Possible causes:
   i. Corrupted files – Please verify file integrity
   ii. Insufficient system resources – Please contact support
   iii. Make sure the files are sent to only ONE valid and specific email address communicated for the given customer organization

b. Supported file formats
   i. Check the list of supported file formats in our documentation

7.5. Processing errors:

a. Check for error messages and refer to our documentation for troubleshooting steps.

b. Processing Delays
   i. Be patient. Processing times may vary
   ii. Ensure a stable internet connection
   iii. Contact support if delays persist

c. PDF report not generated
   i. Processing failure – review processing logs for errors

ii. Insufficient permissions – ensure you have the necessary permissions – contact administration

7.6. Download issues:

a. Unable to download the PDF report
   i. Browser issues – Try alternative browser if issue persists. Check your browser settings to allow file downloads
   ii. Network problems – Ensure a stable internet connection during the download

7.7. Incomplete or Incorrect Reports:

a. If reports are incomplete or inaccurate, verify file integrity, re-run the analysis or contact support

7.8. General performance issues:

a. Slow response to frequent timeouts
   i. Heavy system load – Check system status and resources
   ii. Browser compatibility issues – Use a supported browser
b. Session Timeouts
   i. Be aware of session timeout limits
   ii. Re-login if necessary

7.9. Hazardous situations resulting from IT-network failure:

a. Causes:
   i. Network infrastructure issues – Problems with routers, switches or other network components may disrupt communication between IVD devices and laboratory information system.
   ii. Cybersecurity incidents: Malicious activities such as cyberattacks or unauthorized access could compromise the network and disrupt communication between IVD devices in the laboratory information system
b. Potential consequences:
   i. Data loss or corruption – network failure may result in the loss or corruption of patient data stored in the laboratory system.
   ii. Disruption of workflow – healthcare professionals may be unable to access or transmit critical patient information, leading to delays in diagnosis and treatment.
   iii. Missed results – The failure might prevent the IVD device from transmitting test results to the laboratory system causing some results to go unrecorded or overlooked.

7.10. Contact Support:

a. EMAIL ADDRESS: HER2DX@reveal-genomics.com
b. PHONE NUMBER – +34626938257
c. If issues are not resolved by the troubleshooting steps, please contact support and provide detailed information about the issue.
d. *When leaving the application unattended, you must manually log-out!*
e. Tip for avoiding common issues:
   i. Regularly update browsers and ensure system compatibility.

ii. Periodically check for software updates.

The HER2DX® software will identify failing samples and no samples results will be reported. The HER2DX® results will be reported in the case of passing quality control metrics.

8. HER2DX® SOFTWARE RESULTS

The HER2DX® Software incorporates a series of quality control metrics that are systematically applied to each sample and control reference samples during processing. These metrics assess the software's performance to verify whether the outcomes align with anticipated values.

Following the successful evaluation of these quality control metrics, the HER2DX® software generates the subsequent results:

8.1. Relapse Risk score:

This score provides the patient's long-term risk of recurrence during follow-up at 10 years after (neo)adjuvant chemotherapy. Patients are classified as low or high risk.

Relapse risk score: Ranges from 1 to 99. Scores from 1 to 49 indicate a low risk of recurrence group, while scores from 50 to 99 signify a high risk of recurrence group.

- **Low risk of recurrence**: Low risk of recurrence following curative intent therapy. Consider less intensive systemic therapy regimens.
- **High risk of recurrence**: High risk of recurrence following curative intent therapy. Consider intensive systemic therapy regimens, likely including multi-agent chemotherapy.

8.2. pCR likelihood score:

This score provides the patient´s probability of achieving a pathological Complete Response (pCR) if treated with neoadjuvant therapy. Patients are classified as low, moderate or high chance of pCR.

pCR likelihood score: Ranges from 1 to 99. It is categorized into low chance of pCR (1 to 32), moderate chance of pCR (33 to 67), and high chance of pCR (68 to 99).

- **Low chance of pCR**: The likelihood of the tumor completely disappearing after undergoing neoadjuvant trastuzumab-based chemotherapy is low. The pCR rates are below 30%, regardless of the treatment administered. High sensitivity to endocrine therapy when hormone receptor positive.
- **Moderate chance of pCR**: The likelihood of the tumor completely disappearing after undergoing neoadjuvant trastuzumab-based chemotherapy is intermediate. In the context of dual HER2 blockade, pCR rates seem to be higher with multi-agent chemotherapy than with a single taxane.

- **High chance of pCR**: The likelihood of the tumor completely disappearing after undergoing neoadjuvant trastuzumab-based chemotherapy is high. In the context of dual HER2 blockade, a single taxane appears to achieve comparable pCR rates to multi-agent chemotherapy.

8.3. *ERBB2* mRNA score:

This score provides levels of *ERBB2* gene expression and this feature is linked to the tumor's responsiveness to anti-HER2 targeting in the absence of standard chemotherapy. Patients are classified as low, intermediate or high levels.

***ERBB2* mRNA score**: Ranges from 1 to 99. This score divided into low *ERBB2* mRNA expression (1 to 32), Intermediate *ERBB2* mRNA expression (33 to 50), and high *ERBB2* mRNA expression (51 to 99) groups.

- **Low *ERBB2* mRNA**: Low expression of *ERBB2* mRNA. These tumors show low sensitivity to anti-HER2 targeting in the absence of standard chemotherapy. Consider these results in the context of complex cases. This result cannot be used as a replacement for clinical HER2 status according to guidelines.
- **Intermediate *ERBB2* mRNA**: Intermediate expression of *ERBB2* mRNA. These tumors show low sensitivity to anti- HER2 targeting in the absence of standard chemotherapy. Consider these results in the context of complex cases. This result cannot be used as a replacement for clinical HER2 status according to guidelines.
- **High expression of *ERBB2* mRNA**. These tumors show high sensitivity to anti-HER2 targeting in the absence of standard chemotherapy. Consider these results in the context of complex cases. This result cannot be used as a replacement for clinical HER2 status according to guidelines.

9. PERFORMANCE PRINCIPLES

HER2DX® Software processes gene expression data (RCC files from the nCounter® platform) and clinical information (tumor size and nodal status).

The software performs preprocessing steps such as quality control, housekeeping normalization, and log2 transformation. It then calculates gene expression signatures, which are used in pre-trained machine learning models to generate three scores that reflect risk of relapse, likelihood of pCR, and *ERBB2* mRNA expression level.

9.1. Quality controls

The file obtained from the nCounter® Analyses System, with extension .RCC, is either automatically transmitted via email to the file storage AWS S3 which will then retrieve corresponding T and N information from the Web Ordering Application database, hosted on the same AWS account, corresponding T and N information, or the customer has the option to manually upload them together with the T and N on a .txt file format.

Controls

Controls included in the HER2DX assay ensure accuracy and reliability of the gene expression assay:

1. 6 External RNA Controls Consortium (ERCC) as **positive controls**. Their concentrations range linearly from 128 fM to 0.125 fM and are referred to as POS_A to POS_F, respectively. These positive controls are typically used to measure the efficiency of the hybridization reaction, and their stepwise concentrations are also useful in checking the assay's linearity performance.
2. 6 External RNA Controls Consortium (ERCC) as **negative controls**; used to set background thresholds.

The ERCC spike-in controls are provided as part of the NanoString nCounter assay reagents and Tagset system used for gene expression analysis. Two synthetic RNA reference samples (RNA-SINT) are added in every run to monitor the efficiency and consistency of the test process. Commercially produced **synthetic RNA samples** are synthesized according to a customized design that includes the 27 HER2DX genes and 5 endogenous controls (housekeeping genes).

These RNA-SINT are assay-specific quality control materials used within the HER2DX analytical workflow. They are not part of the standard nCounter Analysis System procedure and are included in each HER2DX run to verify assay performance before interpretation by the HER2DX® Software.

The RNA-SINT reference samples are prepared and provided by the manufacturer and are included in each HER2DX assay run as reproducibility controls within the HER2DX workflow.

Assay Quality

The HER2DX® Software evaluates results from the exogenous (positive controls and negative controls), endogenous controls (housekeepers), and synthetic RNA samples to evaluate assay quality and sample quality of each run.

- Background is estimated from exogenous negative controls by the mean of the count. All genes in the synthetic RNA samples must exceed the background estimate for evaluation to continue.
- Control variability is assessed by calculating the geometric mean of 27 HER2DX genes for each synthetic RNA, then averaging these values and calculating the ratio. This ratio must fall within the range of 0.2-5, ensuring consistency and representativeness.
- Linearity of quantification is evaluated by regressing abundance estimates from the exogenous positive controls against their expected concentration. ERCC positive control A count exceeding 500 and slope exceeding 0.95 indicates a strong linear relationship and certifies the technical performance of the assay.
    - ERROR: The reference samples (synthetic RNA) exhibited inconsistent reproducibility or detection in this run.
      This error affects the reference samples, meaning the error will appear in all samples if this QC fails.

RNA sample quality

RNA sample quality is assessed using endogenous positive controls (housekeepers) to ensure RNA abundance estimates of the sample are adequate for model evaluation. Empirical evaluation from Real-World Evidence (RWE) cohorts (1-10) evaluated a number of estimators for RNA sample quality. Analysis revealed a pronounced decrease in effect size for predicting both pCR and Survival as the endogenous control RNA abundance approached background. We integrate this statistic as a sample QC measure to mitigate bias in reporting.

- Sample RNA abundance is estimated from the mean of the endogenous controls and compared to the background estimate. The ratio of sample RNA abundance to background is required to exceed 2.86 or a process error terminates further reporting.
    - ERROR: The expression levels of the housekeeping genes are nearly indistinguishable from background noise values.

Abundance estimates may fall below background due to low abundance of the target in the sample, or due to a technical failure of the target. Performance of the assay is evaluated under both scenarios to evaluate stability of the classification and the score.

- Comparison of diagnostic scores (relapse risk score and pCR likelihood score) and classification is performed under two alternative scenarios for low abundance targets. Samples are rejected if the score changes more than 10 points and if the diagnostic classification changes.

    - ERROR: Numerous critical genes were undetectable, and the application of gene weight-based corrections for pCR and Risk Scores yielded highly inconsistent results.

- Linearity of quantification is evaluated by regressing abundance estimates from the exogenous positive controls against their expected concentration. count exceeding 500 and slope exceeding 0.95 indicates a strong linear relationship and certifies the technical performance of the assay.

    - ERROR: Spiked-in positive controls (ERCC) were detected; However, their linearity did not meet the expected standards ($R2 < 0.95$).

The samples that do not pass these QCs are removed. After that we processed with the normalization of the raw data using 5 housekeeping genes and log base 2 transformed.

9.2. Score and gene signatures calculation

The HER2DX® Software uses pre-trained statistical models to generate the reported scores. These models are fixed and remain unchanged during use, and no adaptive learning or iterative processing is performed at runtime.

For the scores calculation, the first step is to calculate the 4 gene signatures by taking the mean of the genes that comprise each signature. Each score is compose for some of these signatures as explained below:

9.2.1. HER2DX® Relapse risk score (for the prognosis of patients with early-stage HER2+ breast cancer):

The relapse risk score is derived from a penalized Cox proportional hazards model that was trained on the SHORT-HER dataset (n=434) during development and remains locked for all versions of the software, with fixed model coefficients.[1]
The score is based on the following features:

- **Immune signature (IGG)** (14 genes): *CD27, CD79A, HLA-C, IGJ, IGKC, IGL, IGLV3-25, IL2RG, CXCL8, LAX1, NTN3, PIM2, POU2AF1* and/or *TNFRSF17*.
- **Tumor cell proliferation signature** (PROLIF) (4 genes): *EXO1, ASPM, NEK2* and/or *KIF23*.
- **Luminal differentiation signature** (LUM) (5 genes): *BCL2, DNAJC12, AGR3, AFF3 and/or ESR1*.
- **Tumor size (T stage):** T1 vs. T2–T4
- **Nodal status (N stage):** N0 vs. N1 vs. N2–3

Each variable contributes to the score through a fixed coefficient, and the total score is scaled between 1 and 99. The score is interpreted categorically as follows:

- 1–49: Low risk of relapse
- 50–99: High risk of relapse

9.2.2. HER2DX® pCR likelihood score (for the prediction of response to anti-HER2 therapies):

The pCR likelihood score is derived from a fixed penalized logistic regression model that was trained in a cohort of pre-treated tumours from 116 patients during development and remains locked for all versions of the software, with fixed model coefficients..[1] The model uses the following features:

- **Immune signature (IGG)** (14 genes): *CD27, CD79A, HLA-C, IGJ, IGKC, IGL, IGLV3-25, IL2RG, CXCL8, LAX1, NTN3, PIM2, POU2AF1*, and/or *TNFRSF17*.
- **Tumor cell proliferation signature** (PROLIF) (4 genes): *EXO1, ASPM, NEK2*, and/or *KIF23*.
- **Luminal differentiation signature** (LUM) (5 genes): *BCL2, DNAJC12, AGR3, AFF3*, and/or *ESR1.*
- **HER2 amplicon signature** (HER2) (4 genes): ERBB2, GRB7, STARD3, and/or TCAP.
- **Tumor size** (T stage): T1 vs. T2–T4
- **Nodal status** (N stage): N0 vs. N1–3

Each variable contributes to the score through a fixed coefficient, and the total score is scaled between 1 and 99. The score is scaled from 1 to 99 and interpreted as:

- **1–32:** Low probability of pCR
- **33–67:** Intermediate probability of pCR
- **68–99:** High probability of pCR

9.2.3. HER2DX® *ERBB2* mRNA score (for the prediction of the tumor's responsiveness to anti-HER2 targeting in the absence of standard chemotherapy):

The *ERBB2* mRNA score provide levels of *ERBB2* gene expression and this feature is linked to the tumor's responsiveness to anti-HER2 targeting in the absence of standard chemotherapy patients are classified into low, medium and high levels. To build an *ERBB2* mRNA expression assay that predicts clinical HER2 status, a cohort of 637 patients with primary invasive breast cancer and known HER2 status according to the ASCO/ CAP guidelinesClick or tap here to enter text.was evaluated using the HER2DX assay and used as the training dataset.[1]

The result is expressed as a continuous score (1 to 99) and interpreted categorically as:

- **1–32:** Low *ERBB2* mRNA expression
- **33-50:** Intermediate *ERBB2* mRNA expression
- **51-99:** High *ERBB2* mRNA expression

Once the individual patient's three scores are calculated, they are extrapolated by referencing databases that store the outcomes collected during the test's development phase. These databases serve as the foundation for the algorithm, which relies on them in the final step to compute the three numerical scores, presented as percentiles (ranging from 1 to 99 and categorized into low, medium (for pCR likelihood and *ERBB2* mRNA scores), or high levels.

9.3. Interpretation methodology

The HER2DX® Software algorithm utilizes a scoring methodology to simplify the interpretation of the scores results. The scoring levels are outlined as follows:

HER2DX® Relapse risk score: ranges from 1 to 99. Scores from 1 to 49 indicate a low risk of recurrence group, while scores from 50 to 99 signify a high risk of recurrence group. The high risk group is identified with a CMYK color code of C 100 - M 80 - Y 6 - K 32, or in RGB as R 0 - G 45 - B 114, and the low risk group indicated by CMYK: C 88 - M 8 - Y 0 - K 0, or RGB R0-G163-B224.

HER2DX® pCR likelihood score: ranges from 1 to 99. It is categorized into low probability of pCR (score from 1 to 32), medium probability of pCR (33 to 67), and high probability of

pCR (68 to 99). The high probability group is identified with a CMYK color code of C 100 - M 80 - Y 6 - K 32, or in RGB as R 0 - G 45 - B 114. The medium probability group is represented by CMYK: C100 - M47 - Y0 - K34, or in RGB R0-G89-B167, and the low probability group is indicated by CMYK: C 88 - M 8 - Y 0 - K 0, or RGB R0-G163-B224.

HER2DX® *ERBB2* mRNA score: ranges from 1 to 99. This score divided into low *ERBB2* mRNA expression (1 to 32), medium *ERBB2* mRNA expression (33 to 50), and high *ERBB2* mRNA expression (51 to 99) groups. The high expression group is identified with a CMYK color code of C 100 - M 80 - Y 6 - K 32, or in RGB as R 0 - G 45 - B 114. The medium expression group is represented by CMYK: C100 - M47 - Y0 - K34, or in RGB R0-G89-B167 and the low expression group indicated by CMYK: C 88 - M 8 - Y 0 - K 0, or RGB R0-G163-B224.

9.4. Reporting system

HER2DX® Software calculates 4 genomic signatures tracking immune activation, tumor cell proliferation, luminal differentiation, and expression of the HER2 amplicon (including *ERBB2*). The information obtained from the genomic signatures is combined with clinicopathological information (T and N) to build a relapse risk score, a pCR likelihood score, and an *ERBB2* mRNA score. These data are displayed in the format of a five-page PDF document, with a specific summary of the results on the first page.

Additionally, there is a general explanation of the IVD MDSW, followed by three pages covering the explanation and validation studies for the three reported scores:

- **HER2DX® Relapse risk score** indicates the long-term risk of recurrence during follow-up after (neo)adjuvant trastuzumab based chemotherapy.

- **HER2DX® pCR likelihood score** indicates the probability of achieving a pCR following neoadjuvant trastuzumab-based chemotherapy.

- **HER2DX® *ERBB2* mRNA score** reflects the expression levels of *ERBB2* in HER2+ breast cancer, and this feature is linked to the tumor's responsiveness to anti-HER2 targeting in the absence of standard chemotherapy.

The report obtained from HER2DX® Software provides information regarding the observed rates of recurrence and pCR for the groups, and the association between the HER2DX® *ERBB2* mRNA score and drug therapy response within each validation study. In addition to the HER2DX® Software scores, the report generated by HER2DX® Software includes details on the clinical significance of the results and the validation studies that support them.

At the header of the report, it is provided: the software version, the date, the sample identifier, REVEAL GENOMICS, S.L., own identifier, and the patient's T and N values.

10. LIMITATIONS OF THE PROCEDURES

- HER2DX® Relapse risk score was developed from primary tumor specimens and staging was based on surgical pathology reports. This approach is different from the neoadjuvant setting where a core biopsy is the only available tissue and staging is based on imaging. Despite this limitation, HER2DX® Software performed well in core biopsies in the validation prognostic dataset, where all patients received neoadjuvant therapy and clinical staging was used instead of pathology reports.

- HER2DX® Relapse risk score development is based on Short-HER cohort that was powered for a particular primary endpoint, which was to compare DFS between two arms distinguished by the duration of trastuzumab (i.e., 9 weeks versus 1 year). Due to the low sample size and number of events in each arm, this cohort was not used to evaluate the value of HER2DX® Software to predict the benefit from adjuvant trastuzumab according to its duration.

- Separately, several patient cohorts used for the clinical validation have a limited sample size.

- Some cohorts collected from trials do not follow standard treatment strategies, like the administration of letrozole as neoadjuvant treatment.

- The IVD MDSW HER2DX® Software cannot be considered in any case as a substitute for the physician's activity or for the required medical surveillance in any treatment to the patient. The healthcare professional must be a licensed physician specialist (mainly medical oncologists but also gynecologists, surgeons, radiologists, among others specialist responsible for prescribing treatment for patients with breast cancer) and they have the ultimate responsibility for all therapeutic decisions based on the patient diagnosis, medical history and family medical history, the indications of the treatment, concomitant medications, other individual characteristics of the patient, and a comprehensive interpretation of the report.

- The interpretation of HER2DX® Software (including the Relapse risk score, pCR likelihood score and *ERBB2* mRNA score) should be evaluated within the context of other clinicopathological factors and the patient's medical history.
- There is currently no evidence to support the use of HER2DX in patients under 18 years old, as all development and validation studies were conducted in adult populations.
- Only FFPE samples are validated. Frozen tissue is not supported for clinical use.
- Currently, only data derived from the nCounter® platform are validated. The use of alternative platforms is under investigation but not supported for clinical use.
- The performance of HER2DX® Software depends on the quality of the input data derived from FFPE tumor samples. Therefore, pre-analytical acceptance criteria for both tissue and RNA must be fulfilled to ensure a valid result. Tissue samples

must meet minimum requirements, including tumor surface area ≥ 4 mm^2 and tumor cellularity ≥ 10%. A minimum number of sections is required depending on tumor size (e.g., one section if ≥100 mm^2, up to three sections for smaller tumor areas). In addition, RNA samples must meet minimum quality criteria, including RNA purity (OD260/280) between 1.7 and 2.5 and RNA concentration ≥ 12.5 ng/µL. Failure to meet these criteria may result in insufficient or unreliable gene expression data and, consequently, no reportable HER2DX® result.

- Validation datasets included only samples obtained prior to systemic treatment, in order to avoid confounding transcriptomic signatures. Therefore, HER2DX® Software should not be applied to post-treatment samples.
- Only 12 samples can be run at a time, which includes 10 samples and 2 reference controls.

11. ANALYTICAL AND CLINICAL PERFORMANCE CHARACTERISTICS

11.1. Analytical Validation Summary

HER2DX® Software has been analytically validated with results published in a peer-reviewed article (Marín-Aguilera et al., ESMO Open, 2024)[4]. This validation included a comprehensive set of experiments to assess the precision, reproducibility, robustness, and cross-platform consistency of the HER2DX® algorithm and test procedure.

The validation demonstrated the following analytical performance characteristics:

11.1.1. Precision and Reproducibility

- Intra-laboratory precision: Testing 10 FFPE tumor samples showed a maximum standard error of 0.94 units on the 1–99 HER2DX score scale, with 99.4% agreement between replicates.
- Inter-laboratory reproducibility: 29 FFPE samples and 20 purified RNA samples were tested across three laboratories. All HER2DX scores showed Pearson correlation >0.97 and a score difference ≤5 units in 94.8–98.2% of cases. This confirms high reproducibility across labs and instruments.

11.1.2. Robustness

- The HER2DX assay maintained consistent results under variations in:
  - RNA input quantities (100–500 ng),
  - Instrument platforms,
  - Reagent lots and TagSet thaw/freeze cycles,
  - RNA extraction kits (Roche vs. Promega),
  - Tumor cellularity as low as 10%.

- Concordance of score classification across these variations was >91% for all scores.

11.1.3. Intratumor and Intra-patient Variability

- Evaluation of 208 biopsies from 104 tumors showed <4.0% variability in HER2DX scores across different regions within the same tumor.
- Paired samples taken at different time points from the same patients (pre- and post-surgery) showed consistent HER2DX results, with standard error <6.1 units.

11.1.4. Platform Concordance

- HER2DX scores were compared across nCounter and RNAseq (Illumina) platforms using 30 RNA samples:
  - Score group concordance was 96.7% for all three HER2DX outputs.
  - Pearson correlation for gene expression between platforms: mean r = 0.89.

11.2. Clinical performance

To evaluate the clinical performance of HER2DX® Software, several validation studies were conducted:

11.2.1. Relapse risk score:

The prognostic value of HER2DX® has been independently validated in 739 patients in two different clinical scenarios:

Clinically low-risk disease: 284 patients (i.e., 85% stage 1) treated in the APT clinical trial with adjuvant trastuzumab and paclitaxel alone. [5]HER2DX® exhibited a significant association with the relapse-free interval (RFI) when assessed both as a continuous variable and using the pre-established cutoff (i.e., <50 versus ≥50), as well as an exploratory cutoff (i.e., <32 versus ≥32). Similar results were obtained in 187 patients treated in the ATEMPT clinical trial. [6]

Clinically high-risk disease: 268 patients (i.e., 78% cT2-4, 45% cN-positive) treated with (neo)adjuvant trastuzumab-based therapy (i.e., 96% received chemotherapy, 56% dual HER2 blockade, and 14% adjuvant T-DM1 in patients not achieving a pCR). HER2DX® was significantly associated with event-free survival (EFS) and overall survival[7] when assessed both as a continuous variable and using the pre-established cutoff (i.e., <50 versus ≥50), and this association remained regardless of pCR status.[7] The main results of this cohort are shown in the plot below.

Clinical significance:
Low-risk: The low-risk category identified by HER2DX® is associated with a low long-term risk of recurrence following (neo)adjuvant trastuzumab-based chemotherapy. Within this context, HER2DX® offers an opportunity to consider a less intensive systemic therapy approach, which may encompass various alternatives, such as the utilization of a single taxane instead of multi-agent chemotherapy.

High-risk: The high-risk category identified by HER2DX® is associated with a significantly elevated long-term risk of recurrence following (neo)adjuvant trastuzumab-based chemotherapy. Within this context, HER2DX® encourages thoughtful consideration of adjuvant systemic therapy, likely involving multi-agent chemotherapy. Furthermore, the high-risk category can help guide the choice between neoadjuvant therapy and primary surgery.

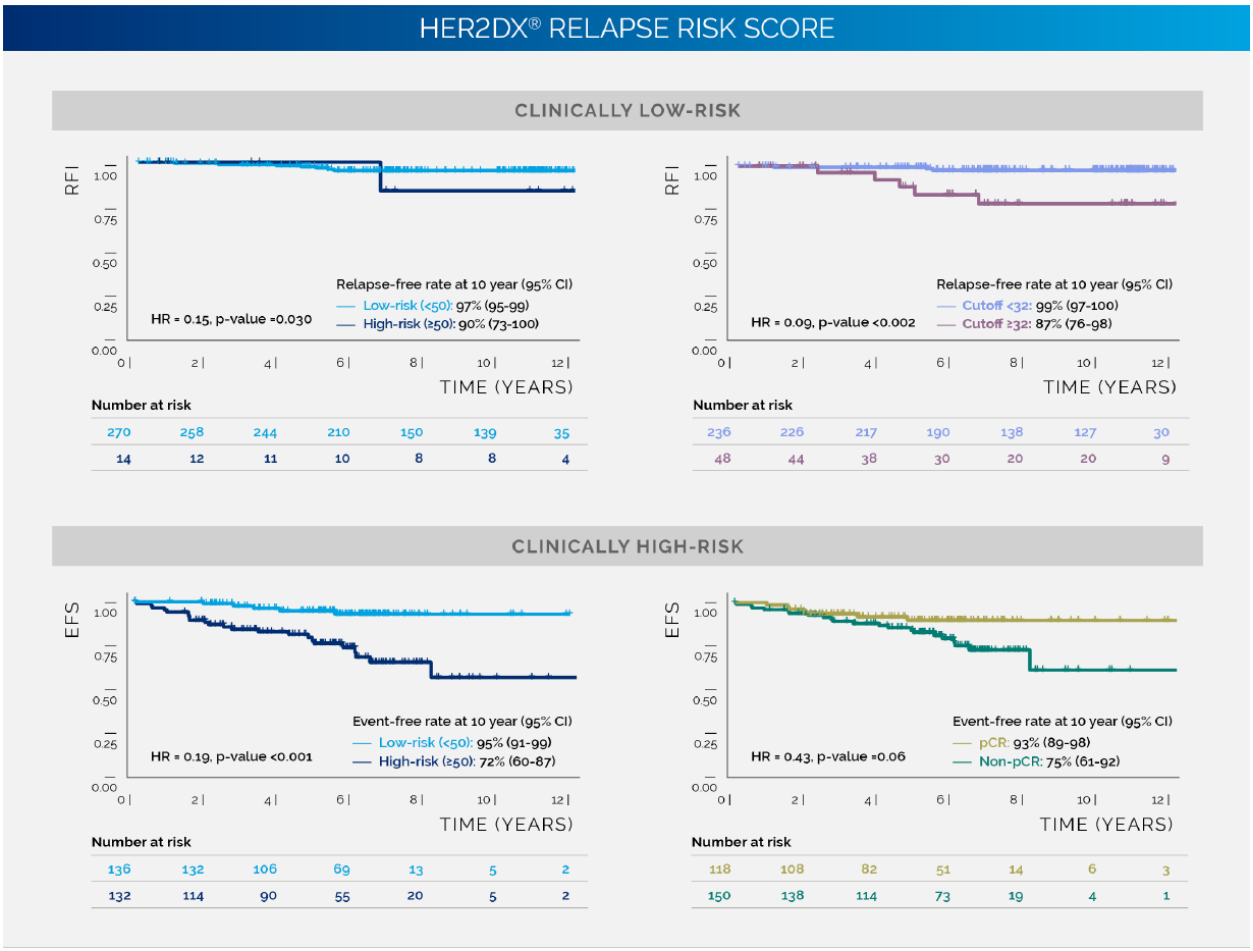


Figure 51 Relapse Risk Score

11.2.2. pCR likelihood score:

The association of the HER2DX® pCR likelihood score with pCR following trastuzumab-based chemotherapy has been evaluated retrospectively in 765 patients with HER2+ breast cancer across 7 independent studies[1,7–9]. Overall, the HER2DX® pCR likelihood score (as a continuous variable and as group categories) is associated with pCR regardless of hormone receptor status and treatment regimen. A patient-level meta-analysis of HER2DX® has linked the HER2DX® pCR likelihood score with neoadjuvant response to dual HER2 blockade and multi-agent chemotherapy.[7]

The association of the HER2DX® pCR likelihood score with endocrine therapy sensitivity has been evaluated in 55 patients with hormone receptor-positive/HER2+ breast cancer treated with 2 weeks of letrozole monotherapy.[10] The rate of endocrine sensitive disease (i.e., Ki-67 relative reduction >20% from baseline) in low, medium, and high HER2DX® pCR score groups was 89.7%, 65.0% and 16.7%, respectively.

Clinical significance

pCR-low: The pCR rates are below 30%, regardless of the treatment administered. In this group, dual HER2 blockade does not seem to increase the pCR rates over trastuzumab, and multi-agent chemotherapy does not seem to increase the pCR rates over a single taxane. Conversely, this group is highly endocrine sensitive if hormone receptor-positive.

pCR-medium: The likelihood of the tumor completely disappearing after undergoing neoadjuvant trastuzumab-based chemotherapy is intermediate. In the context of dual HER2 blockade, pCR rates seem to be higher with multi-agent chemotherapy than with a single taxane.

pCR-high: The likelihood of the tumor completely disappearing after undergoing neoadjuvant trastuzumab-based chemotherapy is high. The highest pCR rates (i.e., >80%) are obtained if dual HER2 blockade is administered with chemotherapy. In the context of dual HER2 blockade, a single taxane appears to achieve comparable pCR rates to multiagent chemotherapy.

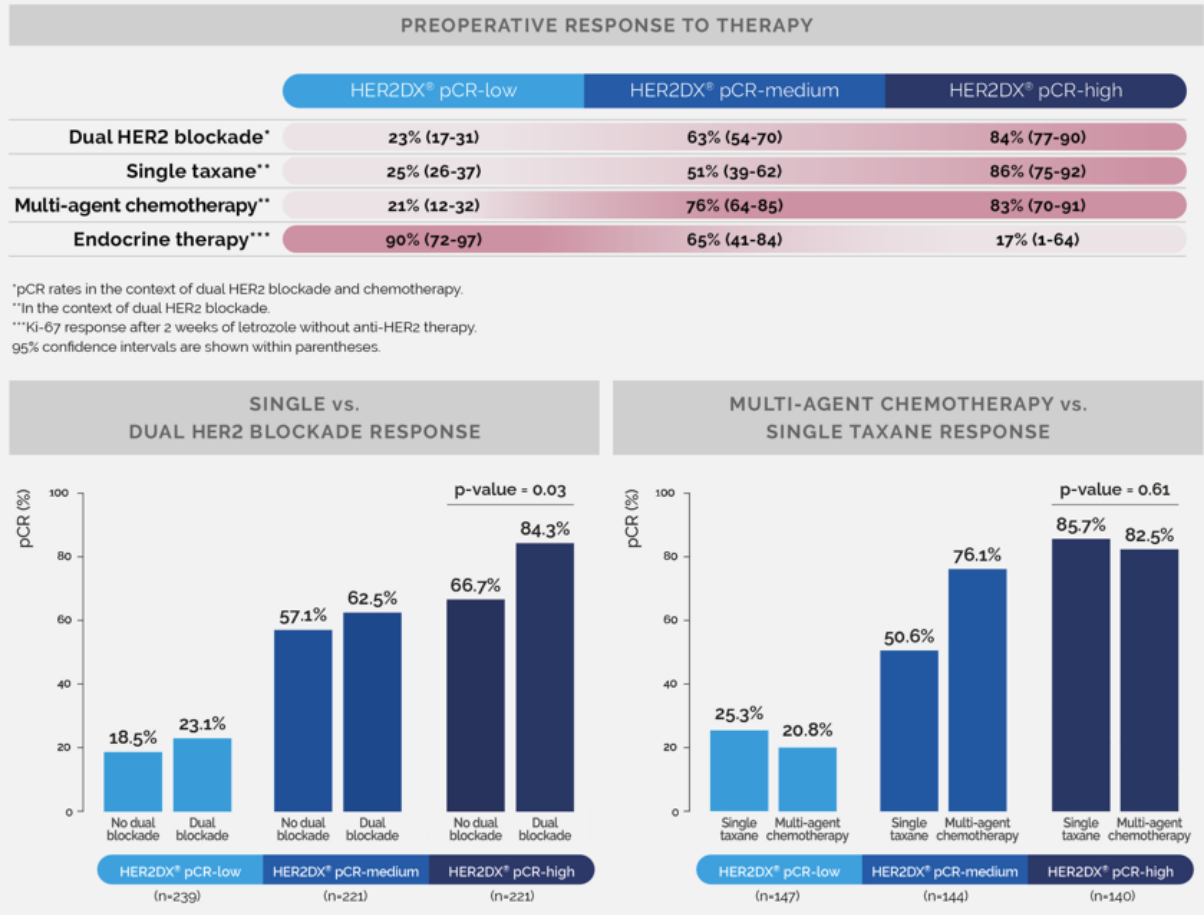


Figure 522 pCR likelihood score

11.2.3. *ERBB2* mRNA score:

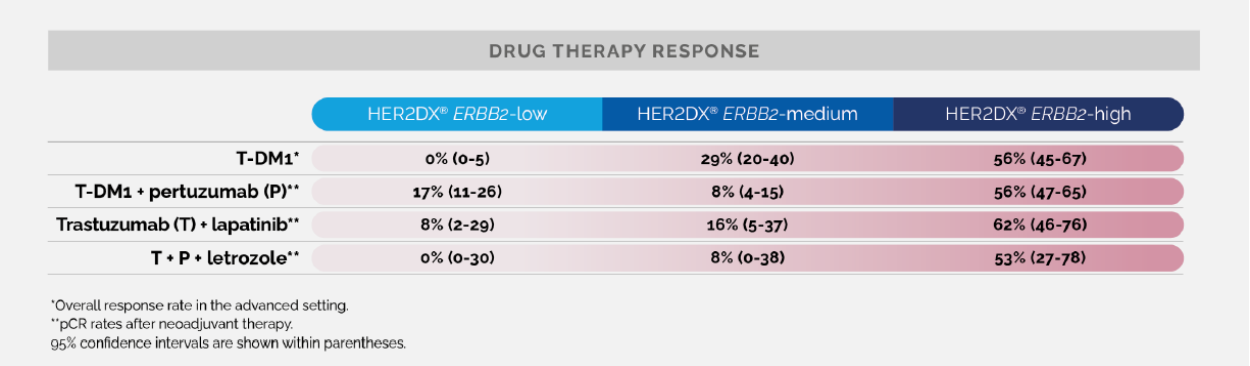
To validate the findings, we applied the optimal *ERBB2* cutoff to an independent cohort consisting of 353 cases of HER2-negative and HER2+ breast cancers. The Receiver Operating Characteristic Area Under the Curve (ROC AUC) for *ERBB2* expression in predicting clinical HER2 status was 0.96, demonstrating a sensitivity of 84%, and a specificity of 100%. None of the HER2-negative cases were categorized as *ERBB2*-medium/high, while 16.4% of HER2+ cases were classified as *ERBB2*-low.[1]

HER2DX® *ERBB2* score and drug therapy response: The association between the HER2DX® *ERBB2* score and drug therapy response has been investigated in various studies.[1,10,11] In the PAMELA phase II trial (n=91), patients treated with 16 weeks of trastuzumab and lapatinib (with additional endocrine therapy for hormone receptor-positive cases) exhibited of 8%, 16%, and 62% for HER2DX® *ERBB2* low, medium, and high groups, respectively.[1] In the PER-ELISA phase II trial (n=40), among hormone receptor-positive/HER2+ patients responding to letrozole (i.e., drop in Ki-67 at week 2), those treated with 18 weeks of trastuzumab and pertuzumab had pCR rates of 0%, 8%, and 53% for HER2DX® *ERBB2* low, medium, and high groups, respectively.[10] Within the NCT02326974 phase II trial (n=121), patients receiving 18 weeks of T-DM1 and pertuzumab

showed pCR rates of 17%, 8%, and 56% for HER2DX® *ERBB2* low, medium, and high groups, respectively.[1] Lastly, in 87 patients with advanced HER2+ breast cancer treated with T-DM1 monotherapy,[12] the overall response rates were 0%, 29%, and 56% for HER2DX® *ERBB2* low, medium, and high groups, respectively.

Clinical significance

***ERBB2*-low**: Identifies HER2+ tumors with low expression of *ERBB2*. The proportion of *ERBB2*-low in HER2+ breast cancer is ~10- 20%. Clinically, the tumor shows low sensitivity to anti-HER2 targeting in the absence of standard chemotherapy.
***ERBB2*-medium**: Identifies HER2+ tumors with intermediate expression of *ERBB2*. The proportion of *ERBB2*-medium in HER2+ breast cancer is ~10-20%. Clinically, the tumor shows low sensitivity to anti-HER2 targeting in the absence of Standard chemotherapy.
***ERBB2*-high**: Identifies HER2+ tumors with high expression of *ERBB2*. The proportion of *ERBB2*-high in HER2+ breast cancer is ~60- 70%. Clinically, the tumor shows high sensitivity to anti-HER2 targeting in the absence of standard chemotherapy.



DRUG THERAPY RESPONSE

	HER2DX® *ERBB2*-low	HER2DX® *ERBB2*-medium	HER2DX® *ERBB2*-high
T-DM1*	0% (0-5)	29% (20-40)	56% (45-67)
T-DM1 + pertuzumab (P)**	17% (11-26)	8% (4-15)	56% (47-65)
Trastuzumab (T) + lapatinib**	8% (2-29)	16% (5-37)	62% (46-76)
T + P + letrozole**	0% (0-30)	8% (0-38)	53% (27-78)

*Overall response rate in the advanced setting.
**pCR rates after neoadjuvant therapy.
95% confidence intervals are shown within parentheses.

Figure 533 ERBB2 mRNA score

11.3. Summary of Diagnostic and Predictive Performance Metrics

This section summarizes the diagnostic and predictive performance of the HER2DX® scores, including the ERBB2 mRNA score, the pCR likelihood score, and the relapse risk score. The metrics shown include sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), area under the receiver operating characteristic curve (AUC), and accuracy when applicable. All values are derived from independent validation studies and meta-analyses, as cited.

11.3.1. HER2DX® Relapse Risk Score – Prognostic Performance

The relapse risk score is a prognostic model designed to estimate long-term recurrence risk. As a time-to-event outcome, binary diagnostic metrics (sensitivity, specificity, etc.) are not applicable. Performance is assessed using C-index values:

Outcome	Cut-off (percentile)	C-index	95% CI
EFS	50th percentile	0.75	0.43–0.92
OS	50th percentile	0.81	0.58–0.93

11.3.2. HER2DX® pCR Likelihood Score – Predictive Performance

The pCR score was validated using pathological complete response (pCR) as the clinical endpoint. Binary classification metrics were derived using a predefined threshold:

Outcome	Cut-off (predefined)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	AUC	95% CI	Accuracy (%)
pCR	Predefined threshold	78.9	75.1	74.1	80.8	0.75	0.72–0.79	76.9

11.3.3. HER2DX® *ERBB2* mRNA Score – Diagnostic Performance

The *ERBB2* mRNA score was developed to approximate HER2 status based on gene expression and has been validated using IHC/ISH-based HER2 classification as reference. Results from two independent validation datasets are presented below:

Dataset / Study	Cut-off (percentile)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	AUC	95% CI	Accuracy (%)
Prat et al., EBioMedicine 2022[1]	33rd percentile	84.0	100.0	100.0	65.9	0.96	0.93–0.99	**87.8**

ESMO Breast Cancer 2023 (Poster)[11]	33rd percentile	90.1	99.2	99.9	52.9	0.98	0.97–0.99	**92.4**

Note: Accuracy was calculated post-hoc based on reported sensitivity, specificity, and class prevalence

12. ADDITIONAL RESOURCES

In order to correctly visualize HER2DX® IFU in electronic format as well as a download a pdf copy of results reports, it is necessary to have a PDF Viewer (such as Adobe Acrobat Reader) installed.

This IFU will be delivered in PDF format by default. However, the user can request its delivery in paper form at any time and no additional cost by phone at +34 626938257 or by e-mail to HER2DX@reveal-genomics.com. Consequently, the requested IFU will be provided in paper form at the latest within 7 calendar days of receiving a request from the user.

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