



RheumaKit is a solution for early differential diagnostic of patients with undifferentiated arthritis.

User instructions

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1. Intended purpose

RheumaKit¹ is a first step in the introduction of biomarker-based in vitro diagnostic (IVD) tools in rheumatology to be used by rheumatologists and orthopaedic surgeons (both in hospitals or private practices). RheumaKit is a solution for the early differential diagnosis of patients suffering from undifferentiated arthritis. Undifferentiated arthritis (UA) is a subclass of early arthritis (EA) and is a condition in which synovial inflammation is present in one or more joints, and no definite diagnosis can be made using presently available diagnostic criteria. RheumaKit is based on a transcriptomic signature and three clinical/biological factors.

The aim of RheumaKit is to help obtain an early differential diagnosis for patients with undifferentiated arthritis.

It is of primary importance to detect the correct underlying disease in UA patients as early as possible for two reasons. First, the earlier the disease is addressed, the more likely its progression and irreversible damages to the joints will be limited. Second, a mistake in differential diagnosis leads in most cases to inappropriate treatments, resulting in useless expenses and probable serious side effects, unjustified.

RheumaKit will provide a differential diagnosis between rheumatoid arthritis and other types of arthritis, such as osteoarthritis or seronegative spondyloarthropathy. Septic arthritis is not part of the differential diagnosis proposed by the kit, and this condition needs to be carefully considered by the caring physician before any further processing.

The solution is only validated for adult patients.

2. Intended users

RheumaKit is an In Vitro Diagnostic (IVD) tool² to be used by health care professionals (typically rheumatologists or orthopaedic surgeons).

3. Minimal requirements

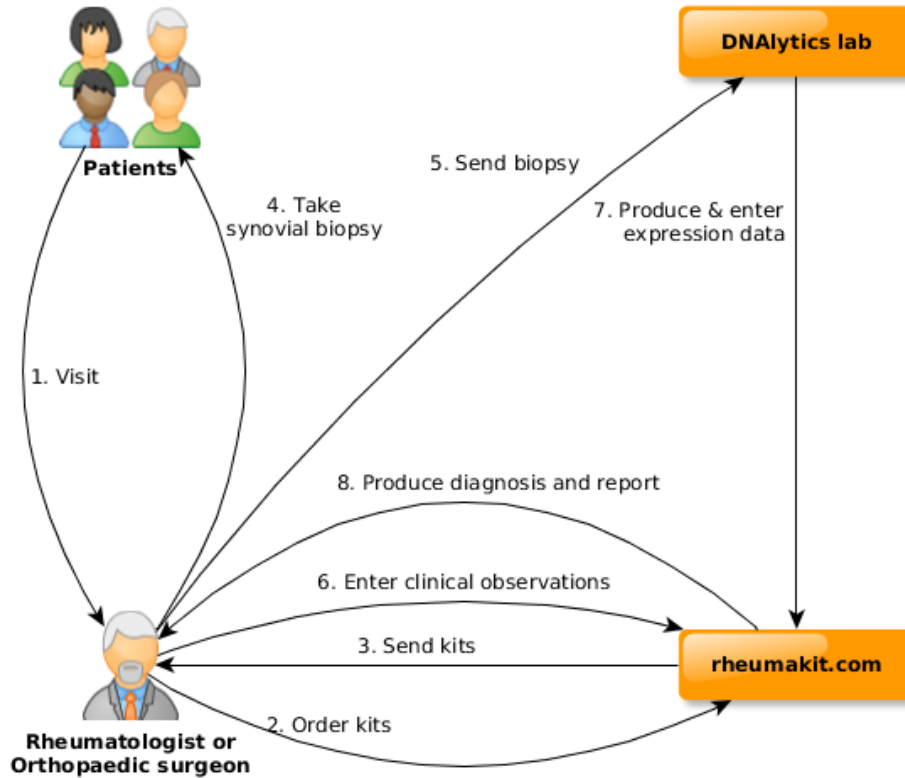
From a functional point of view, the correct use of RheumaKit platform requires:

- A device (laptop, desktop, tablet, smartphone) connected to the Internet
- A web browser installed on this device, with Javascript enabled. The following browsers are compatible with RheumaKit:
 - Mozilla Firefox version 2 or later
 - Microsoft Internet Explorer version 9 or later
 - Apple Safari version 4 or later
 - Google Chrome version 1 or later
 - Opera version 1 or later
 - All recent mobile browsers (2014 and later)

¹Helleputte et al. RheumaKit, a new early diagnostic tool for patients with arthritis. Winner of the Inspiring Young Scientist Award, Knowledge for Growth, Ghent, Belgium, 2013.

²Falling into the 98/79/EC IVD Directive of the European Parliament, categorized as a Non-Annex II IVD tool for professional use.

4. Overview of the standard flow for RheumaKit



This section presents an overview of the overall RheumaKit process. You will find more details about each step in the various below sections.

- 1- The patient visits his/her doctor who makes a diagnosis of undifferentiated arthritis
- 2- The doctor or his agent (like the hospital pharmacist) orders a biopsy collection and transportation kit from the RheumaKit website
- 3- The doctor receives the kit(s)
- 4- The doctor performs a synovial biopsy
- 5- The doctor sends the biopsy to a laboratory certified by RheumaKit
- 6- The doctor encodes the three required clinical observations on the RheumaKit website
- 7- The laboratory produces the gene expression data and the doctor is notified when they are available
- 8- A differential diagnostic between Rheumatoid Arthritis and other type of arthritis such as Seronegative Spondyloarthropathy or Osteoarthritis is available on the RheumaKit website

5. Manage your account

5.1 Register

Create your account by clicking on the "Signup" button and provide an email address and a password. A confirmation email will be sent to you. It contains a link to this document, that you are invited to read. Click on the link inside the email and you will be redirected to the RheumaKit website.

Once the account creation is confirmed, complete your account information and your shipping and billing address. You will be able to modify those items later on.

Note that RheumaKit is not intended to be used directly by patients (see section "Intended users").

5.2 Login

If you never created an account, read first section 5 "Register".

If you have already created an account, open the «www.rheumakit.com» website and click on the «login» button. Then, enter your Email and password.

If you forgot your password, click on «login», then enter your Email address and click on 'I forgot my password'. An email with a link to change your password will be sent to your Email address.

If you encounter any issue with the login contact us at customer-support@rheumakit.com.

5.3 Order transportation kits

You or your agent (hospital pharmacist for instance) can order sample collection and transportation kits by going to the menu "Kits" and then by clicking on the "Order new kits" button. You can also request kits directly from the patients menu by clicking on the "Order new kits" button. Choose the number of kits you would like to receive. The kits will be sent to the specified address.

5.4 Invoicing and payment

Once you order one or several kit(s) on www.Rheumakit.com, an invoice is generated, and you get charged for the full service. You can choose between addressing the invoice to your institution or directly to the patient. It is your responsibility to respect your country legislation in this regard (some countries might not allow direct patient invoicing). In both cases, you can also choose your payment method: by wire transfer or credit card (we use Stripe, one of the most efficient and secured online payment services worldwide).

Two types of account exist: regular account and RheumaKit Partner status account.

With a regular account, the reception of the sample collection and transportation kit is

conditional to the reception of the payment. By default, any new user will be considered as a regular user and will follow the contractual relationship specified in the RheumaKit terms and conditions and standard invoices should be paid within the payment terms.

With a RheumaKit Partner status account, the reception of the sample collection and transportation kit is not conditional to the reception of the payment. This means that your patients can be diagnosed faster. With a partner account, you can also order larger batches of transportation kits than with a regular account.

From a regular account, you can upgrade to RheumaKit Partner status. To benefit from that status, you or (preferably) your institution has to sign a general convention with RheumaKit. RheumaKit remains free to accept, reject and revoke applications to this status.

5.5 View existing patients

Click on the button "Patients", you view a list of your current patients.

5.6 Modify the account information

If you wish to modify your account information, your address or password, click on your name in the top right menu, then on 'settings' in the drop-down menu.

6. Initiate a RheumaKit diagnosis

6.1 Create patient file

Once the account creation is confirmed and once you are connected to the website, click on the button "Patients". Note that a demo patient is created automatically to test the RheumaKit functionalities.

Add patients to your account by clicking on the "Add a patient" button. Enter the patient first and last name, your internal ID number, the patient birth date, gender, language and any useful notes.

6.2 Assign kits to patients

If you have ordered the sample collection and transportation kits, they already will appear in your "Kit stock", regardless of the fact that the delivery already occurred or not. You can then assign any currently unassigned kit to your patients.. A kit corresponds to a biopsy, and, as a result, to the measurement of gene expressions.

If your agent (hospital pharmacy or any other third party) has ordered the sample collection and transportation kits, you can assign a kit to a patient by using the unique kit identification (ID)

number appearing on the box. You need to enter the kit ID number in the patient file.

When you assign a kit to a patient, you are asked for the three clinical observations. See section 12 "Provide clinical information" to find out how to answer the question in an appropriate manner. Those can also be provided later (see section 16 "Obtaining and interpreting the results").

You can assign several sample collection and transportation kit ID numbers to a single patient, for example if you want to perform a follow-up in time. Note however that the tool is dedicated to undifferentiated arthritis only.

6.3 Provide clinical observations

Two types of information is required to obtain a probable diagnosis for your patients via the RheumaKit application : clinical observations, and gene expressions. On one hand, the gene expressions are provided by the certified laboratory based on the biopsy that you will send. On the other hand, you need to provide three pieces of information about a patient for whom you want a diagnosis:

- Hand arthritis : Answer yes if your patient has one or more swollen metacarpo-phalangeal or inter-phalangeal joints. Answer no otherwise.
- Skin Psoriasis : Answer yes if your patient shows cutaneous psoriasis, either presently or in the past. You can also answer yes in the case a first-degree relative of the patient has/had psoriasis. Answer no otherwise.
- Rheumatoid factors : Answer yes if your laboratory test are above the upper value of normal (Waller-Rose, Latex or both). Answer no otherwise.

Later on, in case of error, you can still change the clinical /biological information you have previously provided by clicking on "Change my clinical observations" button.

6.4 Instructions about biopsy collection

The detailed instructions about the biopsy collection come with the sample collection and transportation kits.

6.5 Return biopsy for analysis

Transportation kits are validated for air transportation of biological samples (UN3373) and contain vials filled with a RNA-preserving solution to collect the biopsies. The return material is delivered with the kit, and the shipment is pre-paid. A phone call to the shipping company has to be made to get the kit (including the biopsy) picked up for analysis. The more detailed instructions about the biopsy shipment come with the transportation kits.

7. Obtaining and interpreting the results

7.1 Quality control

Once our laboratory receives your sample, a quality control is performed. If this control is successful, you will be notified by mail and the gene expression analysis will occur. If the quality control is negative, a probable cause of the failure is provided by mail when possible.

7.2 Diagnostic results

If the kit has been assigned to a specific patient and you have provided the 3 clinical observations, you will receive an email to which a pdf report will be attached, containing the diagnosis and pathways analysis already computed, as soon as the gene analysis has been performed.

Some information might be missing:

- If the kit is assigned to a patient but the clinical information is missing, you will receive a notification email when the gene analysis has been performed and you will need to fill in the missing clinical information in order to compute the diagnosis and the pathways analysis.
- If the kit is not assigned to a patient, the notification email will be sent to the person who has effectively purchased the kit (see section 11 "Assign kits to patients").

Once you have filled the missing information, you are ready for diagnosis computation. Go to your patient file (menu 'Patient', then click on the patient) on «www.rheumakit.com» and click on the "Compute diagnosis" button. Note that this button only appears once all required information has been provided. The diagnosis and pathways analysis will be computed within a few seconds only and be available online. A notification email is also sent to you when they are available.

Once generated, the results are available in your patient file on the platform. This patient file is composed of one or several blocks of information. Each block represents either a particular differential diagnostic computation or a pathways analysis.

The differential diagnosis block reports on the left the clinical and biological information entered by the healthcare professional. On the right, the main result is displayed. It consists of a color rectangle showing the most probable disease behind the undifferentiated arthritis of the patient. The diagnosis will either be Rheumatoid Arthritis or Not Rheumatoid Arthritis. In the latter case, a trend toward Osteoarthritis or Seronegative Spondyloarthritis is also provided. Note also that RheumaKit as most diagnostic tools is not a perfect predictor, although it significantly outperforms well-known diagnostic criteria like ACR/EULAR2010. Refer to Section 17 (Expected performances) for more details.

7.3 Information on Pathways

The pathways analysis block shows on the left a numerical summary of the level of activity of seven metabolic pathways. On the right, the same results are provided in a more graphical manner. The pathways considered are Chromatin Remodeling, T and B Cells Activation, Tumor Necrosis Factor (TNF), Type 1 Interferon, Extracellular Matrix, RAS-GTPase Activation, Hypervascularity and JAK-STAT. You can learn more about these pathways by reading the corresponding descriptions online. For each pathway, the given value is the average of the expression of the genes belonging to the pathway (after a preprocessing step making all genes comparable to each other). A linear scaling is then applied to this value so that on the reference cohort, the 5% percentile corresponds to a value of 5, and the 95% percentile corresponds to a value of 95. Finally, the values outside the [0,100] interval are reduced to this range (to the nearest boundary). In concrete terms, for example, a value of 50% for the TNF pathway means that the patient is located midway between the 5 and 95 percentiles of the reference cohort for that pathway.

The metabolic pathways analysis is provided to the clinician as a complementary information based on the transcriptomic analysis of a synovial biopsy. It should not be interpreted as a clear recommendation for treatment orientation nor any other form of patient management recommendation. The description of each pathway provided results from a review of the literature, not from RheumaKit developments and studies.

In each block, information is provided about the RheumaKit sample and transportation kit used for the patient for the corresponding analysis.

A PDF report can also be generated by clicking on "Download report" in your patient file.

7.4 Quick Access to Results

Without having to login to your rheumaKit account, you can view the diagnosis and pathways analysis for a specific kit ID by going to the "quick access" box on www.rheumakit.com and by entering the Kit ID number. However, in order to print out the pdf report, you will need to enter your login information.

8. Technical details and Expected Performances

The technology behind RheumaKit is a mathematical model combining a signature of about 100 gene expressions³ with three clinical observations. RheumaKit has been trained to discriminate between patients suffering from rheumatoid arthritis (RA), seronegative spondyloarthropathy (SNSA) or osteoarthritis (OA) at a very early stage. RheumaKit relies on Machine Learning and Cloud Computing.

³ Adapted from Lauwerys, B. and Van, D.E.B. and Houssiau, F. and Gutierrez-Roelens, I., Method for the determination and the classification of rheumatic conditions, 2008, WO Patent App. PCT/EP2008/052,532. <http://www.google.com/patents/WO2008104608A1?cl=en>

RheumaKit accuracy is above 90% for RA diagnosis, a better performance than any other diagnostic algorithm designed until now, including the ACR/EULAR 2010 criteria for the diagnosis of RA. This was measured during a clinical trial⁴ with a cohort of 31 patients with Undifferentiated Arthritis (UA) used as validation cohort. The mathematical model of RheumaKit was evaluated on those patients based on biopsies and clinical information collected while arthritis was still undifferentiated. After follow-up, a diagnosis could be established for all patients in the cohort. The average duration of follow-up was 10.9 months (standard deviation: 10.7 months).

Detailed performances, evaluated by cross-validation, are reported in the following table. A comparison with the ACR/EULAR2010 criteria on the same patients is provided.

Model	Sensitivity			Positive predictive value ⁵		
	RA	OA	SNSA	RA	OA	SNSA
RheumaKit	93.5 ± 16.3	73.5 ± 29.2	80.0 ± 26.5	84.9 ± 17.0	79.5 ± 22.7	100.0 ± 0.0
ACR/EULAR 2010 criteria	51.0 ± 33.1			72.3 ± 27.2		

A sensitivity of 93.5 for RA means that among all patients finally diagnosed as RA, 93.5% were predicted RA by RheumaKit. A predictive value of 84.9 for RA means that among all patients predicted RA by RheumaKit, 84.9% effectively developed later symptoms of RA. Similar explanations hold for the other diseases.

By comparison, the ACR/EULAR 2010 criteria have a sensitivity of 51% on the same patients. RheumaKit thus misses 7.5 times less RA patients than these criteria. The comparison of positive predictive values is also in favor of RheumaKit (84.9% vs 72.3%).

The repeatability of the metabolic pathways analysis was assessed by running experiments in duplicates on 21 samples. The average replication error on the pathways expression levels is 10.0%.

9. Contact Details

RheumaKit website offers several ways of communication:

- We keep our users informed through a dedicated mailing list. Each user is automatically added to this list.
- Contact us through the online form in the 'Contact us' page available on the website or send us an email to info@rheumakit.com
- If already registered, send an email to customer-support@rheumakit.com

⁴ See Bernard Lauwerys et al., Heterogeneity of synovial molecular patterns in patients with arthritis, PLoS ONE, 2015.

⁵ The reported positive predictive values (also called "clinical efficacy") assume that RA, OA and SNSA have equal prevalences in the population of interest.