

EDIT-B[®] Instruction for use

RNA blood test for differential diagnosis of bipolar disorder and unipolar depression

This document present the general instruction for use for EDIT-B[®] test. The detailed protocol to use EDIT-B[®] test is provided in the Handbook available on the EDIT-B[®] Platform.

EDIT-B[®] is not a self-diagnostic test.

EDIT-B[®] is not a companion diagnostic test.

EDIT-B[®] is solely intended to be used by qualified professionals.

<u>Identification of the IVD device</u>	<u>Legal manufactured</u>
Product name: EDIT-B[®] Product reference: 0100	ALCEDIAG Cap Gamma 1682 rue de la Valsiere 34790 GRABELS FRANCE Email: support.edit-b@alcediag-alcen.com Website: https://www.alcediag-alcen.com

EDIT-B[®] is an in vitro diagnostic medical device CE marked according to the european directive 98/79/CE.



The IFU is available in three languages (FR, EN and IT) in electronic format.

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1. Test description

Intended use

EDIT-B® is a qualitative In Vitro Medical Device (IVD) intended for the differential diagnosis between bipolar disorders and major depressive disorders (unipolar depression).

EDIT-B® combines RNA sequencing technology to measure RNA editing targets (from a panel of biomarkers using whole blood) and patient individual data (age, sex, treatment(s), consumptions or addiction [tobacco, alcohol])^[1].

EDIT-B®'s prescription is exclusively intended for physicians authorized to establish a diagnosis of psychiatric illnesses.

EDIT-B® is part of the diagnostic process for mood disorders, in addition to the usual diagnostic methods, such as the DSM-5 and ICD-11 criteria and clinical scales (MADRS, HDRS, BDI, etc.)^[2,3]. The results of the test should complement the clinical and behavioural arguments usually used by the physician to make its final diagnosis.

EDIT-B® is intended to be used on patients aged 18 years and older, male or female, with a current major depressive episode (moderate or severe) and being treated* for that depression at the moment of the test.

**According to ATC classification, five treatment classes are considered: Antiepileptics, Antipsychotics, Anxiolytics, Hypnotics/Sedatives and Antidepressants.*

Overview of the test

From a blood sample collected in a PAXgene® Blood RNA tube at usual collection points, the biological analyses are carried out by medical biology laboratories accredited for RNA sequencing and approved by the company ALCEDIAG.

Then a score calculation is carried out by a software developed by ALCEDIAG, which contains modules for quality control and an algorithm to determine the patient result. The score calculation algorithm is hosted on a SaaS (Software as a Service) type platform, accessible via the website <http://edit-b.alcediag-alcen.com/>. The platform offers dedicated access to medical biology laboratories (access authorization administered by the company ALCEDIAG). It complies with European security, GDPR (General Data Protection Regulation) and health data management standards. The analysis and algorithm calculation processes are patented.

2. Scientific and technical principles of the test

The biological component of the EDIT-B® test falls under a specific sub-category of molecular biology, called epigenetics. While genetics is the study of genes and heredity, epigenetics focuses on a complementary aspect, in particular how environmental factors turn on/off or regulate genes, and affect gene expression ^[4, 5]. Epigenetic processes are reversible and dynamic, as they evolve over time. They are affected by environmental factors in the broad sense (pathologies, nutrition, physical activity, stress, medication, etc.) ^[6, 7]. Thus, epigenetic biomarkers allow a dynamic approach to diagnosis ^[8], taking into account the condition of the patients, the potential progression of the disease as well as the impact of treatment ^[9, 10].

RNA editing is one of the epigenetics phenomena. This is a physiological mechanism that occurs in any individual, and that is – among other factors – influenced by the pathology and/or the medication ^[11-13]. It consists of the substitution, in specific places on the RNA of an adenosine by an inosine, under the specific action of enzymes ^[11, 14]. Several studies have shown that RNA editing is involved in many physiological functions and can affect proteins and regulation mechanisms ^[4].

One of the most studied processes occurring at the RNA level is the Adenosine to Inosine (A-to-I) deamination, that has been shown to be modified in several neuro-psychiatric disorders. In particular, RNA editing regulates some synaptic functions via an alteration of the functionality of key receptors, leading to a direct impact on synaptic transmission ^[15]. RNA editing is a mechanism linking inflammation and neuropsychiatry ^[11, 16-19], changes in RNA editing being associated with some mental illnesses (such as depression, suicidal tendencies, etc.), but also with some inflammatory diseases ^[20] and certain cancers ^[21]. ALCEDIAG used targeted RNA sequencing on 8 genes selected through several scientific and clinical studies combined with artificial intelligence ^[46-50], to objectify and quantify the RNA editing mechanism and to develop a tool for the differential diagnosis of unipolar depression and bipolar disorder ^[47].

3. Terms of use

EDIT-B® must be prescribed by a physician authorized to characterize a depressive episode. The medical prescription must mention general patient information, therapeutic classes of the treatment taken by the patient, and any consumptions or addictions (tobacco/alcohol).

The necessary details to be provided when prescribing are described in the EDIT-B® user manual.

The test must be performed during the depressive episode, subject to the medical consultation.

As an aid of diagnosis, EDIT-B® result brings supplementary data on the patient for the physician.

EDIT-B® is carried out according to a standardized method requiring strict compliance with pre-analytical, analytical and post-analytical procedures, as described in the EDIT-B® protocol provided to the laboratory by ALCEDIAG. To perform the test, the laboratories need to be approved by ALCEDIAG.

4. Precautions for use

EDIT-B® pre-analytical precautions:

- The blood sample should be taken soon after the prescription (in the days that follow), to ensure that the patient is still in the same state of depression.
- The laboratory should respect the pre-analytical and analytical recommendations provided in the EDIT-B protocol.

Precautions for interpreting EDIT-B® results for the healthcare professionals:

- A result indicating a unipolar depression profile does not necessarily exclude the presence of bipolar disorder. It is mandatory to establish the diagnosis taking into account all the clinical and biological factors related to the patient, and to maintain regular patient monitoring.
- A result indicating a bipolar disorder profile does not formally mean that the patient is affected with bipolar disorder. It is mandatory to establish the diagnosis taking into account all the clinical and biological factors related to the patient, and to maintain regular patient monitoring.
- In the event of a discrepancy between the EDIT-B® result and other diagnostic tools (DSM-5, ICD-11, MADRS, HDRS, BDI, etc.), it is imperative to refer to the conclusions of the prescriber.
- EDIT-B® cannot replace the clinical diagnosis of the prescriber. The causes of these discrepancies may be of pre-analytical, analytical or post-analytical origin, due to non-compliance with the terms of use of the diagnostic test and/or non-compliance to the protocol and/or associated with the percentages of false positives and false negatives.

5. Limits of use

EDIT-B® is not intended for the patient profiles detailed below:

- Patient under the age of 18;
- Patient with manic symptoms;
- Patient with a contraindication to having a blood test.

6. Collection and storage of samples

EDIT-B® is performed from a whole blood sample taken in a 2.5 mL PAXgene® Blood RNA tube. Collection of blood samples can be performed at any time of the day.

The sample should be shaken and stored according to the instructions for use of PAXgene® tubes, namely:

- The sample should preferably be stored at -20°C and below.
- If the sample has to be stored at temperatures below -20°C, freeze it first at -20°C for at least 24 hours, then transfer it to a freezer at -70°C or -80°C.

7. Sample analysis method

EDIT-B® library preparation protocol is provided to laboratories accredited by ALCEDIAG to perform EDIT-B®.

The Instructions for Use (IFU) of EDIT-B® are intended for the professionals who will perform the test.

EDIT-B® is intended to be used with an automated RNA PAXgene® extraction device, followed by a manual process until the sequencing step.

The EDIT-B® protocol is described in the EDIT-B® Handbook available on the EDIT-B® platform (<http://edit-b.alcediag-alcen.com/>).

8. EDIT-B® Platform

The access to the EDIT-B® platform is provided to laboratories accredited by ALCEDIAG to perform EDIT-B® test.

9. EDIT-B® test results

At the end of the EDIT-B® test, a medical report consisting of the results provided by the software is sent to the medical biology laboratory via the EDIT-B® platform. The latter will validate this report and then send it to the physician who issued the prescription.

The results are presented as a qualitative result (Bipolar / Unipolar / No result). See decision rules in the following paragraph.

Decision rules for EDIT-B® test results

EDIT-B® provides an aid to the physician in diagnosing bipolar or unipolar depressed patients.

<u>Outcomes</u>	<u>Description</u>
✓ Bipolar	The RNA editing profile measured by EDIT-B® corresponds to a bipolar patient profile.
✓ Unipolar	The RNA editing profile measured by EDIT-B® corresponds to a unipolar patient profile.
✓ No result	Requirements to run the EDIT-B® test are not fulfilled.

No Template Control (NTC) (Water quality control)

Water quality control allows the detection of potential contamination or non-specific amplification in the flow cell being analysed.

EDIT-B® specifications

In order to successfully deliver a diagnostic aid report, the EDIT-B® test must meet several quality criteria and specifications. These criteria are described in the EDIT-B® Handbook.

10. EDIT-B® clinical performance

Two prospective clinical studies were performed to determine the clinical performances of EDIT-B®.

Sample type: Whole blood collected with PAXgene® Blood RNA tubes

EDIT-B® performance	<u>First clinical study¹</u>	<u>Second clinical study²</u>
✓ Population size	245	94
✓ Sensibility (%)	82.1	85.7
✓ Specificity (%)	80.0	81.8

Clinical study carried out with CHU of Montpellier
2 Clinical study carried out with Swiss psychiatric centres, les Toises

11. Abbreviation

ATC: Anatomical Therapeutic Chemical Classification System
BDI: Beck Depression Inventory
DSM-V: Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition
EN: English
FASTQ: Text-based format for storing both a biological sequence and its quality scores
FR: French
GDPR: General Data Protection Regulation
HDRS: Hamilton Depression Rating Scale
ICD-11: International Classification of Diseases eleventh revision
IFU: Instructions for Use
IT: Italian
IVD: In Vitro Diagnostic device
MADRS: Montgomery–Åsberg Depression Rating Scale
MAPQ: MAPping Quality
NTC: No Template Control (water quality control)
Phred: Quality score to measure the quality of the identification of the nucleobases sequenced
RNA: RiboNucleic Acid

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