Title:	**ALEX² - Summary of Safety and Performance (SSP)**
Document type:	**Technical Documentation (v2.0)**
Document ID:	**TD-9**
Author:	Thomas Göls-Manninger
Owner:	Thomas Göls-Manninger
Approver(s):	Birgit Wahlmüller *approved at 2024-09-23 15:11 (UTC +0200)*
Approval date:	2024-09-23
Effective date:	2024-09-23
Next periodic review date:	2026-09-23

ALEX² - SUMMARY OF SAFETY AND PERFORMANCE

1. Device identification and general information

Device trade name(s)	ALEX² Allergy Xplorer
Reference Number	02-2001-01 02-5001-01
Basic UDI-DI	91201229202JQ
European Medical Device Nomenclature (EMDN) description / text	W01020205
Risk class of device	Class C
Is it a device for near-patient testing and/or a companion diagnostic?	No
Year when the first certificate was issued under Regulation (EU) 2017/746 covering the device	2024

Manufacturer's name and address	MacroArray Diagnostics Lemböckgasse 59, Top 4 1230 Vienna, Austria
Manufacturer's single registration number (SRN)	AT-MF-000030541
Authorized representative if applicable; name and the SRN	n.a.
NB's name (the NB that will validate the SSP) and the NB's single identification number	QMD Services GmbH NB Number: 2962

2. Intended Use of the device

2.1. Intended Purpose

The ALEX² Allergy Xplorer is a test kit used for in-vitro examination of human serum or plasma (exception EDTA-plasma) to provide information to aid the diagnosis of patients suffering from IgE-mediated diseases in conjunction with other clinical findings or diagnostic test results.

The IVD medical device detects allergen-specific IgE (sIgE) quantitatively and total IgE (tIgE) semi-quantitatively. The product is used by trained laboratory personnel and medical professionals in a medical laboratory.

2.2. Indications and target populations

ALEX² is for trained laboratory personnel and medical professionals only.

Any human serum or plasma (exception EDTA-plasma) from any target population can be used for analysis as there are no restriction on the target population. When developing IgE assays, age and sex are typically not considered as critical factors because IgE levels, which are measured in these assays, do not significantly vary based on these demographics.

2.3. Limitations and/or contra-indications

A definitive clinical diagnosis should only be made in conjunction with all available clinical findings by medical professionals and shall not be based on results of a single diagnostic method only.

In certain areas of application (e.g. food allergy), circulating IgE antibodies may remain undetectable although a clinical manifestation of food allergy against a certain allergen may be present, because these antibodies may be specific to allergens that are modified during industrial processing, cooking or digestion and hence do not exist in the original food for which the patient is tested.

Negative venom results only indicate undetectable levels of venom specific IgE antibodies (e.g. due to long term non-exposure) and do not preclude the existence of clinical hypersensitivity to insect stings.

In children, especially up to 2 years of age, the normal range of tIgE is lower than in adolescents and adults (Martins TB, Bandhauer ME, Bunker AM, Roberts WL, Hill HR. New childhood and adult reference intervals for total IgE. J Allergy Clin Immunol. 2014 Feb;133(2):589-91). Therefore, it is to be expected that in a higher proportion of children younger than 2 years the total IgE-level lies below the specified detection limit. This limitation does not apply to specific IgE measurement.

Anti-IgE therapy is a contra-indication for the use of IgE-based in-vitro diagnostic tests.

3. Device description

3.1. Description of the device

ALEX² is a solid-phase immunoassay. Allergen extracts or molecular allergens, which are coupled to nano-particles, are deposited in a systematic fashion onto a solid phase forming a macroscopic array.

First, the particle bound allergens react with specific IgE that is present in the patient's sample. After incubation, non-specific IgE is washed off. The procedure continues by adding an enzyme labelled anti-human IgE detection antibody which forms a complex with the particle bound specific IgE. After a second washing step, substrate is added which is converted to an insoluble, coloured precipitate by the antibody-bound enzyme. Finally, the enzyme-substrate reaction is stopped by adding the Stop Solution. The amount of precipitate is proportional to the concentration of specific IgE in the patient sample. The test procedure is followed by image acquisition and analysis. The test results are reported in allergen IgE response units (kU_A/l) for the specific allergen and in IgE response units (kU/l) for total IgE (tIgE).

The product is used in a medical laboratory by trained laboratory personnel and medical professionals only.

Processed ALEX² array in cartridge

3.2. In case the device is a kit, description of the components

Components	Amount (REF 02-2001-01)	Amount (REF 02-5001-01)	Description	Regulatory Status (IVD, Medical Device)
ALEX² cartridge	20	50	Polystyrol cartridge containing a solid-phase immunoassay	component of IVD
ALEX² Washing Solution**	2 x 50 ml	1 x 250 ml (4 x conc)	1 x TBS-Tween Buffer, < 0,1 % Sodium Azide	IVD
ALEX² Sample Diluent	1 x 9 ml	1 x 30 ml	1 x TBS-Tween Buffer, CCD Blocker, < 0,1 % Sodium Azide	component of IVD
ALEX² Detection Antibody	1 x 11 ml	1 x 30 ml	Anti-human IgE detection antibody dissolved in Conjugate Buffer with Additives. The anti-human IgE detection antibody is enzyme labelled and forms a complex with the allergen-bound specific IgE	component of IVD

ALEX² Substrate Solution	1 x 11 ml	1 x 30 ml	NBT/BCIP Substrate (NBT: 4-Nitro blue tetrazolium chloride, solution, BCIP: 5-bromo-4-chloro-3-indolyl-phosphate, 4-toluidine salt)	component of IVD
ALEX² Stop Solution* **	1 x 2.4 ml	1 x 10 ml	Ethylenediaminetetraacetic acid (EDTA)-Solution	IVD

* The cartridges comprise an array (=immunoassay) with 305 spots, as follows:

- 300 different allergens (thereof 5 for research-use-only (RUO)),
- 4 spots for plausibility check (QC), named “Guide Dots”,
- 1 spot with anti-total IgE antibodies for the semi-quantitative determination of total-IgE,
- a 2-dimensional QR-Code which is used to identify the cartridge during the scanning process

** The “ALEX² Washing Solution” corresponds to and is synonymous with the “Washing Solution”.

*** The “ALEX² Stop Solution” corresponds to and is synonymous with the “Stop Solution”.

3.3. A reference to previous generation(s) or variants if such exists, and a description of the differences

Name: ALEX Allergy Explorer

REF: 01-2001-01

ALEX² was developed based on the predecessor product ALEX - Allergy Explorer. For ALEX², the allergen panel has been reworked and several improvements were implemented. Concerning the allergen panel the following improvements have been implemented:

- new relevant allergens were added
- extracts were exchanged by new molecular allergens and

Additionally other improvements were added:

- substrate incubation is performed without shaking in order to avoid spot bleeding
- in this course the substrate solution was adopted and
- the plausibility check of the test results has been reworked
- the stop solution is now provided in one container and not in two vials anymore
- as more space was needed for the additional allergens on the array, the test cartridge was enlarged and to avoid a change of the outer cartridge

dimensions, the barcode area was therefore reduced

- looking into the future for a more automated manufacturing process, the cartridges are packed into blisters containing 10 cartridges each and are sealed with the re-sealable zipper pouches
- finally, the labelling was changed completely to lay the foundation for the implementation the UDI requirements from the IVDR. In this course, new labels were purchased and a 1D (Code128) barcode was added to the components indicating the lot and the expiry date.

3.4. Description of any accessories which are intended to be used in combination with the device

- Array holder (optional): for an easier handling of the cartridges during the manual test procedure
- Lab Rocker (vertical rocker, WxDxH – 28x15x33 cm, inclination angle 8°, required speed 8 rpm): needed for the manual test procedure
- Incubation chamber (WxDxH – 35x25x2 cm): needed for the manual test procedure
- PC/Laptop
- Pipettes and pipette tips

3.5. Description of any other devices and products which are intended to be used in combination with the device

- **RAPTOR SERVER Analysis Software** (IVD): for the acquisition and analysis of images taken by the ImageXplorer or MAX devices
- **ImageXplorer** (IVD): an imaging device specifically built to acquire images of ALEX technology-based test cartridges for the manual test procedure
- **MAX devices - MAX 45k, MAX 9k** (IVD): instruments for the automated processing of ALEX technology-based test cartridges

4. Reference to any harmonized standards and CS applied

EN ISO 13485:2016, EN ISO 13485:2016/A11:2021	Medical devices - Quality management systems - Requirements for regulatory purposes (ISO 13485:2016)
EN ISO 13485:2016, EN ISO 13485:2016/A11:2021, EN ISO 13485:2016/AC:2018	Medical devices - Quality management systems - Requirements for regulatory purposes (ISO 13485:2016)
EN ISO 14971:2019, EN ISO 14971:2019/A11:2021	Medical devices - Application of risk management to medical devices (ISO 14971:2019)
EN ISO 15223-1:2021	Medical devices - Symbols to be used with information to be supplied by the manufacturer - Part 1: General requirements (ISO 15223-1:2021)
EN ISO 17511:2021	In vitro diagnostic medical devices - Requirements for establishing metrological traceability of values assigned to calibrators, trueness control materials and human samples (ISO 17511:2020)

5. Risks and warnings

5.1. Residual risks and undesirable effects

One residual risk involves the utilization of samples containing EDTA, which may have a detrimental effect on the test results. Although the complete exclusion of EDTA-containing samples cannot be assured, the Instructions for Use (IFU) provide appropriate guidelines for the samples used, thus leading to the assessment of this risk as acceptable.

Another residual risk pertains to the inhomogeneous distribution of the stop solution on the array. Nevertheless, the IFU offers clear procedural instructions to address this concern, resulting in the evaluation of the risk as acceptable.

5.2. Warnings and precautions

Warnings and precautions are described in the IFU and the Safety Data Sheet of ALEX², both available at www.madx.com/extras.

5.3. Other relevant aspects of safety, including a summary of any field safety corrective action (FSCA including FSN), if applicable

No FSCAs were initiated since launching ALEX².

6. Summary of the performance evaluation as referred to in Annex XIII, and relevant information on the PMPF

6.1. Summary of scientific validity of the device

Using ELISA for the detection of sIgE and tIgE in serum is state of the art, moreover multiplex in vitro assays have several advantages against the widely used skin prick test:

Serum IgE testing entails no risk to the patient other than a blood draw and is preferable if the patient

- has an unstable or uncontrolled medical condition,
- is at high risk of anaphylaxis,
- is taking essential medication that interferes with testing,
- is very young such that the procedure would be unduly stressful, or
- has a skin condition that limits available skin for testing.

The development of screening tests with multiple allergens or multiplex tests that identify multiple specific IgEs with a small blood volume is especially beneficial for testing very young children.

> *IgE allergy diagnostics and other relevant tests in allergy, a World Allergy Organization position paper*
>
> *Ansotegui et al. World Allergy Organization Journal (2020) 13:100080*
> *http://doi.org/10.1016/j.waojou.2019.100080*

6.2. Summary of performance data from conducted studies of the device prior to CE-marking

6.2.1. Precision (Lot-to-Lot Variation) with ImageXplorer

The lot-to-lot variation was determined on 3 cartridge lots in three sperate runs. Multi-sensitized samples were included in the study. The study comprised 319 allergen per sample combinations covering 191 individual allergens at 3 different levels (> 10 kU_A/l, 1-10 kU_A/l and 0.3-1 kU_A/l).

	0.3 - 1 kU_A/l	1 - 10 kU_A/l	> 1 kU_A/l	> 10 kU_A/l
Total CV%	24.7	12.1	11.3	9.6

6.2.2. Precision with MAX Devices

The variation between different MAX devices in the ALEX² assay was determined on three MAX 45k and three MAX 9k devices in three separate runs (same ALEX² lot). Three selected multi-sensitized samples were tested covering the majority of priority components at 3 different levels (>10 kU_A/l, 1-10 kU_A/l and 0.3-1 kU_A/l).

For the selected allergen components, the CV (in %) was calculated between runs and between instruments (= total CV).

	0.3 - 1 kU_A/l	1 - 10 kU_A/l	> 1 kU_A/l	> 10 kU_A/l
Total CV% MAX 45k	24.0	11.0	10.6	9.1
Total CV% MAX 9k	20.6	10.1	9.4	8.8

6.2.3. Repeatability (within-run Precision) with ImageXplorer

In the repeatability study, multi-sensitized samples were tested 10 times by the same operator on different days. The study comprised 319 allergen per sample combinations covering 165 individual allergens at 3 different levels (>10 kU_A/l, 1-10 kU_A/l and 0.3 - 1 kU_A/l).

	0.3 - 1 kU_A/l	1 - 10 kU_A/l	> 1 kU_A/l	> 10 kU_A/l
Total CV%	25.6	13.8	13.5	10.7

6.2.4. Homogeneity for MAX Devices

The homogeneity of the ALEX² results within a MAX test run was tested on three separate MAX 45k and three MAX 9k devices. A single multi-sensitized positive test sample was tested at all positions of the cartridge carousel.

	0.3 - 1 kU_A/l	1 - 10 kU_A/l	> 1 kU_A/l	> 10 kU_A/l
Total CV% MAX 45k	33.6	12.3	11.5	9.2
Total CV% MAX 9k	28.1	10.3	9.8	9.3

6.2.5. Analytical Sensitivity

The Limit of Detection (LOD) was determined in accordance with CLSI guideline EP17-A for representative allergen components and was below 0.3 kU_A/l for all allergen components and all allergen extracts.

6.2.6. Analytical Specificity

There is no detectable cross-reactivity with other human Immunoglobulins (IgA, IgG1, IgG2, IgG3, IgG4 and IgM) at normal physiological concentrations.

6.2.7. Interference

There is no detectable interference with bilirubin, cholesterol/triglycerides and hemoglobin at normal physiological concentrations. Neither is there an interference with tIgE which was tested in concentrations of up to 3000 kU/l.

6.3. Summary of performance data from other sources, if applicable

6.3.1. Data from Clinical Study (MADMAX)

A clinical study called "Diagnostic Accuracy of the MADx Multi Array Xplorer (MAX 45k) Automated Laboratory System and the MADx Allergy Explorer Version 2 (ALEX²) - IgE Multiplex Test for the Diagnosis of Pre-defined Groups of Specific High-priority Allergens (MADMAX)" (reference number: NCT04435678) was successfully completed in April 2022.

The primary objective of the study was to assess the diagnostic accuracy (sensitivity, specificity) of the MAX 45k/ALEX² IgE multiplex test in comparison to clinical symptoms. Furthermore, the usability as well as the processing duration (incl. hands-on time) were assessed.

In total, 111 birch pollen allergic patients, 113 grass pollen allergic patients and 107 cat allergic patients were included in the study conducted from July 2020 to April 2022, leading to a total number of 839 patients.

6.3.1.1. *Sensitivity – primary outcome*

It was expected that the MAX 45k/ALEX² IgE multiplex test can achieve sensitivities of 75% (bee venom), 95% (house dust mite), 96% (grass pollen and vespid venom), or even 98% (birch pollen and cat), depending on the allergen. In relation to clinical symptoms, the ALEX² test was in the range of all expected sensitivities.

	Sensitivity (95% Confidence interval)
Birch pollen allergy (N=111)	94.6% (88.6-98.0)
Grass pollen allergy (N=113)	98.2% (93.8-99.8)
House dust mite allergy (N=148)	91.2% (85.4-95.2)
Cat allergy (N=107)	92.5% (85.8-96.7)
Bee venom allergy (N=104)	76.0% (66.6-83.8)
Vespid venom allergy (n=106)	94.3% (88.1-97.9)

All patients with the respective positive personal clinical history have been included in the analysis.

6.3.1.2. *Specificity*

The specificity of the ALEX² test was expected to be 95% for healthy controls. This assumption was confirmed for the comparison with clinical symptoms.

	Specificity (95% Confidence interval)
ALEX² test compared to clinical symptoms (N=146)	95.9% (91.3-98.5)

Patients with asymptomatic bee and/or vespid venom sensitization were included in the analysis.

6.3.1.3. *Likelihood ratio*

The likelihood ratio was calculated from the sensitivity and specificity data according to the following formula: Sensitivity / (1 - Specificity).

	Calculated Likelihood ratio
Birch pollen allergy (N=111)	23.1 (0.946/(1-0.959))
Grass pollen allergy (N=113)	24.0 (0.982/(1-0.959))
House dust mite allergy (N=148)	22.2 (0.912/(1-0.959))
Cat allergy (N=107)	22.6 (0.925/(1-0.959))
Bee venom allergy (N=104)	18.5 (0.760/(1-0.959))
Vespid venom allergy (n=106)	23.0 (0.943/(1-0.959))

6.3.1.4. *Usability*

To analyze the quantitative usability of the MAX system, we calculated the System Usability Scale (SUS). The SUS is a standardized questionnaire designed to assess perceived usability (Brooke, 1996, 2013; Sauro, 2011). We performed a standard questionnaire of 10 items. It is a mixed-tone questionnaire in which the odd-numbered items have a positive tone, and the even-numbered items have a negative tone.

A SUS value of 97.5% was achieved, which represents an excellent usability.

6.3.2. Data from Literature

A systematic review to assess the diagnostic accuracy of the IgE multiplex diagnostic test ALEX Allergy Xplorer, compared with the ImmunoCAP ISAC microarray and ImmunoCAP single-plex was performed. PubMed, Google Scholar and internal sources were searched until July 25th, 2023, for publications regarding the diagnostic accuracy of the ALEX Allergy Xplorer. 910 studies were screened. A total of 36 cohort studies, 34 published and 2 unpublished, were included. Diagnostic test accuracy information for ALEX components was available for pollen (tree, grass, weed), food, animal dander, mites, cockroaches, moulds, yeasts, and insect venoms. Specificity, sensitivity, positive predictive value (PPV) and negative predictive value (NPV) of ALEX Allergy Xplorer were generally high. Comparison studies with ImmunoCAP test systems (single- and multiplex) demonstrated that, with few exceptions (bee venom & sunflower seed extract), ALEX showed similar or better diagnostic accuracy (better for shrimp-, fish-, and house dust mite allergens).

6.4. An overall summary of the performance and safety

ALEX² is an innovative product that improves allergy diagnosis through the ability to detect specific IgE against a wide range of molecular allergens and allergen extracts, as well as total IgE. ALEX² demonstrates a high specificity, positive/negative predictive values and sensitivity. Safety measures have been implemented to protect patients, users and all persons involved. Through risk assessment, all known and foreseeable potential risks associated with the use of ALEX² have been determined to be acceptable given the significant benefits it provides to patients.

Overall, ALEX² is considered a reliable, safe and sensitive tool for supporting the diagnosis of a wide range of allergies.

6.5. Ongoing or planned post-market performance follow-up

In regular intervals customer inquiries and complaints are systematically evaluated. Additionally, MADx participates in ring trials:

- CAP (College of American Pathologists): Proficiency Testing - Diagnostic Allergy
- UK NEQAS for ALLERGEN COMPONENT TESTING

and several clinical and scientific studies are ongoing and planned:

Ongoing studies:

Topic/Title	Category	Status
AIT Monitoring-Study Peru	New application	Ongoing
tIgE-Standard new	Technical Comparison Study	Ongoing
RAPTOR SERVER data mining	Data Mining	Ongoing
Northern Italy comparison ALEX² ISAC	Technical Comparison Study	Ongoing
Euroimmune/Polycheck/ImmunoCAP/ ALEX²	Technical Comparison Study	Ongoing

Planned studies:

Topic/Title	Category	Status
Molecular Mapping Latin America	Other	Resource planning (Feasibility)
Cannabis-Study	Evaluation of new Allergens/new markers	Resource planning (Feasibility)
Local IgE-production study	New application	Resource planning (Feasibility)
Berlin AIT cohort study	Other	Ongoing

7. Metrological traceability of assigned values

7.1. Explanation of the unit of measurement, if applicable

Total IgE results are reported in IgE response units (kU/l). The sIgE (specific IgE) results are reported in kU_A/l, whereas 1 kU/l correlates to 2.42 ng/ml.

During development a master calibration curve was established. The master calibration curve is stored in the RAPTOR SERVER Analysis Software. The customer performs a measurement and the RAPTOR SERVER Analysis Software takes the Signal-to-Noise data and calculates, based on the master calibration curve, the calibrated data in kU_A/l and kU/l, respectively.

7.2. Identification of applied reference materials and/or reference measurement procedures of higher order used by the manufacturer for the calibration of the device

There is no reference material for sIgE available. For tIgE a WHO reference preparation 11/234 is available.

MADx uses sera which are tested with ImmunoCAP (Thermo Fisher), which at the moment is perceived as “reference method” for in vitro IgE testing (WAO position paper).

The validity of the master calibration curve is controlled with control sera with known sIgE levels, e.g. Lyphochek Allergen sIgE, Panel A by Bio-Rad Laboratories or the recently launched QualityXplorer by MADx.

8. Suggested profile and training for users

The ALEX² test system is for trained laboratory personnel and medical professionals only.

9. Revision history

SSP Revision number	Date issued	Change description	Revision validated by the Notified Body
1.0	23.09.2024	First compilation	☑ Yes Validation language: English ☐ No (only applicable for class C (IVDR, Article 48 (7)) for which the SSP is not yet validated by the NB)
			☐ Yes Validation language: ☐ No

MacroArray Diagnostics (MADx)
Lemböckgasse 59, Top 4
1230 Vienna, Austria
+43 (0)1 865 2573
www.madx.com

Document number: TD-9 ALEX² - Summary of Safety and Performance
Version number: 1.0
Released: 09-2024