


# BMJ Open Investigating nanomotion-based technology (Resistell AST) for rapid antibiotic susceptibility testing among adult patients admitted to a tertiary-care hospital with Gram-negative bacteraemia: protocol for a prospective, observational, cross-sectional, single-arm study

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## ABSTRACT

**Introduction** Effective treatment of bloodstream infections (BSIs) is relying on rapid identification of the causing pathogen and its antibiotic susceptibility. Still, most commercially available antibiotic susceptibility testing (AST) methods are based on monitoring bacterial growth, thus impacting the time to results. The Resistell AST is based on a new technology measuring the nanomotion caused by physiologically active bacterial cells and detecting the changes in nanomotion caused by the exposure to a drug.

**Methods and analysis** This is a single-centre, prospective, cross-sectional, single-arm diagnostic accuracy study to determine the agreement of the Resistell AST on Gram-negative bacteria isolated from blood cultures among patients admitted to a tertiary-care hospital with the reference method. Up to 300 patients will be recruited. Starting with a pilot phase, enrolling 10%–20% of the subjects and limited to *Escherichia coli* BSI tested for ceftriaxone susceptibility, the main phase will follow, extending the study to *Klebsiella pneumoniae* and ciprofloxacin.

**Ethics and dissemination** This study has received ethical approval from the Swiss Ethics Committees (swissethics, project 2020-01622). All the case report forms and clinical samples will be assigned a study code by the local investigators and stored anonymously at the reference centre (Lausanne University Hospital). The results will be broadly distributed through conference presentations and peer-reviewed publications.

**Trial registration number** ClinicalTrials.gov Registry (NCT05002413).

## STRENGTHS AND LIMITATIONS OF THIS STUDY

- ⇒ Rapid antibiotic susceptibility testing recording fluctuations of metabolically active bacteria responding to drug exposure.
- ⇒ Outcome comparison with both clinical and diagnostic endpoints.
- ⇒ Real-life clinical data on a target hospital population.
- ⇒ Preliminary agreement data obtained in a preclinical phase.
- ⇒ Non-randomised observational clinical study.

## INTRODUCTION

### Background and rationale

Bloodstream infections (BSIs) represent the main cause of sepsis and septic shock, associated with high morbidity and mortality.<sup>1 2</sup> Previous studies showed an incidence of BSIs up to 190 per 100 000 population, with increasing trends both in Europe and the USA.<sup>1 3–5</sup> Effective treatment of BSI is relying on the ability to promptly identify the causing pathogen and its antibiotic susceptibility.<sup>6 7</sup>

Timely antibiotic prescription and reduction of the antimicrobial spectrum are the cornerstones of antimicrobial stewardship programmes, as they reduce the selective pressure on bacteria limiting the spread of multidrug-resistant pathogens.<sup>8</sup>

Most commercially available antibiotic susceptibility testing (AST) methods are based on monitoring bacterial growth, representing the main limitation for the speed of

such tests.<sup>9 10</sup> Other molecular technologies, including sequencing analysis (Sanger, pyrosequencing), are available but their use is mainly limited by the higher costs, for being determined by mutations or resistance-associated genes and due to changes in gene expression. Only few rapid phenotypical technologies have been developed (such as Accelerate Pheno, FASTinov AST) but not necessarily available in each country or not yet commercialised. The current gold standard are broth microdilution or disk diffusion test (Kirby-Bauer Test), which both take about 16–48 hours to yield results.<sup>11</sup> Automated systems, such as VITEK 2 (bioMérieux, France) and BD Phoenix (Becton Dickinson, USA), have the advantage of being directly connected to an advanced expert system software for interpretation of results and online data processing, therefore improving the turnaround time to 8–12 hours.<sup>12</sup>

The Resistell AST is a diagnostic tool based on a new nanomotion-based technology for rapid AST. It consists of a device (ie, Resistell Phenotech), single-use disposables, micro-electro-mechanical sensors—microcantilevers (ie, Phenotech Sensors), and software for data acquisition, processing and classification. Resistell AST characterises the bacterial reaction to a given drug by recording low-frequency fluctuations of microcantilevers.<sup>13–15</sup> These fluctuations are caused by the nanomotion due to metabolically active bacteria and may change upon exposure to a drug. These changes in nanomotion as a response to a drug are growth independent and can be detected long before bacteria replicate.

For the development of the AST, drug concentrations need to be predetermined and introduced in the algorithm, which will render susceptibility results. Categories will be classified into ‘susceptible, standard dose (S)’, ‘resistant (R)’ and ‘susceptible, increased exposure (I)’; Resistell classification into ‘I’ will depend on the number of drug concentrations tested and the reference outcome. In the absence of ‘I’ category for Resistell AST, samples resulting ‘I’ on the reference test will be classified as ‘S’ when compared with Resistell AST.

Resistell AST is expected to decrease the time to AST result to 2–4 hours, depending on the bacteria and antibiotic combination, thus, potentially reducing the time to results by 6–46 hours.

### Preclinical development and validation

Before the start of the pilot phase, Resistell AST was developed for *Escherichia coli* and ceftriaxone. The method was optimised to work with bacterial pellets directly from positive blood cultures (PBCs) artificially inoculated (spike cultures) with clinical isolates from the Lausanne University Hospital as well as reference strains acquired from the American Type Culture Collection (ATCC), IHMA Europe Sàrl society, respectively. Direct attachment pellet preparation was used (see the Methods section for details of the procedure), with all experiments performed with 32 µg/mL ceftriaxone. The typical recording for a susceptible strain shows a stagnant or decreasing variance of the cantilever deflection over

time, while resistant strains exhibit an increase upon exposure to an antibiotic. The data can be fitted to an exponential equation resulting in the slope. Extremely susceptible or resistant strains can be differentiated based on a single feature such as the slope of the variance. However, in the clinical setting, we operate with strains of a wide minimum inhibitory concentration (MIC) spectrum and genetic diversity. To classify these strains, reliable classification algorithms sought out by machine learning are necessary as the slope is insufficient to deal with the various nanomotion curves resulting from the strain complexity in the clinic. To be able to classify antibiotic susceptible and resistant isolates, features or sets of features need to be extracted from the nanomotion signal. These features have a single mathematical value for each measured sample and a combination of them can be used to classify all the different susceptible and resistant isolates correctly. In one of the preclinical steps of developing the nanomotion-based AST, we required machine learning to calculate hundreds of thousands of features, select a few and validate these selected features in a procedure called 300 times repeated threefold cross-validation. In this validation (using spiked blood cultures), the dataset is repeatedly split in nanomotion recordings used for training and those used for validation (ratio 2:1). The resulting classification algorithm employs only a few general features and is further tested on a smaller set of anonymised PBCs from patients that were performed in replicates. This step is fast and comparable with the analysis applied in the clinical study.

The initial algorithm for *E. coli* and ceftriaxone was developed in the preclinical phase (concluded April 2021) and trained and validated on a batch of data comprising 706 experiments performed on spike cultures (step 1). This batch of data included two *E. coli* reference strains (‘S’: ATCC-25922 and ‘R’: BAA-2452) and 32 clinical isolates of *E. coli* with various MICs (‘S’: RN-01, RN-02, RN-03, RN-04, RN-08, RN-13, RN-15, RN-19, RN-23, RN-26, RN-27, RN-36, RN-37, RN-42, RN-43, RN-49, RN-52, RN-59, RN-61; and ‘R’: B1, B3, B4, B11, B12, B13, B14, B15, RN-28, RN-29, RN-35, RN-55, RN-64). Reference AST methods were Kirby-Bauer Test and Epsilon-meter Test (E-Test). The training and validation dataset was hence balanced among ‘S’ and ‘R’ isolates with 20 and 14 strains, respectively. Each strain was measured in replicates from the same spiked blood culture and repeatedly on different dates from a different culture. The method’s accuracy on the training dataset estimated using the 300 times repeated threefold cross-validation procedure amounted to 88%, with 89% sensitivity and 86% specificity.

In the second step, the algorithm was tested on a blind batch of data, using bacterial pellets isolated directly from PBCs originating predominantly from the Lausanne University Hospital’s emergency room. The test dataset included 23 pellets of PBC samples from different patients directly measured in technical triplicates or quadruplicates, resulting in 90 Resistell AST experiments.

Twenty of these samples were confirmed as ‘S’ and three as ‘R’ for ceftriaxone using the standard AST methods Kirby-Bauer Test and E-Test, with no strains in the ‘I’ category. For the 90 experiments, the performances were 94.4% category agreement (CA), 1.1% very major errors (VMEs, resistant pathogen classified as susceptible) and 4.4% major errors (MEs, susceptible pathogen classified as resistant) rate, corresponding to 94.4% accuracy, 94.9% sensitivity and 90.9% specificity. However, after implementation of a majority-based voting system between replicates (in case of a tie between replicates, the sample was classified ‘R’), the performance for the 23 PBC samples increased to 95.7% accuracy, 95% sensitivity (95% CI 75.1% to 99.9%) and 100% specificity (95% CI 29.2% to 100%), corresponding to a CA of 95.7% with 0% VME and 4.3% ME.

In this preclinical period, the algorithm has been further improved to cope with additional strains and species/antibiotic combinations that will be addressed in the main phase of the study. Adding more strains in this process of machine learning will result in a more comprehensive and general classification algorithm.

### Objectives

This study aims to clinically evaluate the performance of the Resistell AST compared with the reference. The primary endpoint is the correct classification of susceptible samples: ME/sensitivity. For this purpose and to also reach the secondary endpoints, CA, VME rate, mE (minor error, pathogen with an intermediate MIC classified as susceptible or resistant) and turnaround time of the Resistell AST will be evaluated on prospectively collected clinical routine PBC samples by comparison with reference methods. We hypothesise (1) a CA rate  $\geq 95\%$ , with a VME rate  $\leq 3\%$ , an ME rate  $\leq 5\%$  and an mE rate  $< 10\%$  compared with the reference method; (2) a gain in time to result of at least 6 hours (from blood culture positivity for Gram-negative bacteria to obtainment of susceptibility results) compared with the reference method (VITEK 2).

The performance will be based on the comparison with standard comparators, such as Kirby-Bauer and VITEK 2. In addition to ME (which, in absence of ‘I’ category reflects the test ‘sensitivity’) and for a better comparison with other AST methods approved by EUCAST and CLSI, we will also report CA (reflecting the overall ‘accuracy’) and VME (which, in absence of I category, reflects the test ‘specificity’). Using Resistell AST, we anticipate a gain in time to result (TTR, from blood culture positivity to AST results) of at least 6 hours compared with VITEK 2. Hence, we expect a more rapid and narrowed-down effective antibiotic spectrum with the Resistell AST that will potentially aid patient treatment and curb the spread of antimicrobial resistance.

### Primary objective

The primary goal of this study is the assessment of ME/sensitivity of the Resistell AST on prospectively collected



**Figure 1** The red arrow points to the measurement head, visible and accessible for the operator. The measurement head and chamber are covered with a removable lid (blue arrow).

clinical routine PBC samples by comparison with the reference methods.

### Secondary objectives

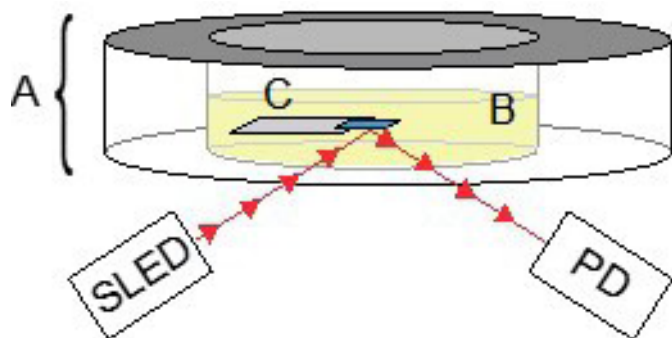
We will also assess: (1) CA/accuracy, VME/specificity and mE of the Resistell AST on prospectively collected clinical routine PBC samples by comparison with the AST reference methods; and (2) evaluate the differences in TTR with these methods. This last endpoint is further defined in: (a) time from blood culture positivity to Resistell AST results, (b) time from MALDI-TOF MS strain identification to Resistell AST results, (c) time from start to return of Resistell AST results.

## MATERIALS, METHODS AND ANALYSIS

### Investigational device and materials

The Resistell Phenotech system comprises: (1) a measurement head with a measurement chamber holding the Phenotech Sensor; (2) a stand for the measurement head and a vibration damping/isolation set-up; (3) a light source with a driver; (4) data acquisition system; and (5) a laptop with control software and access to data archiving server (figure 1).

The measurement head holds the measurement chamber in which the Phenotech Sensor, measuring microbial nanomotion, is located. The Resistell Phenotech head works similarly to measurement heads used in atomic-force microscopy (AFM). However, it is inverted compared with standard AFM set-ups, which allows (1) a better sensor and liquid handling during experiments without affecting the optical set-up, and (2) the investigation of the sensor from the top using an optical microscope. The light beam generated by a superluminescent light-emitting diode enters the measurement chamber through a sealed window. The beam is then reflected by the Phenotech Sensor’s microcantilever towards the



**Figure 2** Schematic of the components in the measurement head of the Resistell Phenotech device, with the removable measurement chamber (A) forming a 2 mL reservoir for liquid culture media (B) in which the Phenotech sensor (C) with the cantilever (in blue) is placed. Though the measurement chamber is made of stainless steel, it contains light-permissive windows in its bottom, allowing the light beam emitted by the superluminescent light-emitting diode (SLED) to enter and the beam reflected from the cantilever to exit, measured by the photodetector (PD).

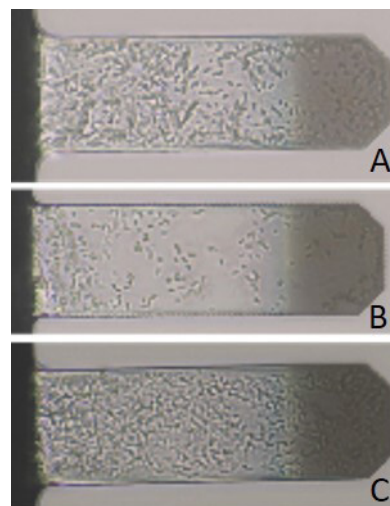
position-sensitive photodetector (figure 2). The Phenotech Sensor is fully submerged in a culture medium that may or may not contain drugs. Microcantilever vibrations induced by attached cells are transformed into electrical signals recorded by a data acquisition system and further processed by dedicated software.

### Procedures

Bacterial pellets are obtained from PBCs as follows: (1) for the direct attachment protocol, 1 mL of anaerobic (ANH) or aerobic (AEH) PBC is filtered using a 5 µm or 1.2 µm membrane filter, respectively (to separate bacteria from blood cells and debris), subsequently centrifuged for 3 min at 2000 g. (2) Alternatively, the MBT Sepsityper IVD Kit (Bruker, USA) can be used, according to the manufacturer's protocol, to obtain pellet for ANH and AEH PBCs. Briefly, 1 mL of blood culture is mixed with 200 µL of Lysis buffer and centrifuged at 12 000 g for 2 min. Supernatant is discarded and pellet is resuspended in 1 mL Washing buffer, centrifuged again at 12 000 g for 1 min. For both methods, the obtained pellet is resuspended in 200 µL of Thickening buffer A. This cell suspension is then used for cell immobilisation on a Phenotech Sensor (figure 3) pretreated with the Functionalizing Buffer A for 45 s at room temperature. After aspiration of the suspension and a short wash with phosphate buffered saline (PBS), the sensor can be placed into the Phenotech device and the nanomotion recording be commenced.

### Study design

This is a prospective, observational, cross-sectional, single-arm study investigating nanomotion-based technology (Resistell AST) for rapid AST by comparison with one of the following reference methods: (1) disk diffusion (Kirby-Bauer Test), (2) VITEK 2 or (3) BD Phoenix system.



**Figure 3** (A–C) Phase contrast images (40× objective) of *Escherichia coli* isolated from a positive blood culture and attached on a microcantilever using the Resistell Attachment Kit after a 45-second incubation at room temperature. The dimension of the cantilever is 130×40×0.75 µm and made of silicon-like material. The tip is coated with a reflective material for a better signal-to-noise ratio in the optical measurement system.

The study is divided into a pilot phase and the main study. In the pilot, only prospectively collected routine *E. coli* PBCs will be analysed and tested for ceftriaxone susceptibility using Resistell AST. *E. coli* was prioritised, representing the most frequently isolated pathogen in BSIs<sup>16</sup> and considering the continued increase in third and fourth-generation cephalosporin resistance in Switzerland from 0.9% in 2004 to 11.4% in 2019.<sup>17–19</sup>

About 10%–20% of all the required samples need to be analysed (30–60 samples). The recruitment rate and proportion of resistant strains will be assessed after the pilot. If at least 10% samples could be analysed, with at least 10% resistant, an interim analysis will be performed. However, if major amendments are required, the recruitment will be temporarily stopped.

Moreover, if after 3 months 10% recruitment has not been achieved (such as in case of high proportion of consent refusal, low prevalence of resistance or, overall, of *E. coli* BSIs, patients' transfer, patients' death), further samples will be analysed until 10% of the required samples (n=30) are reached.

In the main phase of the study, nanomotion-based Resistell AST of *E. coli* and *Klebsiella* spp (most common *Enterobacteriaceae* isolated from blood cultures<sup>16</sup>) will be analysed for ceftriaxone and ciprofloxacin. Single or multiple antibiotic concentrations will be experimentally determined for other antibiotic molecules (such as cefotaxime, ceftazidime, piperacillin–tazobactam, meropenem, gentamicin, amikacin and cefepime), as well as for other Gram-negative bacteria (such as *Enterobacteriaceae* and *Pseudomonas aeruginosa*) in a further multi-centre international study, which will include centres

with a higher rate of resistance according to the country epidemiology.

To control for selection bias, we planned, (1) a main 'per-protocol' analysis (included patients) and (2) an 'intention-to-test analysis', with all patients, including those initially excluded for reasons such as technical problems (eg, (1) bent/damaged Phenotech Sensor, (2) extensive cell elongation after drug exposure, (3) technical problem during recording, in particular loss of signal).

CA between Resistell AST method and reference method will be interpreted according to ISO 20776-2:2007 guidelines.<sup>20</sup>

### Study setting

The study is conducted at Lausanne University Hospital, a tertiary care centre with over 1500 beds and over 50 000 patients every year. The microbiology laboratory receives about 50 000 blood culture samples every year, out of which about 3% are true positives. Of these, *E. coli* constitutes about 28%, and all Gram-negative about 62%.

### Sampling

All PBC samples will be treated according to the microbiology laboratory procedure in place. Each PBC containing Gram-negative bacteria (identified by MALDI-TOF MS) will be screened for eligibility.

### Recruitment

If the sample passes the eligibility screening, a written informed consent form (ICF) is gathered from the patients or from next in kin/legal representative (if the patient cannot sign). Alternatively, a signature from an independent physician is obtained and an a posteriori ICF will be submitted to the patient once they regain the ability to sign (see the Informed consent and participant withdrawals section).

More than one specimen may be collected for AST. In such case, the absolute number of specimens analysed will be considered within the scope of this study, but only one consent will be obtained per patient per hospitalisation.

### Inclusion criteria

Patients meeting all the following criteria can be recruited in the study: (1) patients over the age of 18 years, or their legal representatives, who sign the ICF; (2) patients with *E. coli* or *Klebsiella* spp bacteraemia; (3) patients hospitalised at Lausanne University Hospital at the time of blood culture inoculation; (4) patients whose PBCs were not older than 24 hours at time of AST start.

### Exclusion criteria

Patients meeting any of the following criteria will be excluded: (1) patients with polymicrobial bacteraemia; (2) samples undergoing technical errors during the Resistell AST analysis (eg, bent or damaged Phenotech Sensor; extensive cell 'elongation' after drug exposure; technical problem with a device during recording, in

particular loss of signal); (3) patients refusing to sign the ICF.

If the maximum capacity of the Resistell AST is reached, no more ICFs will be obtained for that day.

### Interventions

Fraction of the pellets is analysed for AST with Resistell AST and the reference methods: (1) VITEK 2, (2) BD Phoenix system and/or (3) Kirby-Bauer Test. In case of a discrepancy between the Kirby-Bauer Test and VITEK 2/BD Phoenix system, an E-Test will be performed. Only AST results obtained with the reference method will be used for patients' care.

### Outcome measures

CA is considered as the number of bacterial isolates with the same AST category as the reference method category result.<sup>20</sup> VME is defined when classifying the test method as 'S' and the reference method being 'R'; ME is defined as when classifying the test method as 'R' and the reference method being 'S'; and mE represents the test in which either method is reported as 'I' while the other being 'S' or 'R'.<sup>20</sup>

Summary statistics for the duration of tests (mean, SD and 95% CI) will be calculated for the TTR, collected in minutes as continuous variables. The difference of TTR between Resistell AST, VITEK 2/BD Phoenix systems and Kirby-Bauer Test will be assessed by one-way analysis of variance with Tukey's HSD post hoc comparison. Continuous variables will be compared using the Student's t-test and Mann-Whitney U test for normally and non-normally distributed variables, respectively. The  $\chi^2$  test or Fisher's exact test will be used to compare categorical variables.

### Other assessments

Summary statistics on data gathered from clinical charts (if available) will be performed, along with 95% CI. In addition, the following variables will be collected: age, gender, site of infection, height, body mass index, drug allergies and antibiotic history.

### Timeline schedule

After the first site initiation visit on 25 May 2021, the first patient for the pilot study was enrolled on 7 June 2021, and recruitment was stopped in December 2021 (figure 4). Six months from the recruitment of the first patient, the recruitment rate and proportion of resistant strains were assessed and further samples were collected up to 10% of the required samples were reached. An interim analysis is ongoing to assess potential problems, confounders or critical factors to adjust before the main study. The main study will start after this evaluation and will continue until the optimal sample size calculated is reached (or the study stopped for other reasons).

### Sample size calculation and statistical methods

Based on the preclinical validation, the Resistell AST is expected to be at least 95% accurate.

## Study Timelines



**Figure 4** The study started in June 2021 with the pilot phase, which was completed in December 2021. RMV performed by the external CRO for quality assessment. RMV, routine monitoring visit. FPFV = first patient first visit. CRO = contract research organization, i.e a company that provides clinical trial management services for biotech industries.

To this aim, a total sample size of 240 (with anticipated 209 susceptible samples) achieves 90% power to detect a change in sensitivity from 0.97 of the ‘gold standard’ to 0.8 of Resistell AST using a two-sided binomial test, with target significance level at 0.05. The actual significance level achieved by the sensitivity test is 0.0377. About 87% of the PBCs analysed with Gram-negative bacteria at Lausanne University Hospital are susceptible to antibiotics. Therefore, the sample size was rounded to 300 to account for withdrawals, with an inflation of about 20%. The sample size was calculated using PASS 2019, V.19.0.5 (NCSS, Kaysville, Utah, USA).

### Data storage and retention

A unique anonymised identifier will be used to track patients, samples and their clinical data. A list will be maintained at the investigational site with this unique identifier, along with the patient’s hospital number and the sample identification code; only the investigators and the study nurse will have access to it. Another list will be included in the electronic case report forms (eCRFs) with the patient’s unique identifier and medical record only accessible to investigators, study nurse and the contracted research organisation (see the Roles and responsibilities section). All records will be stored on a secured electronic data capture platform ([www.Smart-Trial.com](http://www.Smart-Trial.com)).

### Traceability

During the clinical investigation, traceability of the Resistell AST will be achieved according to Resistell quality management, that is, each device is identified by a unique serial number, while nanomotion sensors are identified by lot numbers. All device service activities are kept in a ‘Revision Track’ online system. The model name and serial number of the device are displayed on the device label. Full traceability is established using unique device serial numbers, delivery notes, incoming inspection, installation and calibration.

### Roles and responsibilities

The manufacturer of the investigational device (Resistell AG, Switzerland) is the sponsor of the study, who is responsible to competent/health authorities for taking all reasonable steps to ensure the proper conduct of the clinical trial as regards ethics, clinical trial protocol compliance, and integrity and validity of data recorded in the eCRF. The sponsor contributed to the study’s design, together with the investigators, and will take part in the report for publication. The ultimate authority over the decision to submit the work for publication and samples’ collection, study management, analyses, interpretation of data and writing the report is reserved to the investigators. The sponsor reserves the right to discontinue the study at any time for any reason.

The coordinating centre (Lausanne University Hospital) is composed of the coordinating investigator, two subinvestigators, two study nurses/coordinators and two operators. The investigators will allow inspections of the study documentation and maintain patient confidentiality during all audits and inspections from the sponsor, the sponsor representative, the ethical committee (EC), external auditors or representatives of the regulatory authorities. Furthermore, they will ensure that the investigational device and the study documentation are stored in a secure area under recommended storage conditions and in accordance with applicable regulatory requirements. Investigators are also responsible for safety reporting to their local EC, following its reporting requirements and timelines.

An external contract research organization (CRO) has been commissioned to help the investigators and the sponsor maintaining a high level of ethical, scientific, technical and regulatory quality in all aspects of the clinical trial. It will be part of the steering committee, which participates in endpoint adjudication. It will have access, at any time, to the source documents, signed consent

**Table 1** Categories of adverse events

Adverse events	Non-device related	Device related	
In the scope of this study, the device may cause direct harm to the user or another person; as the study is performed on previously collected samples and is non-interventional, no harm is anticipated to the patients.			
Non-serious	Adverse event	Adverse device effect	
Serious	Serious adverse event	Serious adverse device effect	
		Anticipated	Unanticipated
		Anticipated serious adverse device effect	Unanticipated serious adverse device effect

forms and all other study-related documents. The external monitoring team will perform routine monitor visits monthly and will be responsible for reporting the reportable events (severe adverse events (SAEs), device deficiencies that might have led to SAEs and new findings/updates linked to already reported events) to the competent authority completing appropriate adverse events (AEs) forms.

An independent Data Monitoring Committee (DMC) will monitor the safety of the study and advise the sponsor. The DMC will be formed and composed of three medical doctors; the members of the DMC are otherwise not actively involved in the trial. The primary responsibilities of the DMC are the following: (1) to assess results of the pilot phase in the interim analysis, (2) to periodically review and evaluate the accumulated study data for effectiveness, (3) to make recommendations concerning the continuation, modification or termination of the study. In addition, the DMC considers study-specific data and relevant background knowledge about the disease, test agent or subject population under study.

### Safety reporting

AEs will be summarised by severity and by relationship to the study device. All safety events will be coded according to Annex G of ISO EN 20916:2019.

All applicable AEs will be routinely monitored by the DMC and reported in interim and final study reports. The applicable categories are described in [table 1](#).

Both investigator and Resistell/CRO will make a causality assessment of the event for the Resistell AST. Device deficiencies that might have led to an SAE are always related to the device. All AEs judged by either the reporting investigator or the sponsor as having at least a 'possible' causal relationship to the study device qualify as an adverse reaction or as an adverse device effect (or both). All SAEs will be evaluated, regardless of whether they are related to the investigational medical device or anticipated.

### Informed consent and participant withdrawals

The lead researchers or a delegated member will obtain informed consent at the start of the study visit directly from the patient or from next of kin/legal representative (online supplemental files 1–3). Alternatively, a signature from an independent physician (treating the patient)

will be obtained (online supplemental file 4). A further a posteriori ICF will be submitted to the patient when again able to sign it (online supplemental file 5). The investigators will explain the nature of the study, its purpose, the procedures involved, the expected duration, potential risks and benefits and any discomfort it may entail. All those obtaining consent will have received informed consent training as well as Good Clinical Practice training. Participants have the right to withdraw from the study at any time, without giving a reason and without affecting their future care. If withdrawn from the study, all gathered information will be destroyed.

Study-related procedures and documentation should end on the day of the patient's study termination. However, SAEs directly related to the investigational device, which are not resolved on the date of the patient's study termination, will be followed until termination of the study, if not resolved before. In addition, any harm to the user arising due to the device or the investigation will be followed up until the conclusion.

Subjects will receive standard medical care arising from SAEs after completion of the investigation.

### Patient and public involvement

No formal patient advisory committee was established, and there was no patient or public involvement in the design and planning of the study.

### Ethics and dissemination

The study will be conducted according to the ethics and regulatory principles. All local regulatory requirements will be adhered to. Before initiating the study, the investigators obtained a written and dated favourable opinion from the national ethics committee (swissethics, project 2020-01622). All the CRFs and clinical samples will be assigned a study code by the local investigators and stored anonymously at the reference centre. The results will be broadly distributed through conference presentations and peer-reviewed publications.

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**Contributors** GG conceived the idea and devised the project and its main conceptual ideas assisted by GC, AK, DC, MPV, AS, JD, ED and AL. GC and GG supervised the clinical study. GC also wrote the manuscript, assisted by AL, ED, AS, DC and GG. AK, AV, AS, GJ and JD provided editorial input. AV, AL and ED had hands-on conduct of the experiments. GC, assisted by AK, performed patients' inclusion and supervised the patients' clinical data collection. SK supervised the ethical, scientific, technical and regulatory quality aspects.

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**Competing interests** GG is medical advisor for Resistell. AV, AL, ED, AS, DC and GJ are employed by Resistell. GC, AK, SK and JD have no conflict of interest.

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**Patient consent for publication** Not required.

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