(PROTOCOL)

Tuberculosis screening with AeoNose and CAD4TB in Paraguayan prisons

PriNose Study

December 2019

PROTOCOL TITLE

Tuberculosis screening with AeoNose and CAD4TB in prisons in Asunción, Paraguay

Acronym: PriNose

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LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

AE	**Adverse Event**
AR	**Adverse Reaction**
CCMO	**Central Committee on Research Involving Human Subjects; in Dutch: Centrale Commissie Mensgebonden Onderzoek**
CAD	**Computer Aided Detection**
CRF	**Case Report Form**
CXR	**Chest X-ray**
EDC	**Electronic Data Capture**
EU	**European Union**
TB	**Tuberculosis**
GCP	**Good Clinical Practice**
GDPR	**General Data Protection Regulation; in Dutch: Algemene Verordening Gegevensbescherming (AVG)**
IC	**Informed Consent**
MGIT	**Mycobacteria Growth Indicator Tube (BD Products)**
MREC	**Medical research ethics committee (MREC)**
NAAT	**Nucleic Acid Amplification Test, for example GeneXpert®**
PIF	**Participant Information Form**
PNCT	**Programa Nacional de Control de la Tuberculosis (National TB Program)**
PPL	**Personas Privadas de Libertad / people deprived of liberty, prisoners**
POC	**Point-of-care**
PRONASIDA	**Programa Nacional de Control de VIH-sida (National HIV Program)**
Sponsor	**The sponsor is the party that commissions the organisation or performance of the research, for example a pharmaceutical company, academic hospital, scientific organisation or investigator. A party that provides funding for a study but does not commission it is not regarded as the sponsor, but referred to as a subsidising party.**
WMO	**Medical Research Involving Human Subjects Act; in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen**
WHO	**World Health Organization**

SUMMARY

Rationale: Despite being a curable disease, each day thousands of people die of tuberculosis (TB) worldwide. TB diagnosis is often delayed, increasing the likelihood of transmission. The aim of the World Health Organization (WHO) “End TB Strategy” is to end the global pandemic by 2035. To be able to succeed, better point-of-care (POC) tests are urgently needed to improve screening of high-risk populations. Prisons are recognized worldwide as high risk environments for the concentration, amplification and transmission of TB among prisoners and their communities outside. Paraguayan penal institutions are known to have very high incidence rates of active TB (3000-5000/100.000, according to Paraguayan Ministry of Justice). Two diagnostic tools for TB screening in high risk groups need additional validation: the AeoNose™, an ‘electronic nose device’ for breath sampling, and digital chest X-ray (CXR) with computer aided detection with CAD4TB® software. This study will systematically screen prisoners and its’ employees for TB, test the diagnostic performance of AeoNose™ and CAD4TB® and assess prison *Mycobacterium tuberculosis* epidemiology through several objectives: **Objective**: 1. Assessment of the sensitivity and specificity of the AeoNose™ and its utility for mass TB screening in high incidence settings. 2. Evaluation of the feasibility and utility of CAD4TB® digital CXR as a mass screening tool for TB. 3. Identification of factors that affect diagnostic accuracy of AeoNose™ and CAD4TB®. 4. Assessment of TB epidemiology in Paraguayan prisons and identification of mycobacterial strains. 5. Assessment of cost effectiveness of different screening algorithms. 6. Characterization of transmission dynamics and potential measures for transmission reduction. **Study design:** Diagnostic cohort study. **Study population:** Detainees (PPL) of Paraguayan penal institutions as well as their employees. **Main study parameters/endpoints:** 1. Number of TB cases diagnosed with GeneXpert® and/ or culture of Mycobacterium tuberculosis complex. 2. Number of TB cases correctly identified by AeoNose™. 3. Number of TB cases correctly identified by CAD4TB®. 4. Costs and effectiveness of different screening algorithms. 5. *Mycobacterium tuberculosis* complex strains identified with Whole Genome Sequencing. **Nature and extent of the burden and risks associated with participation, benefit and group relatedness:**
Individual burden: all participants will perform one visit with a medical doctor for medical history, physical exam, digital X-ray (with CAD4TB®) and AeoNose sampling. Participants will be offered voluntary HIV testing and counseling. In case of presumptive TB (estimated 20-30% of participants) two sputum samples will be taken, either spontaneous or saline-induced. One sample is tested with GeneXpert and the other with liquid mycobacterial culture. Cases of presumptive TB with both negative GeneXpert® and culture results will be followed-up after three and six months for repeat testing.

Individual and group health benefit: all participants will have a medical consultation and CXR. In case of an alternative diagnosis, the prison health service will be notified. People diagnosed in the study with active TB will be notified and referred to receive treatment through the PNCT according to national guidelines. All participants will eventually benefit from less exposure to infectious TB cases through optimized infection control measures and treatment of infected individuals. The study will provide the PNCT with epidemiological data of this vulnerable group to optimize future TB control strategies.

Long-Term Benefit:

After the study, the Ministry of Health will have an accurate update on the incidence of TB in the three involved prisons. Based on this information, more effective strategies can be designed for TB elimination in Paraguayan prisons.

The Ministry of Health (department of telemedicine - PNCT) will receive the mobile digital X-ray machine and CAD4TB software that can be used throughout the country, especially in vulnerable groups and people living in remote areas.

1. INTRODUCTION AND RATIONALE

Every day, over 4700 people die of tuberculosis (TB). The aim of the World Health Organization (WHO) “End TB Strategy” is to stop the global TB pandemic by 2035. (WHO, Geneva 2010) TB is a curable disease, but frequently remains undiagnosed, leading to ongoing transmission, infection and suffering. More accurate and affordable point-of-care (POC) tests are urgently needed that are suitable for the low resource settings in which TB is most prevalent. The pandemic is driven by transmission, which occurs not only from known TB patients but also from persons with unsuspected TB or ineffective TB treatment. The former WHO strategy of screening only symptomatic persons with (low-sensitive) sputum smear microscopy needs to be changed drastically as many TB cases remain undiagnosed.

Target product profiles for new TB screening tools and diagnostics were devised stressing the high priority of a systematic screening test for active case finding. (WHO Consensus meeting report, Geneva 2014). New POC screening tests should rapidly identify high likelihood individuals to select them for subsequent laboratory tests that can confirm active disease. A cheap and rapid POC test can improve the efficacy and efficiency of screening algorithms by optimizing the use of expensive and time-consuming tests such as nuclear acid amplification tests (NAAT) or sputum mycobacterial culture.

A promising POC diagnostic tool is the AeoNose™; an electronic nose device. (The eNose Company, Zutphen, the Netherlands). The AeoNose™ works with three metal oxide sensors which gather ‘smell prints’ of volatile organic compounds in the subject’s breath. The collected vectors are sent via internet to a central computer and then compared to a neural network of TB breath profiles. After establishing a validated neural network, new breath profiles can be analyzed within minutes. The device is battery-powered, handheld and lightweight, making it very suitable for POC use.
The AeoNose™ showed promising results as a TB diagnostic tool in previous studies in Bangladesh and Paraguay. (Bruins, Tuberculosis 2013; Coronel Teixeira, J Infect 2017) Like digital CXR and CAD, an electronic nose device could be implemented in a screening algorithm to “rule in” subjects for further testing. This could bring improvements of case finding and infection control, efficiency and reduced costs. Before the AeoNose™ can be brought to the market the technology needs to be further tested and the neural network needs to be validated in a large high-incidence population.

Abnormalities corresponding with TB can be detected early on CXR, even in asymptomatic participants. The WHO now recommends to add CXR to screening algorithms (WHO,

Geneva 2013). Conventional X-ray technology is available even in many low-income countries, although imaging quality is often poor and systems need expensive X-ray films and skilled personnel to interpret results. Digital X-ray with computer-aided detection (CAD) has many advantages over conventional X-ray and is considered very promising for TB mass screening programs. A mobile digital X-ray laboratory with CAD has been developed and has already been used in several screening programs in high-incidence settings. (E.g. Philipsen, Sci Rep 2015) CXR is used to identify participants with presumptive TB and subsequently sputum is obtained for culture or a rapid molecular test (NAAT/GeneXpert®). Figure 1, adapted from Khan et al. (Eur Resp J 2017), illustrates the logistics of screening with CXR in either symptomatic participants (a) or mass screening in a vulnerable group or community (b).

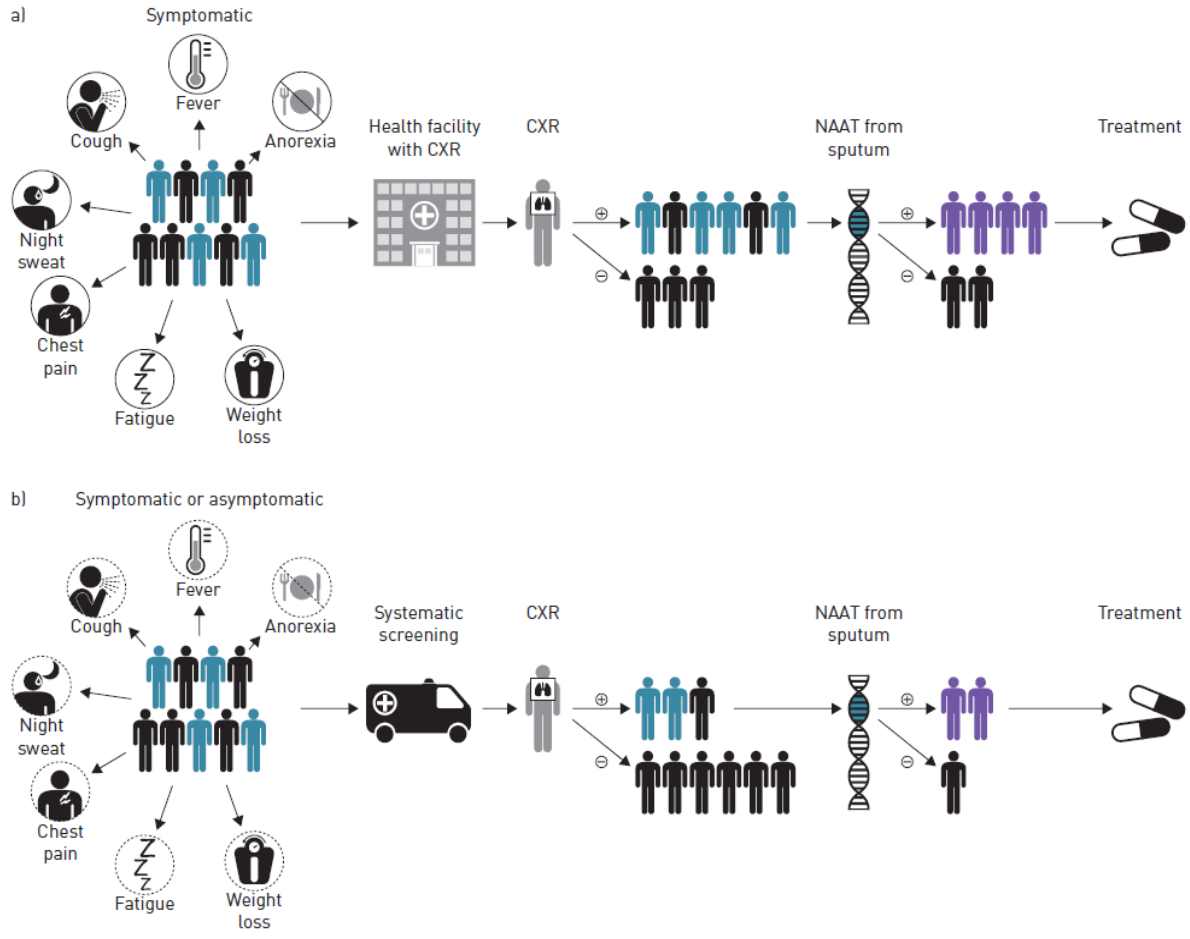


Figure 1. Screening in (a) symptomatic subjects or (b) a vulnerable group or community. Source: Khan et al. Eur Resp J 2017.

Currently, there are three commercially available CAD systems to detect TB on CXR that show no significant differences in performances. (Qin et al., NatureResearch-scientific reports 2019) CAD4TB® by Delft Imaging Systems (Veenendaal, the Netherlands) was

developed in collaboration with the Diagnostic Image Analysis Group from Radboud University Medical Centre (Prof. dr. B. van Ginneken). The CAD4TB® software has already been adopted for screening purposes, including studies in prisons. The newest software version (version 6) performs as good as skilled human readers, with a high sensitivity of 95% and excellent negative predictive values (NPV) as well as specificity of 70% , depending on the selected threshold value of the output score. (Melendez, Int J TB Lung Dis 2018) Still, there is evidence lacking for WHO-endorsement for CAD as part of large screening programs. (Khan, Eur Resp J 2017) Most importantly, an optimal threshold value of the output score must be identified for different population and patient characteristics. Additionally, CAD needs to be compared against skilled human readers, and safety and ethical issues need to be addressed concerning the incidental detection of lung malignancies and other non-TB pathology. Finally, the large-scale implementation of such technology needs to be evaluated from a health-economic viewpoint.

Setting: Prisons worldwide are high-risk environments for the concentration, amplification and further transmission of TB among prisoners, employees and the communities outside the prison. Sanitary conditions and healthcare services in prisons are often poor. Addressing the unmet healthcare needs of prisoners is crucial to achieve the goal of global tuberculosis control. (Kamarulzaman, Lancet 2016; Dara, Int J ID 2015) Paraguayan penal institutions also count with very high estimated incidence rates of active TB (3000-5000/100.000). Currently, since the assembly meeting in New York in 2018, reduction of TB incidence and transmission is a top priority for the Paraguayan Ministry of Health and the PNCT. In this study proposal, we aim to perform a mass TB screening in Paraguayan prisons using AeoNose™ and CAD4TB® and assess their utility for future screening algorithms.

2. OBJECTIVES

Primary Objective:

1. Assessment of the sensitivity and specificity of the AeoNose™ and its utility for mass TB screening.
2. Evaluation of the feasibility and utility of CAD4TB® digital CXR as mass screening tool for TB in high incidence settings.
3. Identification of factors that affect diagnostic accuracy of AeoNose™ and CAD4TB®.

Secondary Objective(s):

1. Assessment of TB epidemiology in Paraguayan prisons and identification of mycobacterial strains.
2. Assessment of cost-effectiveness and cost-efficiency of mass screening programs integrating digital CXR with CAD4TB®, AeoNose™ or both techniques in conjunction.
3. Characterization of transmission dynamics and potential measures for transmission reduction.

3. STUDY DESIGN

Diagnostic prospective cohort study: mass screening through conventional symptoms screening by a doctor, a physical exam and with testing of CAD4TB® and AeoNose™ as well as bacteriological testing of presumptive cases.

An estimated 5000 individuals will be invited to participate. All prisoners and employees will be eligible for inclusion. The sample size is based on an estimated incidence of 3000-5000/100.000 and a presumptive number of 150-250 active TB cases to be found in order to have enough cases to first train the neural network (with at least 500 - 1000 healthy individuals and a 100 active TB cases) and then perform the blind predictions on the rest of the participants to establish sensitivity and specificity of the AeoNose™.

The study consists of one primary visit and, in case of suspicion of presumptive TB but no confirmed TB or alternative diagnosis, two follow-up visits after three and six months respectively. The estimated total study duration will be from February 2020 to May 2021. The study settingwill be the Buen Pastor, Esperanza and Tacumbú prisons in Asunción, Paraguay Required condition to perform the study in each of these three institutions is safety for the study team and also the guards and prisoners. In case of unstable conditions we will look for an alternative penal institution.

Start

The entire prison population and all employees will be informed on the upcoming screening operation by prison authorities, prison health care workers and trained prison health promotors. Participants will be educated on the topic of TB and upcoming study through an awareness campaign before the start of the study.

Visit 1.

1. Participants will watch an animation (video) with voice-over in Spanish, Guarani or Portuguese with general information on TB and an explanation of the study procedures. Each participant will be offered to be tested voluntarily for HIV by PRONASIDA. An independent volunteer witness is present in the room to guarantee that the participant has understood the information correctly and is able to ask questions if necessary. Participants may then decide to sign informed consent using signature or fingerprint. Individuals can also decline participation, or take time to consider their participation and return later.
2. After providing informed consent, a study nurse will take vital signs (blood pressure, pulse and axillary temperature) and measure the participants weight, height, temperature, middle upper arm circumference, as well as ask for smoking habits, alcohol and/or drug. Co-morbidities, medication and food intake (4 hours prior to testing) will be recorded in the digital case record form. If the participant agrees a HIV rapid test will be performed using a finger prick blood sample. Participants with a positive result will be notified and referred to the national HIV program (PRONASIDA) for final diagnosis and treatment according to national HIV guidelines.
3. At the third station, a technician will perform the CXR and will process the digital image with CAD4TB™ software. A TB likelihood output score is presented within minutes.
4. At the fourth station, a technician will take a breath sample with an AeoNose™ device during 5 minutes in a room free of gasses and odours.
5. At the fifth station, a doctor will first take a history of symptoms and will then examine the participant for signs of TB:
    - Healthy / ill
    - Cough (15 days or longer)
    - Sputum or haemoptysis
    - Weight loss
    - Fever (continuous or intermittent)
    - Night sweats
    - Chest pain

- Dyspnea / shortness of breath
- Loss of appetite
- Lymphadenopathy
- Pulmonary auscultation
- Abdominal distension
- Anaemic conjunctivae

Also the dental status of the participants will be assessed.

The digital CXR image will be analysed by CAD4TB® and the doctor will interprete the CAD4TB® output score and check the CXR for alternative diagnosis. All the findings by the study doctor will be recorded in the digital case record form. At the end of this visit the doctor will decide on health status of the participant: either the participant is considered healthy or a presumptive TB case.

For healthy participants the study ends after this 5th station.

6. Presumptive TB cases (*see 6.1.1 Case definitions*) will be asked to produce two sputum samples outside, preferably in the open air. Sputum can be either be produced spontaneously or via sputum induction with nebulized saline. Saline solution will be nebulized using a portable nebulizer. Sputum samples can deposited in standard containers. Samples will be stored in a cool box and transported to the laboratory the same day. Samples will then be processed and analysed by the National Reference Laboratory, Laboratory San Alfonso and IICS using GeneXpert® molecular testing and MGIT mycobacterial culture.
7. All participants will be educated about the importance of seeking medical care if symptoms compatible with TB emerge at any time.
8. In case a participant is passively identified with TB during the study period by the prison health services or PNCT, the study researchers will be notified. Clinical and microbiological data will be shared based on informed consent given by the participant.

Follow-up of presumptive TB cases

Participants who are considered presumptive cases of TB at Visit 1, but with negative sputum results or unable to produce sputum, will be followed-up after three and six months.

<u>Visit 2 and 3 (at three and six months after Visit 1)</u>

Study procedures are the same as Visit 1 steps 1 through 5. Participants will be asked for symptoms, have a physical exam, undergo CXR with CAD4TB® and AeoNose™ breath sampling. At visit 2 and 3, all participants will be asked to produce two sputum samples. In case of positive results for *Mycobacterium tuberculosis*, confirmed cases will be notified to

the PNCT. After visits 2 and 3 a decision will be made by the study team on the final health status of the participant (either considered healthy or a TB case). In case of a high suspicion of TB the prison health authorities and the PNCT may decide to treat this individual for active TB. The result of this anti-tuberculosis treatment will be communicated with the study team.

Referral of TB cases and non-TB illness

In case of a positive culture result or GeneXpert® with *Mycobacterium tuberculosis*, the participant will be informed and the PNCT will be notifiedthrough the prison authorities. GeneXpert® will provide a test result within 24 hours.

In compliance with the current Paraguayan national TB treatment guidelines, established TB cases will start anti-tuberculous therapy and will be followed-up by the PNCT and prison health service.

For presumptive TB case with signs of severe illness but a negative GeneXpert® result, the PNCT and the prison health service will be notified. The prison health service and PNCT may decide whether it is justified to start empiric treatment for TB. This decision will be made available to the investigators who will review these cases afterwards to categorize them according to the formal case definitions.

If a participant presents to the study doctors with signs and symptoms compatible with severe illness, the prison health service will directly be notified in order to take an appropriate action.

If the digital CXR shows pathological abnormalities not being consistent with tuberculosis, the participant will be informed and referred to the prison health service.

4. STUDY POPULATION

4.1 Population (base)

Persons deprived of liberty (PPL) adults (≥18 years) and personnel of the three penitentiary centers of Paraguay (Buen Pastor, Esperanza and Tacumbú prison) in Asunción. Both male and female (estimated 90% vs. 10%). Ethnicity is mostly mixed / Latin-American as well as indigenous from several different ethnicities.

4.2 Inclusion criteria

1. All persons, either being a prisoner or employee, of the involved penal institutions after providing informed consent, including those with known active TB disease and/or currently on TB treatment.

1. Exclusion criteria

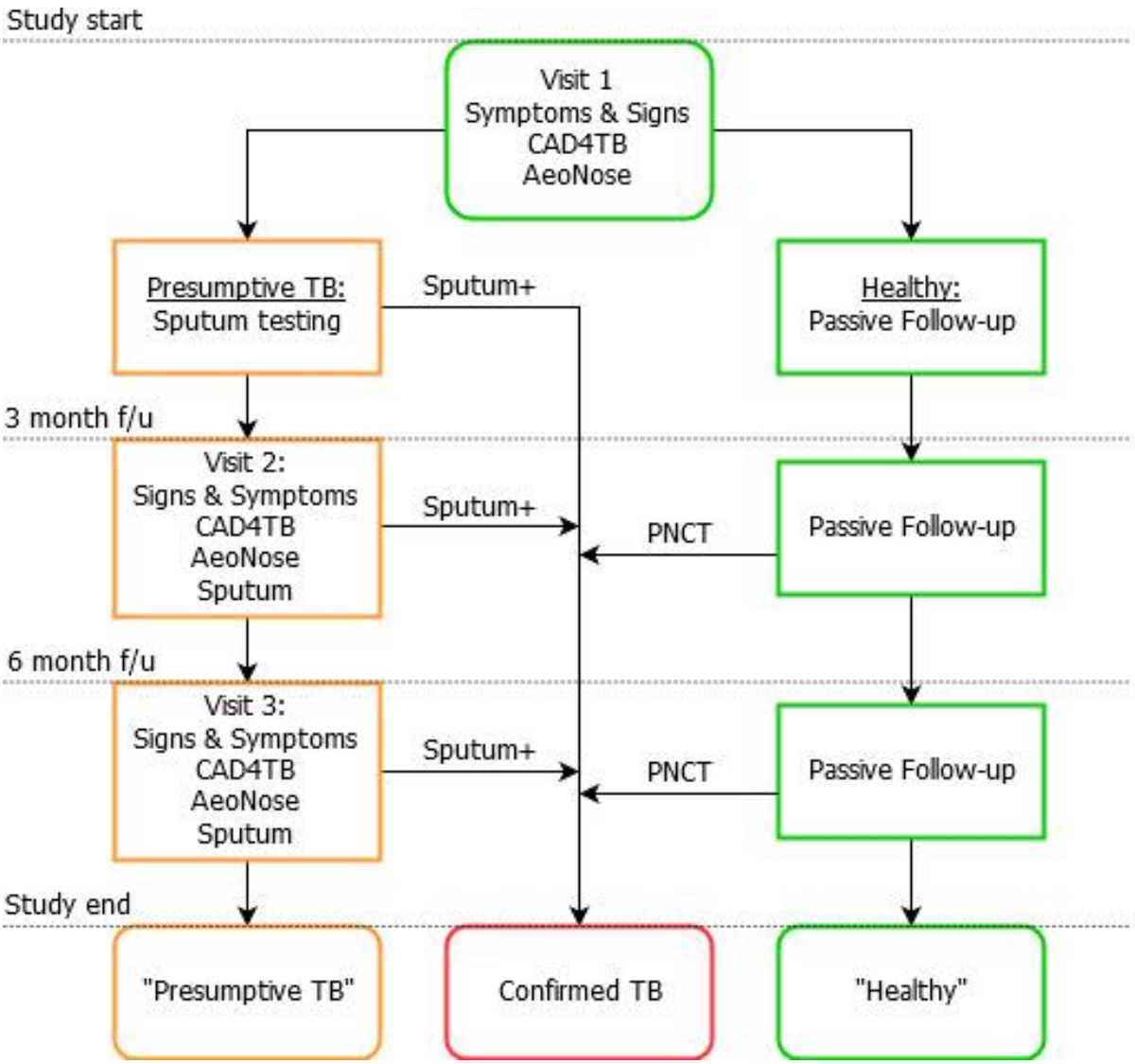
A potential participant who meets any of the following criteria will be excluded from participation in this study:

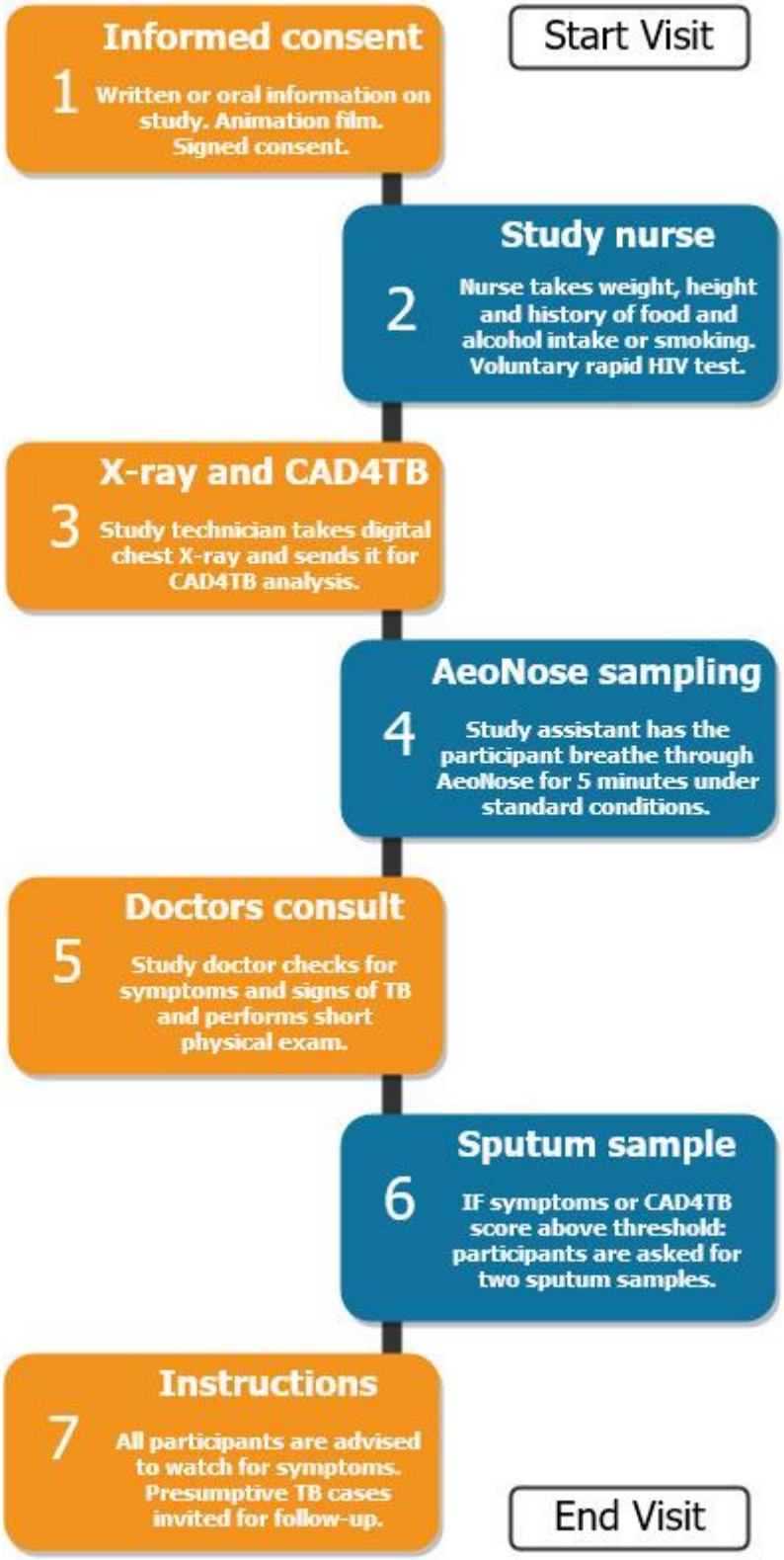
1. Unable to exhale breath through the AeoNose™ during five minutes due to respiratory insufficiency
2. Unable to stand in an upright position for the CXR
3. Unable to communicate and comply with the instructions of the study team

4.3 Sample size calculation

Based on WHO definitions, mass screening includes every person in a vulnerable community. The combined prisons of Tacumbú, Esperanza, Buen Pastor house around 4700 PPL. An additional 500 employees (estimated number, to be confirmed) who are in direct contact with the PPL will also be included. Based on calculations we expect to detect around 200-240 cases of active tuberculosis in prisoners (3-6% of 4000 PPL) and 0-5 cases in employees (estimated 0.2% of 1000 employees). The number of expected cases in the latter group could be an underestimation as these people are in constant contact with PPL and have a higher risk than the general population of active TB.

The study sample size is based on the need for at least 100 cases with active TB to build a robust AeoNose™ neural network (calibration model) and subsequently another 100-150 cases to make a blind prediction (validation model). A systematic literature review of acceptability of TB screening among risk groups showed an average percentage of screening consent of 72% among prisoners. (range 18-100%, Mitchell et al. 2012)


Study start
Visit 1
Symptoms & Signs
CAD4TB
AeoNose
Presumptive TB:
Sputum testing
Sputum+
Healthy:
Passive Follow-up
3 month f/u
Visit 2:
Signs & Symptoms
CAD4TB
AeoNose
Sputum
Sputum+
PNCT
Passive Follow-up
6 month f/u
Visit 3:
Signs & Symptoms
CAD4TB
AeoNose
Sputum
Sputum+
PNCT
Passive Follow-up
Study end
"Presumptive TB"
Confirmed TB
"Healthy"


Start Visit
Informed consent
1
Written or oral information on study. Animation film. Signed consent.
Study nurse
2
Nurse takes weight, height and history of food and alcohol intake or smoking. Voluntary rapid HIV test.
X-ray and CAD4TB
3
Study technician takes digital chest X-ray and sends it for CAD4TB analysis.
AeoNose sampling
4
Study assistant has the participant breathe through AeoNose for 5 minutes under standard conditions.
Doctors consult
5
Study doctor checks for symptoms and signs of TB and performs short physical exam.
Sputum sample
6
IF symptoms or CAD4TB score above threshold: participants are asked for two sputum samples.
Instructions
7
All participants are advised to watch for symptoms. Presumptive TB cases invited for follow-up.
End Visit

5. INVESTIGATIONAL PRODUCT

5.1 Name and description of investigational product(s)

- AeoNose™ (the eNose Company, Zutphen, the Netherlands):
  The Aeonose™ is a diagnostic device for exhaled-breath analysis. It is portable and requires a participant to breathe through the device for five minutes at tidal breathing. Volatile organic compounds (VOC) in the breath are captured by metal-oxide sensors. The unique 'smell print' of TB can be identified by an algorithm that is built on a de-blinded sample of the population in the calibration phase using an artificial neural network.
- CAD4TB® (Delft Imaging, Veenendaal, the Netherlands):
  The CAD4TB® software analyses an X-ray image and detects the many different abnormal structures that may be related to TB. The system has been trained in the detection of these abnormalities by applying machine learning to thousands of healthy and diseased X-rays from over 15 countries, through which it has become accurate and reliable. The output of the CAD4TB® software is a score between 0 and 100, indicating the likelihood that the participant, above a threshold score, has possible, probable or definite (active) TB. The CAD4TB® software also outputs a 'heatmap' – a colour-coded image of the lungs indicating regions of abnormality.

5.2 Summary of findings from clinical studies

- *AeoNose™:* In recent years we performed several studies to test the AeoNose™ as a POC screening test, as well as a biomarker of treatment response. In a pilot study in 194 subjects in Bangladesh, a sensitivity of 93.5% and a specificity of 85.3% was detected discriminating healthy controls from TB patients. (Bruins, Tuberculosis 2013) In a pilot study in 110 subjects in Asuncion, Paraguay, the AeoNose™ showed a sensitivity of 88% and a specificity of 92%. (Coronel Teixeira, J Inf 2017) The device showed ease of operation and no adverse effects were observed. A field study in 2016 in an indigenous community in Paraguay (Puerto Casado) showed a specificity of 92% (IndiPaNose study, manuscript under revision), with no sensitivity calculated because of no actual active TB cases in the community. A pilot study assessing changes after initiation of therapy showed a fall in AeoNose™ signal correlating with treatment outcome (clinical signs and symptoms, CXR and sputum smear), demonstrating its potential as a biomarker for treatment response. (Elnosur study, poster presentation ERS congress 2015). An AeoNose™ study in 327 Indonesian patients differentiating pulmonary TB from suspected pulmonary TB demonstrated a lower sensitivity and specificity of 78-85% and 42-55%, respectively. The study indicated

the need for a large calibration group for better pattern recognition. The authors concluded that the AeoNose™ is better suited for screening purposes than clinical diagnosis. (Saktiawati, PLOS One 2019). The utility of the AeoNose™ for screening purposes was recently confirmed by researchers from Peru who demonstrated the possibility of using the AeoNose™ to identify high-risk patient who should receive confirmatory TB testing as part of a FAST strategy (Find cases Actively, Separate safely, and Treat effectively). AeoNose™ demonstrated a sensitivity of 85-93% and specificity of 63-73% in a population of 629 patients that underwent AeoNose™ analysis on admission to the hospital. (Nathavitharana et al., accepted abstract, The Union conference 2019)

- *CAD4TB®:* CAD4TB® has been widely adopted over the past years, currently analyzing over 5000 CXR every day in clinics all over the world, including in prison facilities. The CAD4TB® software is going through continuous improvements. In a systematic review of CAD for detection of tuberculosis, version 1 and 3 of the software achieved AUC of 0.71-0.87 for sputum confirmed cases. (Pande, Int J TB Lung Dis. 2016) Version 5 of the software demonstrated an AUC of 0.90 in 47510 images with the clinical decision to treat as reference. (Melendez, Int J TB Lung Dis. 2018) Sensitivity and specificity is dependent on the selected threshold score. Threshold scores achieving high sensitivity (≥95%) can be reached with moderate-to-high specificity, performing similarly to expert radiologists. Version 6 of CAD4TB® was released in 2018. In a validation data set of 5665 CXR images the software attained an AUC of 0.987 for radiological reference and 0.885 for bacteriological reference (Murphy et al. Arxiv pre-print March 2019). CAD4TB® has already been tested as a triage tool to select presumptive TB cases for GeneXpert® testing. In a study in 388 South African presumptive TB cases, pre-screening with CAD4TB® version 3 led to an almost 50% reduction in cost per screened subject and cost per notified TB case, and more than doubled the number of subjects screened per day, at the expense of only a small number of missed cases. (Philipsen, Sci Rep 2015) These benefits were realized by quickly identifying TB negative subjects and reducing the total number of expensive and time-consuming NAAT tests.

6. METHODS

6.1 Study parameters/endpoint

6.1.1 Case definitions:

Presumptive TB:

Any participant with symptoms or signs suggestive of TB as judged by the study doctor.

AND / OR

Any participant with CAD4TB output score above the threshold level (to be determined), or presenting with CXR abnormalities as judged compatible with TB by the study doctor.

Respiratory symptomatic

Any participant presenting with cough or phlegm of duration of more than 14 days.

TB case:

A participant in whom TB has been confirmed bacteriologically, or a participant who started treatment empirically and showed response to treatment after the initial phase of TB treatment, as documented by the PNCT.

TB Case Classification

1. A bacteriologically confirmed case of pulmonary TB is defined by either GeneXpert® OR mycobacterial culture (MGIT™) positive for *M. tuberculosis complex*
2. A bacteriologically unconfirmed case is defined by:
    1. Not meeting definition for bacteriologically defined case
    2. Decision by the PNCT of the necessity for empiric treatment with anti-TB chemotherapy

    A TB case is categorized by anatomical site of disease:
    3. Pulmonary TB (PTB): The disease affects the lung parenchyma. Pulmonary TB is the most common form.
    4. Extrapulmonary TB (EPTB): The disease affects other sites including lymph nodes, pleurae, meningeal, pericardial, peritoneal, spinal, intestinal, genitourinary, larynx, spine, bones and joints, and skin.
    5. A patient diagnosed with both pulmonary and extrapulmonary localisations of TB is classified as PTB + EPTB.

Classification based on history of Previous Treatment (in accordance with Paraguayan National TB guidelines):

Cases will be defined to whether or not the participant has previously received TB treatment.

- New treatment: A participant who has never had treatment for TB or who has taken anti-TB medicines (for treatment of active TB) for less than one month at time of enrolment.
- Current treatment: A participant who is currently taking anti-TB medicines (for treatment of active TB) for one month or more at time of enrolment.
- Previous treatment: further classified according to result of last treatment:
    - Relapse: A participant previously treated for TB, who has been declared cured or has completed treatment up to the final stage of treatment and currently diagnosed with a new episode of active TB, whether a reactivation or new episode of TB (re-infection)
    - Failure: A participant who has treatment failure at the end of his/her most recent treatment.
    - Lost to follow up: A participant declared lost to follow-up during his/her most recent treatment.
    - Other previously treated participants: All participants who have been previously treated but with unknown or undocumented treatment outcome of the most recent treatment.
    - Unknown history of previous treatment: participants who relate having been treated but without documentation, only verbal reference.

Classification based on patterns of drug resistance:

- Monoresistant, polyresistant, MDR, XDR TB (in accordance with Paraguayan National TB guidelines)

Classification based on HIV status

- Case of TB with HIV
- Case of TB without HIV
- Case of TB with unknown HIV status

Bacillary load and Severity of TB:

Bacillary load, as determined by:

- GeneXpert® cycle time (Ct) value
- MGIT™ culture time-to-positivity

Radiological severity

- CAD4TB score
- Unilateral, bilateral, pleural, miliary

- Presence of cavitations
- Percentage of lung affected as defined by a posteroanterior X-ray projection (<33%, 33-66%, >66%)

- Clinical severity (TBscore, Wejse et al. 2008 and TBscoreII, Rudolf 2014)
  Severity according to TBscore 2008: Class I (0-5 points)
- Class II (6-7 points)
- Class III (8 or more points)

Parameter	Points assigned *TBscore*	Points assigned *TBscoreII*
Self-reported:		
Cough	1	1
Haemoptysis	1	-
Dyspnea	1	1
Chest pain	1	1
Night sweating	1	-
Anaemic conjunctivae	1	1
Tachycardia (>90/min)	1	-
Positive finding at lung auscultation	1	-
Axillary temperature >37.0	1	-
BMI <18	1	1
BMI <16	1	1
MUAC <220	1	1
MUAC <200	1	1

BMI: Body Mass Index; MUAC: Middle Upper Arm Circumference.

6.1.2 Main study parameter/endpoint

1. The total number of TB cases found by GeneXpert® and/ or culture of Mycobacterium tuberculosis complex.
2. Number of TB cases correctly identified by AeoNose™.
3. Number of TB cases correctly identified by digital X-ray with CAD4TB®.
4. Factors affecting or influencing sensitivity and specificity of AeoNose™ and CAD4TB®, e.g. medication use, smoking and drugs, co-morbidities, BMI, dental status

6.1.3 Secondary study parameters/endpoints

1. Costs of different (theoretical) screening algorithms using AeoNose™, CAD4TB® or both in conjunction.

2. Signal differences and diagnostic performance of AeoNose™ and CAD4TB® in:
    a. HIV cases (and CD4 count if available)
    b. Patients with syphilis (and disease stage if available)
    c. Diabetes cases (insulin dependent or non-insulin dependent)
    d. Cases of EPTB

3. Number of incidental CXR findings and the effect on CAD4TB® output score.

4. Numbers of *Mycobacterium tuberculosis complex* bacterial strains identified.

6.1.4 Other study parameters

- Body weight (kilograms)
- Height (centimetres)
- Blood pressure and heart frequency
- Body temperature (axillary)
- Upper arm circumference
- Date of birth
- Place of birth
- Place of residence
- Gender (male/female)
- Sexual orientation
- Nationality
- Level of education (none, primary school, secondary school, vocational, higher education, unknown)
- Occupational history
- Civil state (single, married, widowed, separated, divorced, unknown)
- Ethnicity
    - If indigenous: specific indigenous ethnicity
- Dental state (good / plaque / inflamed gums / broken or missing teeth)
- Symptoms (including onset and intensity)
    - Cough
    - Sputum or haemoptysis
    - Weight loss

  - Night sweats
  - Chest pain
  - Dyspnea
  - Other
- Medical history and co-morbidities
- Previous diagnosis/treatment for TB (year and duration of treatment in months)
- Location in prison (cell, hallway, yard, others)
- Date of current prison entry, previous prison entries
- Medication (especially antibiotics)
- Smoking
- Drug use
- Factors influencing breath signal: intake and timing of ingestion of food, drinks, cigarettes
- CAD4TB® output score
- Radiological severity of TB
- AeoNose™ breath profiles
- GeneXpert® cycle time (if positive)
- MGIT™ culture time-to-positivity (if positive)
- Clinical severity of TB (TBscore, Wejse et al. 2008 and TBscoreII, Rudolf 2014)
- CXR abnormalities as described by study doctor (infiltrate, cavity, lymphadenopathy, mass lesion, pleural abnormality, etc.)

6.2 Study procedures

AeoNose™:
Participants will be asked to produce a breath sample by tidal breathing through the AeoNose™ device for 5 minutes. In case of interruption (e.g. cough) the interval between stop and re-start will be recorded. Participants will use a nose clamp to avoid inhaling unfiltered air. The air sample collection will be performed under standardized conditions in a room free of gases with no small molecules (such as alcohol) that can influence the breath profile of the participant. The exhaled-breath signals will be stored on the study computer and later sent to The eNose Company for analysis.

Digital CXR and CAD4TB®:
X-ray image will be taken using a digital X-ray system connected with CAD4TB® software. Participants will be asked to stand in front of the panel while one posterior-anterior X-ray image is taken. Images will be processed by the CAD4TB® software. A TB likelihood output score is presented to the doctor within minutes. Study doctors will also view and interprete the digital CXR during the consultation.

Sputum induction:

Participants with presumptive TB will be asked to produce two sputum samples. If necessary sputum can be induced using saline nebulisation sputum. The technique of sputum induction consists of inhaling an aerosol of saline over period of two minutes. This procedure will be preferably carried out in open air using a portable nebulizer and saline solution provided by the researchers. Participants are then instructed to expectorate in a standard sputum container.

6.3 Collection of data

Data collected during the screening will be recorded using electronic CRF's on the Castor EDC (electronic data capture) platform. (Castor EDC, Amsterdam, the Netherlands) Data will be inserted immediately and stored securely in the Castor study database. Information will be linked to a unique study ID number and contain no personal identifying information.
AeoNose™ breath profiles data, CXR images and CAD4TB® output will be securely stored and linked to the participant ID number.
After the breath profiles have been shared with The eNose Company they will be stored indefinitely (anonymized and encrypted) on a server in Amsterdam, the Netherlands.

6.4 Withdrawal of individual participants

Participants can leave the study at any time for any reason if they wish to do so without any consequences. All participants included for follow-up will be educated about their presumptive diagnosis and encouraged to follow-up either inside or outside the prison. The investigator can decide to withdraw a participant from the study for medical reasons (e.g. hospitalization for a non-TB illness).

6.5 Replacement of individual participants after withdrawal or lost to follow-up

Not applicable

6.6 Follow-up of participants withdrawn from treatment

Not applicable

6.7 Premature termination of the study

Potential reason for (a temporarily interruption or) premature ending of the study are if the security of participants and researchers or quality of the study can not be guaranteed in the Paraguayan prison environment. In case the study is ended prematurely, the sponsor will notify the accredited MREC within 15 days, including the reasons for the premature termination.

7. SAFETY REPORTING

7.1 Temporary halt for reasons of participant safety

In accordance to section 10, subsection 4, of Dutch Medical Research Involving Human Participants Act (WMO), the sponsor will suspend the study if there is sufficient ground that continuation of the study will jeopardise either the study team or participants health or safety. The sponsor will notify the accredited MREC (Laboratorio Central de Salud Publica, LCSP, Asunción, Paraguay) without undue delay of a temporary halt including the reason for such an action. The study will be suspended pending a further positive decision by the accredited MREC. The investigator will take care that study participants are kept informed.
In this study the safety risk is primarily the potential for aggressive or violent behaviour by PPL against other PPL, employees or members of the study team. The prison officials will ensure the safety of the study team and participants during the study.

7.2 AEs, SAEs and SUSARs

7.2.1 Adverse events (AEs)

Adverse events are defined as an undesirable or harmful experience occurring to a participant during the study. All adverse events, whether or not considered related to study procedure, which are reported spontaneously by the participant or observed by the investigator or his staff will be recorded. Potential adverse events are not expected in this study.

7.2.2 Serious adverse events (SAEs)

There is no risk of (serious) adverse events involved in participation in this study.

7.2.3 Follow-up of adverse events

Not applicable

7.2.4 Data Safety Monitoring Board / Safety Committee

Because the study investigations do not carry a health risk for the participants no Data Safety Monitoring Board will be installed.

8. STATISTICAL ANALYSIS

All data will be analysed using SPSS software. (IBM, United States) The data of the participants will be analysed cross-sectional. Follow-up of participants with presumptive TB but negative microbiological testing will be completed until 6 months after Visit 1. After a

follow up period of 6 months a final diagnosis will be made (either healthy or TB case and the participant will be offered treatment if indicated). A p-value (two-sided) < 0.05 is considered to indicate a statistically significant difference in all comparisons. Multivariate analysis will be performed to analyse influences of different combinations of participant characteristics.

8.1 Primary study parameter(s)

Primary study parameters are the number of TB cases cases by CAD4TB® CXR analysis and AeoNose™ breath sampling.
A binary assessment of the presence or absence of illness has four possible outcomes: true positives (TP), false positives (FP), true negatives (TN) and false negatives (FN). The relationship of the four outcomes is the basis of sensitivity and specificity analysis which determines the accuracy and predictive value of a diagnostic tool.

True positive: A positive test result in a confirmed TB case.
False positive: A positive test result with negative sputum results and no confirmed TB during follow-up.
True negative: A negative test result with no confirmed TB during follow-up.
False negative: A negative test result with confirmed TB during follow-up.

Receiver operating characteristics (ROC) will be generated using an ROC package, and bootstrapping to generate confidence intervals in the ROC plot. T-testing or McNemar's test will be used to compute P values when comparing the sensitivity of conventional screening and CAD4TB® and AeoNose™ at assumed levels of specificity. We will also use multivariate analysis to analyse influences of different combinations of participant characteristics.

CAD4TB®: The collection of digital CXR images will be analysed and compared to presumptive and confirmed cases. The number of true/false positives and negatives will be used to calculate sensitivity and specificity as described above. The predictive value of the test will be analysed.

AeoNose™: After the end of the study, a subset of inclusions will be used as 'calibration set' for the neural network. This set will be selected randomly and from participants throughout the whole study period to avoid bias from the AeoNose™ conditions over time, bias from the first volunteering participants who might be more ill than the others and bias from different penal institutions. To build a reliable calibration model we try to include at least 30-40% of all TB cases in the calibration set. After calibrating the neural

network, the breath profiles of the remaining participants will be tested for validation of the calibration model. A proprietary 'hybrid' tucker-tensor decomposition is used to compress the breath signal data in four dimensions (TP, FP, TN and FN) using PARAFAC22 and TUCKER323 kernels. The software used is customized for this specific data set-up. The compressed data is thereafter used to build a classification model using an artificial neural network (ANN) and the 95% confidence interval is calculated using the Wilson score interval. The result of the ANN is a similarity indication and the chosen threshold value determines the ratio between the outcomes.

8.2 Other study parameters

Not applicable

9. ETHICAL CONSIDERATIONS

9.1 Regulation statement

This study will be conducted according to the principles of the Declaration of Helsinki (8th revision, 2013) and in accordance with the Dutch Medical Research Involving Human Participants Act (WMO). Principles of Good Clinical Practice (GCP) will be adhered. The Medical Research Ethics Committee (MREC) of the National Reference Laboratory (LCSP) in Asuncion, Paraguay will approve the study. Permission to conduct the study has already been obtained from the prison authorities, the Paraguayan Ministry of Justice, and the Ministry of Health has expressed their special interest, emphasized the importance of the project and has called on their employees to cooperate with the project to let it succeed. The study is in accordance with the WHO Operational Guide on Tuberculosis Screening (2015) and Red Cross Guidelines for Control of TB in Prisons (2009).

9.2 Recruitment and consent

Participants will be recruited from employees and prisoners of three Paraguayan penal institutions:Tacumbú, Buen Pastor, Esperanza.

The study will be integrated in a wider prison public health campaign which will inform on tuberculosis, how to recognize the disease, how to prevent transmission and how to seek care.

During the study visit, participants will be informed about tuberculosis and the study procedures by an independent staff member who can answer questions. This staff

member will be trained to understand the study procedures and advantages and disadvantages of participation. An educational animation video with voice-over will be shown to all potential participants (Annex I). Participants will be informed of the test procedures and test results, including the potential benefits and harms of screening and the potential harms of not being screened. This information will be given in either Spanish, Guarani or Portuguese and adapted to a primary school educational level to be understood by all participants. The information will be presented in the presence of an independent witness, e.g. a prison or church volunteer. The participant can confirm his/her agreement with a signature or fingerprint.

At informed consent, participants will be asked for permission to exchange medical data between PNCT & PRONASIDA program and the study researchers.

In case of presumptive TB the participant will be informed immediately. Sputum and HIV test results will be communicated with the highest priority. Patients will then be referred to the indicated national program (PNCT and/or PRONASIDA).
In case of suspicion of other pathology, referral will be arranged to the prison health service.
See also the participant information form and informed consent form in *Appendix.*

9.3 Benefits and risks assessment, group relatedness

Prior to this study, none of the prisons perform the entry and/or annual TB screening recommended by WHO and ECDC (WHO, Geneva 2013; ECDC Stockholm/Lisbon 2018). TB cases are currently detected actively by trained prison health promotors and passively by seeking medical consultation due to symptoms. As TB often manifests with a prolonged period of mild or unspecific symptoms, many patients remain undiagnosed, untreated and contagious for a long period. Participation in the screening offers the participant an opportunity to be checked for TB. The mass screening offered in this study is an opportunity for the PNCT to comply with WHO recommendations for prison screening and thus improving the prisoners' healthcare and reducing the continuous spread of TB within and from the prisons. After this study the PNCT will possess accurate information on the prevalence of TB within Paraguayan prisons which can serve as the basis for future policy and program enhancements. CAD4TB® analysis brings no burden to the participant. Breath sampling with the AeoNose™ device is risk-free and a minimal burden for the participant. Group-relatedness is inherent in a mass screening study of a known high risk population. For an accurate assessment of the prevalence of TB in the prisons and

the utility of the CAD4TB® and AeoNose™ as a screening tool the entire group needs to be investigated without any inadvertent selection of participants.

9.4 Compensation for injury

Compensation for injury is not applicable as the study does not include any invasive procedures or other risk of injury. The sponsor requests the MREC for dispensation of the obligation to provide insurance for participants.

9.5 Incentives for participants

The participants will not receive an individual monetary compensation or equivalent for their participation. The only perceived incentive is to identify and address a potentially life-threatening disease that can be transmitted to fellow staff or prisoners, family and associated communities.

10. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

10.1 Handling and storage of data and documents

Participant data will be handled confidentially and anonymously. For each participant a unique identification code will be used to link the study data to the participant's personal characteristics. The personal data will be handled according to the European General Data Protection Regulation (GDPR).

Data will be recorded using electronic CRF's on the Castor EDC (electronic data capture) platform. (Castor EDC, Amsterdam, the Netherlands) Castor is compliant with all relevant regulations including ICH E6 Good Clinical Practice, GDPR, HIPAA, FDA 21 CFR Part 11, ISO 27001, and ISO 9001. Castor is also dedicated to the FAIR principles for data storage. (https://www.force11.org) Data will be inserted immediately and stored securely on the Castor servers using a dedicated study computer. Information will be linked to a unique study ID number only known to the study investigators and contain no personal identifying information. AeoNose™ breath samples as well as digital CXR and CAD4TB® output will be stored securely on the study computer and linked to the unique study ID. All data will be stored for ten years.

10.2 Amendments

Amendments are changes made to the research after a favourable opinion by the accredited MREC has been given. All amendments will be submitted to the MREC for approval.

A 'substantial amendment' is defined as an amendment to the terms of the MREC application, or to the protocol or any other supporting documentation, that is likely to affect to a significant degree:

- The safety or physical or mental integrity of the participants of the trial;

- The scientific value of the trial;
- The conduct or management of the trial; or
- The quality or safety of any intervention used in the trial.

All substantial amendments will be notified to the MREC and to the competent authority. Non-substantial amendments will not be notified to the accredited MREC and the competent authority, but will be recorded and filed by the sponsor.

10.3 Annual progress report

The sponsor/investigator will submit a summary of the progress of the trial to the accredited MREC once a year. Information will be provided on the date of inclusion of the first participant, numbers of participants included and numbers of participants that have completed the trial, serious adverse events/ serious adverse reactions, other problems, and amendments.

10.4 Temporary halt and (prematurely) end of study report

The investigator/sponsor will notify the accredited MREC of the end of the study within a period of 8 weeks. The end of the study is defined as the last patient's last visit.
The sponsor will notify the MREC immediately of a temporary halt of the study, including the reason of such an action.
In case the study is ended prematurely, the sponsor will notify the accredited MREC within 15 days, including the reasons for the premature termination.
Within one year after the end of the study, the investigator/sponsor will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited MREC.

10.5 Public disclosure and publication policy

The study will be registered at https://clinicaltrials.gov/, as well as https://eudract.ema.europa.eu/. Research data and results will be disclosed/published in the medical literature unreservedly (Open Access).

11. STRUCTURED RISK ANALYSIS

Participation in the study does not carry any health risk for the participant.

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13. ADNEXES

I. Approval Ministry of Justice
II. Approval Ministry of Public Health
III. PIF
IV. Case Record Form (CRF)