**Document**: Protocol Document

**Brief Title**: Point-of-Care rapid LFI (Lateral Flow Immunoassay) for Human Plague in Madagascar

**Project Title:** Evaluation of Diagnostic Tools " Lateral Flow Immunoassay (LFI) for Human Plague in Madagascar" (Testing Multi-Echelon Diagnostics in a Resource Limited Endemic Setting: Human Plague in Madagascar

**NCT Number**: *NCT04562012*

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August 19, 2020

Dear Clinical Trial Reviewer,

We are registering this novel research proposal conducted in Madagascar as a clinical trial study. It is our goal to present a description of this study in a straightforward way so that you can effectively evaluate the logic of the scientific design and how compliance to regulatory rules are met.

This is an investigational medical device study of a non-FDA cleared in vitro diagnostic (IVD) device/assay called Lateral Flow ImmunoAssay (LFI) for plague detection using a design concept similar to a home pregnancy test https://www.brimrosetechnology.com/detection-diagnostics. The study has been approved by an accredited Institute Review Board (Northern Arizona University FWA00000357) as a non-significant risk device study and minimal risk to human subjects (project ID 1582884). Since this study involves human subjects in Madagascar, this study was also approved by the local Malagasy ethics committee (CERBM - Comite D'Ethique de la Recherche Biomedicale FWA # 00000330). NAU is the IRB of record therefore is the overall lead institute for the human subject research component for this study. To conform to the requirements of the IRB regulations and gain the approval of two IRB boards, adequate measures are built in this study to ensure maximum protection of human subjects. This includes proper consenting, minimizing risk of participant harm (physical, psychological, social), preservation of confidentiality, data security, and a response plan.

Submitted by,

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**STUDY PURPOSE**

The **purpose** of this study will be to generate the data required to thoroughly validate the ability of plague LFI assay to accurately diagnose human infections with *Y. pestis*. These validation data will eventually be presented to the FDA (along with data from other types of studies not from human subjects) to seek approval for commercial license.

**STUDY OBJECTIVE**

The objective will be to validate this assay on the capillary blood of humans suspected to have plague.

**SPECIFIC AIMS**

The specific aims of this study are to enroll up to 300 participants who present clinical signs of illness based on specific inclusion criteria. We will collect two types of blood samples from enrolled participants 1) capillary blood from a finger prick and 2) venous blood. The capillary blood will be used for direct testing on the LFI assay and the venous blood will be used to perform independent validations using FDA approved methods. This study is designed as a correlation study to understand 1) how LFI assay results compare with results from traditional diagnostic methods based on DNA detection methods and bacterial culture isolate and 2) effectiveness of capillary blood to serve as a diagnostic clinical sample as compared with traditional biological samples (venous blood, bubo and sputum). The study is designed to evaluate the outcome of LFI (*SMART™-PRT*, New Horizons Diagnostics Corp. https://www.nhdiag.com/index.htm) and how LFI results correlate with the standard plague diagnostics methods used in Madagascar and two other FDA-approved methods. We are not examining the relationship between the results of the LFI and health outcomes of the participants. Decision of participant's medical treatment is solely based on the clinical judgment of the physician and guidelines set forth by Madagascar National Plague Control Program (PNLP). All participants who are tested by LFI will have received medical treatment prior to the start of the study and the continuation of their medical treatment is guided by PNLP and physician judgment only.

**BACKGROUND**

*Yersinia pestis*, which causes the disease plague, is ecologically established in rodent populations worldwide, including on all continents except Australia and Antarctica. Plague is a rapidly progressing highly feared infectious disease that historically was responsible for the deaths of millions world-wide due to natural outbreaks during three major pandemics. Within the last century, several nation states developed plague as a biological weapon and the US government currently lists *Y. pestis* as a potential bioweapon (1) that could be used against US war fighters. As a natural disease, plague continues to be a threat in modern times with the World Health Organization (WHO) classifying it as a re-emerging infectious disease . Human plague outbreaks occur naturally on an annual basis worldwide with the vast majority of cases (4,5) located in rural remote low-resource settings of Madagascar, an island nation off the east coast of Africa. Madagascar has more human plague cases than any other country in the world. In 2015 alone, WHO reported 85.93% of global cases came from Madagascar (2). Over a five-

year period, there have been more than 3,500 suspected human plague cases in Madagascar (Rajerison et al., unpubl).

In nature, humans acquire plague by being bitten by fleas carrying *Y. pestis*. The bacterium travels to the nearest lymph node (LN) from the wound site and causes LN swelling, known as a bubo (hence, bubonic plague) within 3-6 days post infection with a 50%-70% mortality rate if untreated. If bubonic plague is not medically treated, it can further progress to infect the lungs (pneumonic plague) for which person to person transmission can ensue. Pneumonic plague, is a rapidly progressing disease (1-3 days) with 100% mortality rate; for this reason, aerosolized *Y. pestis* is of concern as a potential biological weapon. Importantly, there are no licensed vaccines currently available for plague, so antibiotics are the only means to medically defeat this disease. Key to preventing mortality and further transmission in rural settings is the ability to rapidly diagnose at the very early acute phase of the disease. Such a diagnostic assay would need to be highly simple and usable in a low-resource setting to effectively service the human populations most affected (local rural inhabitants and possibly warfighters).

The existing rapid diagnostic assay generally relies on detection of the F1 antigen of *Y. pestis* or serodiagnosis of anti-F1 antibody (6). A rapid assay, F1RDT (3) is not licensed for commercial use but is used in low-resource settings worldwide. This assay uses non-blood clinical samples (bubo aspirates and sputum) to diagnose. Procuring bubo aspirate for diagnosis requires an invasive collection procedure that is significantly more painful than blood sample collection. The research here aims to evaluate the diagnostic effectiveness of a blood-based rapid diagnostic assay. This is a US-developed assay based on a Lateral Flow ImmunoAssay (LFI) design concept similar to a home pregnancy test. The biological sample target is capillary blood from a finger prick. The simplicity of design enables this assay to be used in low-resource settings. We will evaluate the expression levels two *Y. pestis* proteins in the capillary blood of humans suspected to have plague and determine how these levels correlate with results from traditional diagnostic assays.

***Lay Summary*:** Plague is a deadly but highly treatable disease caused by the bacterium *Y. pestis*. Due to the historical development of *Y. pestis* as a bioweapon by several nation states, it is listed by the US as a potential bioweapon that could be used against US warfighters. Although this bacterium is ecologically established worldwide, it mostly affects impoverished people who live in rural low-resource areas of Madagascar. Plague is acquired directly from bites of infected fleas but, if left untreated, it can progress to the highly lethal pneumonic form that can result in human to human transmission. With the dangers of pneumonic plague in the context of both natural outbreak and as a bioweapon used against warfighter, the goal of this study is to investigate a diagnostic test that is able to rapidly and locally diagnose this disease in low-resource settings. This study aims to evaluate a US-developed new LFI assay intended for capillary blood to diagnose humans infected with *Y. pestis*. We will rigorously validate with assay on human populations from active plague sites and correlate the results with the results of paired clinical samples used in standard medical workup using existing diagnostics tests.

**RESPONSIBLE INSTITUTES**

Brimrose is the sponsor for this clinical trial study and will be the entity to manufacture the LFI device. David Trudil, Director Biotechology Division at Brimrose, will be responsible for overseeing the device component of this entire study. He will take the data generated from this study to the FDA for clearance request. Northern Arizona University (NAU) is the lead institute for overseeing the field work conducted in Madagascar which involve enrolling human subjects suspect to be plague positive and test the LFI device on their clinical samples. NAU has partnered with Institut Pasteur de Madagascar (IPM) to perform this field work. Madagascar is the region with the most active plague site in the world documented by the WHO and, for this, IPM is the world's expert on managing human plague outbreaks, and they have the field work expertise necessary to successfully validate this LFI assay. The technical expertise for the LFI assay will come from the Naval Health Research Center (NHRC). They will provide quality training and will serve as a technical liaison. As the lead institute, NAU (https://in.nau.edu/pmi/) will oversee the work performed by our International Partner (IPM) and will coordinate the training of IPM personnel. Dr. Wagner will be responsible for the global scientific direction for the field work and Dr. Birdsell will be responsible for the coordination of the collaboration for the human subject research that occur in Madagascar.

***International Partner*:** IPM is the lead plague research organization in Madagascar and has collaborated with NAU on several previous plague studies in Madagascar. Diagnosing and reporting of human suspect plague cases is mandatory by Madagascar national law under Plague National Control Program (PNLP). The Madagascar Ministry of Public Health (MMPH), as part of PNLP, requires declaration of all suspected human plague cases and mandates the collection of biological samples (sputum and bubo aspirates, but not blood) from these cases as part of the confirmation procedure. Human plague outbreaks primarily occur in rural low-resource settings. These cases are treated at local rural health centers located throughout Madagascar. Local physicians collect clinical specimens (bubo aspirates and/or sputum), which are then transported to IPM, located in Antananarivo, where sample workup is performed. IPM facility serves as the national plague reference laboratory, and has stored 90% of human samples and bacterial isolates from recent cases across five years. Drs. Minoarisoa Rajerison (head of the plague unit – Science officer) and Voahangy Andrianaivoarimanana (senior scientist - PI) are key IPM personnel. Personnel conducting the field study include: Angeltine Lalao Ralafiarisoa and Soloandry Rahajandraibe. All the IPM field investigators are medically and/or paramedically trained and have $\geq$ 8 years of field experience. IPM personnel, Jerry Rakotoniaina, will serve as study coordinator and will handle highly sensitive medical information. Because IPM is charged by the Madagascar government to oversee all operations involved with managing active plague outbreaks, IPM personnel are well connected with rural local public health officials. They communicate regularly with rural health officials to provide consultations (via email and cell phone) and support (medical supplies and personnel).

**MANAGEMENT PLAN**

The Directors and PIs at each of the participating institutions are well experienced and will be responsible for their respective entity's participation in the project. David Trudil from Brimrose will serve as the sponsor for this clinical trial study. Dr. Wagner will serve as the overall PI (and co-PI for the human subjects research) and will be responsible for the global scientific direction

and coordination of the collaboration for the field work in Madagascar. Dawn Birdsell will serve as PI for the human subjects research component for this project. The PIs of the respective institutions will meet bi monthly by videoconference and also regularly communicate via email. In addition, NAU, and IPM will meet together in person annually or biannually at agreed location site once the COVID pandemic subsides.

**TRAINING ON LFI ASSAY IMPLEMENTATION**

A formal training on LFI device will be given. The NAU group will meet with the Naval Health Research Center (NHRC) to develop LFI assay protocol and train on the LFI assay. NHRC will provide training for the assay and will serve as a technical liaison for both NAU and IPM, with NAU overseeing training of IPM personnel. NHRC will ensure that overall protocols developed by NAU are being implemented correctly. This training includes proper use of the LFI assay, accuracy in reading of LFI results, and proper method of data collection. NHRC will provide NAU and IPM with onsite training at both facilities (NAU and IPM) at a sufficient timing to meet the requirements of the statement of work.

**FIELD INVESTIGATION PROTOCOL**

IPM has developed a field sampling protocol which closely mirror those from past field missions conducted for retrospective plague investigations. IPM and NAU will work together to finalize the field sampling protocol that will involve enrolling up to 300 participants across two plague seasons (Table 1). Five IPM personnel with field mission experience were trained according to IRB requirements.

***Timeline of study activity:*** Human plague in Madagascar has a distinct seasonal component and is associated with specific environmental conditions. The human plague season typically starts to take off in September, peaks from October to January, and tapers off in March.

**Table 1**: Timeline for the study divided across three performance periods (Base period = 18 months; Option Period 1 (OP1) = 12 months; Option Period 2 (OP2) = 6 months). Months are aligned with plague seasons, performance periods and study activities.

| Month | Plague Season | Period | Study Activity |
|---|---|---|---|
| Dec-19 | 2019-2020 plague season | Base | |
| Jan-20 | 2019-2020 plague season | Base | Get Human Subjects Approval in place (NAU IRB, IPM IRB) |
| Feb-20 | 2019-2020 plague season | Base | |
| Mar-20 | 2019-2020 plague season | Base | |
| Apr-20 | 2019-2020 plague season | Base | |
| May-20 | | Base | |
| Jun-20 | | Base | Earliest time that we can expect human subjects approval |
| Jul-20 | | Base | |
| Aug-20 | 2020-2021 plague season | Base | 1. Consent and Enroll; 2. LFI Testing and confirmatory testing |
| Sep-20 | 2020-2021 plague season | Base | |
| Oct-20 | 2020-2021 plague season | Base | |
| Nov-20 | 2020-2021 plague season | Base | |
| Dec-20 | 2020-2021 plague season | Base | |
| Jan-21 | 2020-2021 plague season | Base | |
| Feb-21 | 2020-2021 plague season | Base | |
| Mar-21 | 2020-2021 plague season | Base | |
| Apr-21 | 2020-2021 plague season | Base | |
| May-21 | | Base | Confirmatory testing |
| Jun-21 | | OP1 | |
| Jul-21 | | OP1 | |
| Aug-21 | 2021-2022 plague season | OP1 | 1. Consent and Enroll; 2. LFI Testing and confirmatory testing |
| Sep-21 | 2021-2022 plague season | OP1 | |
| Oct-21 | 2021-2022 plague season | OP1 | |
| Nov-21 | 2021-2022 plague season | OP1 | |
| Dec-21 | 2021-2022 plague season | OP1 | |
| Jan-22 | 2021-2022 plague season | OP1 | |
| Feb-22 | 2021-2022 plague season | OP1 | |
| Mar-22 | 2021-2022 plague season | OP1 | |
| Apr-22 | 2021-2022 plague season | OP1 | |
| May-22 | | OP1 | Confirmatory testing |
| Jun-22 | | OP2 | |
| Jul-22 | | OP2 | |
| Aug-22 | | OP2 | |
| Sep-22 | | OP2 | |
| Oct-22 | | OP2 | |
| Nov-22 | | OP2 | |

***Location*:** The study will be carried out mostly in endemic plague outbreaks regions in Madagascar (i.e., the Central Highlands region) but can extend to any region in Madagascar where local clinical health officials report human plague.

***Study population*:** Active plague sites tend to be located in low resource rural settings. The study population will be comprised of local people presenting signs of illness consistent with plague. They will be recruited at rural health centers located throughout Madagascar. The Madagascar Ministry of Public Health requires declaration of all suspected human plague cases and mandates the collection of biological samples (sputum and/ or bubo aspirates) from these cases as part of the medical workup for official confirmation. Our proposal will work within this existing mandatory confirmation and reporting system to obtain the requisite enrollment number. Since blood is a novel biological sample for plague diagnostics, we will include a traditional clinical sample type as a positive control for plague detection. We will test the LFI on residual de-identified clinical samples (bubo and/or sputum) from participants who are enrolled in our study. This would thoroughly document the diagnostic capabilities of the LFI assay as compared to paired capillary blood.

We plan to enroll up to 300 participants across the entire study which encompasses two plague seasons (Aug 2020-March 2021; Aug 2021-March 2022). We aim to enroll at least 150 participants per plague season but if the opportunity presents to enroll more within the first plague season, then we will proceed to enroll up to 300 participants.

- The **inclusion criteria** for this study
    - Adults 18 to 75 years old (male and female)
        - Be able to receive and give verbal communication
    - Children 5 to 17 years old (vulnerable population)
        - Parents or legal guardian be available to give permission.
        - Parents or legal guardian to consent for children (5-6 years)
    - Identified as a suspect human plague case by the local medical professional

    - Will include at least one of the following symptoms:
        - For bubonic plague: High fever, chills, and/or the presence of a painful bubo
        - For pneumonic plague: High fever, chills, cough for less than 5 days, bloody sputum, and/or chest pains.
            - Patients may be recruited from both plague surveillance program and non-plague surveillance programs.
                - For this study, patients from the plague surveillance program will have higher priority for enrollment than patients from the non-plague surveillance programs.

- The **exclusion criteria** for this study
    - Children under the age of 5 years old
    - Children between the age of 5 years to 17 years without a parent or legal guardian
    - Not compliant with the study procedure (blood sampling)

Adults 18 to 75 years old (male and female), Children 5 to 17 years old with parent or legal guardian, Malagasy nationals across all ethnic groups can participate. This study does not exclude pregnant women but they are not considered vulnerable population because their pregnancy status is not a selection criterion. For this reason, Appendix B is not included in this application.

Participants will initially learn about the study from their physician during their medical consultation. They will then receive an information sheet by the physician who will then be referred to an IPM personnel to receive more information about the study.

***When will recruitment occur?*** Recruitment will most likely occur during the regular plague season (Aug 2020-April 2021 and Aug 2021-April 2022) when plague activity is high but it can happen any time of the year as sporadic cases can arise outside of the normal season. Local physicians will alert IPM of a suspect plague case at their health center. IPM personnel will be dispatched to this local health center and begin the informing, consenting, and enrolling process.

***Where will recruitment take place?*** The recruitment will take place at the site of regional and local health centers throughout Madagascar where local people seek medical assistance. It is local practice for IPM research personnel to visit these healthcare facilities to provide additional medical assistance (during outbreaks) at the invitation of the local physicians and perform plague investigations to fulfill Malagasy government mandates.

***Identifying partnering healthcare centers.*** During the low plague season (May – Aug), IPM will contact the local physicians from regional health center to inform them of this study. Physicians will learn about the study's purpose, design, and the referral role for which they would serve. From these communications, IPM will know in advance which health centers plan to participate.

***Identifying suspect plague case and providing immediate medical treatment.*** When the local physician has identified a suspect plague case based on clinical presentation, he/she will perform the national protocol for declaring suspect plague case. This includes collecting medical workup samples (sputum and/or bubo aspirates) and completing a TLO declaration form. The sample and TLO form are to be given to IPM for medical workup for official confirmation, irrespective if the patient enrolls in the study. The physician will then provide standard medical care according to PNLP. This includes administration of antibiotics. Blood collection is not part of the standard medical workup.

***Role of physicians as providing referrals.*** The physician is not part of the research but will provide prospective subjects with information about the research (which, in this case, includes the information section of the informed consent document but will not obtain subjects' consent for the research or act as representatives of the investigators. This practice is in alignment with guidance on engagement described here: https://www.hhs.gov/ohrp/regulations-and-policy/guidance/guidance-on-engagement-of-institutions/index.html. After the start of medical

treatment, the local physician will alert the Plague-IPM Unit of suspect plague case(s) at their health center. IPM will immediately dispatch field investigators who will arrive as soon as possible. The physician will give a leaflet to the patient that will provide information about the study conducted by IPM. If the patient is illiterate, then the physician will read the leaflet aloud. The local physician will then will refer them to IPM personnel for more information. This is a process approved by the local ethics committee. The physician will not participate in the consenting and enrollment process but rather refer patients to the study. Medical treatment already started before introduction of the study and will continue regardless of study enrollment status.

***Consenting participants***. The informing, consenting, enrolling, and sample collection process will be performed in a room at the local health center and will require two to three hours total. If the participant meets the inclusion criteria, the potential participant will undergo the consenting process. He/she will meet face to face with an IPM personnel who will verbally take them through the study information (the objectives of the study, proposal to participate in the study, and the type of sample to be taken – as laid out in the flyer leaflet. He/she will be provided a hard copy of the informed consent form that contains the signature and give them time for review. For those who are illiterate, the IPM personnel will verbally communicate the consent form information and the signature page at a language level that is understood by the participant. After confirming that each participant understands the nature and details of the study, they will be given the opportunity to ask questions and to receive answers. Part of this confirmation is to ensure they understand that entities outside of Madagascar may review their data records and health information (age, gender, location, clinical form of the disease, medical treatment). No identifiable information will be provided to outside entities. FDA and DoD are not specifically mentioned in the consent form due to a lack of familiarity of this organization for the typical participant in this study. After this comprehension check, each person will be asked if they would like to enroll in the study. To be enrolled, each participant must sign the informed consent form. For those who are illiterate, they will sign by marking an X or provide a finger print on the signature line. For minor children ages 5-17, the parents or legal guardian will sign the consent form to authorize permission. The minors ages 7-17 will provide assent by signing the assent form. For children ages 5-6, a parent or legal guardian signs the assent letter in their place. Two duplicate consent form (and assent form if applicable) will be signed, with one copy be given to the patient or parent / legal guardian and a second copy retained by IPM) for long-term storage for five years.

***Post Enrollment Procedure***. Once a participant is enrolled, the IPM personnel will assign a numeric code to represent, a unique identifier code (TLO #) to track data collected to preserve the participant confidentiality. Then the IPM personnel will measure the body temperature using an axillary thermometer and proceed to perform a one-time collection of two types of blood (venous and capillary) that will total less than 5 ml. This is equivalent to approximately one teaspoon of blood. Each blood sample type will be labeled with the participant's TLO #. Approximately 4 ml of venous blood will be drawn out of the vein of an arm into a storage tube designed for blood preservation and ~ 0.1 ml of capillary blood will be captured from a finger prick into a capillary tube. These blood collection procedures are done using standard medical

practices performed by medically or paramedically trained IPM personnel. Specimen sampling protocols are designed in such a way as to not endanger the health of patients or IPM personnel. These practices are safe, minimally invasive, and the blood volume falls below the minimal risk category. The participants may experience some level of discomfort but this level does not exceed discomfort experienced with these low risk medical procedures. When collecting blood from the participants, IPM personnel will wear proper protective gear (gloves and masks) and, when appropriate, prophylactically take antibiotics when needed. These are standard practices of IPM research personnel when conducting field investigations.

Once the blood collection is complete, the enrolled participant will be given their compensation and congratulated for completing the study. Immediately afterwards, in a separate room or at the IPM facility, the capillary blood will be tested on the LFI assay. Venous blood along with the residual medical workup samples (bubo or sputum) will be transported to IPM facility for further testing.

Prior to leaving the health care center, IPM personnel will collect the medical workup specimen (bubo aspirate or sputum) from the physician along with the completed TLO declaration form. The medical workup specimens are normally shipped to IPM facility but this step will be bypassed for enrolled patients. Instead IPM personnel will transport the medical workup specimen and TLO declaration form during their return to IPM facility. IPM is responsible for conducting the medical workup for all suspect plague cases to provide official confirmation. The TLO declaration form, containing highly sensitive information (including patient's full name), is stored at IPM in a secured location according to Madagascar protocol. Every medical workup sample is de-identified by assignment of a unique TLO # which is recorded on the TLO declaration form to link samples and resulting data to the patient. This is the normal procedure of formal confirmation mandated by Madagascar Law. TLO# will be used as ID for collected blood samples and data of LFI results from capillary blood. In addition, each enrolled patient will also be assigned a unique Study ID#. From the TLO form, a limited metadata will be collected to populate a data collection form (Table 2). No identifiable metadata will be included. To further safeguard the confidentiality and privacy of enrolled participants, data will be further de-identified using the unique study ID# when shared with entities outside of Madagascar (including NAU). The TLO# will be removed.

**Table 2:** Data collection form.

| Study ID | *TLO# | Date of enrollment | Age | Gender | **location | Date of symptom onset | Temperature on TLO form | Body Temperature after enrollment | symptoms | Antibiotic treatment & duration | time elapse between treatment to blood draw | Clinical form | sample collection |
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| *TLO # is the unique numeric code assigned to patient's TLO official declaration form to de-identify the patient name from sample and data. IPM personnel will delete the column prior to transferring these data to NAU. | | | | | | | | | | | | | |
| **location refer to GPS coordinates, or Community name, or other geographical description | | | | | | | | | | | | | |

***Diagnostic testing of biological samples***. For the venous blood, these tests include two primary confirmation method (Table 3): 1) Real-time qPCR and 2) Culture for bacterial isolation. Venous blood samples will be processed for secondary confirmatory methods: 1) DNA testing using FilmArray NGDS Warrior Panel (FDA-approved), 2) testing for presence of plague antibodies, and 3) DNA analysis for genetic studies and genome discovery. FilmArray warrior panel will serve as one of several independent diagnostic confirmation methods to compare LFI results. The medical workup samples will be largely consumed for confirmation tests mandated by Madagascar law (Table 3 – gray highlights). Remaining residual amounts will be utilized to test on LFI and extract DNA for genetic studies.

**Table 3**: Four human specimen types collected in the field and their respective analytical fates. Each field sample will be tested directly or after further processing "Analyzed Sample". Each analytical fate is classified as the new device diagnostic test (LFI = Lateral Flow ImmunoAssay), primary confirmation (qPCR = DNA tests; Culture = bacterial isolate), and secondary confirmation (RDT = Rapid diagnostic test; ELISA = antibody detection in blood; FilmArray Warrior Panel PCR, canSNP = Genetic typing; WGS = Whole genome sequencing to discover novel regions). Region in gray indicate standard medical workup confirmatory testing mandated by Madagascar law. Region in black are tests not subject to IRB regulations due to the use of de-identified residual medical workup specimens.

| | | Work done at Madagascar | | | | | | Work done at NAU - USA | |
|---|---|---|---|---|---|---|---|---|---|
| **Field Sample** | **Analyzed Sample** | **LFI** | **2 target qPCR** | **Culture** | **IPM F1 RDT** | **ELISA** | **FilmArray** | **canSNPs** | **WGS** |
| Capillary blood | Capillary blood | YES | - | - | - | - | - | - | - |
| Venous blood | Venous blood | - | - | YES | YES | - | YES | - | - |
| Venous blood | DNA extracted from venous blood | - | YES | - | - | - | - | YES | - |
| Venous blood | Sera obtained from venous blood | - | - | - | - | YES | - | - | - |
| Venous blood | Isolate obtained from venous blood | - | - | - | - | - | - | - | - |
| Venous blood | DNA extracted from blood isolate | - | YES | - | - | - | - | YES | YES |
| Bubo aspirate | Bubo aspirate | YES | - | YES | YES | - | - | - | - |
| Bubo aspirate | DNA extracted from bubo aspirate | - | YES | - | - | - | - | YES | - |
| Bubo aspirate | Isolate obtained from bubo aspirate | - | - | - | - | - | - | - | - |
| Bubo aspirate | DNA extracted from bubo isolate | - | YES | - | - | - | - | YES | YES |
| Sputum sample | Sputum sample | YES | - | YES | YES | - | - | - | - |
| Sputum sample | DNA extracted from sputum sample | - | YES | - | - | - | - | YES | - |
| Sputum sample | Isolate obtained from sputum sample | - | - | - | - | - | - | - | - |
| Sputum sample | DNA extracted from sputum isolate | - | YES | - | - | - | - | YES | YES |

**Description of assay**

- New investigative *in vitro* (outside of human) diagnostic assay: Lateral Flow ImmunoAssay (LFI) (Fig 1)
    - Not FDA approved

        - This Madagascar study will inform decision on FDA clearance path for the LFI.
        - Until FDA approval – the device risk determination was determined as non-significant by the sponsor (Brimrose) and the IRB of record (NAU).
    - Biological Sample Type and Volume:
        - Human biological samples (capillary blood, sputum, bubo aspirate)
            - Volume (30 ul - 50 ul) is equivalent to 3-5 eye drops of liquid
    - Device characteristics: Design concept is very similar to a home pregnancy test
        - Result will be read by eye after 15 minutes
        - This assay was developed by New Horizons Diagnostics Corp. and targets two plague proteins, F1 and LcrV. (https://www.nhdiag.com)
            - The F1 protein is well characterized but the characteristics of LcrV protein in terms of expression and stability in the context of human infection is not known.
                - Results from this study can provide knowledge in this area.

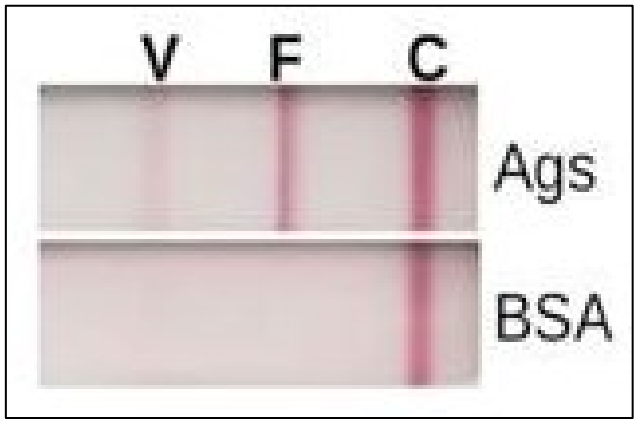


**Figure 1.** New Horizons Diagnostics Corp has developed a rapid point-of-care lateral flow immunoassay (LFI) to diagnose plague infections. This assay (SMART™-PRT) is a multiplex LFI capable of detecting two Y. pestis antigens; F1 and LcrV. The picture illustrates results with laboratory-purified antigens (top) and control (bottom). The test requires a single drop of blood from a finger stick and is read in 15 minutes.

Data Analysis:

These human specimen samples will then be used to test the LFI assay directly at the remote field locations and IPM will read the results by eye after 15 minutes.

***Statistical analysis of the data***

- For evaluation of the LFI compared to the reference test (culture on the same sample), a 2 * 2 contingency table will be constructed from the laboratory results.

The sensitivity of the test LFI sera evaluated against the reference test (bacteriology). It expresses the proportion of confirmed plague cases correctly identified by the reference test.

- Results data from other standard diagnostic tests (Table 3) will be compared with LFI results to determine the diagnostic effectiveness of the LFI device.

- A professional statistician will be employed for data analysis as a subcontractor from the University of Maryland-Baltimore, Department of Mathematics & Statistics.

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