Demonstration of NOPERSIST results leading to novel, validated diagnostic tests for active human and bovine tuberculosis

Reporting

Project Information

**DEMO-NOPERSIST**

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Coordinated by
LIONEX GMBH
Germany

Final Report Summary - DEMO-NOPERSIST
(Demonstration of NOPERSIST results leading to novel, validated diagnostic tests for active human and bovine tuberculosis)

Executive Summary:
Tuberculosis (TB) in humans and bovine TB in farm animals are global health problems of immense social and economic importance.

Diagnosis of human and bovine TB is extremely difficult, time-consuming and in-efficient. No efficient, cost effective tests are available for an early diagnosis of these infections. Furthermore, there is no test on the
market which can differentiate the more than 500 million infected people (LTBI) from the active TB patients. A test which can differentiate between healthy infected and the TB patient shall, without doubt, have an annual World-market of several hundred million Euro.

The main objectives of DEMO-NOPERSIST project are
1. Development and evaluation of prototype test kits for active human TB (LIONEX, SME). This test shall be the World’s first blood test for discriminating latent from active TB in humans.
2. Development and evaluation of prototype test kits for active Bovine TB (PRIONICS, SME)
3. To develop marketable, improved diagnostic products for human and bovine TB within a period of 2-3 years.

PROGRESS: All experimental work was completed as planned and optimised coating conditions for the commercial anti-IL2 antibodies. The Elispot test was successfully developed for detecting IL2 in blood. In the next step LIONEX generated its own mAbs against human IL2. The LIONEX produced anti-IL2 clones proved superior than the commercial ones. LIONEX further continued the work on test optimisation varying several parameters for increasing the intensity of spots in the newly developed Active TB test kit. All components needed for the diagnostic kit were put together and stored for stability studies. Detailed IVD confirm documentation was prepared by LIONEX so that CE labelling could be done at the end of the project. LIONEX also designed and created a professional Active TB Kit format with kit insert. The product name "LIOSpot® TB" was registered as trade mark for the Elispot test. LIONEX also developed a IL2 ELISA kit and a IL2 complete kit for measuring IL2 in whole blood. In addition "LIOKine®" registered as trade mark for tests which detect cytokines in ELISA format. 400 Tests were provided to partner UNIFI who performed evaluations of the tests. In addition to the work described above, LIONEX also developed a rapid test for bovine TB. The trade name "LIODetect®" was registered for rapid test formats. 300 LIODetect®-Animal TB tests were manufactured and provided to the partner AHVLA who performed evaluations using well characterised bovine samples. Following amendment, PRIONICS activities were transferred to LIONEX in March 2014. In this case also a product for bovine TB called Prionics-Strip test was developed, manufactured and provided by LIONEX to partner AHVLA (APHA) for evaluation.

Results of evaluations: UNIFI evaluated LIONEX LIOSpot® TB test kits using panels of well defined PBMC from different cohorts: BCG vaccinated, ESAT6 IGRA positive, TST positive, healthy. The kits were found to produce excellent results matching our previously reported sensitivity of more 92% and specificity of more than 95%.

AHVLA evaluated the LIODetect® Animal TB tests using sera from cattle stored at AHVLA. Unexpected high specificity of almost 100% was obtained. Sensitivity was in the expected range of about 60%. The test is very simple to use and can be performed on site at the farm. AHVLA also evaluated the Prionics-Strip test which also showed high specificity but lower sensitivity than the LIODetect® Animal TB tests All documentations on the human and bovine TB products developed during DEMO-NOPERSIST are now available for registration purposes.

Project Context and Objectives: Tuberculosis (TB) in humans and bovine TB in farm animals are global health problems of immense social and economic importance.

Human TB: Globally, there are more than 14 million persons dually infected with TB and HIV. Drug resistance to HIV-treatment and appearance of multiple-drug resistance (MDR) and off late of Extra-Drug
Resistance (XDR) strains of M. tuberculosis, the causative agent of human TB is steadily leading to a hopeless situation as far as the therapy is concerned. To make things worse, there is no effective vaccine available against HIV. M. bovis BCG, the only vaccine available against TB, has shown highly variable efficiency and has been very often ineffective. Its use has been discontinued in several countries.

Bovine TB: The principal hosts for bovine tuberculosis are cattle and buffalo, however many other domestic and wild animals can become infected e.g. goats, cervids, pigs, wild boars, dogs, cats, camels, badgers, primates, hares, amongst others. Bovine tuberculosis is found worldwide. All developed countries currently have a TB eradication program in place for many years. These programs have been largely successful; however, incidences are increasing in many countries (e.g. UK, Ireland, France, Austria, Germany).

Diagnosis of human and bovine TB is extremely difficult, time-consuming and in-efficient. No efficient, cost effective tests are available for an early diagnosis of these infections. Furthermore, there is no test on the market which can differentiate the more than 500 million infected people (LTBI) from the active TB patients. A test which can differentiate between healthy infected and the TB patient shall, without doubt, have an annual World-market of several hundred million Euro.

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All documentations on the human and bovine TB products developed during DEMO-NOPERSIST are now available for registration purposes.

LIONEX completed all management and coordination activities without any complications. Regular meeting and telephone conferences were organised. The emphasis was on serious discussions on project activities. All issues on consortium management, IPR were properly managed by the coordinator and a new patent was filed.

The DEMO-NOPERSIST website was modernised and several press releases were made presenting the results of DEMO-NOPERSIST to public and to the specialists in areas of Medicine and Health.

The LIOSpot® TB kit was demonstrated at several sites to public at BIOTECHNICA exhibition, practitioners, laboratory doctors, regional health departments, pharmacy.

DEMO-NOPERSIST has achieved all its goals and has produced real products for TB diagnosis which shall be ready for marketing within the next 20 months. The final results are:

1. Development and manufacture of LIOSpot® TB , a novel active TB test for humans TB diagnosis
2. Development and manufacture of LIODetect® Animal TB test for bovine TB diagnosis
3. Securing new IPR (Patent) on novel ELISPOT technology developed by LIONEX.

Project Results:
Context and main objectives

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3. Securing new IPR (Patent) on novel ELISPOT technology developed by LIONEX.

IMPACT: The results and products developed in DEMO-NOPERSIST are expected to have immense socio-economic and societal impact, not only in the European Union, but also world-wide since TB is a global disease. The LIONEX LIOSpot® TB kit is expected to diagnose, for the first time, active TB accurately. Since it is an immunological test, it shall detect both pulmonary and extra-pulmonary TB, the latter is much more difficult to diagnose than pulmonary TB. Unfortunately, extra-pulmonary TB is highly neglected, even though it accounts for about 40% of all TB cases world-wide. Women are main sufferers due to extra-pulmonary TB. Improving the quality of life of women in all countries is the declared commitment of international organisations such the WHO and the UN.

The LIODetect® Animal TB test is also valuable for increasing the sensitivity of the currently used skin tests. The test may help reduce TB in farmers who are in regular contacts with cattle. Since the test can be performed on site, it is very useful for rapid and routine diagnosis on farms. Due to the reasons described above, the project results are expected to have strong economic impact by reducing the cost of health budget in member states and other TB endemic countries. It should lead to creation of new jobs for LIONEX as manufacturer and owner of IPR on the products. This project has established strong collaborations between partners which shall be continued in future. Previous observations suggest a possible role of IL-2-based ELISPOT assay in addition to the IFN-gamma-based assay in discriminating active from latent TB, as only cells from individuals with LTBI and not those from individuals with active TB has been found to secrete IL-2 after specific stimulation. These results probably reflect the increased number of IL-2 secreting and IL-2/IFN-gamma secreting central memory T cells and the reduced number of IFN-gamma effector T-cells in LTBI patients, associated with the low bacterial replication and low antigen load. In the present study we prospectively evaluated the performances of Liospot IL-2 based ELISPOT assays by the use of a panel of Mycobacterium tuberculosis antigens in patients consecutively referred to infectious disease unit and at risk for TB infection with the aim of investigating possible immunological markers for a differential diagnosis between LTBI and active TB, as well as in uninfected.

LIONEX has developed LIOspot® TB Kit, using as antigen Ala-DH, ESAT-6, or CFP-10. Furthermore LIONEX has developed HUMAN IL-2 ELISA and LIOkine TB kits.

Kit manual and procedure for LIOSpot TB kit:

A total no. of 23 kits of LIOSpot Active TB are produced by Lionex and send to UNIFI for evaluation. Analyzing the results observed for the 1st and 2nd batch of kits it was obvious, that sensitivity of the kit was too low, therefore modifications on kit composition are done. After addition of co-stimulating substance and modifying antibody concentrations a 3rd batch of kits is send to UNIFI which has shown excellent discrimination of active and latent TB without loss of specificity by using antigen A (AlaDH). In IL-2 ELISpot kit (LIOSpot Active TB) the antigens B and C (ESAT6 and AlaDH) were able to detect latent-
and active TB.
So far a total no. of 100 samples from TB- and LTBI patients are measured. Negative sample panel consists of 50 from healthy individuals.

Selection of patients: Following approval by Florence Ethics Committee of the study, and after informed consent to each patients, we studied the response of LioSpot Kit to several mycobacterial antigens in tuberculosis patients, latent tuberculosis and healthy controls. In a first series of experiments (coded as kit 150108) we studied 22 patients (median age 54, 11 males, 11 females) with active tuberculosis, 16 patients with latent tuberculosis (median age 47, 8 males and 8 females), and 10 age-matched uninfected controls. In a second series of experiments (coded as kit 150421) we studied 16 patients (age 52, 8 males and 8 females) with active tuberculosis, 6 patients with latent tuberculosis (age 48, 3 males and 3 females), and age-matched 9 uninfected controls. In a third series of experiments we studied 62 patients (median age 55, 32 males, 30 females) with active tuberculosis, 78 patients with latent tuberculosis (median age 51, 40 males and 38 females), and 31 age-matched uninfected controls. Each patient underwent chest radiograph, TST test, sputus examination, physical examination and medical history.

Collection and Storage of samples: BBMC from blood of each subject shall be isolated as described in the LIOSpot Kit protocol and stored under liquid nitrogen til further use.

Evaluation of the LIOSpot®-TB human: LIOSPOT assay detecting IL-2 responses were performed using recombinant M. tuberculosis antigens (ESAT-6, CFP-10, AlaDH), provided by Lionex Diagnostics & Therapeutics GmbH (Braunschweig, Germany), following the manufacturer protocol. Briefly, peripheral blood mononuclear cells (PBMCs; 1×106 cells/mL) of each patient were stimulated with M. tuberculosis recombinant antigen (5 µg/mL), and seeded in triplicate in 96-well plates coated with anti–IL-2 antibody. Cells stimulated with medium alone served as negative controls. Cells stimulated with phytohemoagglutinin (PHA) served as positive controls. LIOSpot®-TB human microplates were then incubated at 37°C in 5% CO2 for 24 hours. At the end of the culture period, plates were washed and incubated for 3 hours with the appropriate biotinylated anti–IFN-γ or anti–IL-2 monoclonal antibody. Streptavidin-HRP complex was then added for 2 hours, followed by the substrate solution. Spot forming colonies (SFCs) were counted using an automated ELISPOT reader (Autoimmune Diagnostika GmbH) and results expressed as number of SFCs per million PBMCs. Laboratory workers were blind to the clinical status of participants. Categorical data were compared using the Chi-squared test (or Fisher’s exact test, when expected cell sizes were smaller than five). The Wilcoxon-Mann-Whitney test was used for continuous measurements to test relationships in unpaired analysis, when assumed that the dependent variable is a not normally distributed interval variable.

250 adults were included in the study. 50 hundred were classified as uninfected, 100 as LTBI and 100 as active TB case, according to the TB guidelines of the Centers for Disease Control and Prevention.

On the basis of the results obtained so far using the LIOSpot®-TB human kit, a cut-off of 15 spot /106 examined cell can be used to discriminate patients with active tuberculosis and latent tuberculosis.

• Highlights:

1. Development of a test specific to active TB called LIOSpot®-TB human. This is fort the first time that such a test has been developed in the history of tuberculosis.
2. LIOSpot®-TB human is the first test showing high sensitivity and specificity, both in children and in adults.

3. Manufacturing of LIOSpot®-TB human was completed and all documents prepared for CE marking.

Evaluation of Active-TB-ELISA Kits

LIONEX performed extensive work on mAb screening and isolation anti-IFN-gamma producing clones. Kit manual and procedure IL-2 ELISA and LIOkine TB kit:
Lionex has produced a total no. of 34 IL-2 ELISA kits and LIOkine kits for evaluation and validation. A total no. of 13 kits were sent to UNIFI for evaluating TB, LTBI and healthy patent samples according to instruction manual. The remaining kits were used for validations at Lionex (stability tests and determinations of intra- and inter assay variations ect.). Furthermore 3 IFNγ ELISA- and LIOkine TB/LTBI kits were produced by using MABTECH antibody pair and sent to the corresponding institutes.

Figure: LIOkine TB kit (IL2) and LIOkine LTBI (IFN-G), test procedure.

LIOkine kits are Human whole blood cytokine release assays consisting of vials pretreated by antigens and Phaeosolus vulgaris Phytoheamagglutinin (PHA). The human blood samples are collected with the LIOkine vials and the cells are stimulated to produce cytokines such as IL-2 and IFN-γ in vitro at 37°C. After separation the plasma from red blood cells the cytokine levels in the stimulated samples are quantified by Human IL-2 and / or IFN-γ detection kits (Human IL-2 and IFN-γ ELISA kits of LIONEX).

Human IL-2 ELISA (Cat.-no. LIO-IL201) and Human IFN-γ ELISA (Cat.-no. LIO-IFN01), kit components.

Evaluation of Active-TB-ELISA Kits is done according to the kit manual provided with the kit. Specialized blood collection tubes are used to collect whole blood (LIOkine kits). The tubes are incubated at 37°C as soon as possible and within 16 hours of collection. After incubation of the blood in the tubes is finished, plasma separated and analyzed for the presence of IL-2 produced in response to the respective antigen by using HUMAN IL-2 ELISA kits. Negative control tubes and PHA-containing tubes are used as negative and positive controls. The cytokine level of negative tube are subtracted from the cytokine level for TB antigen tube.

Results of anti-human IL-2 detection ELISA kit evaluation (LIO-IL-2):
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The Mabtech antibodies are replaced by LIONEX own antibody pair and new kits are produced for Evaluation in 2015. Positive signal was strong enough in most of the samples and the reference curves looked well, but no or very low signal is observed in the TB samples. Detection limit is determined at 5 pg/mL, concentrations of IL-2 higher than 5 pg/mL are observed in only 3 out of 20 of the samples from TB patients measured (table, sensitivity = 15 %). After measuring TB and LTBI patients and healthy individuals, it was obvious, that the test was not sensitive enough to detect IL-2 in the TB-samples.
Highlight: In contrast to the excellent results obtained with the LIOSpot TB, measurement of IL2 in blood shows low sensitivity. It is known that IL2 is consumed by the growing T cells in blood.

Production of Pilot lots and Field Validation

As planned, LIONEX manufactured both PRIONICS Strip test as well as a new Animal TB rapid test format of LIONEX as shown below:

Prionics Check-TB Strip test and the procedure:

Development of the prototype kits of M. bovis antibody detection assay based on the LFD Comb technology was completed after selection of the most sensitive and specific antigens and after coating parameters, buffer compositions and sample volumes required were optimized.

The final test format is a comb consisting of 8 strips (see fig below). Three antigen lines are sprayed on the sheets which are used for preparation of the comb. The prototype kits contain membrane coated by MPB70 and MPB83. The conjugate is supplied as liquid as well as the diluent buffer (running buffer). Furthermore the kit contains an untreated 96 well microtiter plate for mixing sample, conjugate and diluent buffer. Each kit box contains 80 determinations (10 combs with 8 test strips each). The kit components are packed into cardboard boxes (outer dimensions: 11 x 15 x 17 cm).

Figure 3: Prionics® TB-STRIP assay,

Components of prototype kits.

The final prototype kit was evaluated at Lionex by a total number of 183 samples from cattle by following the test protocol established. A color card is provided with each set of test, which allows the user to rate intensity of the test lines (Figure 4).

Figure 4: colour card provided with each set of tests, Prionics® TB-STRIP assay. The user shall rate intensity of the test line(s) by comparing the lines by the colour card.

The positive panel includes 69 samples from cow, which were skin test and IGRA-positive. The 114 negative samples includes 50 samples from skin test and IGRA negative cow (AHVLA), 18 samples from the negative panel of Prionics (samples from a UK negative herd, defined historical records of > 5 years TB free history based on skin test and BOVIGAM negative test results) and 46 samples from a herd with only 3 infected animal (France). It is observed, that low-intensity MPB83 test lines (intensity ≤ 1) appear more often in the AHVLA-negative group than in the other groups evaluated. If the low-intensity MPB83 test line is rated as “negative” (intensity ≤ 1), specificity was 95.61 % (all samples, including AHVLA). For the negative panel with AHVLA-samples (68 individuals, France and Prionics) the specificity was 100 %. Sensitivity was 71.01 % (69 samples, MPB83 low-intensity test lines are rated as “negative”).

In addition to the manufacture and validation of Prionics® TB-STRIP Kit, LIONEX also developed and
manufactured single test Cassette-formats. This LIONEX kit was named “Animal TB Test”

LIONEX Animal TB Rapid test and procedure:

The LIONEX ANIMAL TB Rapid test kit is suitable for fast and reliable detection of antibodies against mycobacterial antigens in serum or plasma samples from cattle or other animals (e.g. elephant, sheep, goat, badgers).

Each kit contains test devices (cassettes) and a dropper vial with diluent buffer. The unopened kit is stable at room temperature until expiry date.

INTERPRETATION OF RESULTS

For test interpretation a pattern attached which shows the positions of the test- and control lines on the membrane (see below).

ANIMAL TB Rapid Test

Pattern for reading the test line(s):

- Cell wall antigen
- Recombinant antigen 2
- Recombinant antigen 1

The test line(s) may appear distinct, weak positive or strong positive. If no control line appears, repeat the test!

NEGATIVE:

One strong line appears in control zone “C” and no test line in "T" ZONE (Fig. 1, left).

POSITIVE: Up to 4 pink-purple lines appear – one appears in control zone “C” and the others in test zone “T”.

If one or more test lines appear (figure 2), the result is POSITIVE, regardless on the position of the test line (“Test line 1”, “Test line 2”, “Test line 3”).

Examples of POSITIVE test results are shown below:

Figure 2: Examples of POSITIVE test results. Left hand: one

Validation results at AHVLA (now APHA)

Serum samples. Samples used were derived from three different animal populations (see table 1).

Samples from infected animals were collected from two populations, infection was confirmed in all cases by the presence of visible lesions typical of bovine tuberculosis and the culture of M.bovis from tissue samples. Firstly, samples were collected from skin-test positive reactor animals. These animals were not tested with the interferon-gamma release assay (IGRA), population 1. These animals were included as they provide reference to studies with other serological tests that employed animals of this nature. It needs to be stated that samples from such animals are the least relevant as they have been detected already by tuberculin skin testing and therefore will be removed without the need for auxiliary tests such as serology.
or IGRA. To contemplate replacing the skin test with a serology test is unrealistic at present, not only due to the lower sensitivities and specificities of serology but also because serum responses in cattle with bovine tuberculosis are crucially dependent on prior tuberculin skin testing to boost serum responses and thus to increase test sensitivity. Thus, the most relevant group included in this study are animals with bovine tuberculosis that escaped detection with the tuberculin skin test. Hence, the second group of animals is composed of such animals, population 2. They tested skin test negative and underwent ancillary testing and were all Bovigam IGRA-positive. The last group was composed of samples from TB-free animals (Long-term, > 5 years, TB-free herds, located in TB non-endemic area of GB), population 3.

Table 6. Study populations

Serological assays were performed according to the suppliers’ instructions. Relative sensitivities and specificities and 95% CI values of the tests studied in this project were calculated using GraphPad Prizm (GraphPad, San Diego, CA).

Results

Develop Prototype lateral flow serology test.

APHA provided 304 serum samples from M. bovis infected cattle to Prionics and Lionex to support development of their test kits.

Evaluation of prototype lateral flow test Lionex test kit and Lionex ELISA kits

Lionex Rapid TB test

The Lionex Animal TB test kit, a lateral flow device, was supplied to APHA and tested using sera from all three population groups (n=50 each group). The test is composed of three bands (1 – 3) with reactions to any band being deemed positive. The results, summarised in table 2, show that, when reactions to either of these bands are taken into account, the test displays a high degree of sensitivity in population 1 (86 %), which decreased to 52 % when the most relevant population of skin test negative yet infected cows were considered (population 2). However, the specificity was severely compromised (62 %) when population 3 was considered. Thus, this interpretation does not represent a useful test as; too many false-positive reactions would be found. We investigate the cause of this low specificity by assessing the reactions to individual bands. This analysis demonstrated (table 2) that the cause of this low degree of specificity was almost exclusively due to reactions to band 1. Therefore the data was reanalysed only considering responses to bands 2 or 3 as giving a positive responses. This results in a minor reduction in sensitivity when population 1 was considered 84 % compared to 86%. However, the sensitivity when tuberculin skin test negative cattle were studied was reduced to 38 % compared to 52 %, whilst the specificity was increased substantially to 98 %, which is comparable to other similar tests.

Table 7. Results obtained with the Lionex Animal TB Rapid test.

Lionex MPB70 and MPB83 ELISA kits

In addition to the rapid lateral flow test discussed in the previous section, Lionex also provided us with two conventional ELISA tests measuring recognition of the sero-dominant M. bovis antigens MPB70 and MPB83. These kits were applied to the same samples that were tested with the rapid TB test to allow direct head-to-head comparisons. Figure 1 present the raw data observed when the three target
populations were tested, expressed of OD450 readings. As expected, responses to both antigens in samples from infected animals (skin test –positive or skin test-negative, populations 1 or 2) were higher than those observed with the uninfected cattle (population 3). Mean serum responses against either antigens of samples from infected animals were similar. Mean responses in the uninfected group (TB-free, population 3) were low with more false-positive responses detected against MPB83 (Figure below).

Serum responses against MBP70 and MPB83 measured by Lionex ELISA kits. Results are expressed as OD450 values of individual samples, horizontal bar indicates mean OD values. Groups: SICCT+ = population 1, SICCT- = population 2, TB-free = population 3. VL = visibly lesioned animal

Using these data, we undertook a ROC analysis to define optimal cut-off values for the different target populations (populations 1 and 2). Specificities were set at the same level as displayed by the Lionex Rapid test (98%). As the data shown in table 3 indicate, sensitivities were lower compared to the Rapid TB test format in population 1 samples, with higher sensitivities observed with MBP70 (30%) compared to MPB83 (18%). Interestingly, when population 2 (tuberculin skin test negative animals) were considered, 48% of animals responded to MBP70, which give a higher sensitivity than using the Lionex Animal TB Rapid test (bands 2 or 3) in this important sample population that constitute animals that escaped tuberculin skin testing.

Table 8. Serum responses to MPB50 and MPB83 detected by Lionex ELISA kits.

VL animals with visible lesions

Production of pilot lots and field testing (Prionics, APHA)

In this task, we evaluated the performance to the Prionics® TB-STRIP Kit provided by LIONEX in the same populations of animals. In these experiments we assessed 80 animals per group. The results are summarised in table 4. We considered a sample to be positive if either band 1 or band 2 were positive, and also if both bands had to be recognised by the test sera. If we considered band 1 reacting sera, we determine the test specificity in population 1 (skin test positive reactors) to be around 59% with a specificity of 100%. This indicated comparable test performance compared to other serology assays for bovine TB. However, when we considered performance in the group of tuberculous animals that escaped skin testing, sensitivity dropped to 1.25%. Responses to band 2 were not detected in population 1 animals and only in 5% of population 2 animals (at 100% specify. Combining responses to bands 1 or 2 in a parallel interpretation approach did not increase sensitivity in this group as the one animal recognising band 1 in population 2 also recognised band 2.

Table 9. Responses determined using the Prionics TB Strip Test.

2. Discussion and conclusions

• In conclusion, we could demonstrate that the specificity of the LIONEX Animal TB Rapid lateral flow test could be improved by disregarding reactions to band 1 without loss of sensitivity to detect skin test positive reactor animals. Compared to other similar tests, both sensitivity and specificity of this test are comparable, with the sensitivity on the higher end of the reported result spectrum.
• However, when the more relevant group of skin test negative cattle with confirmed bovine tuberculosis
were considered only a mediocre to low degree of sensitivity could be observed (38%). Nevertheless these animals would have all escaped detection with the tuberculin skin test and thus these data highlight that serological ancillary will improve overall test sensitivity when used in combination (parallel) with the tuberculin skin test. However, one also needs to state that all these skin test negative animals were detected by routine IGRA testing confirming that these were truly infected animals.

- The sensitivities of the Lionex ELISA tests detecting MPB70 and MPB83 were lower in the animals presenting with tuberculin skin reactions (population 1) compared to the Rapid TB test. However, when MPB70 was used, the sensitivity of the ELISA test in the important population 2 of animals that escaped skin testing, was higher (although not statistically) than determined for the same animals with the Rapid TB test (48 % versus 38 %).
- Thus, using either test in parallel to the tuberculin skin test would detect a proportion of animals that had escaped skin test detection.
- Whilst the Prionics TB Strip test promised reasonable sensitivity comparable to other similar test in population 1, tuberculin skin test positive reactors, its performance in the more relevant target population was too low to be of any practical relevance (5%). These results in particular highlight the need to test serological tests in the most relevant target population, namely the tuberculous animals that have escaped tuberculin skin testing to avoid unsupportable claims and expectations on test performances generated in animals that are tuberculin skin test positive.

Highlights: 1. Serological ancillary tests such as LIONEX Animal TB will improve overall test sensitivity when used in combination (parallel) with the tuberculin skin test.
2. A further patent application/publication is being prepared on the bone TB rapid test achievements mentioned above
3. Press release made

PUDK and LIONEX-Prionics-Product registrations

1. Risk management

All documents for Registration of Lionex Active-TB tests are prepared. The product is designed in that way, that the possible the risk of infection to the user or others is eliminated or reduced as far as possible according to DIN EN 13641. All kit components selected are respecting safety for the user.

So far the Lionex Active-TB tests meets the essential requirements of IVD directive 98/79/EG and corresponding harmonized norms. The products are designed in that way, that the possible the risk of infection to the user or others is eliminated or reduced as far as possible according to DIN EN 13641. All documentations are prepared by using blank forms, which are part of the Lionex QM system according to DIN EN ISO 13485. The following documents are prepared for product registrations:
- Essential requirements of the products to demonstrate conformity to IVD directive 98/79/EG “DO 209 Grundlegende Anforderungen”.
- Risk management according to DIN EN ISO 14971: Failure Mode and Effects Analysis (FMEA) is prepared using blank form “DO 211 FMEA”.
- A fully risk analysis including final risk management report is prepared and recorded by using the corresponding blank form (“DO-208 Risikoanalyse”).
- Material Safety Data Sheets (MSDS) are prepared according to REACH Regulation (blank form “DO 619 MSDS”).

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- Product design and process of production: design of labelling and instructions for use have been done by using symbols according to DIN EN 15223. According to DIN EN ISO 18113-1 and DIN EN ISO 18113-2 labels and instructions for use contain all information required for safe and reliable use of the product to the user. All SOP, MBR and blank forms (DO) required to produce the product under controlled conditions are finished.

- Performance evaluations are done according to DIN EN 13612 and the results are reported by using the corresponding blank form (DO PI-050 performance evaluation study IVD).
- Stability tests according to DIN EN 23640 are done and in progress to determine shelf life of the IVD devices and the results are recorded by using the corresponding blank form (DO PI-047 stability testing of IVD).
- The technical file dossiers are prepared which summarizes all test characteristics (corresponding blank form: “DO 207 Technische Dokumentation”, refer to “Del-4_7 Technical file dossier”).

The blank form for declaration of conformity to the IVD directive 98/79/EG is available and is finished as soon as all files are completed and updated. Finally, after all documents are finished, the manufacturer product registration to the corresponding authority is planned.

Secondary packaging of HUMAN IL-2 ELISA, closed and opened box

LIOkine® Active TB, outer box with blood incubation vials.

Secondary packaging of LIOSPOT®-Active TB, closed and opened box.

Furthermore all documents required to produce the Prionics® TB-STRIP TEST test under controlled conditions are available. This includes instructions for use, kit labels and protocols.

Prionics® TB-STRIP assay, components of prototype kits.

LIONEX has prepared all documentations required for producing the product under controlled conditions and for registering the Bovine TB test.

www.laves.niedersachsen.de

Dissemination and/or exploitation of project results, and management of intellectual property

1. LIONEX has prepared a new and updated website during PR2. Address of the website is www.demo-nopersist.com
2. A press release was submitted in PR2 on LIOSpot TB test out of DEMO-NOPERSIST

Another press release was made on Animal TB Rapid Test

Type: Company News
Category: Medical | Health | Wellness

Task 10: Formulation of an exploitation plan considering commercialisation

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Following is our main exploitation plan:

1. Secure strong IPR and trade mark registration: LIONEX already has a patent on the Elispot test based on ALADH as a patented antigen. Another patent has been filed which shall cover a novel Elispot technology application to all types of Elispot tests on the market. This is a completely new development and is expected to lead to extensive commercialisation by Lionex GmbH. LIONEX shall manufacture kit components under its own brand of Elispot tests. To the best of our knowledge, no patents or existing knowledge inside the consortium ("background") and outside the consortium is known to us that shall be detrimental to the exploitation of the project results by the SMEs.

LIONEX has already registered LioSpot, LIOKine and LIODetect as trade marks covering the different types of tests developed during the DEMO-NOPERSIST project.

2. Licensing and Distribution of products: LIONEX has established contacts with Oxford Immunodiagnostic, a large diagnostic companies selling the T-Spot TB test world-wide. Their test cannot differentiate between active TB and LTBI which is a big problem for them currently. Our LIOSpot TB test shall be highly attractive to them. A NDA has been signed between LIONEX and Oxfordimmundiagnostic. Both parties shall initiate deliberations very soon. Highly intense discussion are also in progress with Omega Diagnostic, U.K. LIONEX has good relations with Omega Diagnostics for the last 10 years. Omega is interested in distributing LIONEX TB products. A US company is another partner for distribution in North and South America. For Europe, our plans to initiate marketing ourselves, first in Germany and later in other European countries. We have about 1000 contact addresses from customers including Big distribution companies (Pharma Grosshandel) in Germany. Partnerships with distributors in India and Bangladesh, China and Mangolia has been established.

3. Registration. LIOSPot TB registration is planned for April 2016. For this, extensive stability studies are needed which are in progress. Prionics is now part of Thermo Fisher, one of the largest companies in this area. All decisions on Prionics TB products shall be made by Thermo who are in an excellent position to market any product, if they wish to go for it.

4. Advertising: LIONEX has already made press releases. This activity shall be extended further via EUROVISION, a company publicising the EU project results through films. Further press releases shall be made within the year 2016. A press release on our Animal TB test was also been done. All these products were presented at the BIOTECHNICA, an International Exhibition of Biotech. This year, LIONEX shall present the products at the MEDICA, the World’s largest exhibition on medical products.

5. Market penetration: Market penetration is achievable within 2-3 years since LIONEX has established business cooperation with distributor’s world-wide (India, USA, S. Korea, Japan, S. America, S. Africa, Bangladesh, Mangolia, USA, Europe).

Patent continuation issues:
LIONEX has continued with its PCT application. Final decisions from the EPO are expected by the end of 2015. Once granted, we plan to select countries based on the potential market volume which in turn shall depend on the population size, economic strength, health system, re-imbursement system etc. Depending
on the project budget we plan to go for Russian Federation countries, India, China, Bangladesh, Pakistan, China, USA, Canada, Mexico, Brazil, Argentina, Colombia, Venezuela, most of the European countries, Turkey, Tanzania, Nigeria, S. Africa, Kenya, Senegal, Uganda, Morocco, Tunisia, UAE, Saudi Arabia. Intellectual property rights ownership and user rights (e.g. licenses, royalties)
All background (Foreground from previous project) is owned by partners in this project.

Background and Foreground IPR
Access rights to Background IPR: The partners have signed the consortium agreement on a list of Background IPR to which they are ready to grant access rights to the other partners. Access rights to Background IPR needed to carry out the project will be granted royalty free by the partners, unless fair and reasonable conditions have been agreed prior to the signing of the grant agreement. Access rights to Background IPR needed to use own Foreground IPR will be granted by the RTD performer and by the SMEs on fair and reasonable conditions.

Current status of patent
LIONEX patent EP 12176506.9 (Background) is being continued further. To the best of our knowledge no patents or existing knowledge inside the consortium ("background") and outside the consortium is known to us that shall be detrimental to the exploitation of the project results by the SMEs.

Foreground IPR: All Foreground IPR shall belong to the respective SMEs. The SME shall have the right to file patents on such Foreground IPR in their own name. Access rights to Foreground IPR will be granted on a royalty-free basis if needed for a partner to carry out its work under the project. The RTD performers can use the Foreground IPR developed by the respective RTDP and owned by the respective outsourcing SME royalty-free for further research (noncommercial exploitation). Besides such research use, the RTD performers can publish the results of their research after obtaining the agreement of the respective outsourcing SME. If a SME is not interested in exploiting certain Foreground IPR developed by a RTDP, it shall grant the RTDP that developed such Foreground IPR a license to use such Foreground IPR for all purposes.

Dissemination by SMEs to other enterprises is at present not foreseen and shall depend on the type of IPR/ product developed.

To the best of our knowledge no patents or existing knowledge inside the consortium ("background") and outside the consortium is known to us that shall be detrimental to the exploitation of the project results by the SMEs.

Details of specific IPR exploitation rights
IPR exploitation rights:
1. LIONEX is the sole owner of the background from IPR from NOPERSIST concerning the human TB part.
2. LIONEX shall own all results of the human TB part of the DEMO-NOPERSIST.
3. LIONEX shall grant a non-exclusive license to UNIFI for the use of the human-TB tests developed in DEMO-NOPERSIST for research use.
4. PRIONICS shall be the sole owner of all results from the bovine TB part. Prionics shall grant a non-exclusive license to AHVLA for the use of tests developed in DEMO-NOPERSIST for research use.
5. LIONEX and PRIONICS already have agreements on the manufacture and commercialization of bovine TB rapid tests of Prionics.

6. Background from previous project NOPERSIST on human TB part shall be the responsibility of LIONEX. LIONEX has filed an EU patent application which shall be further pursued till granting.

7. All rights for the bovine TB tests shall be the property of Prionics.

8. Access conditions of any background which is owned by partners from the previous project but who no longer participate in the DEMO project: NA

9. Access is guaranteed: NA

10. Indication on time to market: 2016/2017

All background (Foreground from previous project) is owned by partners in this project.

Regulatory requirements or constraints

We do not see any serious regulatory requirements with which we are not familiar. We have ample experience in dealing with such requirements in a large number of countries world-wide.

Human active TB-tests, regulatory requirements

Final goal is to verify, that the Lionex products meet the essential requirements of IVD directive 98/79/EG and corresponding harmonized norms. All documentations were prepared by using blank forms, which are part of the Lionex QM system according to DIN EN ISO 13485. The essential requirements are recorded by using “DO 209 Grundlegende Anforderungen”. The declaration of conformity to the IVD directive 98/79/EG and the product registration was completed.

Finally all documents are prepared for registration to the corresponding authority.

The products are designed in that way that the possible of the risk of infection to the user or others is eliminated or reduced as far as possible according to DIN EN 13641.

Product design and process of production: design of labelling is done by using symbols according to DIN EN ISO 15223. According to DIN EN ISO 18113-1 and DIN EN ISO 18113-2 labels and instructions for use supply all information required for safe and reliable use of the product to the user. All SOP and blank forms (DO) required to produce the product under controlled conditions are prepared.

Performance evaluation was done according to DIN EN 13612.

Finally a technical file dossier was prepared which summarizes the test characteristics (corresponding blank form: “DO 207 Technische Dokumentation”). The file contains product descriptions, technical requirements, short risk statement with FMEA, statement of demonstration compliance of labels and instructions for use with the IVD directive 98/79/EG and harmonized norms DIN EN ISO 18113-1 and DIN EN ISO 18113-2, results of performance evaluation, results of stability tests, short statement of manufacturing process, specifications for quality control procedures, instructions for complaint management and mandatory problems and references.

Bovine TB-tests, regulatory requirements
Regulatory requirements or constraints: We do not see any serious regulatory requirements with which we are not familiar. Market launch in Europe will require product licensure by the Friedrich-Löffler Institute (FLI). It was planned that Prionics will seek regulatory approval from the FLI as a first step towards market entry. But the plan changed due to the merger of Prionics with a large industrial company Thermo Fischer. Following this LIONEX performed most of the experimental work and all documentation for registration. According to the regulatory guidelines, LIONEX must first obtain production licence for the veterinary product before any registration can be done. Discussions with Prionics are in progress regarding this issue.

- Highlight of significant results:

1. Development of a test specific to active TB called LIOSpot®-TB human. This is for the first time that such a test has been developed in the history of tuberculosis.

2. LIOSpot®-TB human is the first test showing high sensitivity and specificity, both in children and in adults

3. Manufacturing of LIOSpot®-TB human was completed and all documents prepared for CE marking.

4. Development of animal TB Rapid test was completed. Such serological ancillary tests will improve overall test sensitivity when used in combination (parallel) with the tuberculin skin test for the diagnosis of bovine TB.

5. Two press releases made: on LIOSpot TB and on Animal TB Rapid

6. Further patent application and two publications are being prepared on LIOSpot TB and Animal TB Rapid validation results

Potential Impact:
IMPACT: The results and products developed in DEMO-NOPERSIST are expected to have immense socio-economic and societal impact, not only in the European Union, but also world-wide since TB is a global disease. The LIONEX LIOSpot® TB kit is expected to diagnose, for the first time, active TB accurately. Since it is an immunological test, it shall detect both pulmonary and extra-pulmonary TB, the latter is much more difficult to diagnose than pulmonary TB. Unfortunately, extra-pulmonary TB is highly neglected, even though it accounts for about 40% of all TB cases world-wide. Women are main sufferers due to extra-pulmonary TB. Improving the quality of life of women in all countries is the declared commitment of international organisations such the WHO and the UN.

The LIODetect® Animal TB test is also valuable for increasing the sensitivity of the currently used skin tests. The test may help reduce TB in farmers who are in regular contacts with cattle. Since the test can be performed on site, it is very useful for rapid and routine diagnosis on farms.

Due to the reasons described above, the project results are expected to have strong economic impact by reducing the cost of health budget in member states and other TB endemic countries. It should lead to
creation of new jobs for LIONEX as manufacturer and owner of IPR on the products. This project has established strong collaborations between partners which shall be continued in future.

Following is our main exploitation plan:
1. Secure strong IPR and trade mark registration: LIONEX already has a patent on the Elispot test based on ALADH as a patented antigen. Another patent has been filed which shall cover a novel Elispot technology application to all types of Elispot tests on the market. This is a completely new development and is expected to lead to extensive commercialisation by Lionex GmbH. LIONEX shall manufacture kit components under its own brand of Elispot tests. To the best of our knowledge, no patents or existing knowledge inside the consortium ("background") and outside the consortium is known to us that shall be detrimental to the exploitation of the project results by the SMEs.

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6. Background from previous project NOPERSIST on human TB part shall be the responsibility of LIONEX. LIONEX has filed an EU patent application which shall be further pursued till granting.
7. All rights for the bovine TB tests shall be the property of Prionics.
8. Access conditions of any background which is owned by partners from the previous project but who no longer participate in the DEMO project: NA
9. Access is guaranteed: NA
10. Indication on time to market: 2016/2017

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4. Regulatory requirements or constraints

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Finally all documents are prepared for registration to the corresponding authority.

The documents involved are described below:

Risk management: A parts of the work according to DIN EN ISO 14971 a Failure Mode and Effects Analysis (FMEA) has been completed. Finally, to reach all requirements for regulatory purposes of the IVD device, a full risk analysis was prepared and recorded by using the corresponding QM documents (document blank form: “DO-208 Risikoanalyse”). The risk analysis completes with the risk management report. Material Safety Data Sheet was prepared according to REACH Regulation.

The products are designed in that way that the possible of the risk of infection to the user or others is eliminated or reduced as far as possible according to DIN EN 13641.

Product design and process of production: design of labelling is done by using symbols according to DIN EN ISO 15223. According to DIN EN ISO 18113-1 and DIN EN ISO 18113-2 labels and instructions for use supply all information required for safe and reliable use of the product to the user. All SOP and blank forms (DO) required to produce the product under controlled conditions are prepared.

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List of Websites:
www.demo-nopersist.com

Last update: 15 September 2016
Record number: 189154

Permalink: https://cordis.europa.eu/project/id/606577/reporting

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