General information	
PPP-number	TKI-AF-16138
Title	Multi-analyte diagnostic methods to detect (sub)clinical mastitis in cows
Theme	Food safety
Implementing institute	Wageningen Food & Biobased Research (WFBR)
Project leader research (name + e-mail address)	Dr. Aart van Amerongen, aart.vanamerongen@wur.nl
Coordinator (on behalf of private partners)	Dr. Holger Eickhoff, eickhoff@scienion.de
Project-website address	x
Start date	01-01-2017
Final date	31-12-2019

Approval by the coordinator of the consortium The final report must be discussed with the coordinator of the consortium. The "TKI's" appreciate additional comments concerning the final report.	
Assessment of the report by the coordinator on behalf of the consortium:	x Approved ☐ Not approved
Additional comments concerning the final report:	

Consortium	
Mention any changes in the composition of the project partners:	There has been a change in the consortium: Global DX has replaced ELDC. This has been reported in June 2018 (Adapted project plan dated the 9th of October 2018).

Summary of the project	
Problem definition	Mastitis is an inflammation of the mammary gland and is the most significant cause of economic losses in the modern dairy industry [1]. Direct losses occurring due to mastitis include: costs of medication, veterinary services, discarded milk, and additional care of diseased animals, and in some severe cases, the death of the animal. Indirect losses include: decreased milk yield, decreased milk quality due to changes in milk chemical content, shorter productivity duration of dairy cows, earlier onset of the dry period, and decreased subsidies due to inferior milk quality. Mastitis is also associated with other health disorders in cows, such as reproductive disorders and decreased feed intake, which can be reflected in decreased milk yield. At the laboratory level several diagnostic methods and technologies are available to detect these risk factors. In most cases one factor is determined in a single test (e.g. ELISA). In general, user-friendly at-line (onsite, or point-of-care) tests allow the detection of one factor as well. Both in view of the time needed to perform a test and with respect to high costs this is far from efficient. In addition, the joint results of single-factor

[1] Miroslav Benić *et al.* Bovine mastitis: a persistent and evolving problem requiring novel approaches for its control - a review. VETERINARSKI ARHIV 88 (4), 535-557, 2018.

	tests will generally be available at a later stage which is undesirable if animal health and food quality and safety is concerned. Farmers of milk-producing cows require a proactive diagnostic strategy with respect to their animal's health. User-friendly, onsite and multi-analyte (multi-factorial) diagnostic devices could greatly contribute to monitoring cow's health status with respect to (sub)clinical mastitis. Furthermore, the application of multi-analyte tests will yield a substantial efficiency improvement and costs reduction for farmers. The dairy diagnostic sector is, therefore, looking for multi-analyte diagnostic tests that can be produced at a large scale and in an economically costs-effective way.
Project goals	In this project onsite point-of-care tests for 3 acute phase proteins (APPs) will be developed at WFBR. The format of the assays is a lateral flow microarray immunoassay format. Milk samples will be collected by Global DX. The HAN and Scienion will be involved in the development of a portable reader, and furthermore, Scienion will be involved in the larger scale preparation of multi-analyte tests.

Results

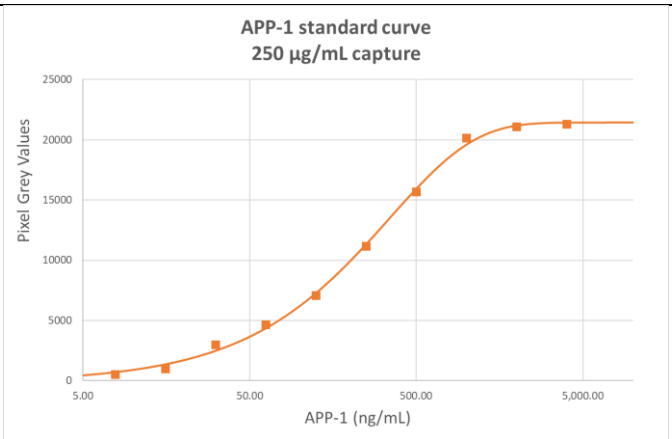
Planned results in the original project plan

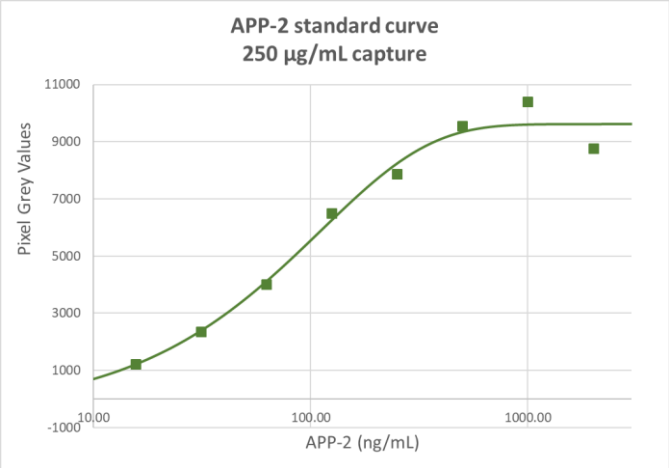
Technology
• Optimised protocol for the development of multi-analyte lateral flow, flow through and ELISA methods.
• Technical conditions for the large scale production of multi-analyte lateral flow tests by ultra low volume dispensing.
• Data acquisition system for real-time videorecording of multi-analyte test data.
• Technology demonstrator of multi-analyte data acquisition and processing.
Software
• Algorithms for spot tracking, feature extraction, and classification, as well as validation of such algorithms.
Diagnostics
• Rapid multi-analyte lateral flow tests for detection of acute phase proteins that correlate with (sub)clinical mastitis in cows.
• Multi-analyte diagnostic test platforms for the easy set up of new diagnostic combinations.

Achieved results

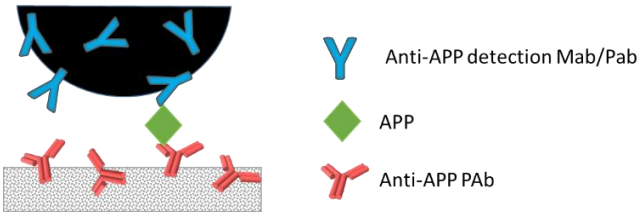
Acute phase proteins literature search
An extensive literature search was performed. This literature search resulted in a list of possible acute phase proteins (APPs) that could be markers of the subclinical and/or clinical state of mastitis.

Development of APP lateral flow tests
In the one year of project time left, for two of the APPs a lateral flow microarray assay was developed. Based on the literature search, the combined quantitative results of these lateral flow assays will be sufficient to be able to discriminate between a healthy cow, a cow with subclinical mastitis and a cow with mastitis. Therefore, the next, post-project, step for these assays will be a combination of these two assays on one lateral flow micro array immunoassay. Below the dose response curves for these two APP's can be found.

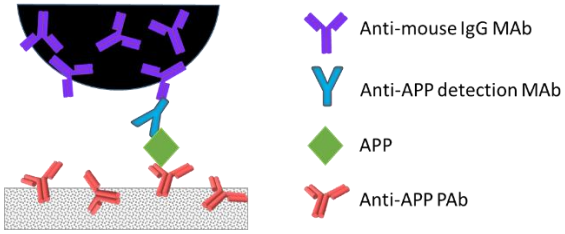


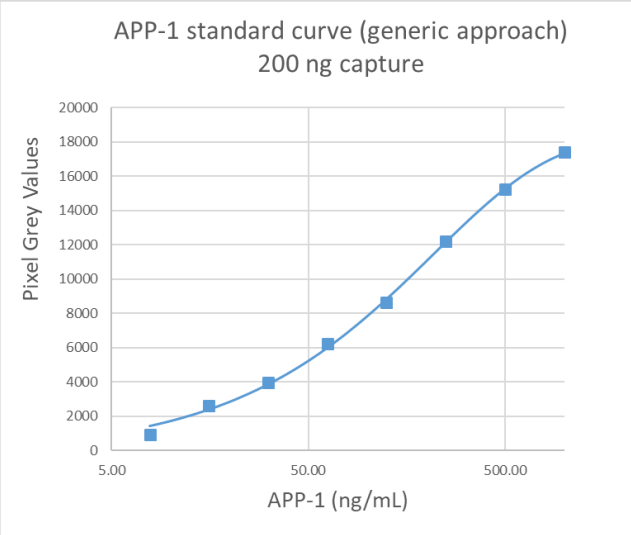


The setup of these assays was a conventional sandwich immunoassay as depicted below.



Generic format for a multi-analyte diagnostic test platform
For APP-1 an immunoassay with a generic format was set up as well. The setup and the dose response curve of these assay can be found below. In this generic setup the detection uses an anti-mouse IgG monoclonal (Mab) and the sandwich will be formed with two different antibodies directed against the APP. The detection antibody should be a mouse Mab that will be recognized by the anti-mouse IgG Mab.



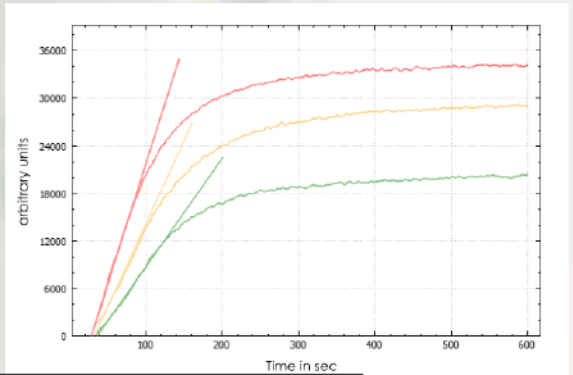


Unfortunately, for APP-2 the mouse Mab was out of stock until summer 2020 and no other mouse Mab could be found. If for APP-2 the generic approach can be set up after summer 2020, one carbon conjugate with an anti-mouse monoclonal can be used to analyze both APP-1 and APP-2 in one lateral flow microarray immunoassay.

Reader technology for lateral flow immunoassays

- Prototypes are available of the real-time sciREADER LF1 and Scienion is at the point of getting a CE label for the electronics, for which several tests have been performed. It is expected that the reader will soon be available on the market. With this reader the pixel grey value of spots can be measured kinetically. For quantification the slope of the line can be used (see figure below for an example). These prototypes use an open platform, are fully customizable, are small and portable, and can make use of wireless and wired connections.

- In the last year of the project experiments have been performed for line recognition. The general processing architecture appears to operate correctly, based on the processing of artificial images. More characterization experiments are required to find the limits of the algorithms.



Explanation of changes relative to the project plan	• Due to the change of subject in October 2018, there was only one year left for new test development. • In some cases particularly antibodies could not be obtained (e.g. APP-3), and therefore some specific tests could not be developed.

What was the added value created by the project for:

Participating "Knowledge Institutes" (scientific, new technologies, collaboration)	• Good collaboration with the participating companies • Innovative approach for a generic format for a multi-analyte diagnostic test platform • In some parts of the reader development students were involved giving them the possibility to collaborate in the contest of a commercial project.
Participating private partners (practical application of the results, within which period of time?)	**Global DX**: For two of the APPs a lateral flow microarray assay was developed. These assays have to be tested with milk samples and with dried carbon conjugates. If successful, the tests can be marketed afterwards. **Scienion:** It is expected that the reader will be on the market this year.
Society (social, environment, economy)	Application of the developed tests will be beneficial for the animal welfare and health and will reduce the loss of income for farmers due to mastitis.
Possibly other stakeholders (spin-offs)	The generic, multi-analyte approach would enable farmers to easily diagnose cows for (sub)clinical mastitis on-site.

Follow-up	
Did the PPP result in one or more patents (first filings)?	Title: Lateral flow immunoassay technique Application No.: 16001697.8 - EP3279662A1 Applicant: Scienion AG Inventors: Jeroen Veen, Hugo Antonie Arends, Aart van Amerongen, Maatje Koets
Are there any follow-up projects planned? If yes, explain. (Contract research resulting from this project, additional funding, or new PPP projects)	Scienion and WFBR are discussing follow-up projects

Deliverables/products during the entire course of the PPP (provide the titles and/or a brief description of the products/deliverables or a link to a website.
Scientific articles:
External reports:
Articles in professional journals/magazines:
(Poster) presentations at workshops, seminars or symposia. Scienion and WFBR provided a workshop at the 12th Rapid Methods Europe (RME) conference 5-7 November 2018 in Amsterdam, The Netherlands https://www.rapidmethods.eu/programme.php
TV/ radio / social media / newspaper:
Remaining deliverables (techniques, devices, methods, etc.):

https://www.wur.nl/en/Research-Results/kennisonline/AF16138-Multi-analyt-diagnostic-methods-to-detect-food-pathogens.htm

https://www.wur.nl/nl/Onderzoek-Resultaten/Onderzoeksprojecten-LNV/Expertisegebieden/kennisonline/Multi-analyst-diagnostic-methods-to-detect-food-pathogens.htm