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Towards the Measurement of Acute-Phase Proteins in Saliva in Farm Conditions: Development and Validation of a Lateral Flow Assay for the Measurement of C-Reactive Protein in Pigs

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Abstract: Point-of-care diagnostic tests, such as lateral-flow immunoassay (LFIA), have emerged as a fast diagnostic tool in both human and veterinary medicine. In this paper, a gold nanoparticle-based LFIA device was developed for the measurement of C-reactive protein (CRP) in porcine saliva, using a monoclonal anti-porcine CRP antibody. The dilution ratio for the saliva samples was optimized at 1:5 with an assay buffer. The reaction time was optimized to 20 min, since this provided a positive signal with high CRP concentration saliva samples, but a negative result with an assay buffer or samples with a low CRP concentration. Linear results were observed when two samples with a high CRP concentration were serially diluted. Also, a linear relationship was observed with a validated quantitative method. The assay was precise when samples with high CRP concentration were measured five times in a single assay run. No overlap was observed when samples from healthy and diseased animals were analyzed. The LFIA allowed the detection of high CRP concentrations in porcine saliva samples. The intensity of the result was proportional to the CRP concentration obtained with the quantitative method, allowing for the possible use of the test for semiquantitative purposes.

Keywords: C-reactive protein; lateral flow; saliva; pig

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1. Introduction

According to the Eurobarometer data, the European population believes that the future of livestock production must seek respect for animal welfare and health as a way to increase its sustainability [1]. Currently, one of the main causes of decreased welfare and health of pig farm animals is the appearance of pathologic conditions, which are usually associated with inflammation, especially due to infectious diseases. They represent

a major problem in porcine farms, producing losses due to lower production and increased mortality rates [2–4], also leading to extensive use of antibiotics. An early detection of inflammatory and infectious diseases could be a very useful aid to avoid economic losses as well as the use of anti-microbials, which has led to the search of suitable biomarkers for this purpose. C-reactive protein (CRP) is a major acute phase protein (APP) with an important role in the immune system's reaction against infection and in systemic inflammation. It increases in a fast and significant way after an inflammatory stimulus, and it also has a rapid decrease after appropriate treatments. Therefore, CRP measurement can be an early biomarker to detect infectious disease and can also have application in treatment monitoring. For this reason, the use of this biomarker is increasing in both human [5] and veterinary [6] medicine.

Point-of-care (POC) diagnostic tests have become substantial for measuring biomarkers in modern healthcare, since they allow the early diagnosis of pathological states and diseases [5]. Among the different systems that could be applied for POC, the lateral-flow immunoassay (LFIA) has emerged as a potential platform [7]. It consists of a horizontal nitrocellulose (NC) membrane assembled on a plastic back. The sample is applied to the sample pad which contains a specific antibody conjugated to a suitable label, and it travels to the absorbent pad by microcapillary flow. The pad has test and control bands which are formed by the further specific antibodies or antigens of the selected biomarker, which are immobilized on the NC membrane. As the sample passes, the biomarker–antibody complex is held by the immobilized antibody and a concentrated line is formed [8,9]. In the particular case of CRP, there are several examples of the development and validation of LFIA devices for its measurement in human serum [10–12], as well as in dogs [13,14]; however, to the authors' knowledge, they have not been developed for other veterinary species, and nor for pigs.

Saliva is a very convenient source of analytical biomarkers since it can be obtained easily, without causing stress or pain to the animals and at reduced costs [15]. Furthermore, there is no need for specialized personnel, so the people in charge of taking care of the animals in farms can do the sampling without the need for participation of outsiders, further reducing stress among the farm animals and the possibility of the introduction of additional infection. This is of particular interest in pigs, where the procedures for obtaining blood are particularly stressful [6]. Among the analytes that can be measured in porcine saliva, biomarkers of inflammation such as APPs, and particularly CRP, have shown to be useful to detect inflammation. The first time that CRP was measured in saliva was in dogs [16]. Since then, CRP has been measured in saliva of humans and several animal species, including pigs, providing a picture of the undergoing inflammatory reaction in the animal [17–19]. Based on the authors' experience, the CRP concentration in saliva of healthy pigs is usually lower than 20.0 µg/L, although the range between 20.0 and 39.0 µg/L could be considered as doubtful in which the presence of inflammation is not clear.

Highly sensitive methods based on time-resolved immune fluorometry (TR–IFMA) and alphaLISA technology have been developed for APPs allowing CRP determinations in porcine saliva [20]. Although these methods have shown high sensitivity, they are not suitable for POC, as they require long sample preparation time and sophisticated reading devices, which preclude their use at the farm level. These methods use 96-well plates and the minimum incubation time required is 2 h. In addition, the samples have to be sent to a reference laboratory in which they are processed and analyzed, which implies the need for transportation and also a delay in receiving the results since it could take up to 3 days (min. 1–2 days). Due to these reasons, it would be of great benefit to have analytical POC systems that could be used at farms. Those systems would be especially relevant in the case of CRP measurement in porcine saliva, since they would permit fast and easy measurement of CRP

in field conditions, avoiding degradation of the analyte due to sample management and storage. This would allow to have a real-time picture of the welfare and sanitary status of the animals and take fast and appropriate management decisions.

The hypotheses of this paper are that CRP can be measured in the saliva of pigs using a quick system based on gold nanoparticles (GNPs) LFIA, and that this system can be used to evaluate the welfare status of pigs. Therefore, the objectives of this research are to develop and validated from the analytical point of view a GNPs-based LFIA for the measurement of CRP in saliva of pigs.

2. Materials and Methods

2.1. Monoclonal Antibody Culture and Purification

A monoclonal antibody (mAb) against porcine CRP was produced from clones of hybridome cells, according to standardized protocols [21,22]. Supernatants containing monoclonal antibodies were filtered through 1.2 and 0.22 μm filters (Merk, Darmstadt, Germany) and the Ab were purified using G protein columns (H TrapTM Protein G HP, Merk), following manufacturer instructions, using an automated liquid chromatography system (ÄKTA pure, GE Healthcare, Chicago, IL, USA). The purity of the mAb suspension obtained after purification was assessed by SDS-PAGE under non-reducing conditions, providing ≥95% purity.

2.2. Development of the LFIA for the Measurement of CRP in Porcine Saliva

For this purpose, previously published protocols were used [23,24]. The LFIA devices were developed as follows:

2.2.1. Preparation of Gold Nanoparticles and Gold Conjugates

The LFIA for the detection of CRP in porcine saliva was developed based on the sandwich-type immunoassay format. Therefore, the anti-porcine CRP mAb was spotted onto the nitrocellulose membrane as the capturing bioligand to form the T-line. The same mAb was conjugated to gold nanoparticles to produce the detection reagent.

The GNPs were synthesized by adding 1 mL of 1% w/v sodium citrate (VWR International, Milan, Italy) was added to 0.01% of boiling tetrachloroauric acid (Sigma-Aldrich, St. Louis, MO, USA) under vigorous stirring.

The optimal amount of mAb to be adsorbed on GNPs was determined by the salt-induced aggregation assay [25], as the amount of antibody that stabilize GNPs and prevent their aggregation. GNPs were conjugated with the stabilizing amount of antibody (40 μg/mL of GNP, OD 10) and with 0.5x and 2x the stabilizing amount (corresponding to 20 and 80 μg/mL of GNP, OD 10). The four conjugates were compared in terms of the maximum signal provided by a *positive solution* (a saliva sample from a sick animal was diluted in buffer until a CRP concentration of 100 μg/L) and the absence of signals for a *negative solution* (buffer). The amount of conjugate to be introduced into the device was also optimized using the same strategy. Finally, the optimal diluent and sample dilution factor for the detection of CRP in porcine saliva was determined by comparing the signals obtained from a porcine saliva sample containing 90 μg/L of CRP. Assays were performed by duplicate.

Once the optimal OD was established, monoclonal anti-porcine CRP antibody was adsorbed onto GNPs by mixing 20 μg of antibody and 1 mL of 20 mM borate buffer (pH 8.0) with 10 mL of GNPs. The mixture was incubated for 30 min at 37 °C. Then, 1 mL of a solution of 1% w/v BSA was added to passivate the uncoated residual surface of the GNPs. The mAb conjugate was recovered by centrifugation (15,000× g, 15 min), washed twice with borate buffer with 0.1% BSA, and once with a storage buffer solution (borate buffer

supplemented with 2% w/v of sucrose, 0.25% v/v of Tween20 and 0.02% w/v of sodium azide). The concentrated GNP–mAb conjugate (20x×) was stored at 4 °C until use.

2.2.2. Preparation of LFIA Devices

Membrane and pad types were determined based in previous publications of LFIA in saliva [23]. The GNP–mAb were diluted 1:5 with the storage buffer and adsorbed (0.1 mL/cm) onto the conjugate pad (Merck Millipore, Billerica, MA, USA), pre-saturated with the same storage buffer solution. It was dried for 3 h at room temperature. The monoclonal anti-porcine CRP antibody was applied to a nitrocellulose (NC) membrane (MDI Membrane Technologies, Ambala, India) to form the test line (T-line, 1 mg/mL), whereas an anti-mouse IgG antibody produced in rabbit (Merck Millipore, Billerica, MA, USA) was used as the capture antibody at the control line (C-line), by using a XYZ3050 platform (Biodot, Irvine, CA, USA), keeping a distance of 4 mm between control and test lines. NC membranes were dried at 37 °C for 60 min under vacuum. The sample pad, conjugate pad, membrane, and absorbent pad were overlapped, and strips were cut (3 mm width) by means of a CM4000 guillotine (Biodot). The strips were inserted into plastic cassettes (Kinbio, Shanghai, China). Cassettes were stored in the dark in plastic bags containing silica at room temperature until use. Figure 1 summarizes the basis of this assay.

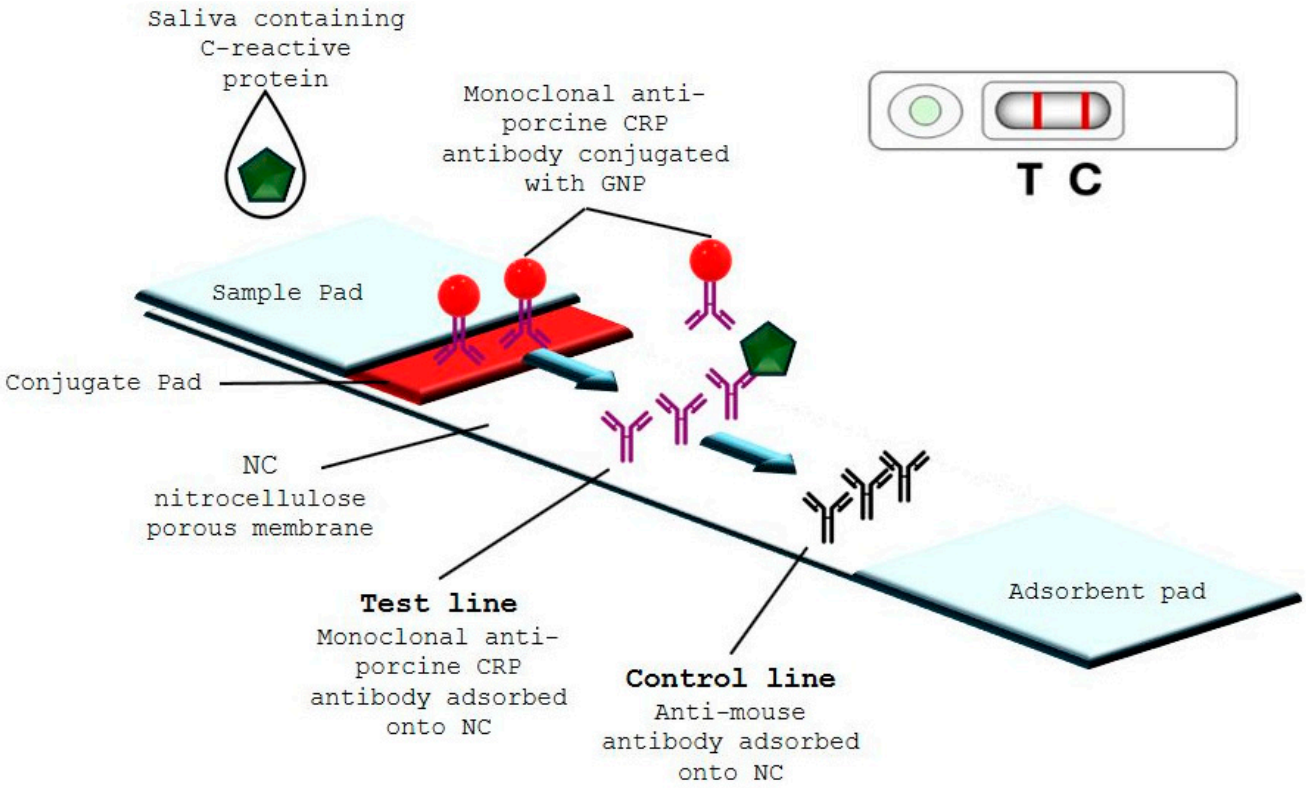

Figure 1. Scheme of the LFIA device used for the rapid measurement of the C-reactive protein (CRP) in porcine saliva. The strip is composed of a nitrocellulose membrane (NC), onto which a monoclonal anti-porcine CRP antibody and rabbit anti-mouse IgG were coated to form the T- and C-lines, respectively. Whereas gold nanoparticles (GNP) conjugated to the anti-porcine CRP were impregned to the conjugate pad. An absorbent pad promotes the capillary flow, removing unbound reagents and thus the background signal. A single visible line (C-line) is expected for buffer diluent or saliva with low CRP concentration. The presence of high CRP concentration is revealed by the specific binding to the monoclonal antibody, generating a second line (T-line).

2.3. Protocol Assay

Different buffers (PBS pH 7.4; Tris-Gly pH 8.2; carbonate–bicarbonate pH 9.0) and several combinations of additives (Tween 20, 0–1–2–5% and BSA, 0–0.5–1%) were checked to find one assuring the highest signal for a positive sample and the absence of background signal. The chosen assay buffer consisted of tris(hydroxymethyl)aminomethane 34 mM, glycine 80 mM, pH 8.2 supplemented with 2% v/v Tween20 and 0.05% w/v sodium azide. For the dilution factor, the optimized buffer was used to check 1:2–1:5–1:10 dilution factors. At 1:2 dilution, matrix interference was not compensated, while at 1:10, the sensitivity was decreased. Then, 1:5 was chosen.

A saliva sample was diluted in a 1:5 proportion with the assay buffer. Then, 60 µL of the dilution were added to the sample pad. The signal was recorded with a strip reader (ESEQuant LR3 Starter Kit, DIALUNOX GmbH, Stockach, Germany). The area of the peaks observed at the T- and C-lines was recorded by multiplying the width of the peak in mm with the height of the peak in mV (mm*mV). The areas obtained at T-line, as well as the T/C ratio, were used for further calculations.

2.4. Animals and Saliva Samples

All samples used in this study were from pigs (*Sus Scrofa domesticus*, Large White) in nursery period from farms located in Southern Spain. Animals were housed according to the European Union guidelines (Directive 2010/63/EU1). Saliva samples were collected by allowing animals to chew a 5 × 2 × 2 cm piece of polypropylene sponge (Esponja Marina, La Griega E. Koronis, Madrid, Spain) attached to a thin, flexible metal rod, until the sponge was thoroughly moistened at around 1–2 min. Then, the sponges were introduced into saliva collection tubes (Salivette, Sarstedt, Aktiengesellschaft & Co., Nümbrecht, Germany), kept on ice, and transported refrigerated (less than 2 h) to the laboratory. Once at the laboratory, the tubes were centrifuged (3000× g, 10 min, 4 °C) and saliva was transferred to 1.5 mL tubes (Eppendorf Ibérica, San Sebastián de los Reyes, Madrid, Spain) to be stored at −80 °C until analysis. In order to avoid the presence of food or dirtiness in the samples, any colored sample was discarded.

All selected animals were at the beginning of the fattening phase (60–65 days old). Two groups of animals were sampled. On the one hand, healthy animals (n = 25) located at the Veterinary Teaching Farm of the University or Murcia (Spain). This farm is free of porcine respiratory and reproductive (PRRS) virus and none of the animals had signs of illness after physical and visual examinations. Also, animals with signs of disease from other farms at Southern Spain were sampled. Diseased animals included pigs from a PRRSv-positive farm with clinical symptoms of meningitis (n = 14), and that were positive for *Streptococcus suis* infection after polymerase chain reaction (PCR) analyses. These samples were used for the validation protocols.

2.5. Validation Protocol for LFIA

The validation of the LFIA devices was based on previously published protocols [23] and included the following parameters:

2.5.1. Optimization of Reading Time

For the assessment of the most appropriate reading time, three samples with different CRP concentrations (230.0, 90.0 and 22.5 µg/L) and two blanks were measured with the LFIA assay and readings were performed at 10, 15, 20, 30, 45, and 60 min after the samples were added to the LFIA wells. Analyses were performed once with each sample. The optimal reading time was selected and used for the rest of the analyses.

2.5.2. Accuracy

Accuracy was indirectly tested by analyzing three saliva samples with high CRP concentrations (110.9 and 445.6 μg/L). Samples were diluted at 1:2, 1:5, 1:10, 1:20, and 1:30 dilution ratio with the assay buffer, as previously stated. All dilutions were analyzed once, as previously indicated. The most appropriate dilution for sample analysis was decided from this approach. Also, 41 saliva samples were measured with two methods: the LFIA device and an immunoassay method based on AlphaLISA technology, previously validated for CRP quantitation in porcine saliva samples [20].

2.5.3. Imprecision

Imprecision was evaluated by measuring one positive sample (CRP concentration 90.0 μg/L) and one negative sample (CRP concentration 0.9 μg/L) five times in a single day for intra-assay imprecision calculation. Also, a blank was measured three times in a single day.

2.5.4. Overlap Performance

LFIA devices were used in saliva samples obtained from healthy (n = 25) and diseased pigs (n = 18). The results obtained from each group were statistically compared.

2.6. Statistical Approach

For linearity under the dilution assay, linear regression analysis was performed for each sample and a curve was constructed by plotting the signal obtained on the Y-axis against the theorical concentrations on the X-axis. Also, the results obtained with LFIA and the quantitative method were compared by linear regression. In the precision assay, the coefficients of variation were calculated as the percentage of the standard deviation of the five replicates divided by the obtained mean value. In the overlap performance, the results obtained in each group were analyzed by a Mann–Whitney U test. Routine statistic calculations were performed by using spreadsheet (Microsoft Windows Excel, Microsoft Corporation, Redmond, WA, USA). GraphPad statistical software (GraphPad Prism, version 9 for Mackintosh (Graph Pad Software Inc., San Diego, CA, USA) was used for statistic calculations and figures preparation. The significance level was set at $\alpha = 0.05$.

3. Results

3.1. Optimization of the GNP–mAb Conjugate

The optimal anti-porcine CRP mAb was determined to be 20 μg/mL of GNP, OD 10, since the highest T-line was observed with no signal with the negative (buffer) sample (Figure 2A). The best compromise between the highest sensitivity and the lowest background signal was achieved by impregnating the conjugated pad with monoclonal anti-porcine CRP GNPs diluted to OD 2 (Figure 2B).

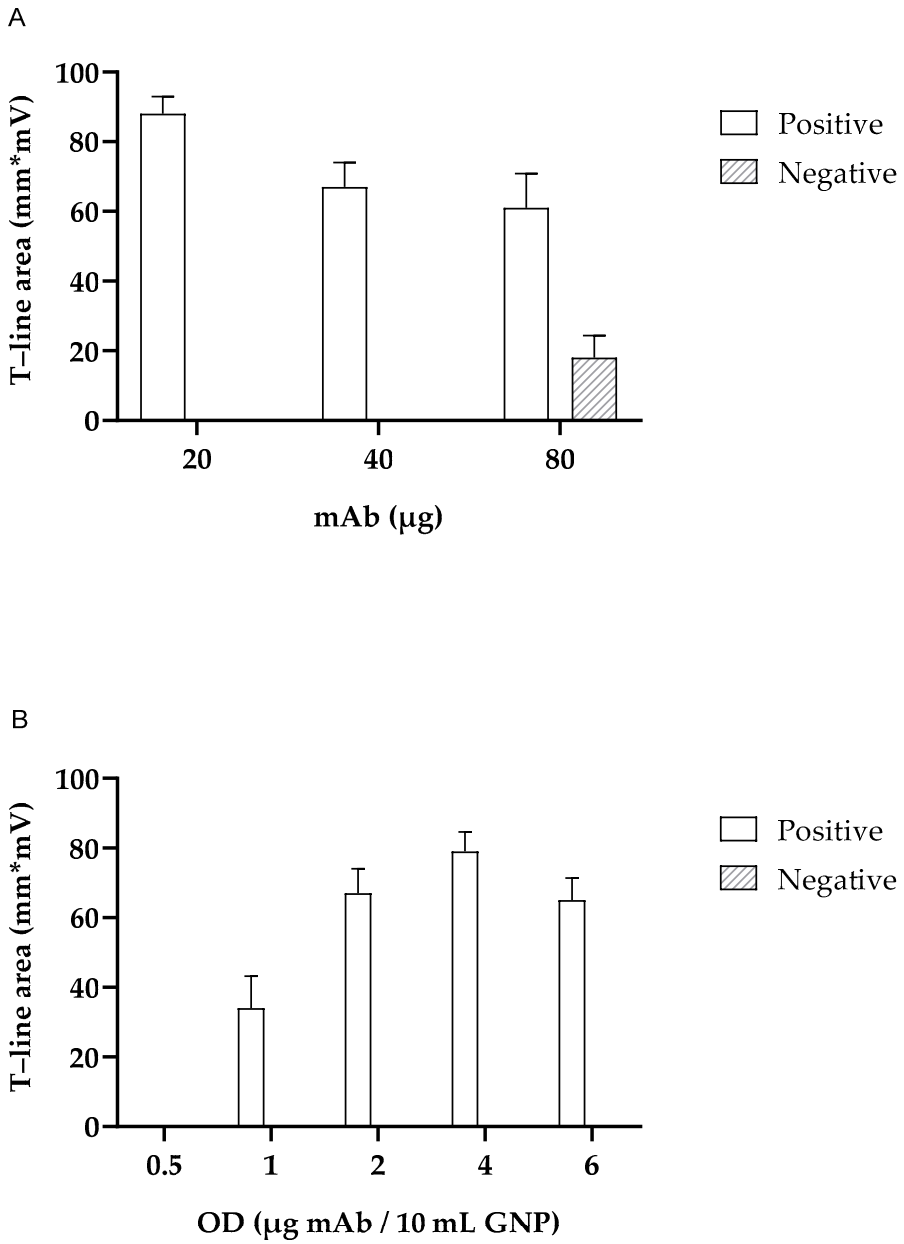

Figure 2. (**A**) Optimization of the monoclonal anti-porcine CRP antibody (mAb) concentration for conjugation with gold nanoparticles (GNP). (**B**) Optimization of the amount of conjugate (OD). Bars indicate the mean value obtained of two replicates, error bars indicate standard deviation. Positive: saliva sample with 100 µg/L CRP concentration. Negative: sample buffer.

3.2. Optimization of Reading Time

The results of the optimization of the reading time are shown in Figure 3. A sample with a CRP concentration of 90.0 µg/L (final concentration of 18.0 µg/L) or higher were positive at 10 min, whereas a sample with 22.5 µg/L of CRP (final concentration of 4.5 µg/L) became positive only after 30 min. Blanks provided negative results after 60 min. Reading time was set at 20 min in order to have a balance between positive and negative samples for the rest of assays as explained in the discussion.

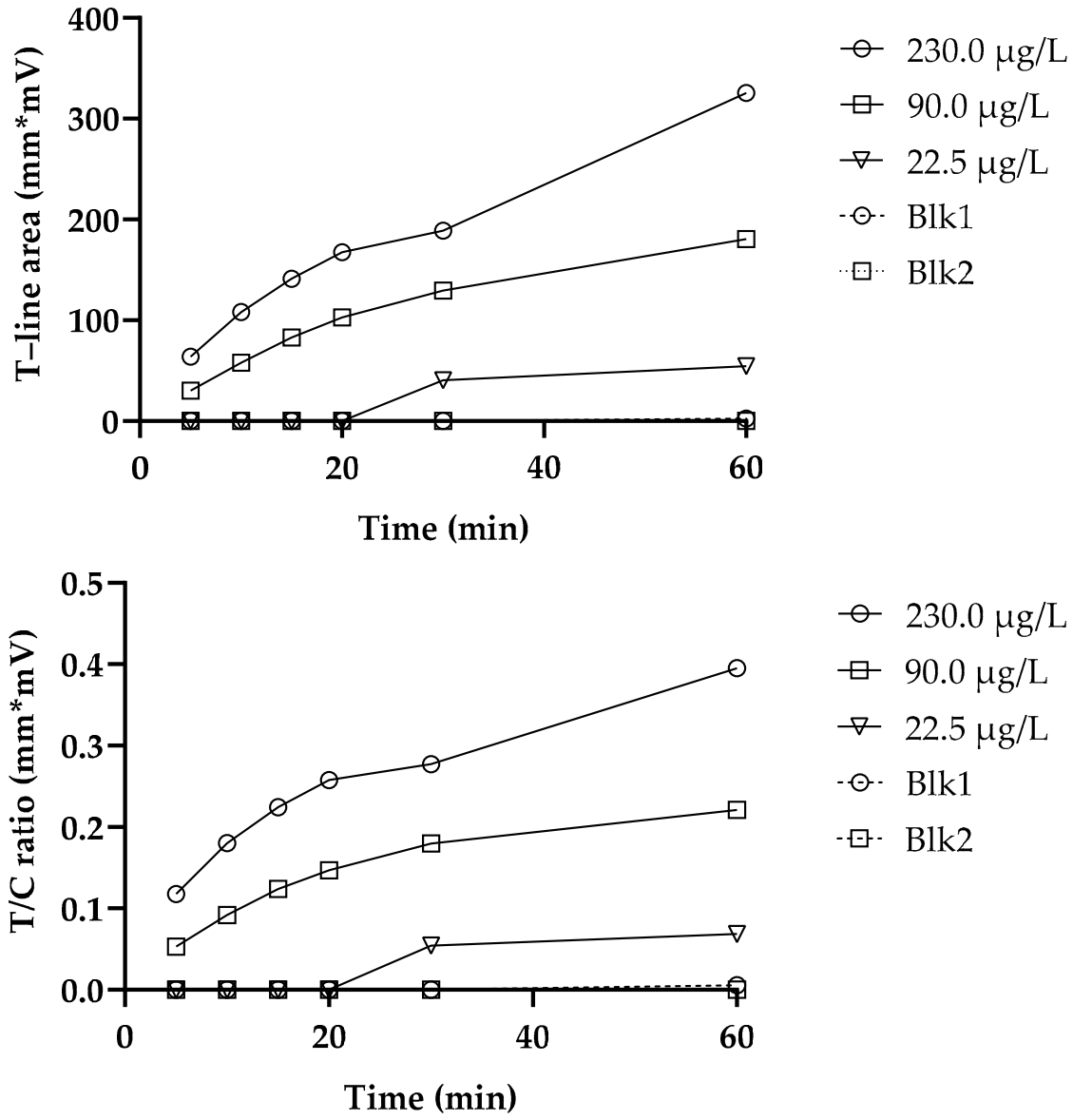

Figure 3. Optimization of reading time with three samples of different C-reactive protein (CRP) concentration and two blanks (Blk) consisting of sample diluent. The Y-axis represents the signal recorded at the T-line and the T-line divided by the control line ratio (T/C ratio), whereas the X-axis indicates the reading time in min.

3.3. Accuracy

The results of the analytical validation of the strips resulted in adequate linearity with coefficients of determination $R^2 > 0.90$ and a slope significantly non-zero ($p < 0.01$). Concentrations lower than 5.5 µg/L yielded a negative result (Figure 4). Also, since a 1:2 dilution ratio resulted in a decrease in linearity, the dilution ratio was set at 1:5 for the rest of the measurements. The regression coefficient between LFIA and the quantitative method provided $R^2 > 0.68$ (Figure 5), and the Spearman correlation coefficient was $r > 0.83$ ($p < 0.001$).

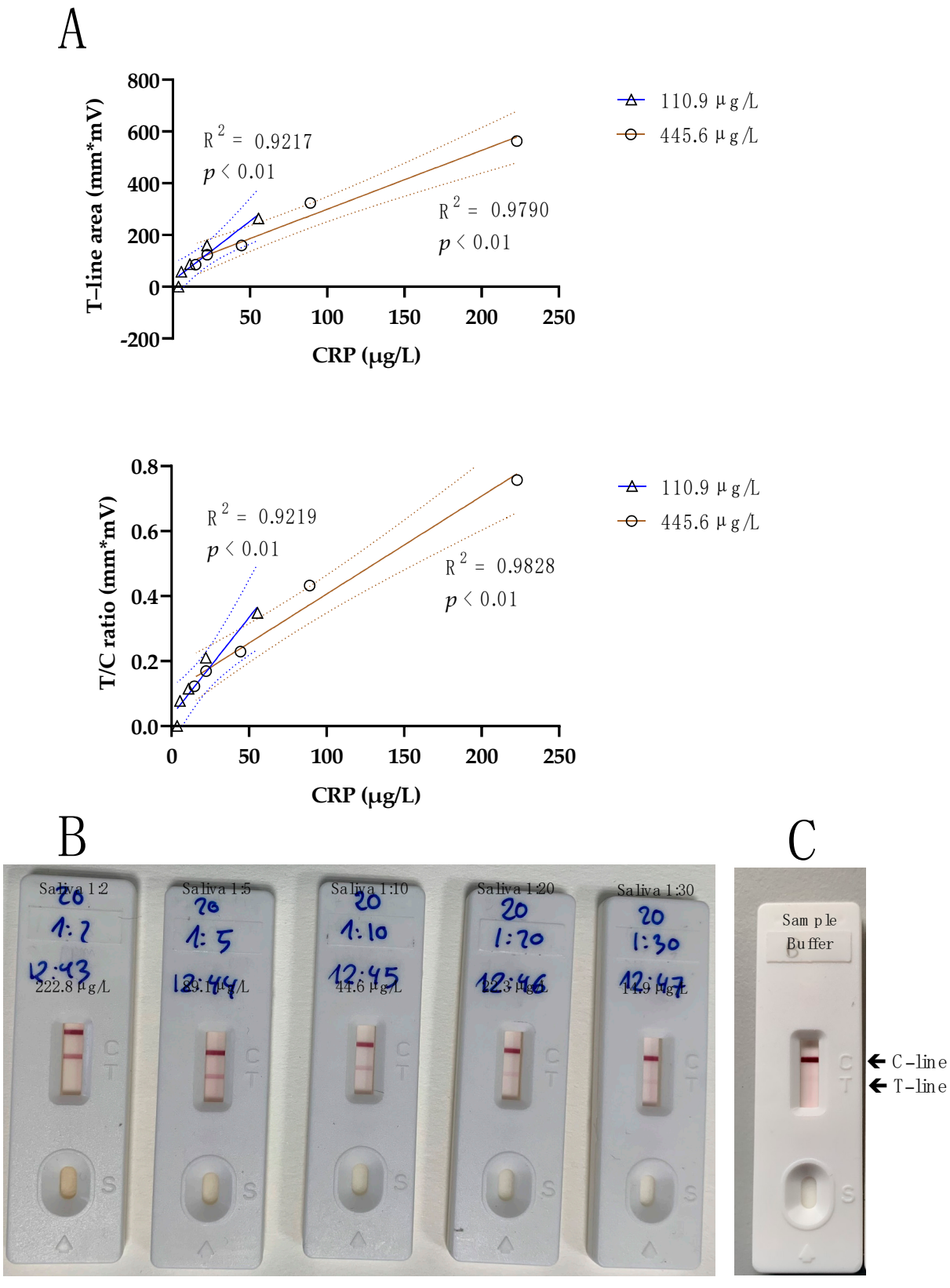

Figure 4. (**A**) Linearity under dilution results obtained with two samples with different C-reactive protein (CRP) concentrations. The Y-axis represents the T-line divided by the control line ratio (T/C ratio), whereas the X-axis indicates the CRP concentration. (**B**) LFIA devices after linearity under dilution assay of the sample with 445.6 µg/L CRP concentration (for each device, the final CRP concentration is indicated). (**C**) A blank consisting of sample buffer.

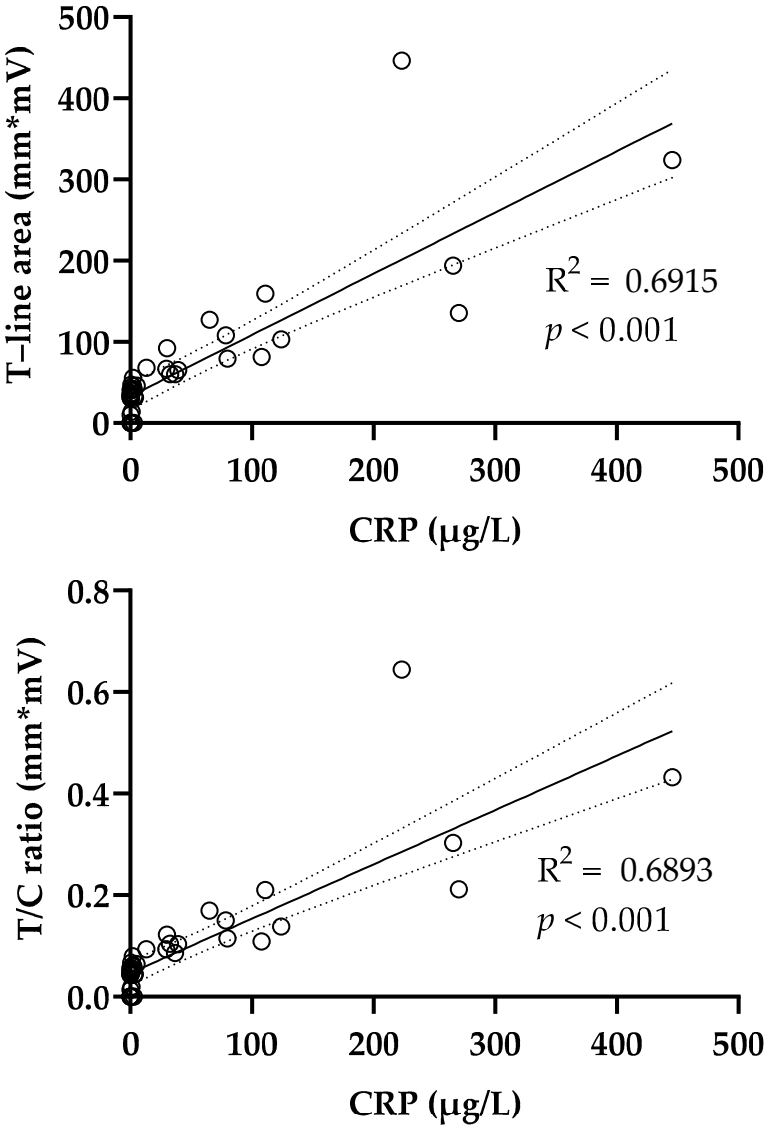

Figure 5. Regression plot obtained between LFIA (Y-axis) and the quantitative method (AlphaLISA, X-axis) after the analysis of 41 porcine saliva samples with different C-reactive protein (CRP) concentrations.

3.4. Imprecision

Precision results are shown in Table 1. The LFIA assay showed intra-CVs with the positive sample lower of 7.5% for the T-line and lower than 6.0% for the T/C ratio. The negative sample provided very high CVs, since the area in the T-line ranged between 0 and 40.0 mm*mV. With blanks, the T-line was always 0.0 mm*mV so the CV was not calculated.

Table 1. Intra-assay imprecision. Two samples with different C-reactive protein (CRP) concentrations were analyzed five times in a single day with the LFIA device. The signal recorded by the reader is indicated.

CRP (µg/L)		Mean (mm*mV)	SD (mm*mV)	CV (%)
0.9	T-line	9.2	19.3	209.1
	T/C ratio	0.01	0.03	204.2
90.0	T-line	42.3	3.1	7.4
	T/C ratio	0.11	0.01	5.9

3.5. Overlap Performance

Overlap performance showed significant differences between the group of healthy animals and the diseased ones (Figure 6). The highest T/C area observed in healthy animals was 0.08 mm*mV (CRP concentration of 2 μg/L), whereas the minimum T/C area in the diseased ones was 0.09 mm*mV (CRP concentration of 36.9 μg/L). No overlap between both groups was found. Also, the T-line area ranged between 0.0 and 55.8 mm*mV in healthy animals, and 60.1 and 446.4 mm*mV in the diseased ones.

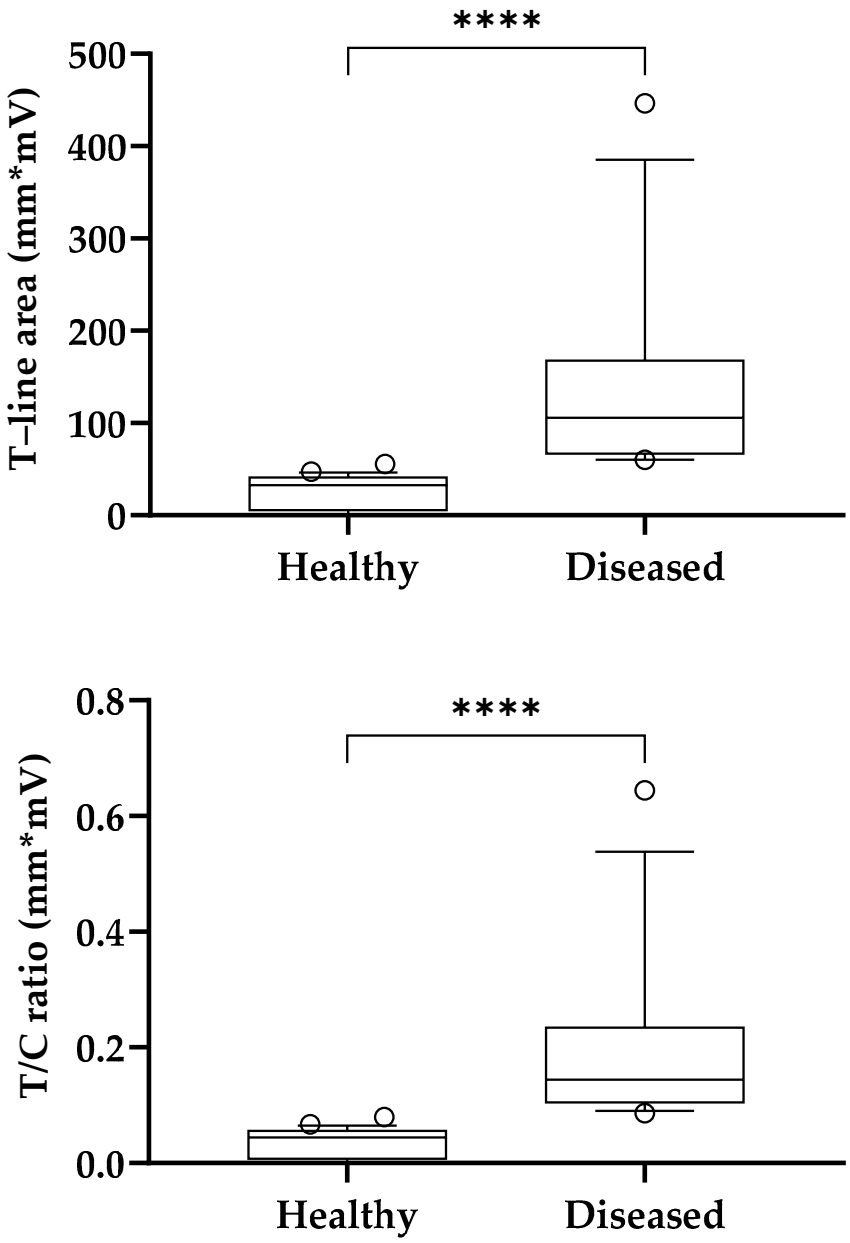

Figure 6. LFIA results observed in healthy pigs (n = 25) and diseased pigs (n = 18), including animals from a positive respiratory and reproductive syndrome (PRRS) farm and positive to *S. suis* infection. Statistical analysis, ****: $p < 0.0001$.

4. Discussion

The use of POC diagnostic testing provides several advantages over traditional laboratory diagnosis. Those advantages derive mainly from the ability of obtaining fast results [26], which allows making rapid decisions. In a previous report, the use of POC testing reduced swine and cattle response time against the appearance of infectious dis-

eases, reducing the spread of the disease and subsequent economic losses. In addition, this strategy was well considered by final consumers, especially within one health concept in case of zoonotic diseases [27]. In swine, POC diagnostic systems have been applied for the diagnosis of enteric coronaviruses [28], encephalitis [29], influenza A [30], or African swine fever [31], in a simple, rapid, and cost-effective way. In contrast with the previous tests that were focused on specific disease diagnosis, the system proposed in this research provides a rapid determination of CRP in porcine saliva that, as a reflect of circulating CRP levels, would allow a general knowledge of the health status of individual animals, discarding the presence of inflammation in case of a negative result [17].

The evaluation of how the test changes over time is an important issue, moreover for negative results since they can trend to show a positive T-line [23]. The T-line was slightly colored for blank only after 60 min. A CRP concentration in saliva of 90.0 μg/L (final concentration of 18.0 μg/L in the LFIA device) or higher provided a positive result in the T-line after 5 min, and a stabilizing trend was observed after 20 min. Meanwhile, a sample with 22.5 μg/L (final CRP concentration of 4.5 μg/L) provided a positive result after 30 min. It is important to notice that the sample with 22.5 μg/L could be considered as a negative one since it is within this doubtful range (between 20.0 and 39.0 μg/L). Based on those results, and in order to achieve the better performance for distinguishing between normal/inflammatory CRP levels, the reading time was set at 20 min since at this time samples with CRP values in saliva within normal range, as well as blanks, will provide a negative or very low result at T-line.

Although the LFIA test was not initially considered for a quantitative assessment, the regression analysis of the measured T-line area and T/C ratio resulted in $R^2 > 0.9$, also reflected in differently colored T-lines. Thus, a more strongly colored T-line would indicate a higher CRP concentration, and vice versa. In addition, the T/C results obtained with the LFIA showed a significant linear relationship with the CRP values obtained with the quantitative method, suggesting the possibility of a semiquantitative determination of the salivary CRP concentration using the LFIA devices. Although a lower-than-ideal correlation was obtained between both methods, probably due to the saliva matrix effect. Further research including a wider number of samples would be desirable in order to check the possible use of this assay for semiquantitative purposes. Precision results provided a CV lower than 20%, which is recommended for immunological methods [32], except for samples with low/normal CRP concentration that have higher CVs. Few studies have been found in which precision had been studied for lateral flow assays in porcine samples. In an assay for African swine virus detection in serum, the CVs were between 7.9 and 14.5% [33], which are in line with our results.

Overlap performance showed a significantly higher T-line and T/C ratio areas in diseased animals compared to healthy ones. The T/C ratio area was always < 0.1 mm*mV in negative samples, and no overlapping was observed between the groups of healthy and diseased animals. Nevertheless, those samples with concentrations below the doubtful value (<39.0 μg/L) had a high imprecision that could provide a high variability, so they could not be fully used in discriminating the values below this concentration. Therefore, it is important to point out that the test developed in this report is not useful for accurately discriminate between low values of CRP, but it has a practical use for detecting values of CRP higher than 39 μg/L, which would indicate that an inflammatory disease is occurring.

This study has some limitations. Due to the low number of samples analyzed, the study should be considered as a pilot one and further assays must be performed in order to corroborate the results obtained. Regarding the observed differences between healthy and diseased animals, it would be worthy studying if the different hydration status could influence them and further studies should be conducted to evaluate this. Also, the use

of the developed LFIA in field conditions by using the devices at farm level, exploring whether skipping the saliva centrifugation step could be applied, as well as the use of reading systems that can be used in these conditions should be explored. This would allow a better knowledge about the test reliability and accuracy, which are considered risks of POC tests [34–36], as well as evaluating the costs associated with test inaccuracy [27]. Another limitation of this assay is the low sensitivity that does not allow discrimination when the CRP concentration is low or within the doubtful range. Furthermore, it must be taken into account that the fact that animals with a CRP concentration within the doubtful range may give a negative result in the test could compromise the detection of animals with a subclinical pathology. Thus, it would be advisable to improve the assay in order to be able to detect these animals, even with the risk of detecting positive healthy animals with a CRP concentration within the said doubtful range. Systems for improving sensitivity can be used to solve this issue, such as the use of weakly ionized AuNP which could amplify the limit of detection to the range of ng/L [37] and could be applied in this assay in future studies.

5. Conclusions

The LFIA test developed in this research allowed the detection of high CRP concentrations in porcine saliva in a reliable, easy, and fast way. By combining the advantages of using saliva and a stand-alone device, it allows CRP measurement in farm and field conditions. In this test, the intensity of the T-line signal and T/C ratio was proportional to the concentration of CRP obtained with a quantitative method, allowing the possible consideration of the test for semiquantitative purposes. Further studies, at farm level, should be performed in order to corroborate the results and also to gain more data about the applicability of the device for POC testing in real practical conditions.

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