J Vet Sci. 2020 Sep;21(5):e79
https://doi.org/10.4142/jvs.2020.21.e79
pISSN 1229-845X·eISSN 1976-555X

Journal of Veterinary Science

Original Article
Biotechnology

Check for updates

Development of a biosensor from aptamers for detection of the porcine reproductive and respiratory syndrome virus

Chakpetch Kuitio [1], **Natchaya Rasri** [2], **Duangnapa Kiriwan** [1], **Sasimanas Unajak** [2], **Kiattawee Choowongkomon** [2,3,*]

[1]Genetic Engineering Interdisciplinary Program, Graduate School, Kasetsart University, Bangkok 10900, Thailand
[2]Department of Biochemistry, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand
[3]Center for Advanced Studies in Nanotechnology for Chemical, Food and Agricultural Industries, KU Institute for Advanced Studies, Kasetsart University, Bangkok 10900, Thailand

OPEN ACCESS

Received: Apr 28, 2020
Revised: Aug 11, 2020
Accepted: Aug 18, 2020

***Corresponding author:**
Kiattawee Choowongkomon
Department of Biochemistry, Faculty of Science, Kasetsart University, 50 Ngam Wong Wan Road, Chatuchak, Bangkok 10900, Thailand.
E-mail: fsciktc@ku.ac.th

ORCID iDs
Chakpetch Kuitio
https://orcid.org/0000-0002-6005-8023
Natchaya Rasri
https://orcid.org/0000-0002-7122-7558
Duangnapa Kiriwan
https://orcid.org/0000-0003-4824-7398
Sasimanas Unajak
https://orcid.org/0000-0002-3242-3978
Kiattawee Choowongkomon
https://orcid.org/0000-0002-2421-7859

Funding
CK was supported by the Research and Researcher for Industries Ph.D. program (Grant No. PHD59I0001), National Research

ABSTRACT

Background: Recently, the pork industry of Thailand faced an epidemic of highly virulent strains of porcine reproductive and respiratory syndrome virus (PRRSV), which spread throughout Southeast Asia, including the Lao People's Democratic Republic and Cambodia. Hence, the rapid and on-site screening of infected pigs on a farm is essential.
Objectives: To develop the new aptamer as a biosensor for detection PRRSV which are rapid and on-site screening of infected pig.
Methods: New aptamers against PRSSV were identified using the combined techniques of capillary electrophoresis, colorimetric assay by gold nanoparticles, and quartz crystal microbalance (QCM).
Results: Thirty-six candidate aptamers of the PRRSV were identified from the systematic evolution of ligands by exponential enrichment (SELEX) by capillary electrophoresis. Only 8 out of 36 aptamers could bind to the PRSSV, as shown in a colorimetric assay. Of the 8 aptamers tested, only the 1F aptamer could bind specifically to the PRSSV when presented with the classical swine fever virus and a pseudo rabies virus. The QCM was used to confirm the specificity and sensitivity of the 1F aptamer with a detection limit of 1.87×10^{10} particles.
Conclusions: SELEX screening of the aptamer equipped with capillary electrophoresis potentially revealed promising candidates for detecting the PRRSV. The 1F aptamer exhibited the highest specificity and selectivity against the PRRSV. These findings suggest that 1F is a promising aptamer for further developing a novel PRRSV rapid detection kit.

Keywords: Porcine reproductive and respiratory syndrome virus; detection; aptamer; l biosensor; gold nanoparticles; QCM

INTRODUCTION

Porcine reproductive and respiratory syndrome (PRRS) has become one of the most important swine diseases worldwide since its appearance in the late 1980s. The disease was first recognized in the United States in 1987 and Europe in 1990 [1,2] and has become endemic in many countries following an epidemic phase. The PRRS mainly causes

Council of Thailand (NRCT). NR, DK and SU were supported by Faculty of Science, Kasetsart University. KC was supported by Faculty of Science, Kasetsart University, and the Center for Advanced Studies in Nanotechnology for Chemical, Food and Agricultural Industries, the KU Institute for Advanced Studies, Kasetsart University.

Conflict of Interest

The authors declare no conflicts of interest.

Author Contributions

Conceptualization: Kuitio C, Unajak S, Choowongkomon K. Data curation: Kuitio C. Formal analysis: Kuitio C, Rasri N, Kiriwan D. Investigation: Kuitio C, Choowongkomon K. Project administration: Unajak S, Choowongkomon K. Supervision: Choowongkomon K. Writing - original draft: Kuitio C, Rasri N, Kiriwan D, Choowongkomon K. Writing - review & editing: Kuitio C, Rasri N, Choowongkomon K.

reproductive failure and respiratory disease in piglets. Therefore, PRRS is the most economically important infectious disease of pigs [3-5], and is caused by the porcine reproductive and respiratory syndrome virus (PRRSV), an enveloped, small single-stranded, non-segmented RNA virus, 45–80 nm in diameter [1,6,7]. The clinical symptoms of PRRS are similar to many bacterial and other viral diseases, which makes it difficult to distinguish among these pathogens. Therefore, PRRS diagnosis platforms are based on the clinical symptoms and biochemical tests. The general symptoms of PRRS disease are respiratory problems, high levels of neonatal mortality, and reproductive failure in pigs of any age [1,8-12]. PRRS can be diagnosed using a wide range of serological tests for the detection of antibodies, as has been done during recent infections. The advantage of an enzyme-linked immunosorbent assay (ELISA) is that it can test many samples in a short time. Nevertheless, these tests only indicate that a pig has been exposed to the virus or vaccinated but cannot tell if the pig is still infected [13-15]. Reverse-transcription polymerase chain reaction (RT-PCR) is recommended for determining the presence of the virus in swine [16]. Confirmation of the PRRSV also includes fluorescent antibody staining and immunohistochemistry staining. Although virus isolation is difficult, it can be attempted from the ascitic fluid, serum, and tissues (spleen, tonsils, lungs, and lymph nodes). All diagnoses of PRRS are complicated by the inability to detect virus particles in pigs [17,18].

Aptamers are alternative choices for the development of biosensors. They are small oligonucleotides, such as single-strand deoxyribonucleic acids, ribonucleic acids (RNA), and small peptides. Aptamers that can bind to the target molecule with high specificity and affinity have been generated from small molecules, such as drugs, to large molecules, such as proteins, and even whole cells, such as viruses or bacteria. The advantages of aptamers include high specificity and affinity, long-term storage, and ease of synthesis and modification. Aptamers show no immune response *in vivo* and could be an alternative to antibodies. Aptamers have been studied as biomaterials in numerous investigations regarding their use as a diagnostic and therapeutic tool. Therefore, aptamers can be developed as a diagnostic of microorganisms and small molecules.

In this research, a new aptamer was developed for the detection of the PRSSV. Colorimetry based on gold nanoparticles and quartz crystal microbalance (QCM) was used to determine the specificity and sensitivity of the new aptamer against the PRSSV. This could be developed further as a biosensor for the on-site detection of the PRSSV in the field.

MATERIALS AND METHODS

Sources of viruses

The PRRSV was the VR-2332 strain. The classical swine fever virus (CSFV) was from a live vaccine of Green Cross Veterinary Products, Co., Ltd. The pseudorabies virus (PRV) was from the Bartha-K61 vaccine strains. These viruses were kindly obtained from B.F. Feed Company, Thailand. The concentrations of viruses were estimated using a Quanta 450 FEI Scanning electron microscope.

PRRSV purification

The PRRSV (ATCC VR-2332) was propagated in the MARC-145 cell line from B.F. Feed Company and purified using a sucrose gradient method. The virus was prepared by 3 freeze/thaw cycles. The cell lysate was centrifuged at 5,000 ×g for 60 min at 4°C and the supernatant

was collected. PEG-8000 (polyethylene glycol-8000) (Sigma-Aldrich, USA) was added to the supernatant, stirred gently at 4°C for 8 h, and centrifuged at 10,800 × g for 20 min. The pellet was collected and dissolved in TNE buffer (10 mM Tris-HCl, 0.1 M NaCl, 1 mM EDTA pH 7.4). The viral solution was overlayed on the top of the sucrose gradient (10%–60%) in the TNE buffer and centrifuged at 110,000 ×g for 12 h at 4°C. The pellet containing the virus particles was collected and dissolved in TNE buffer and stored at −80°C until used. The intact PRRSV particles were confirmed by a scanning electron microscopy (**Supplementary Fig. 1**).

Capillary electrophoresis (CE)

All CE experiments were performed using a Beckman PA 800 plus CE system at Salaya Central Instrument Facility, Mahidol University. The absorbances of the targets were detected in photodiode mode by adjusting the wavelengths in the range of 160–900 nm. The capillary used in all experiments was uncoated fused silica, 50 cm in length and 75 μm diameter. Before starting the CE system, the capillary was rinsed with 0.1 M HCl, 0.1 NaOH, 100% methanol, and distilled water for 5 min in each solution to remove the compounds, proteins, or DNA from the capillaries. The process of CE was controlled using 32 Karat software (version 9.1, Beckman Coulter).

Conditions of capillary electrophoresis for the DNA aptamer library

The DNA aptamer library of 5′-GGC TGG TGT GCG CAG GCT G (40 N) CCC GGT CCG TCG CTC-3′ was developed in house and synthesized by Ward Medic (Bangkok, Thailand). This DNA aptamer library was diluted in distilled water (4 μg/mL) and injected into CE at a voltage of 20 kV for 60 sec. The mobile phase in the CE system was 25 mM sodium phosphate (pH 7.4), or 10 mM phosphate buffer (pH 7.4). The conditions in the capillary included an electric field of approximately 10 kV for 15 min with detection at 260 nm to produce a separated DNA library.

Selection of PRRSV specific aptamers by CE-systematic evolution of ligands by exponential enrichment (SELEX)

The PRRSV at 1.5×10^{13} particles/mL was dissolved in the TNE buffer as a sample for CE. The mobile phase in CE was 25 mM sodium phosphate (pH 7.4). A sample was injected into CE at a voltage of 20 kV for 60 sec. Electrophoresis was carried out for 15 min by the electric field at 10 kV in the capillary, and the target was detected at a wavelength of 205 nm. A 30 μL sample of the 4 μg/mL DNA aptamer library was heated at 95°C for 10 min, and then incubated with 30 μL of 1.5×10^{13} particles/mL PRRSV at 25°C 30 min before being injected into the CE under the same conditions as above but with detection at 205 nm for PRRSV and 260 nm for DNA aptamer. The fraction of the complex between the PRRSV and the DNA aptamer was detected at 205 nm and collected from the capillary.

Polymerase chain reaction and cloning system

The fractions of the complex between the PRRSV and the aptamer were used as a template of amplification by PCR. The forward and reverse primers were 5′-TAA TAC GAC TCA CTA TAG GGC CAG GCA GCG-3′ and 5′-TCT CGG ACG CGT GTG GTC GG-3′, respectively. The reaction buffer consisted of Dream *Tap* buffer (10X, 5.0 μL), *Taq*DNA polymerase (Thermo Fisher Scientific, USA) (1.0 U/mL, 0.2 μL), forward primer (5 μM, 3.0 μL), reverse primer (5 μM, 3.0 μL), and dNTP (2 mM, 5.0 μL) (Thermo Fisher Scientific, USA) for PCR amplification. The PCR sample was first denatured at 95°C for 10 min. Subsequently, 40 cycles of denaturation (95°C/30 sec), annealing (55°C/30 sec), and extension (72°C/10 sec) were performed. The PCR product was analyzed by gel electrophoresis (2% agarose gel) and cleaned up with 3 M sodium acetate. The PCR product was ligated into a pGEM vector

(Promega, USA). The resulting product was then transfected into DH5α *E.coli* (Thermo Fisher Scientific, USA). The white colonies were selected by blue/white colony screening. The plasmid was analyzed further for the aptamer sequences.

Asymmetric polymerase chain reaction

Asymmetric PCR can amplify a single strand of DNA from double-strand templates by the difference in the volume of the forward and reverse primers. In this experiment, the plasmids containing the aptamer were diluted in distilled water to a final concentration of 1 mg/mL for a template in the asymmetric PCR. The primers were the same as in the previous experiment. The ratio of forward: reverse of 30:1 and vice versa was used (5.8 μL:0.2 μL). The reaction used Dream *Tap* buffer (10×, 5.0 μL), *Taq* DNA polymerase (1.0 U/mL, 0.2 μL), forward primer (0.2 μM), reverse primer (0.2 μM), and dNTP (2 mM, 5.0 μL) for PCR amplification. The PCR sample was first denatured at 95°C for 10 min. Subsequently, 40 cycles of denaturation (95°C, 30 sec), annealing (55°C, 30 sec), and extension (72°C, 10 sec) were performed. The PCR product was used for the colorimetric assay.

Screening of the PRRSV aptamers by a colorimetric test

The gold nanoparticles (15 nm) were purchased from Sigma-Aldrich (USA), a product of Germany. The 50–200 μg of purified aptamer were adjusted in distilled water to 50 μL. The aptamers purified from the PCR products were heated at 95°C for 10 min, and put immediately on ice for 10 min to destroy the secondary structure and double stranded DNA. Each 35 μL sample of gold nanoparticles was mixed with the aptamers from both sources and incubated at room temperature for 10 min. Subsequently, 5 μL of 1.5×10^{11} particles/m of PRRSV were added to the reaction and incubated further at room temperature for 10 min. The color of the solution was observed after adding 2 μL of 2 M NaCl. **Fig. 1** presents a schematic diagram of the concept. If the aptamer binds to the target, the gold nanoparticles aggregate after adding NaCl, causing a color change from red to violet. If the aptamer does not bind to the target, it binds with the gold nanoparticles after adding NaCl, resulting in an unchanged color of the solution.

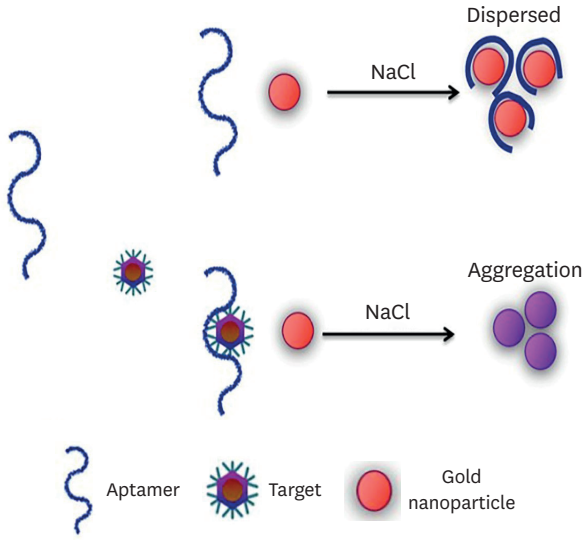

Fig. 1. Schematic illustration of the colorimetric assay by aptamers and gold nanoparticles.
If the aptamer binds to the target, the gold nanoparticles aggregate after adding NaCl, causing a color change from red to violet. If the aptamer does not bind to the target, it binds with the gold nanoparticles after adding NaCl, resulting in the unchanged color of the solution.

https://doi.org/10.4142/jvs.2020.21.e79

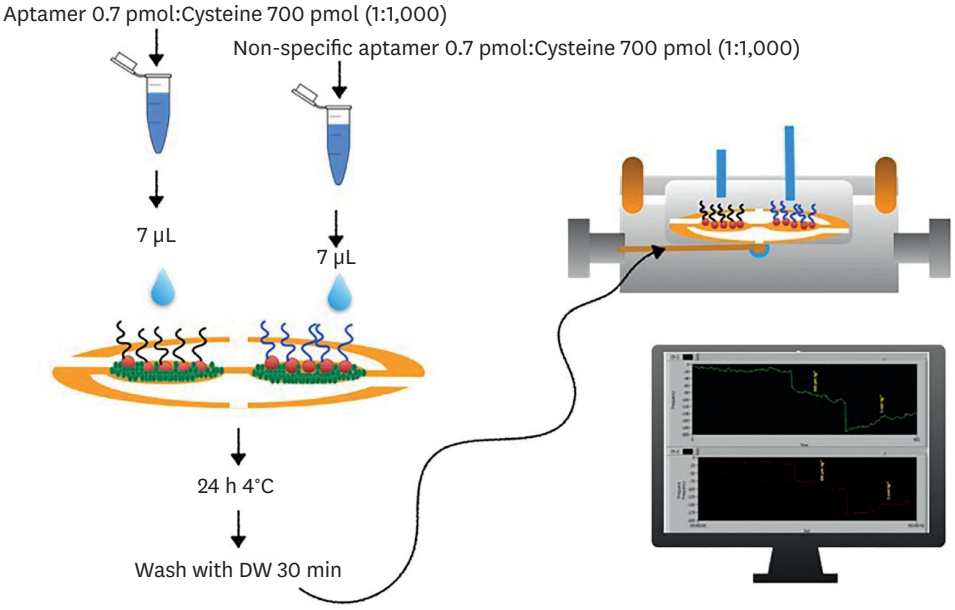

Fig. 2. Instrument setup of the QCM. 0.7 pmol of porcine reproductive and respiratory syndrome virus specific aptamer or non-specific aptamer was mixed with 700 pmol of cysteine and then applied to the working electrode and reference site, respectively. The electrode was dried at 4°C for 24 h and washed with distilled water for approximately 3 h, dried at room temperature, and kept at 4°C for quartz crystal microbalance measurement.

QCM

The synthesized aptamers in this experiment were ordered from Ward Medic (Bangkok, Thailand). The QCM system can be separated into 3 parts. The first part was an electrode and measuring cell. The second part was the electronic equipment, including the oscillator circuit, which was connected to a power supply and a frequency counter for frequency detection. The third part was a computer and software for the control system and results analysis. The quartz for QCM consisted of a 10 MHz AT-cut quartz wafer, 1.8 mm in diameter and 168 μm in thickness. Two gold electrodes with different diameters were positioned on both sides of the quartz wafer. The larger 5 mm electrode was used to test the sample, and the smaller 4 mm electrode was used for signal frequency detection. The electrodes were cleaned with acetone and dried with methyl alcohol at room temperature. The specific aptamer of the PRRSV was diluted in distilled water to 0.7 pmol, mixed with cysteine at a concentration of 700 pmol, and then applied to the working electrode at the big gold site. The non-specific aptamer was diluted with cysteine under the same conditions but was dropped on another site of the electrode, which was the reference site. The electrode was dried at 4°C for 24 h and then washed with distilled water for approximately 3 h, dried at room temperature, and kept at 4°C for the QCM measurement (**Fig. 2**).

RESULTS

Selection of a specific aptamer of the PRRSV by capillary electrophoresis

The PCR products of the DNA aptamer library mixed with the PRRSV were separated to identify the PRSSV-bound aptamer by capillary electrophoresis; **Fig. 3A** shows the chromatogram. The peaks at 8-9 min and 9.5-14 min represent the aptamer-bound PRRSV and unbound aptamers, respectively. The fraction of peaks between 8-9 min was collected and amplified further by PCR. A single band of a 100 bp PCR product was visualized by

https://doi.org/10.4142/jvs.2020.21.e79

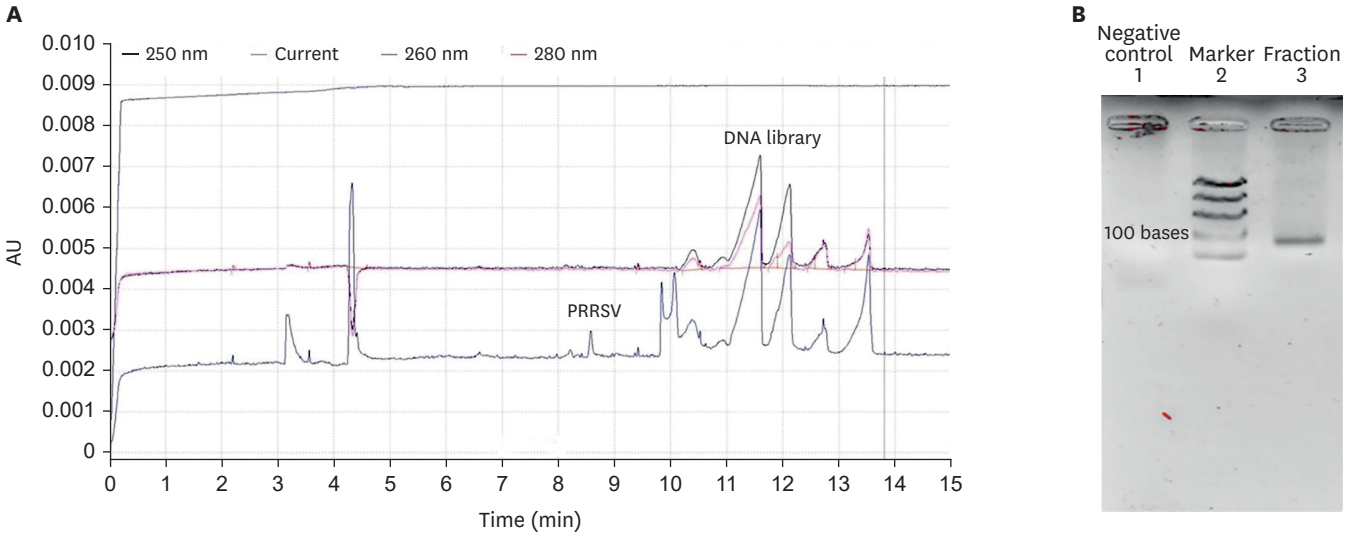

Fig. 3. Selection of the PRRSV specific aptamers by capillary electrophoresis systematic evolution of ligands by exponential enrichment. (A) A capillary electrophoresis chromatogram of the DNA aptamer library mixed with PRRSV. The peaks at 8–9 min represent aptamer-bound PRRSV then collected to perform PCR amplification. The peaks at 9.5–14 min represent the unbound aptamers. (B) PCR product from the PRRSV fraction analyzed by gel electrophoresis (2% agarose gel) (lanes 1, negative control; lanes 2, marker 100 base pairs; lanes 3, fraction of PRRSV from capillary electrophoresis).
PRRSV, porcine reproductive and respiratory syndrome virus; PCR, polymerase chain reaction.

gel electrophoresis, as shown in **Fig. 3B**. This indicates that the aptamers could bind to the PRRSV and be separated by capillary electrophoresis. The PCR product was cleaned up by 3M sodium acetate and ligated into the pGEM vector. The ligated pGEMs were transformed into DH5α *E. coli* for blue/white colony screening. Thirty-six white colonies were screened by colony PCR. This result suggested that all selected colonies had an inserted PCR product of the aptamers in the plasmids (**Supplementary Fig. 2**).

Screening of PRRSV aptamers by the colorimetric test

A gold nanoparticle colorimetric test was used to test if the aptamers could bind to the PRSSV. If the aptamer binds to the PRSSV, it gives a positive result by changing color from red to violet while a negative result remains red. The differences in color between positive and negative were checked using unaided eyes. Eight out of 36 aptamers presented a positive test reaction (gold nanoparticles, aptamer, NaCl, and PRRSV) compared to the negative control reaction (gold nanoparticles, aptamer, and NaCl) (**Supplementary Fig. 3**). These aptamers were numbered 1, 2, 4, 6, 7, 15, 18 and 22 (**Fig. 4**), and the sequences of aptamers were also provided (**Table 1**).

Specificity test of PRRSV aptamers by the colorimetric method

Only aptamer numbers 1, 4, 15, and 18 were selected for further experiments. Each PCR product contains 2 strands of aptamers. To distinguish which strand plays an important role in binding with PRSSV, asymmetric PCR was used to amplify each strand from the PCR product (**Supplementary Fig. 4**). The 4 aptamers, 1, 4, 15, and 18, were analyzed by asymmetric PCR to gain 8 aptamers, 1F, 1R, 4F, 4R, 15F, 15R, 18F, and 18R. The specificity of each aptamer was determined by a gold nanoparticle colorimetric assay using 3 porcine viruses, CSFV (live vaccine from Green Cross Veterinary Products Co., Ltd., Korea), PRV (isolated from cell culture-derived PRV from BF feeds company, Thailand.), and PRRSV as the targets. The CSFV is an RNA virus with a similar shape and size to the PRRSV, while the PRV is a circular particle with a larger diameter of approximately 120-200 nm compared to the PRRSV. **Fig. 5** presents

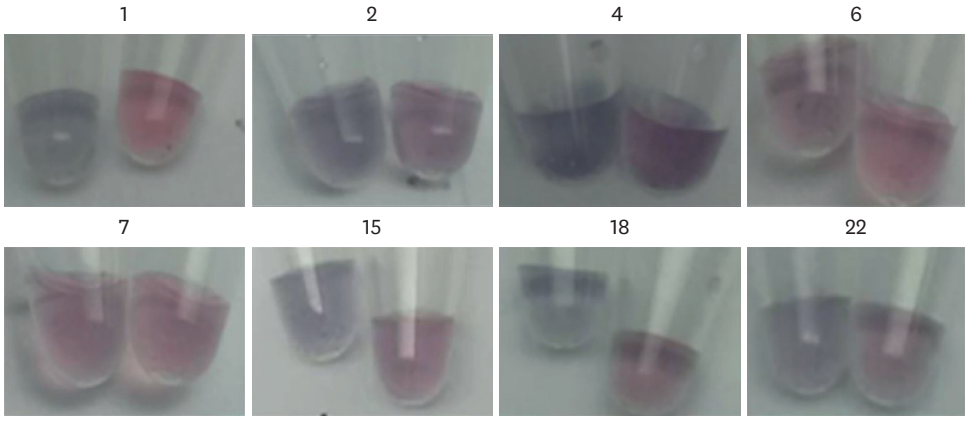

Fig. 4. Positive results of PRRSV aptamers by colorimetric method. From 36 candidate aptamers, screening of the PRRSV bound aptamers by colorimetric test revealed aptamer numbers 1, 2, 4, 6, 7, 15, 18, and 22 to show a positive result (right, test reaction containing gold nanoparticles, aptamer, NaCl, and PRRSV; left, negative control reaction containing gold nanoparticles, aptamer, and NaCl).
PRRSV, porcine reproductive and respiratory syndrome virus.

Table 1. Sequences of the 8 aptamers that bound specifically to the PRRSV

Aptamer No.	Sequence detail
1F	5′-CCCGCGTATGCGCCACAGTAGTTGGCCGTGGAGCTTTAGG-3′
1R	5′-GGGCGCATACGCGGTGTCATCAACCGGCACCTCGAAATCC-3′
4F	5′-AAGTGCATTCCAGACGTAAGACGGAAAGGCGGACTATCAC-3′
4R	5′-TTCACGTAAGGTCTGCATTCTGCCTTTCCGCCTGATAGTG-3′
15F	5′-CGTGGTTTTCAGGTCGCCATATGATACGTGACGCTCCCCC-3′
15R	5′-GCACCAAAAGTCCAGCGGTATACTATGCACTGCGAGGGGG-3′
18F	5′-GCGTTTGCTAGAAGTTATGGTGTCCTAGCAGACGGTACGG-3′
18R	5′-CGCAAACGATCTTCAATACCACAGGATCGTCTGCCATGCC-3′

the results of the colorimetric assay. These results suggest that aptamer 1F was the best for further development as a biosensor for the detection of the PRRSV because the 1F aptamer showed different solution colors when tested with the CSFV, PRV, and PRRSV. Therefore, the 1F aptamer was chosen for further testing with the QCM system.

Sensitivity and specificity test of 1F aptamers by QCM

QCM was used to test the sensitivity of the 1F aptamer by comparing between the working site (1F aptamer) and reference site (non-specific aptamer). After adding the highest concentration of PRRSV particles at 1.5×10^{11}, the frequency of the working site shifted from the baseline to 1,357.11 Hz, while the frequency of the reference site shifted only 268.71 Hz (**Fig. 6A**). The frequency shifted from the baseline at the working site corresponding to the mass of PRRSV in a linear relationship with an R^2 of 0.9972 until the concentration reached 1.87×10^{10} particles. The result from the QCM test of the 1F aptamer suggested that the 1F aptamer can bind with PRRSV with a detection limit at 1.87×10^{10} particles of the PRRSV (**Fig. 6B**).

The specificity test of the 1F aptamer against different viruses, PRRSV, CSFV, and PRV, at the same concentration, was conducted using the QCM system. The result showed that PRRSV caused an approximate 1,400 Hz decrease in frequency, while the CSFV and PRV caused an approximate 100 Hz decrease in frequency and no change, respectively. This suggests that the 1F aptamer has high specificity for the PRRSV compared to the other pig viruses (**Fig. 7**).

https://doi.org/10.4142/jvs.2020.21.e79

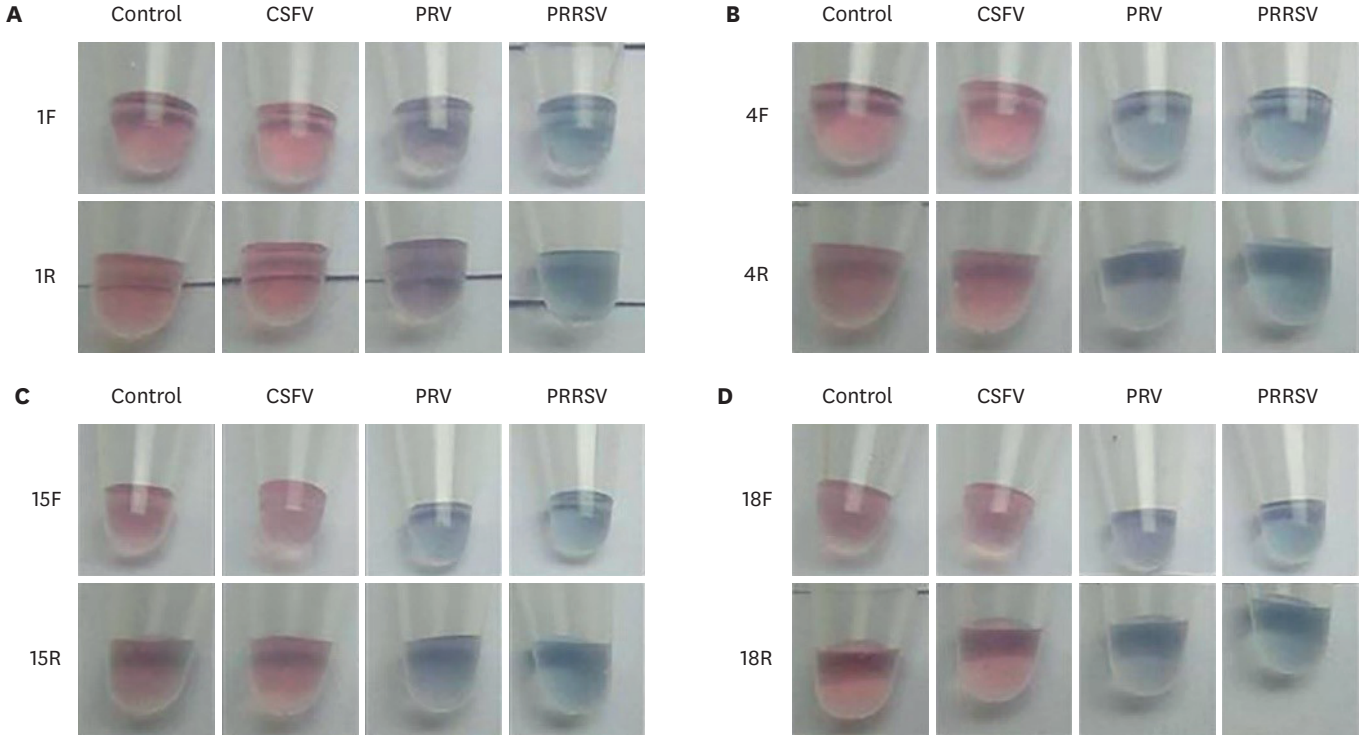

Fig. 5. Specific of the selected PRRSV aptamer colorimetric assays. The 4 aptamers, 1, 4, 15, and 18, were analyzed by asymmetric polymerase chain reaction to gain 8 aptamers and test the specificity against the PRRSV and other viral infected in pigs. (A) 1F, 1R aptamer, (B) 2F, 2R aptamer, (C) 15F, 15R aptamer, (D) 18F, 18R aptamer. These aptamers were tested with the CSFV, PRV, and PRRSV in test reactions containing gold nanoparticles, aptamer, NaCl, and virus, while the control reaction contained gold nanoparticles, aptamer, and NaCl. The positive result was indicated by a color change of gold nanoparticles from red to violet.
CSFV, classical swine fever virus; PRV, pseudorabies virus; PRRSV, porcine reproductive and respiratory syndrome virus.

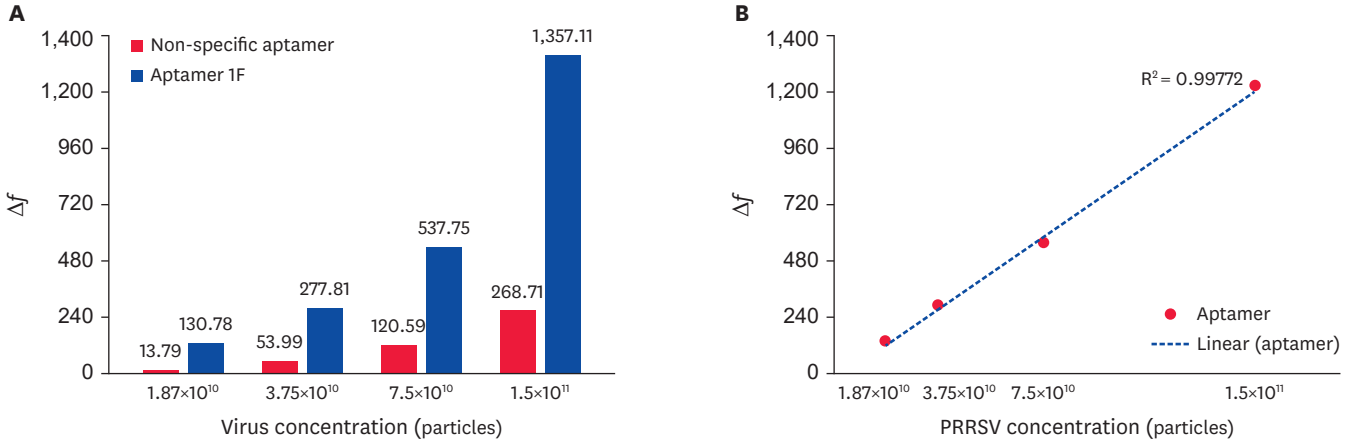

Fig. 6. Sensitivity test of 1F aptamers by QCM. The 1F aptamer was selected to investigate the sensitivity to the PRRSV by QCM. From the principles of QCM, when specific DNA aptamers are immobilized on the quartz surface of s QCM, the binding of the target to the specific aptamer results in a frequency shift. (A) The frequency shift of the 1F aptamer at the working site and non-specific aptamer at the reference site test with the PRRSV $1.87 \times 10^{10} - 1.5 \times 10^{11}$ particles. (B) The linear relationship of the 1F aptamer from QCM presenting the R^2 at 0.99772.
QCM, quartz crystal microbalance; PRRSV, porcine reproductive and respiratory syndrome virus.

DISCUSSION

Previously, the diagnosis of a PRRSV infection used the detection of PRRSV antibodies in oral fluid specimens by the ELISA method [19]. This method is accurate and can test a

https://doi.org/10.4142/jvs.2020.21.e79

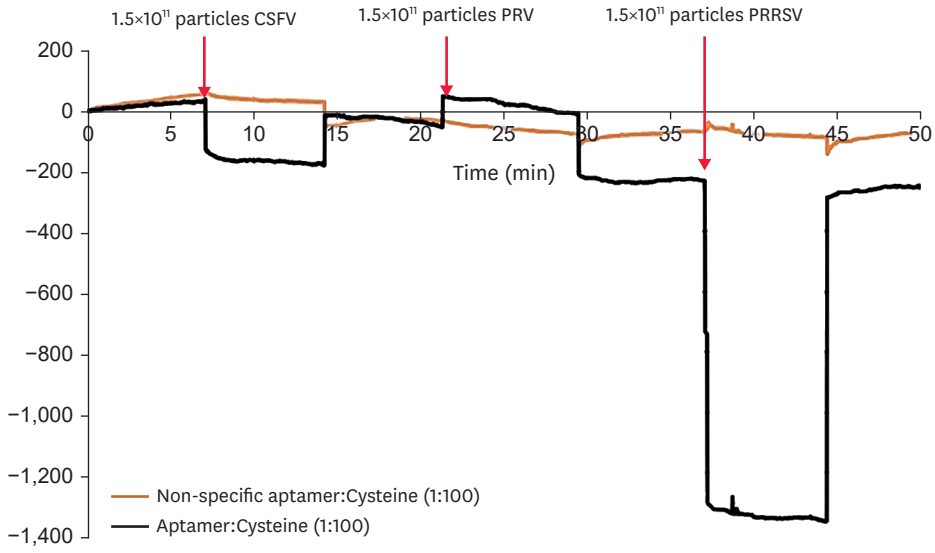

Fig. 7. Specificity test of 1F aptamers by QCM. The frequency from QCM of the 1F aptamer (working site: black line) and non-specific aptamer (reference site: orange line) test with CSFV, PRV, and PRRSV 1.5×10^{11} particles is shown. The frequency of the 1F aptamer and PRRSV showed an enormous difference between the working site and reference, so this result strongly indicated that 1F aptamer specific to PRRSV.
CSFV, classical swine fever virus; PRV, pseudorabies virus; PRRSV, porcine reproductive and respiratory syndrome virus; QCM, quartz crystal microbalance.

large number of samples within a short time, but this test only indicates a prior infection or vaccination but cannot tell if the pig is still infected. On the other hand, PCR or RT-PCR based assays can detect PRRSV circulation. These methods can not only detect the circulation of the virus but also identify the strain. These methods are accurate but can still take several hours to a day to complete the reaction [20,21]. Therefore, this study developed a platform for screening the aptamers for the virus target, starting with SELEX-capillary electrophoresis; this technique can also be applied to other targets to identify the specific aptamers. Recently, SELEX-capillary electrophoresis was used to generate a virus-specific aptamer against the human immunodeficiency virus (HIV) [22] and avian influenza H9N2 [23]. In the present experiments, different retention times of the DNA aptamer library (9.5–14 min) and the aptamer-PRRSV complex (8–9 min) were useful for separating the PRRSV-binding aptamers from the unbound ones. The advantage of capillary electrophoresis is that it uses a smaller sample, in the mL range, for analysis.

The use of gold nanoparticles (AuNPs) is an excellent choice to develop colorimetric assays and biosensors. The aptamer can be adsorbed onto the surface of gold nanoparticles that induce the stability of particles and inhibit aggregation with NaCl. On the other hand, if the solution presents aptamer targets, the structure of the aptamer will be changed, and a new structure will be formed with the target. Therefore, gold nanoparticles lose the aptamer on their surface when induced with NaCl, resulting in nanoparticle aggregation and a visible color change of the solution from red to purple [24-26]. The 1F aptamer was the best aptamer among the candidates and showed specificity against the PRSSV by a colorimetric assay involving 3 different porcine viruses.

A quartz crystal microbalance is an ultra-sensitive weighing technique using the frequency shift of a quartz crystal. Based on this principle, many QCM techniques have been developed to diagnose viral infections, such as immobilized antibodies for the detection of avian

https://doi.org/10.4142/jvs.2020.21.e79

influenza virus H5N1 [27] and maize chlorotic mottle virus [28]. In addition, QCM DNA sensors are used widely to develop rapid and sensitive methods to diagnose viral infections, such as the specific DNA probe to detect a main viral RNA coding G protein in the pathogenic fish virus VHSV [29].

According to the principles of QCM, when specific DNA aptamers are immobilized on the quartz surface of a QCM, binding of the target to the specific aptamer results in a frequency shift. Therefore, this platform was developed to detect the PRRSV using the 1F aptamer. The QCM technique showed that the sensitivity limitation of the 1F aptamer is 1.87×10^{10} particles of PRRSV. Compared to the traditional methods for the diagnosis of PRRS, which are ELISA, PCR, and RT-PCR, the platform developed in this study is more rapid, accurate, and can detect virus particles directly. The advantage of the gold nanoparticle method for the detection of PRSSV is simple, cost-effective, and fast, giving a result within only 15 min. Therefore, this 1F aptamer can be developed further for the diagnosis of PRRS, and as a tool for the rapid detection of PRRSV in porcine farms.

SUPPLEMENTARY MATERIALS

Supplementary Fig. 1

Scanning electron microscopy image of the PRRSV fraction from capillary electrophoresis. PRRSV particles have a circular shape and a size of approximately 60–100 nm.

Click here to view

Supplementary Fig. 2

PCR products from colony PCR of porcine reproductive and respiratory syndrome virus aptamer analyzed by gel electrophoresis from colony numbers 1–12.

Click here to view

Supplementary Fig. 3

Result from the colorimetric method of the PRRSV aptamers number 1–36 (right, positive reaction; left, negative reaction).

Click here to view

Supplementary Fig. 4

Gel electrophoresis with the optimized conditions for asymmetric polymerase chain reaction. The samples were denatured at 95°C/30 sec, annealed at 55°C/30 sec, and extended at 72°C/10 sec for 30 cycles. The reaction used primer volume ratios (forward: reverse) of 10:1, 30:1, and 50:1 (lanes 1, marker 100 base pairs; lanes 2, 1:1 (3 μL:3 μL); lanes 3, 10:1 (5.4 μL:0.6 μL); lanes 4, 30:1 (5.8 μL:0.2 μL); lanes 5, 50:1 (5.88 μL:0.12 μL); lanes 6, 1:1 (.06 μL:0.6 μL); lanes 7, 1:1 (0.2 μL:0.2 μL); lanes 8, 1:1 (0.12 μL:0.12 μL).

Click here to view

REFERENCES

1. Meulenberg JJ. PRRSV, the virus. Vet Res. 2000;31(1):11-21.
PUBMED
2. Rossow KD. Porcine reproductive and respiratory syndrome. Vet Pathol. 1998;35(1):1-20.
PUBMED | CROSSREF
3. Feng Y, Zhao T, Nguyen T, Inui K, Ma Y, Nguyen TH, et al. Porcine respiratory and reproductive syndrome virus variants, Vietnam and China, 2007. Emerg Infect Dis. 2008;14(11):1774-1776.
PUBMED | CROSSREF
4. Kawashima K, Katsuda K, Tsunemitsu H. Epidemiological investigation of the prevalence and features of postweaning multisystemic wasting syndrome in Japan. J Vet Diagn Invest. 2007;19(1):60-68.
PUBMED | CROSSREF
5. Mengeling WL, Lager KM. A brief review of procedures and potential problems associated with the diagnosis of porcine reproductive and respiratory syndrome. Vet Res. 2000;31(1):61-69.
PUBMED | CROSSREF
6. Paul PS, Halbur P, Janke B, Joo H, Nawagitgul P, Singh J, et al. Exogenous porcine viruses. Curr Top Microbiol Immunol. 2003;278:125-183.
PUBMED | CROSSREF
7. Snijder EJ, Kikkert M, Fang Y. Arterivirus molecular biology and pathogenesis. J Gen Virol. 2013;94(Pt 10):2141-2163.
PUBMED | CROSSREF
8. Canning P, Canon A, Bates JL, Gerardy K, Linhares DC, Piñeyro PE, et al. Neonatal mortality, vesicular lesions and lameness associated with Senecavirus A in a U.S. sow farm. Transbound Emerg Dis. 2016;63(4):373-378.
PUBMED | CROSSREF
9. Iseki H, Takagi M, Kawashima K, Shibahara T, Kuroda Y, Tsunemitsu H, et al. Pathogenicity of emerging Japanese type 1 porcine reproductive and respiratory syndrome virus in experimentally infected pigs. J Vet Med Sci. 2016;77(12):1663-1666.
PUBMED | CROSSREF
10. Moennig V, Floegel-Niesmann G, Greiser-Wilke I. Clinical signs and epidemiology of classical swine fever: a review of new knowledge. Vet J. 2003;165(1):11-20.
PUBMED | CROSSREF
11. Segalés J. Porcine circovirus type 2 (PCV2) infections: clinical signs, pathology and laboratory diagnosis. Virus Res. 2012;164(1-2):10-19.
PUBMED | CROSSREF
12. Antunes AC, Halasa T, Lauritsen KT, Kristensen CS, Larsen LE, Toft N. Spatial analysis and temporal trends of porcine reproductive and respiratory syndrome in Denmark from 2007 to 2010 based on laboratory submission data. BMC Vet Res. 2015;11(1):303.
PUBMED | CROSSREF
13. Mengeling WL, Lager KM, Vorwald AC. The effect of porcine parvovirus and porcine reproductive and respiratory syndrome virus on porcine reproductive performance. Anim Reprod Sci. 2000;60-61:199-210.
PUBMED | CROSSREF
14. Zimmerman JJ, Yoon KJ, Wills RW, Swenson SL. General overview of PRRSV: a perspective from the United States. Vet Microbiol. 1997;55(1-4):187-196.
PUBMED | CROSSREF
15. Faaberg KS, Kehrli ME Jr, Lager KM, Guo B, Han J. *In vivo* growth of porcine reproductive and respiratory syndrome virus engineered nsp2 deletion mutants. Virus Res. 2010;154(1-2):77-85.
PUBMED | CROSSREF
16. Bøtner A. Diagnosis of PRRS. Vet Microbiol. 1997;55(1-4):295-301.
PUBMED | CROSSREF
17. Shi Y, Lei Y, Ye G, Sun L, Fang L, Xiao S, et al. Identification of two antiviral inhibitors targeting 3C-like serine/3C-like protease of porcine reproductive and respiratory syndrome virus and porcine epidemic diarrhea virus. Vet Microbiol. 2018;213:114-122.
PUBMED | CROSSREF
18. Wei Y, Li J, Zhang Y, Xue C, Cao Y. Tandem 3′ UTR patterns and gene expression profiles of marc-145 cells during PRRSV infection. Virol Sin. 2018;33(4):335-344.
PUBMED | CROSSREF

19. Kittawornrat A, Prickett J, Wang C, Olsen C, Irwin C, Panyasing Y, et al. Detection of Porcine reproductive and respiratory syndrome virus (PRRSV) antibodies in oral fluid specimens using a commercial PRRSV serum antibody enzyme-linked immunosorbent assay. J Vet Diagn Invest. 2012;24(2):262-269.
PUBMED | CROSSREF

20. Lurchachaiwong W, Payungporn S, Srisatidnarakul U, Mungkundar C, Theamboonlers A, Poovorawan Y. Rapid detection and strain identification of porcine reproductive and respiratory syndrome virus (PRRSV) by real-time RT-PCR. Lett Appl Microbiol. 2008;46(1):55-60.
PUBMED

21. Christopher-Hennings J, Nelson EA, Nelson JK, Hines RJ, Swenson SL, Hill HT, et al. Detection of porcine reproductive and respiratory syndrome virus in boar semen by PCR. J Clin Microbiol. 1995;33(7):1730-1734.
PUBMED | CROSSREF

22. Pavski V, Le XC. Detection of human immunodeficiency virus type 1 reverse transcriptase using aptamers as probes in affinity capillary electrophoresis. Anal Chem. 2001;73(24):6070-6076.
PUBMED | CROSSREF

23. Zhang Y, Yu Z, Jiang F, Fu P, Shen J, Wu W, et al. Two DNA aptamers against avian influenza H9N2 virus prevent viral infection in cells. PLoS One. 2015;10(3):e0123060.
PUBMED | CROSSREF

24. Hu X, Chang K, Wang S, Sun X, Hu J, Jiang M. Aptamer-functionalized AuNPs for the high-sensitivity colorimetric detection of melamine in milk samples. PLoS One. 2018;13(8):e0201626.
PUBMED | CROSSREF

25. Mondal B, Ramlal S, Lavu PS, N B, Kingston J. Highly sensitive colorimetric biosensor for Staphylococcal enterotoxin B by a label-free aptamer and gold nanoparticles. Front Microbiol. 2018;9:179.
PUBMED | CROSSREF

26. Smith J, Chávez J, Hagen J, Kelley-Loughnane N. Design and development of aptamer–gold nanoparticle based colorimetric assays for in-the-field applications. Journal of Visualized Experiments. 2016;2016:179.

27. Li D, Wang J, Wang R, Li Y, Abi-Ghanem D, Berghman L, et al. A nanobeads amplified QCM immunosensor for the detection of avian influenza virus H5N1. Biosens Bioelectron. 2011;26(10):4146-4154.
PUBMED | CROSSREF

28. Huang X, Xu J, Ji HF, Li G, Chen H. Quartz crystal microbalance based biosensor for rapid and sensitive detection of maize chlorotic mottle virus. Anal Methods. 2014;6(13):4530-4536.
CROSSREF

29. Hong SR, Jeong HD, Hong S. QCM DNA biosensor for the diagnosis of a fish pathogenic virus VHSV. Talanta. 2010;82(3):899-903.
PUBMED | CROSSREF