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**Can a radioimmunoassay kit be developed for accurate detection of the S protein of severe acute respiratory syndrome coronavirus 2?**

Yu MM. Radioimmunoassay kit for SARS-CoV-2

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**Abstract**

The coronavirus disease 2019 (COVID-19) pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) at the end of 2019 spread worldwide within only a few months. The screening and timely isolation of infected individuals have been regarded as an effective means of epidemic prevention and control. Therefore, effective screening of infected individuals plays a vital role in epidemic prevention and control. At present, reverse transcription-polymerase chain reaction (RT–PCR) is the main method for the *in vitro* detection of SARS-CoV-2. However, RT–PCR requires certified laboratories, expensive equipment, and trained technicians. Therefore, it is necessary to develop simpler and more convenient methods. Some studies have shown that the PepKAA peptide has a high affinity for the S protein of SARS-CoV-2. The tyrosine in PepKAA is labeled with 125I and used to design a radioimmunoassay kit for the detection of the S protein of SARS-CoV-2, which is of great significance for the early diagnosis of COVID-19.

**Key Words:** SARS-CoV-2; COVID-19; spike protein; detection; radioimmunoassay kit

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**Core Tip:** severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is highly infectious, and early detection of SARS-CoV-2 is the key factor in preventing another epidemic. radioimmunoassay (RIA) exhibits high sensitivity and specificity. The detection of the S protein on the surface of SARS-CoV-2 by RIA is expected to be applied for the early diagnosis of coronavirus disease 2019, which may have a considerable impact on the control of the epidemic.

**TO THE EDITOR**

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is highly infectious, and people are generally susceptible to this pathogen. Coronavirus disease 2019 (COVID-19) has caused a global pandemic and has been categorized as a Class B infectious disease in China, and Class A management measures have been implemented[1]. The early laboratory detection of COVID-19 is a key factor for ensuring the early admission, treatment, and timely control of patients to prevent the development of another epidemic[2].At present, reverse transcription-PCR (RT–PCR) is the main method for the *in vitro* detection of SARS-CoV-2[3]. However, PCR requires certified laboratories, expensive equipment, and trained technicians[4]. The use of a low-cost and simple radioimmunoassay to detect a protein on the surface of SARS-CoV-2 may be of clinical value.

The spike (S) protein is located on the surface of SARS-CoV-2. This protein has a receptor-binding domain (RBD) that can interact with the angiotensin I-converting enzyme 2 (ACE2) receptor in human cells[5]. Souza PFN[6,7] showed that eight antibacterial peptides (*Mo*-CBP3-PepI, *Mo*-CBP3-PepII, *Mo*-CBP3-PepIII, *RcAlb*-PepI, *RcAlb*-PepII, *RcAlb*-PepIII, PepGAT, and PepKAA) can target the S protein of SARS-CoV-2. Of these peptides, *Mo*-CBP3-PepII and PepKAA exhibit the highest affinity. The *Mo*-CBP3-PepII sequence is as follows: Asn-Ile-Gln-Pro-Pro-Cys-Arg-Cys-Cys. The PepKAA sequence is as follows: Lys- Ala- Ala- Asn-Arg-Ile-Lys-Tyr-Phe-Gln. We can label *Mo*-CBP3-PepII or PepKAA sequences using 125I to detect SARS-CoV-2.

***Hypothesis***

To improve the diagnostic sensitivity of COVID-19, the detection of the S protein of SARS-CoV-2 *via* the RIA method was specifically designed as follows.

**The PepKAA sequence is labeled with 125I**: This sequence is characterized by: (1) a high affinity for the S protein of SARS-CoV-2; and (2) the existence of a tyrosine within the sequence for easy 125I labeling. This sequence can be easily synthesized *via* solid-phase polypeptide synthesis, requiring only ten amino acids (Lys-Ala-Ala-Asn-Arg-Ile-Lys-Tyr-Phe-Gln). Thus, synthesis can be achieved at a low cost. PepKAA can be labeled *via* the chloramine-T method, which is simple and constitutes a mature method.

**Preparation of RIA kit**: RIA kits were prepared using following the steps: (1) PepKAA, a peptide targeting the S protein of novel coronavirus, was synthesized by solid-phase peptide synthesis (SPPS); PepKAA was purified by reversed-phase high-performance liquid chromatography (RP-HPLC). After purification, the peptide was analyzed by mass spectrometry; (2) 125I labeling of PepKAA based on the chloramine T method was performed. One hundred and fifty micrograms of PepKAA powder was dissolved in 20 μL DMSO and then added to PB buffer (pH = 7.4) to generate 200 μL of PepKAA solution. After adding 20 μL of chloramine-T solution (5 mg/mL) into a mixed solution of 200 μL PepKAA and 10 μl of Na125I (1.04 mci), the solution was placed in a mixer to react for 50 s at room temperature. Then, 150 μl of sodium metabisulfite (5 mg/mL) was added to terminate the reaction, and 125I-PepKAA was purified using an activated C18 column; (3) preparation of coronavirus-inactivated specimens at six concentrations, including 100 ng/mL, 30 ng/mL, 10 ng/mL, 3 ng/mL, 1 ng/mL, and 0.3 ng/mL; (4) preparation of PEG virus precipitation solution. A 50% PEG solution was first prepared, or solid PEG was directly added to the virus suspension at the required concentration; and (5) the finished solution was packaged, inspected, and stored.

**Detection method**: Sample collection utilized throat swabs or patient serum. All of the samples were inactivated *via* high temperature before detection, and 125I-labeled PepKAA was added to the samples or standards, which were then incubated in a water bath for 30 min. A PEG virus precipitator was added, and then the mixture was allowed to stand. The supernatant was discarded. Then, a gamma counter was used to measure the radioactive count in each tube (including standard tubes and the measuring tube). Finally, the virus concentration in the measuring tube was calculated according to the radioactive counts of the standard tubes with different concentrations.

***Discussion***

Since Yalow and Berson[8] pioneered the development of the first competitive RIA of human insulin in 1959, RIA technology has been applied to a wide variety of fields. RIA has advantages of high sensitivity and specificity[9].

This method exhibits a high specificity. PepKAA was labeled with iodine-125 in this study, and PepKAA can bind specifically to the S protein on the surface of SARS-CoV-2. This method is also highly sensitive. RIA can detect the substance at a level of pg/mL. Other tests cannot achieve this level of sensitivity.

The detection of S protein on the surface of SARS-CoV-2 by RIA is expected to be applied to the early diagnosis of COVID-19, which may have a considerable impact on controlling the epidemic.

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**Footnotes**

**Conflict-of-interest statement:** Dr. Yu has nothing to disclose.

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