Analytica Chimica Acta 881 (2015) 82–89

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

A bare-eye based one-step signal amplified semiquantitative immunochromatographic assay for the detection of imidacloprid in Chinese cabbage samples

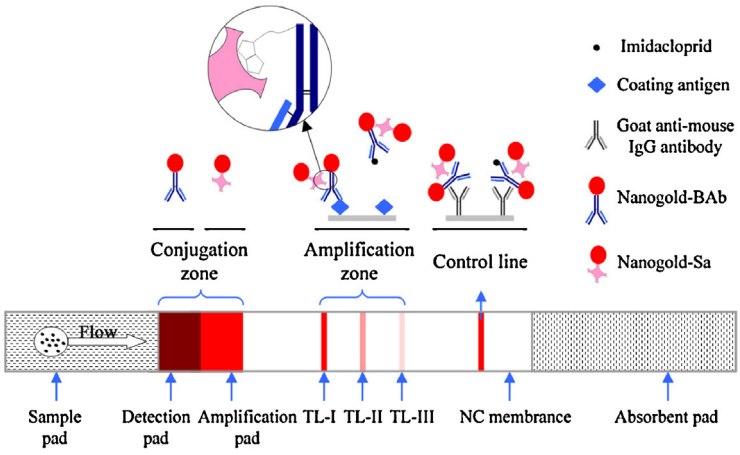
Qingkui Fang [a,1], Limin Wang [a,1], Qi Cheng [a], Jia Cai [a], Yulong Wang [a], Mingming Yang [a], Xiude Hua [a], Fengquan Liu [a,b,*]

[a] College of Plant Protection, Key Laboratory of Integrated Management of Crop Diseases and Pests, Nanjing Agricultural University, Nanjing 210095, PR China
[b] Institute of Plant Protection, Jiangsu Academy of Agricultural Science, Nanjing 210014, PR China

HIGHLIGHTS

- An ICA was developed that used one-step signal amplification.
- The signal amplification was achieved by high affinity biotin-streptavidin probes.
- The sensitivity of the SAS-ICA was significantly improved than that of traditional ICA.
- The method could be developed for field analysis and semi-quantitative screening.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 25 February 2015
Received in revised form 17 April 2015
Accepted 23 April 2015
Available online 25 April 2015

Keywords:
Signal amplification
Bare-eye based semi-quantitative
Immunochromatographic assay
Imidacloprid
Chinese cabbage

ABSTRACT

A novel bare-eye based one-step signal amplified semi-quantitative immunochromatographic assay (SAS-ICA) was developed for detection of the pesticide imidacloprid. This method was based on competitive immunoreactions. Signal amplification was achieved by dual labeling of the test lines (TLs) on the strip using high affinity nanogold-biotinylated anti-imidacloprid mAb (BAb) and nanogold-streptavidin (Sa) probes. The relative color intensities of three TLs (TL-I, TL-II and TL-III) on a nitrocellulose (NC) membrane were used for direct visual analysis of the SAS-ICA strips, and could be used for semi-quantitation of analyte concentrations by observing what TLs disappeared in the amplification zone. Under optimized conditions, the following imidacloprid concentration ranges would be detected by visual examination of the SAS-ICA strip: 0–5 ng mL^{-1} (negative samples), and 5–25 ng mL^{-1}, 25–250 ng mL^{-1}, 250–1000 ng mL^{-1} and >1000 ng mL^{-1} (positive samples). The sensitivity (the visual detection limit (VDL) of TL-III) and semi-quantitative analytical capacity (when TL-III disappeared completely) of the SAS-ICA strip were 10-fold and 160-fold higher than those of traditional ICA, respectively. The developed SAS-ICA strip was applied to the analysis of spiked and authentic

* Corresponding author. Tel.: +86 25 84396726.
E-mail address: fqliu20011@sina.com (F. Liu).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.aca.2015.04.047
0003-2670/

contaminated Chinese cabbage samples in the laboratory and under field conditions, and the results were validated by high-performance liquid chromatography (HPLC). This process could be adopted as a potential generous technique for all ICA-based detection methods.

1. Introduction

Imidacloprid (1-(6-chloro-3-pyridylmethyl)-*N*-nitroimidazolidin-2-ylideneamine) is a systemic, contact and chloronicotinyl neonicotinoid insecticide [1,2]. Imidacloprid acts as an agonist on postsynaptic nicotinic acetylcholine receptors, and modifies insect behavior, resulting in death. It is widely used as an insecticide for controlling aphids, thrips, diamondback moths and white flies [3,4]. Consequently, imidacloprid residues may be found in agricultural products and environmental samples, and these residues are potentially hazardous for consumers [5]. Thus, it is necessary to develop sensitive and rapid methods for the determination of imidacloprid.

Various analytical methods have been used for the determination of imidacloprid in the last decades. Current methods are mainly instrument-based, such as capillary electrophoresis [6], high-performance liquid chromatography (HPLC) [7], HPLC–mass spectrometry (MS) [8], HPLC–MS/MS [9], and gas chromatography (GC) or GC–MS [10]. Although these methods can achieve high detection sensitivity, they can not meet demands for high-throughput on-line screening. Especially, because of the thermolability and high polarity of imidacloprid, GC and GC–MS are difficult because they require derivatization of the analytes before analysis. Some antibody-based enzyme-linked immunosorbent assay (ELISA) have been established for imidacloprid [1–5,11–13], but they are also unsuitable for on-site detection. Therefore, development of a simple, rapid, and inexpensive analytical technique for on-site detection of imidacloprid is of importance for food safety and public health.

An immunochromatographic assay (ICA) is a user-friendly, on-site analytical method that is relatively fast and inexpensive, requires minimal training, and can be used where sophisticated laboratory facilities are not available [14,15]. This method has been applied to rapid detection of toxic or harmful substances in many fields, such as food safety monitoring and on-site diagnostics. Gui et al. [16] and Xu et al. [17] reported a qualitative strip for the detection of triazophos in environmental samples and deoxynivalenol in wheat and maize, respectively. Kuang et al. [18] used a strip reader for detection of lead ions in drinking water, and Kim et al. [19] used a fluorescence scanner for detection of microcystins. Li et al. [20] reported a post-treatment strip for enhanced detection of Avian influenza and Newcastle disease viruses. There are three issues with these ICA strips that need to be overcome. The first of these arises because of the intrinsic properties of traditional ICA methods and the titer of the antibody used, the sensitivity of traditional ICA is not sufficient to meet the detection requirements of particular target analytes at low or trace concentrations. The second issue is that present semi-quantitative or quantitative ICA methods are usually instrument-dependent as they rely on optical detectors, and a more cost-effective detection method is required for on-site analysis. The third issue is that ICA strip test needs further post-treatments, increased test procedure. The recent trend toward semi-quantitation by visual examination of test strips [21,22] and one-step signal amplified assays [23,24] has been driven by a strong demand for rapid diagnosis and on-site detection of toxic or harmful substances, and one-step signal amplification and detection by direct visual examination of the ICA strip are effective solutions to overcome these problems.

Our group has reported an ICA method [25] for the semi-quantitative detection of three pesticides, including imidacloprid. The method is based on the use of four strips. Each strip has three channels, and different channels with different capture reagent are the key to multi-detection. And four strips of ICA by direct visual examination to achieve semi-quantitative detection. In order to

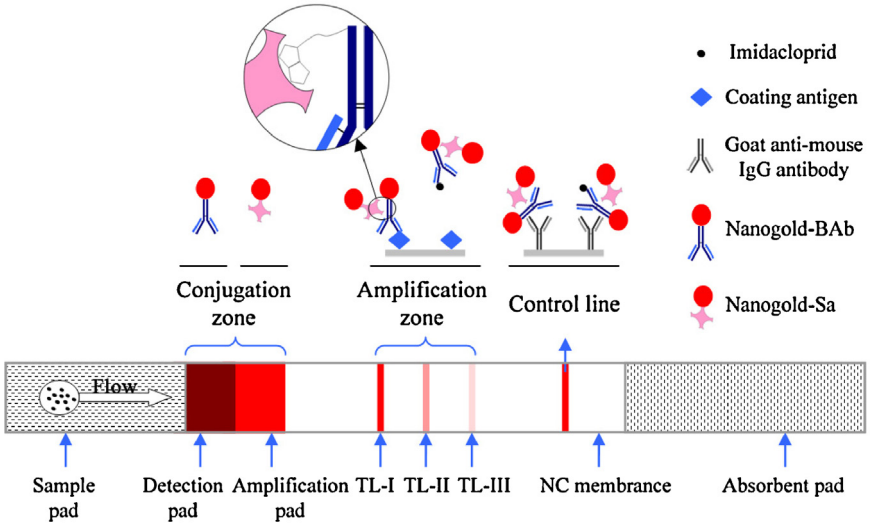


Fig. 1. The schematic diagram of the detection principle of the bare-eye based one-step signal amplified semi-quantitative immunochromatographic assay (SAS-ICA). The SAS-ICA is based on the competitive theory, and included five components: sample pad, detection pad, amplification pad, nitrocellulose (NC) membrane, and absorbent pad. The detection pad contained nanogold-biotinylated anti-imidacloprid mAb (nanogold-BAb) probe and the amplification pad contained nanogold-streptavidin (nanogold-Sa) probe. There were two regions on the NC membrane: the amplification zone and control zone (control line). The control line contained goat anti-mouse IgG antibody; the amplification zone included three test lines (TLs, TL-I, -II, and -III from left to right) which contained the required concentrations of capture reagent (H_2-OVA). The signal amplification was based on the dual labeling of the test lines on the strip using nanogold-BAb probe and nanogold-Sa probe, and the semi-quantitative strategy based on relative color intensity of three TLs.