

## Original Article

# Preliminary screening for pre-pregnancy thyroid dysfunction using magnetic nanoparticle based time-resolved fluoroimmunoassay

Jun Fan<sup>1\*</sup>, Yijuan Cai<sup>2\*</sup>, Biao Huang<sup>3</sup>, Fang Ling<sup>2</sup>, Yun Gao<sup>1</sup>, Bin Zhou<sup>1</sup>, Jue Zhang<sup>1</sup>, Lili Deng<sup>1</sup>, Yi Zhang<sup>1</sup>, Xufeng Ding<sup>2</sup>

<sup>1</sup>Key Laboratory of Nuclear Medicine, Ministry of Health, Jiangsu Institute of Nuclear Medicine, Wuxi, Jiangsu, China; <sup>2</sup>Yixing Maternal and Child Health Care Hospital, Wuxi, Jiangsu, China; <sup>3</sup>School of Life Science, Zhejiang Sci-Tech University, Hangzhou, China. \*Equal contributors.

Received August 30, 2018; Accepted February 11, 2019; Epub May 15, 2019; Published May 30, 2019

**Abstract:** Purpose: Antibodies against thyroid peroxidase (TPOAb) and thyroglobulin (TgAb) have been found to be significant risk factors for hypothyroidism in pregnancies. Screening for TPOAb and TgAb will identify cases missed by measurement of serum thyroid-stimulating hormone (TSH) alone. In this study, a novel immunoassay was established for detection of TPOAb and TgAb in pre-pregnant women. Materials and methods: This new immunoassay method combined time-resolved fluoroimmunoassay (TRFIA) and magnetic nanoparticles. Results: With the advantages of magnetic nanoparticles, the TRFIA immunoassay exhibited broad dynamic assay ranges for TPOAb and TgAb, with detection limits of 0.31 ng/mL and 0.35 ng/mL, respectively. Coefficient variations of the method were lower than 6% and recoveries were in the range of 99-101%. Good correlation levels were obtained in the analysis of 58 serum samples between the proposed method and a commercial kit, with correlation coefficients of 0.9937 and 0.9965 for TPOAb and TgAb, respectively. Conclusion: Present results demonstrate that the developed immunoassay for determination of TPOAb and TgAb is a reliable method for clinical application, with superior sensitivity, specificity, and accuracy.

**Keywords:** Magnetic nanoparticles, time-resolved fluoroimmunoassay, TPOAb, TgAb, thyroid dysfunction

## Introduction

Thyroid hormones are crucial for the growth and maturation of many target tissues, especially the brain and skeleton. Thyroid dysfunction is common in pregnancies and has adverse fetal and maternal health consequences. Women with thyroid dysfunction have an increased risk of fetal-maternal complications, including miscarriages, stillbirths, preeclampsia, and neonatal morbidity [1-3]. Adequate maternal thyroid status in early gestation is critical for fetal brain development, as the fetus relies exclusively on maternal thyroxine sources [4]. Importantly, some complications appear to be preventable by treatment, underpinning the need for prompt correction of gestational thyroid dysfunction. In recent years, with the national population policy adjustment, China has paid more attention to health care in pre-preg-

nant and pregnant women. In many parts of China, free pre-pregnancy care is provided to the pregnant population, playing an important role in improving the quality of the born population and ensuring the safety of mothers and children.

Free pre-pregnancy checkups include many items, in which thyroid function screening plays a key role in female care during the pre-pregnancy period. Clinical diagnosis of thyroid dysfunction during pre-pregnancy and pregnancy is based on serum thyroid-stimulating hormone (TSH) concentrations [5, 6]. TSH in pregnancies is physiologically lower than levels in the non-pregnant population. Due to lower costs of screening for the population, routine TSH detections are currently more available. For those with abnormal TSH, it is necessary to check other indicators. Antibodies against thyroid per-

## Pre-pregnancy thyroid dysfunction screening

oxidase (TPOAb) and thyroglobulin (TgAb) are important indicators of thyroid immune inflammation [7-9]. Even when thyroid function is normal, TPOAb and TgAb positive may have an impact on pregnancies. Thus, using the classical reference range for serum TSH could lead to the underdiagnosis of hypothyroidism and overdiagnosis of hyperthyroidism, missing many cases of thyroid antibodies. Therefore, screening for TPOAb and TgAb is important for detection of thyroid dysfunction.

Currently, detection of serum TPOAb and TgAb mainly depends on enzyme-linked immunosorbent assay (ELISA), chemiluminescence immunoassay (CLIA), and electrochemiluminescence immunoassay (ECLI) [10-12]. However, these methods are either associated with low stability, sensibility, and repeatability or require large instruments and lots of space, with high costs. It is difficult to carry out extensive screening in pre-pregnant and pregnant populations. Magnetic nanoparticles based time-resolved fluoroimmunoassay (TRFIA) is a novel immunoassay method. This method involves primary antibodies immobilized on the surface of magnetic particles and labeled with stable fluorescent chelates. This method combines the advantages of magnetic particles and TRFIA, resulting in high sensitivity, satisfactory specificity, short analytical time, and cost-effectiveness. For this study, researchers developed an immunoassay for measurement of TPOAb and TgAb in human serum, characterized by the use of magnetic particles, as a solid-phase, and detection of Eu chelates. Results indicate that this novel method possesses notable advantages of higher sensitivity, shorter analysis times, and larger linear range. Therefore, this strategy shows significant promise for future application in practical clinical detection.

### Materials and methods

#### Reagents

Magnetic particles were obtained from JSR Life Sciences (Tokyo, Japan). Monoclonal anti-TPOAb antibodies B1 and B7, anti-TgAb antibodies LA001 and CA001, and TPOAb and TgAb standard were purchased from Wason Biotech (China). Eu<sup>3+</sup>-labelling kits were obtained from Perkin-Elmer Wallac (Turku, Finland). PD-10 and Sepharose CL-6B were purchased from Amersham Pharmacia Biotech (Piscataway, NJ,

USA). TPOAb and TgAb commercial immunoassay kits were provided by Roche (Swiss).

Bovine serum albumin (BSA), diethylenetriaminepentaacetic acid (DTPA), 4-morpholineethanesulfonic acid (MES), N-hydroxysulfosuccinimide (NHS), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), proclin-300, and Tween-20 were purchased from Sigma (St. Louis, MO, USA). All other chemicals used were of analytical reagent grade. Ultra-pure water was obtained using a Milli-Q water purification system (Millipore, Bedford, MA, USA). It was used throughout the experiments.

#### Serum samples

A total of 58 serum samples were collected from pre-pregnant women with no history of thyroid disease, from March 2017 to May 2017, in Yixing Maternal and Child Health Care Hospital, Jiangsu Province, China. All patients were diagnosed according to clinical symptoms and laboratory tests. Serum samples were stored at -20°C.

#### Coating conjugate preparation

Magnetic particles (10 µL, 1 mg/mL) were first activated using a mixed solution (pH 7.2-7.6) of EDC (300 µL, 50 mg/mL) and NHS (300 µL, 50 mg/mL). After 30 minutes at room temperature for incubation, the mixture solution was washed three times with phosphate buffer solution (0.05 M, pH 7.2-7.6) containing 1% BSA, 0.1% Tween-20 (PBST), then anti-TPOAb antibody B1 (1 mL, 15 µg/mL) was added. After incubation overnight at 4°C, the magnetic particles were separated by an external magnetic field and washed three times with PBST. This was to remove unbound antibodies with the magnetic separation process. Non-specific sites on magnetic particles were blocked using 2% BSA solution for 30 minutes at 25°C. Obtained magnetic particles-antibody conjugates were stored at 4°C until use.

Anti-TgAb antibody LA001, conjugated using magnetic particles, was prepared using a similar method.

#### Labeling of antibodies

Anti-antibody labeling with Eu chelates was carried out according to manufacturer instructions. The PD-10 column was used to exchange

## Pre-pregnancy thyroid dysfunction screening

buffers to pH 8.5 (50 mmol/L  $\text{Na}_2\text{CO}_3$ - $\text{NaHCO}_3$  0.155 mol/L NaCl). Anti-TPOAb antibody B7 or anti-TgAb antibody CA001 labeling buffer (500  $\mu\text{L}$ , 2 mg/mL, pH 8.5) was added to 0.2 mg Eu-chelate of N1-pisothiocyantobenzyl-diethylenetriamine-N1, N2, N3, N4-tetraacetic acid, then incubated overnight at 4°C. The labeled antibody was isolated from the free chelate and aggregated Mc Abs with Sepharose CL-6B gel filtration column (1 cm  $\times$  40 cm) in an elution buffer containing 50 mmol/L Tris-HCl (pH 7.8 containing 0.9% NaCl and 0.05% sodium azide as a preservative). Fluorescence was measured by dilution with an enhanced solution and the concentration of  $\text{Eu}^{3+}$  in the sample was obtained. Collected protein fractions were rapidly frozen and stored in high vacuum drying after dilution with an elution buffer containing 0.2% BSA as a stabilizer. Labeled Mc Abs were stored at -20°C.

### *Preparation of TPOAb and TgAb standards*

Concentrations of TPOAb in the six mixed standards were prepared by diluting highly purified TPOAb in a standard buffer (50 mmol/L pH 7.8 Tris-HCl buffer containing 2 g/L BSA and 1 g/L  $\text{NaN}_3$ ) to 0, 7, 15, 30, 50, and 100 ng/mL. Concentrations of TgAb in the six mixed standards were prepared by diluting highly purified TgAb in a standard buffer to 0, 10, 50, 100, 200, and 400 ng/mL.

### *Time-resolved fluoroimmunoassay*

Initially, 5  $\mu\text{L}$  of magnetic nanoparticles, coated with anti-TPOAb antibody B1, 75  $\mu\text{L}$  of  $\text{Eu}^{3+}$  labeling anti-TPOAb antibody B7, and serum samples or standards, were added sequentially into the analytes. After 15 minutes of incubation with continuous gentle stirring at room temperature (25°C), the plate was positioned on the magnet for 5 seconds. The supernatant was discarded. After removing the free substances and rinsing with washing buffer six times, 200  $\mu\text{L}$  of enhancement solution was added. Next, the immunocomplexes were re-suspended in the enhancement solution and the mixtures were incubated for 5 minutes with stirring at room temperature. Finally, the fluorescence signal was measured with the AutoDEFLIA<sub>1235</sub> Multi-label Counter. Fluorescence of  $\text{Eu}^{3+}$  was measured at an excitation wavelength of 340 nm and an emission wavelength of 615 nm.

### *Statistical analysis*

Data are presented as mean  $\pm$  standard deviation (SD). Student's t-test was used for comparisons between the two groups. Standard curves were obtained by plotting the fluorescence intensity (Y) against the sample concentration (X) and fitting a linear regression equation. Pearson's linear regression was used to obtain linearity and correlations. Serum samples were measured using the proposed method and a commercial kit (Roche). They were analyzed and compared using the Bland and Altman method.  $P < 0.05$  indicates statistical significance. All statistics were generated using SPSS 17.0.

## Results

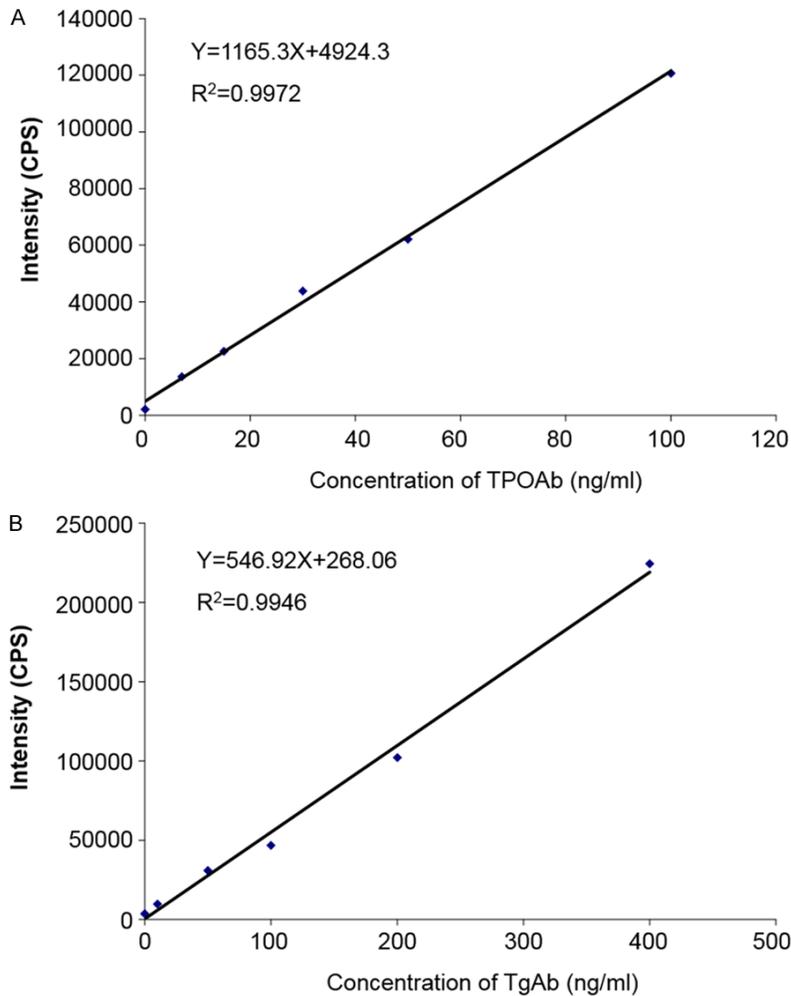
### *Optimization of the assay*

Reaction parameters, including reaction temperature, incubation time, concentration of magnetic particles, and the dilution ratio of  $\text{Eu}^{3+}$ -labeling antibody, were optimized and reported in a previous work. Reaction conditions were optimized as follows: one-stop reaction finished within 15 minutes in a total volume of 50  $\mu\text{L}$  with 5  $\mu\text{L}$  magnetic particles. Optimized magnetic particle-based TRFIA reduced analysis times and minimized the amount of blood samples required.

### *Calibration curves*

Under optimized experimental conditions, standard curves for the TPOAb immunoassay and TgAb immunoassay were generated using a dilution series of standards (0, 7, 15, 30, 50, and 100 ng/mL for the TPOAb immunoassay and 0, 10, 50, 100, 200, and 400 ng/mL for the TgAb immunoassay). Calibration curves were obtained using linear regression. As shown in **Figure 1**, calibration curves for both TPOAb and TgAb were linear over the concentration range, with a correlation coefficient of 0.9972 and 0.9946, respectively. The linear regression equation of TPOAb was  $Y = 1165.3X + 4924.3$ , with a lower detection limit of 0.31 ng/mL (defined as the concentration corresponding to blank fluorescence intensity plus two standard deviations,  $n = 20$ ). The linear regression equation of TgAb was  $Y = 546.92X + 268.06$ , with a lower detection limit of 0.35 ng/mL. No high-dose hook effects were observed within these

## Pre-pregnancy thyroid dysfunction screening



**Figure 1.** Calibration curves for magnetic nanoparticle-based TRFIA measurement of TPOAb (A) and TgAb (B) standards.

**Table 1.** Intra-assay reproducibility

Sample	Theoretical value (ng/mL)	Observed value <sup>a</sup> (ng/mL)	CV (%) n = 10
TPOAb	20	20.83 ± 1.55	3.8
	100	101.42 ± 9.78	4.3
TgAb	50	49.21 ± 1.07	4.7
	400	405.84 ± 10.21	4.9

<sup>a</sup>Mean value ± standard deviation.

linear ranges. Results suggest that sensitivity reached the permitted determination for very low levels of TPOAb or TgAb.

### Precision, specificity, and recovery

Precision of the current method was estimated by inter-assay and intra-assay coefficients of

variation (CV). Precision of inter- and intra-assays for both TPOAb and TgAb met the requirements either at low concentrations or high concentrations (Tables 1, 2). Intra- and inter-precision for TPOAb was 3.8-4.3% and 4.2-5.1%, respectively. Corresponding results for TgAb were 4.7-4.9% and 3.8-6.0%, respectively.

Specificity of the assay for TPOAb was evaluated by measuring the cross-reactivity with TgAb and TSH. Results showed that there was no cross-reactivity with either TgAb or TSH (less than 1%, Table 3). Similar results were found in the assay for TgAb, which had no cross-reactivity with either TPOAb or TSH (Table 4). Present results verify the high specificity of this novel assay, indicating this method could be used for determination of TPOAb and TgAb in human serum.

Analytical recoveries were evaluated via spiking different concentrations of TPOAb and TgAb standards into

maternal serum controls. Results are presented in Table 5. Recoveries of TPOAb and TgAb varied from 99.4-100.1% and 99.2-101.0%, respectively, indicating that recovery of the proposed immunoassay was satisfactory.

### Clinical application of the established assay

TPOAb and TgAb in 58 clinical serum samples were analyzed to evaluate the feasibility of the developed TRFIA based on magnetic nanoparticles in clinical applications. These serum samples were also assayed in parallel, using commercial kits (Roche). Results from the developed method revealed a good correlation with those from Roche kits (Figure 2). The equation of the regression curve was  $Y = 1.096X + 0.2088$  ( $R^2 = 0.9937$ ) for TPOAb and  $Y = 0.9733X + 1.5214$  ( $R^2 = 0.9965$ ) for TgAb. X represents

## Pre-pregnancy thyroid dysfunction screening

**Table 2.** Inter-assay reproducibility

Sample	Theoretical value (ng/mL)	Observed value <sup>a</sup> (ng/mL)	CV (%) n = 10
TPOAb	20	21.04 ± 1.09	4.2
	100	110.54 ± 12.45	5.1
TgAb	50	51.14 ± 1.05	3.8
	400	410.13 ± 10.14	6.0

<sup>a</sup>Mean value ± standard deviation.

**Table 3.** Cross-reactivity for TPOAb

Interfering substance	Concentration (ng/mL)	Observed value (ng/mL)	Cross-reactivity (%)
TgAb	500	0.2	0.04
TSH	250	1.2	0.48

**Table 4.** Cross-reactivity for TgAb

Interfering substance	Concentration (ng/mL)	Observed value (ng/mL)	Cross-reactivity (%)
TPOAb	200	0.1	0.05
TSH	250	0.7	0.28

TPOAb or TgAb concentrations, estimated with the proposed method, and Y represents those obtained using the Roche kit.

### Discussion

Thyroid dysfunction is the second most common endocrine disturbance in women of reproductive age, especially in pregnant women [13]. About 2-3% of pregnant women are diagnosed with hypothyroidism and 10-20% of all euthyroid pregnant women are positive for thyroid antibodies [14]. Hypothyroidism is an important factor in pregnancy outcomes, as well as neonatal and childhood development [15]. Studies have shown that subclinical hypothyroidism and thyroid autoimmunity adversely affect maternal and fetal outcomes [16-18]. Pre-pregnant women with severe hypothyroidism often have difficulty conceiving. Moreover, risks of adverse maternal complications in an untreated hypothyroidism pregnancy are higher than those in normal pregnant women. Wang et al. reported that the case identification screening strategy failed to diagnose 81.6% of pregnant women with hypothyroidism [19]. Ohashi et al. also showed that, in targeted high-risk case pregnant women diagnosed with thyroid disease, only 10% of women with thyroid dysfunction and 90% of women with thy-

roid dysfunction failed to be identified [20].

Thyroid autoantibodies (TPOAb and/or TgAb) are associated with hypothyroidism. TPOAb is a specific indicator of autoimmune thyroid disease and an important indicator of subclinical hypothyroidism. Positivity for TPOAb is common in women of childbearing age and bears a considerable risk for the development of postpartum thyroiditis, with eventual long-term hypothyroidism in the mother. Tg is a thyroid storage protein. Its levels positively correlate with thyroid volume. Tg levels increase in early pregnancy, are maintained at stable levels throughout mid-pregnancy, increase further at 36 weeks of gestation, and decrease to non-pregnant levels after delivery [21]. A recent study reported the assessment of serum Tg levels as a marker for iodine deficiency [22]. Positivity for TPOAb and/or TgAb is common in women of reproductive age in countries with good iodine supplies. The prevalence of positivity for TPOAb among pregnant women has been reported to be between 5.1 and 12.4% [23-25]. Based on these studies, TPOAb and TgAb are believed to be significant risk factors for hypothyroidism in pregnancies.

Currently, laboratory diagnosis of thyroid dysfunction during pregnancy is based on serum TSH concentrations. However, only detecting serum TSH may miss TPOAb- or TgAb-positive cases. Moreover, concentrations of TPOAb and TgAb have been associated with thyroid disease, helping to detect thyroid disease to some extent. Therefore, screening for thyroid disorders in pre-pregnancy and pregnancy women should also include assessment of TPOAb and/or TgAb.

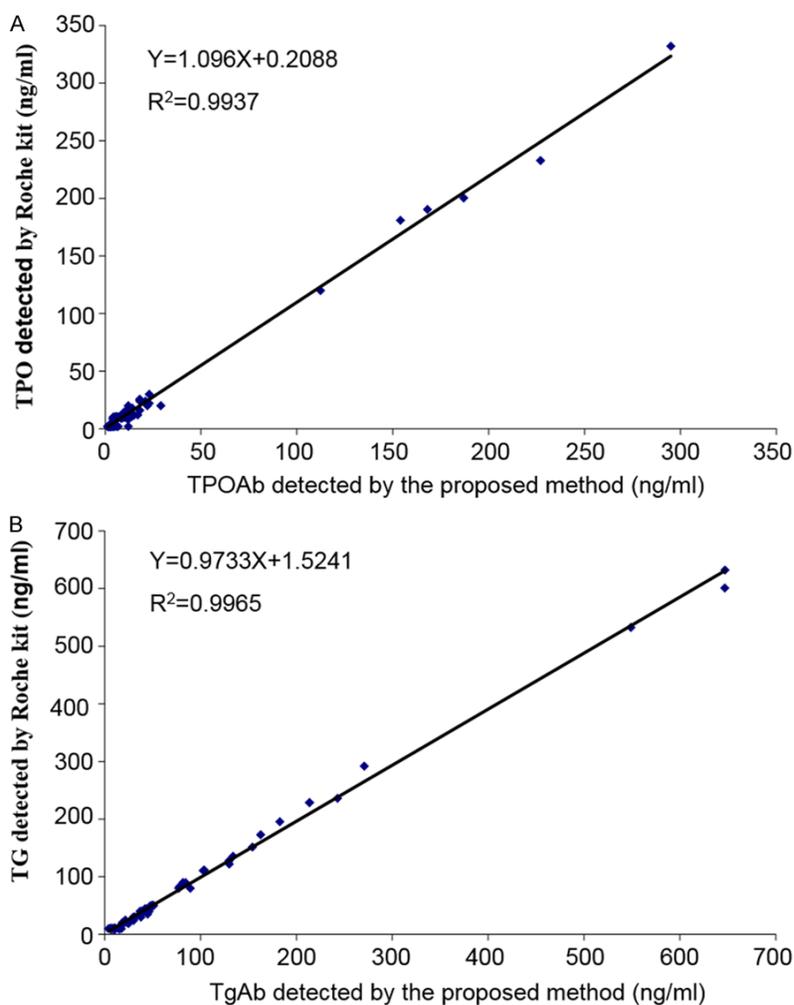
TRFIA is a promising immunoassay method labeled with rare earth metals. This method can generate strong fluorescence with long decay times, large Stokes shifts, and sharp emission profiles [26, 27]. However, some limitations in the conventional TRFIA still remain. Specific antigen or antibody reagents and interactions may not absorb fully (and so be immobilized) on the plastic surface of 96-well microplates. Thus, they could be washed out. Ma-

## Pre-pregnancy thyroid dysfunction screening

**Table 5.** Recoveries determined via spiking different concentrations of TPOAb and TgAb standards into maternal serum controls

Sample	Serum concentration (ng/mL)	Spiked standard (ng/mL)	Observed value <sup>a</sup> (ng/mL)	Theoretical value (ng/mL)	Recovery (%)
TPOAb	25.80	23.95	49.45 ± 0.88	49.75	99.4
	25.80	52.15	78.03 ± 1.06	77.95	100.1
	25.80	87.25	112.60 ± 2.37	113.05	99.6
TgAb	162.48	56.24	220.90 ± 1.63	218.72	101.0
	162.48	120.88	281.09 ± 2.58	283.36	99.2
	162.48	368.45	533.58 ± 1.96	530.93	100.5

<sup>a</sup>Mean value ± standard deviation.



**Figure 2.** Comparison of results obtained using the proposed method versus those obtained using Roche kits for TPOAb (A) and TgAb (B) detection in 58 serum samples.

genetic nanoparticles with bioactive molecules are very useful tools for immunoassays due to large surface areas available for reactions and

less loss of specific antigens or antibodies in the plate-washing process. Combining the advantages of using magnetic nanoparticles with TRFIA, present researchers established a magnetic particle-based TRFIA method. This method is a new generation of sensitive technology, characterized by immobilizing primary antibodies on the surface of magnetic particles and labeling with stable fluorescent chelates. This method achieves high sensitivity, good specificity, high precision, high recovery rate, and stable detection results. It is effective in clinical application. Moreover, this method can possibly replace the electrochemical luminescence detection method.

This novel immunoassay method is applicable to the determination of TPOAb and TgAb. As indicated by present results, the assay procedure is more straightforward than the conventional method, with acceptable reproducibility, specificity, and recovery. Moreover, the cost of the proposed method established is much lower than the commercial kit (Roche kit). Good correlation was found with the Roche kit, sug-

gesting that the new method could be developed into commercially available kits for clinical application.

In summary, the current study developed a TRFIA method for determination of thyroid autoantibodies (TPOAb and/or TgAb) by immobilizing primary antibodies on the surface of magnetic nanoparticles. Present results demonstrate its high sensitivity, specificity, accuracy, and reproducibility, as well as its cost-effectiveness and short assay times. The present novel immunoassay for serum TPOAb and TgAb may be suitable for clinical use in the future.

### Acknowledgements

This work was supported by a grant from Maternal and Child Health Association Project of Jiangsu Province (FYX201614) to Xufeng Ding and a grant from Jiangsu Provincial Maternal and Child Health Research Projects (F201759) to Xufeng Ding.

### Disclosure of conflict of interest

None.

**Address correspondence to:** Xufeng Ding, Yixing Maternal and Child Health Care Hospital, Wuxi 214200, Jiangsu, China. E-mail: 2301906083@qq.com

### References

- [1] Allan WC, Haddow JE, Palomaki GE, Williams JR, Mitchell ML, Hermos RJ, Faix JD and Klein RZ. Maternal thyroid deficiency and pregnancy complications: implications for population screening. *J Med Screen* 2000; 7: 127-130.
- [2] Abalovich M, Gutierrez S, Alcaraz G, Maccallini G, Garcia A and Levalle O. Overt and subclinical hypothyroidism complicating pregnancy. *Thyroid* 2002; 12: 63-68.
- [3] Julvez J, Alvarez-Pedrerol M, Rebagliato M, Murcia M, Forns J, Garcia-Esteban R, Lertxundi N, Espada M, Tardon A, Riano Galan I and Sunyer J. Thyroxine levels during pregnancy in healthy women and early child neurodevelopment. *Epidemiology* 2013; 24: 150-157.
- [4] Moog NK, Entringer S, Heim C, Wadhwa PD, Kathmann N and Buss C. Influence of maternal thyroid hormones during gestation on fetal brain development. *Neuroscience* 2015; 342: 68-100.
- [5] Stricker R, Echenard M, Eberhart R, Chevailler MC, Perez V and Quinn FA. Evaluation of maternal thyroid function during pregnancy: the importance of using gestational age-specific reference intervals. *Eur J Endocrinol* 2007; 157: 509-514.
- [6] Mannisto T, Surcel HM, Ruokonen A, Vaaras-maki M, Pouta A, Bloigu A, Jarvelin MR, Hartikainen AL and Suvanto E. Early pregnancy reference intervals of thyroid hormone concentrations in a thyroid antibody-negative pregnant population. *Thyroid* 2011; 21: 291-298.
- [7] Jensen EA, Petersen PH, Blaabjerg O, Hansen PS, Brix TH and Hegedus L. Establishment of reference distributions and decision values for thyroid antibodies against thyroid peroxidase (TPOAb), thyroglobulin (TgAb) and the thyrotropin receptor (TRAb). *Clin Chem Lab Med* 2006; 44: 991-998.
- [8] Taubner K, Schubert G, Pulzer F, Pfaeffle R, Korner A, Dietz A, Thiery J, Kiess W and Kratzsch J. Serum concentrations of anti-thyroid peroxidase and anti-thyroglobulin antibodies in children and adolescents without apparent thyroid disorders. *Clin Biochem* 2014; 47: 3-7.
- [9] Feldt-Rasmussen U. Analytical and clinical performance goals for testing autoantibodies to thyroperoxidase, thyroglobulin, and thyrotropin receptor. *Clin Chem* 1996; 42: 160-163.
- [10] Nazarpour S, Tehrani FR, Simbar M, Tohidi M, AlaviMajd H and Azizi F. Comparison of universal screening with targeted high-risk case finding for diagnosis of thyroid disorders. *Eur J Endocrinol* 2015; 174: 77-83.
- [11] Yuan S, Yu N, Gao Y, Huang W, He Y, Dong B, Lu G, Li M, Cai X, Peng D, Wang Y, Li T, Huang Y, Guo X and Shi B. Distribution of IgG subclasses of TgAb and TPOAb in sera from patients with Graves' disease, Graves' disease plus Hashimoto's thyroiditis and Hashimoto's thyrotoxicosis. *Zhonghua Yi Xue Za Zhi* 2014; 94: 110-114.
- [12] Li J, Teng X, Wang W, Chen Y, Yu X, Wang S, Zhu L, Li C, Fan C, Wang H, Zhang H, Teng W and Shan Z. Effects of dietary soy intake on maternal thyroid functions and serum anti-thyroperoxidase antibody level during early pregnancy. *J Med Food* 2011; 14: 543-550.
- [13] Vandana, Kumar A, Khatuja R and Mehta S. Thyroid dysfunction during pregnancy and in postpartum period: treatment and latest recommendations. *Arch Gynecol Obstet* 2014; 289: 1137-1144.
- [14] Negro R and Mestman JH. Thyroid disease in pregnancy. *Best Pract Res Clin Endocrinol Metab* 2011; 25: 927-943.
- [15] Gaberscek S and Zaletel K. Thyroid physiology and autoimmunity in pregnancy and after delivery. *Expert Rev Clin Immunol* 2011; 7: 697-706; quiz 707.
- [16] Thangaratinam S, Tan A, Knox E, Kilby MD, Franklyn J and Coomarasamy A. Association between thyroid autoantibodies and miscar-

## Pre-pregnancy thyroid dysfunction screening

- riage and preterm birth: meta-analysis of evidence. *BMJ* 2011; 342: d2616.
- [17] Casey BM, Dashe JS, Wells CE, McIntire DD, Byrd W, Leveno KJ and Cunningham FG. Subclinical hypothyroidism and pregnancy outcomes. *Obstet Gynecol* 2005; 105: 239-245.
- [18] Chen L and Hu R. Thyroid autoimmunity and miscarriage: a meta-analysis. *Clin Endocrinol (Oxf)* 2011; 74: 513-519.
- [19] Wang W, Teng W, Shan Z, Wang S, Li J, Zhu L, Zhou J, Mao J, Yu X, Chen Y, Xue H, Fan C, Wang H, Zhang H, Li C, Zhou W, Gao B, Shang T, Ding B, Ma Y, Wu Y, Xu H and Liu W. The prevalence of thyroid disorders during early pregnancy in China: the benefits of universal screening in the first trimester of pregnancy. *Eur J Endocrinol* 2011; 164: 263-268.
- [20] Ohashi M, Furukawa S, Michikata K, Kai K, Sameshima H and Ikenoue T. Risk-based screening for thyroid dysfunction during pregnancy. *J Pregnancy* 2013; 2013: 619718.
- [21] Zhang X, Li C, Mao J, Wang W, Xie X, Peng S, Wang Z, Han C, Wang D, Fan C, Shan Z and Teng W. Gestation-specific changes in maternal thyroglobulin during pregnancy and lactation in an iodine-sufficient region in China: a longitudinal study. *Clin Endocrinol (Oxf)* 2016; 86: 229-235.
- [22] Bilek R, Cerovska J and Zamrazil V. The relationship between iodine intake and serum thyroglobulin in the general population. *Physiol Res* 2015; 64: 345-353.
- [23] Medici M, de Rijke YB, Peeters RP, Visser W, de Muinck Keizer-Schrama SM, Jaddoe VV, Hofman A, Hooijkaas H, Steegers EA, Tiemeier H, Bongers-Schokking JJ and Visser TJ. Maternal early pregnancy and newborn thyroid hormone parameters: the Generation R study. *J Clin Endocrinol Metab* 2012; 97: 646-652.
- [24] Shields BM, Knight BA, Hill AV, Hattersley AT and Vaidya B. Five-year follow-up for women with subclinical hypothyroidism in pregnancy. *J Clin Endocrinol Metab* 2013; 98: E1941-1945.
- [25] Nambiar V, Jagtap VS, Sarathi V, Lila AR, Kamilanathan S, Bandgar TR, Menon PS and Shah NS. Prevalence and impact of thyroid disorders on maternal outcome in asian-Indian pregnant women. *J Thyroid Res* 2011; 2011: 429097.
- [26] Dickson EF, Pollak A and Diamandis EP. Time-resolved detection of lanthanide luminescence for ultrasensitive bioanalytical assays. *J Photochem Photobiol B* 1995; 27: 3-19.
- [27] Hagan AK and Zuchner T. Lanthanide-based time-resolved luminescence immunoassays. *Anal Bioanal Chem* 2011; 400: 2847-2864.