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# PREPARATION OF MAGNETIC PARTICLE ANTIBODIES FOR RADIOIMMUNOASSAY AND IMMUNORADIOMETRIC ASSAY OF THYROID RELATED HORMONES*

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Silanized magnetic particles have been developed by means of a number of different methods for coupling antibodies for use in radioimmunoassay (RIA) and immunoradiometric assay (IRMA) of thyroid related hormones. Carbodiimide, glutaraldehyde, glutaric anhydride and spacer-arms coupling methods were studied for immobilization of triiodothyronine ($T_3$), thyroxine ($T_4$) and thyroid stimulating hormone (TSH) antibodies on magnetic particles, respectively. The effect of the magnetic particle preparation on the couplings was discussed. The influence of coupling conditions on the preparation of magnetic particle antibodies and the assay performance was also investigated. The storage stability and reproducibility of the magnetic particle $T_3$、$T_4$ and TSH antibodies were good enough for use in total $T_3$, total $T_4$ RIA and TSH IRMA. A comparison of five types of magnetic particles from different sources for coupling TSH antibody for TSH IRMA application was made. Silanized magnetic particles and other types of particles showed comparable results. Five TSH antibodies, including two polyclonal and three monoclonal antibodies coupled to silanized magnetic particles respectively, were used in TSH IRMA. It was found that each of them and a commercial monoclonal TSH antibody as label formed good sandwich partners and gave a high signal to low levels of TSH.

An essential requirement for all reliable RIA and IRMA is an efficient, practical and clean method for separation of the "bound" and "free" ligand fractions. A significant advance in recent years has been the development of solid phase separation method which utilize antibody immobilized on magnetic particles resulting in clean separation using simple operation equipment[1,2]. Magnetic particles combine high surface capacity with fast, efficient separation without the need for centrifugation, thus they provide a solid phase methodology which avoids many of the disadvantages of other solid phase technique while retaining

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their advantages[3]. Magnetic particles made by a variety of ways have been widely used in various RIA, IRMA and other assays[3,4].

The preparation of magnetic particles is one of the most important factors in the development of RIA and IRMA with magnetic separation method, because the perfect performance of magnetic particle antibodies in all magnetic immunoassays in a considerable degree depends on the inherent properties of magnetic particles. However, in developing countries it is very difficult to obtain good quality of magnetic particles with low cost. For this reason, magnetic particles have been developed for the locally produced magnetic particle antibodies.

The present study is emphasized on the preparation of magnetic particles and the couplings $T_3$, $T_4$ and TSH antibodies to magnetic particles for use in RIA and IRMA of thyroid related hormones.

## Materials and methods

Antibodies: Anti-$T_3$, anti-$T_4$ and anti-TSH(IAEpAb) and monoclonal anti-TSH (IAEmAb) were prepared locally. Two TSH monoclonal antibodies (Thai I mAb and Thai II mAb) were obtained by courtesy of Dr. Wiyada (NIH Thailand). A polyclonal anti-TSH (BMSpAb) and a monoclonal anti-TSH (OMBmAb) were obtained from BMS Australia and OMB Finland.

Magnetic Particles: Silanized magnetic particles, polyacrylamide and polystyrene magnetic particles were prepared in our laboratory. Polyacrolein magnetic particles were donated by Dr. Shen Rongsen (IRM China). Magnetizable cellulose and Latex-M particles were purchased from Scipac, U. K. and Rhone-Poulenc, France.

Reagents: N-2-aminoethyl-3-aminopropyltrimethoxysilane, 3-aminopropyltrimethoxysilane, 3-aminopropyltriethoxysilane, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDAC), Glutaraldehyde (GA), 2-(N-morpholino) ethane sulfonic acid (MES), N-(2-hydroxyethyl) piperazine-N'-(2-ethane sulfonic acid) (HEPES) were obtained from commercial sources.

*Preparation of iron oxide particles*[5]

*NaOH precipitation*: 100ml solution of 0.5mol/L $FeCl_2$ and 0.3 mol/L $FeCl_3$ and 100ml of 5 mol/L NaOH solution were mixed and stirred for 1-4 minutes at 50℃. A black, magnetic iron oxide precipitate formed. The precipitate was washed with water until a pH 7 was reached.

*$NH_4OH$ precipitation*: 150ml of 0.2 mol/L $FeCl_2$ and 0.3 mol/L $FeCl_3$ solution were precooled to about 10℃. 100ml of 5 mol/L $NH_4OH$ solution cooled at 10℃ were added at a rate of about 5 ml/s. Precipitation reaction was carried out for 1 hour at 2-4℃ with continuously stirring. A black iron oxide suspension was obtained and washed with 0.9% NaCl until the supernatant was neutral.

*Silanization of iron oxide particles*[1,7]

*An acidic organic silanization*: The methanol replacement was used to remove the most of water in the particles suspension, leaving approximately 0.5% water. 1g orthophosphorous acid and 6ml of N-2-aminoethyl-3-aminopropyltrimethoxysilane were added. The mixture was stirred at about 20000 rpm for 15 minutes and at about 10000 rpm for 2 hours. The contents were then mixed with 100ml glycerol and heated to 180℃. The mixture was allowed to keep at 180℃ for another 1 hour then to cool to room temperature. This process were performed under nitrogen with stirring. The glycerol particles slurry were washed exhaustively with water.

*An acidic aqueous silanization*: 5g iron oxide particles were mixed with 250ml of 10% solution of 3-aminopropyltrimethoxysilane. The pH was adjusted to about 5 with glacial acetic acid. The mixture was heated at 90-95℃ for 2 hours while mixing. After cooling, glycerol dehydration was again used. The silanized magnetic particles were washed 3 times with water, 3 times with methanol and 3 times with water.

*Coupling of $T_3$, $T_4$ and TSH antibodies to magnetic particles*

*Carbodiimide method for coupling anti-$T_4$*: 0.2g magnetic particles were suspended in 8ml of water. 0.2ml $T_4$ antiserum was added. After mixing for 10 minutes, 40mg EDAC were added. The pH was adjusted to 5.6 with 0.1 mol/L HCl or 0.1mol/L NaOH. The mixture was rotated for 24 hours at room temperature. The magnetic particle anti-$T_4$ obtained was washed 3 times with phosphate buffer saline (PBS) containing 0.1% bovine serum albumin (BSA) and 3 times with water, then washed 3 times with PBS and stored at 4℃.

*Carbodiimide method for coupling anti-TSH*: 0.2g magnetic particles or Latex-M particles were washed 2 times with PBS containing 0.1% BSA and 0.1% Tween20. The particles were resuspended in 15ml MES. 2ml purified anti-TSH (caprylic acid precipitation) were added. After rotating for 30

minutes, 40mg EDAC were added. Coupling reaction was carried out overnight at room temperature. The magnetic particle anti-TSH was washed 5 times with PBS containing 0.1%BSA and 1% Tween20, then washed 5 times with PBS containing 0.1% BSA and 0.1% Tween20.

*Glutaraldehyde method for coupling anti-$T_3$*: 0.2g magnetic particles were suspended in 8ml 0.1mol/L phosphate buffer (PB) and 8ml of 5% glutaraldehyde were added. The contents were rotated for 3 hours. Aldehyde magnetic particles were obtained. Unreacted glutaraldehyde was washed with 5 additions of PB. 0.2ml $T_3$ antiserum was added to the activated particles suspension and the mixture was rotated for 24 hours at room temperature. The magnetic particle anti-$T_3$ was washed once with PB and mixed with 15ml 0.2mol/L glycine solution by shaking for 30 minutes. The magnetic particle anti-$T_3$ was washed with PB, ethanol and washed twice with PBS containing 0.1% BSA.

*Glutaric anhydride method*: 2g magnetic particles were washed 3 times with 0.1mol/L $NaHCO_3$. 1.2g glutaric anhydride were added. The particles were mixed for 2 hours. The particles were then washed 2 times and the reaction with glutaric anbydride was repeated. The carboxyl magnetic particles were washed 5 times with water. Carbodiimide method was again used for reaction with antibody.

*Spacer-arms method*: Carboxyl magnetic particles were derivatized with aminoheptane or 6-amino caproic acid by adding EDAC. After reaction for 2 hours, the particles were washed with PB. The glutaraldehyde or carbodiimide method was again used for coupling antibody. Sometimes the derivative procedure was repeatedly used several times.

*Assay protocols*

Total $T_3$, total $T_4$ and blood spot total $T_4$ RIA adopt conventional assay protocols without washing step. The magnetic free $T_3$ and free $T_4$ RIA are based on a two step methodology. In a two-site "sandwich" immunoradiometric assay for TSH, a pair of antibodies, IAE polyclonal anti-TSH linked to magnetic particles and OMB monoclonal anti-TSH as label, were chosen. A assay protocol using two time incubation without rotating the tubes and two times of washing step was used.

Optimal quantity of magnetic particle antibodies for each tube in various assays were selected from titration curves. The amount of magnetic particles was

30-50 μg/tube for total $T_3$, total $T_4$, blood spot total $T_4$, free $T_3$ and free $T_4$ RIA. 300μg/tube and 150 μg/tube for TSH and blood spot TSH IRMA.

Adding appropriate amount of carrier particles to magnetic antibodies may be adopted if the determined magnetic particles were less than 30 μg per tube.

## Results and Discussion

*The application of magnetic particle antibodies to relative RIA and IRMA*

Seven magnetic assays, total $T_3$, total $T_4$, free $T_3$, free $T_4$, blood spot total $T_4$ RIA, TSH and blood spot TSH IRMA, have recently been developed utilizing three types of magnetic particles in our laboratory. $T_3$, $T_4$ antibodies coupled to silanized magnetic particles have been used in total $T_3$, total $T_4$ and blood spot total $T_4$ RIA. The reliability of magnetic separation in these assays has been validated by comparing it with conventional separation methods. The characteristics of these magnetic assays were similar to those of the established non-magnetic assays, polyacrylamide microsphere (ms) and PEG methods. A comparison of three types of total $T_3$ RIA is given in Table Ⅰ[9]. All the results obtained by the magnetic total $T_4$ and blood spot total $T_4$ RIA also pointed to the same conclusion[10] as total $T_3$ RIA shown in Table Ⅰ.

The performance of free $T_3$, free $T_4$ RIA using silanized magnetic particle anti-$T_3$, anti-$T_4$ were also similar to those obtained by antibodies immobilized on magnetizable cellulose using cyanogen bromide or 1,1′-carbonyldiimidazol activation[11].

TABLE Ⅰ. COMPARISON OF THREE TYPES OF $TT_3$ RIA

| Method | NSB (%) | B/T (%) | Standard curve | r | Sensitivity (nmol/L) | Normal value (nmol/l) |
|---|---|---|---|---|---|---|
| Magnetic | 2.0±0.2 | 51.8±5.4 | Y=0.63-2.20LogX | -0.999 | 0.33 | 1.47-2.21 |
| Microsphere | 1.8±0.4 | 47.8±7.5 | Y=0.46-2.17LogX | -0.999 | 0.17 | 1.56-2.40 |
| PEG | 4.8±0.4 | 62.9±4.3 | Y=0.27-2.25LogX | -0.999 | 0.08 | 1.56-2.75 |

Magnetic TSH antibody can be applied to TSH IRMA. The maximum binding ($B_{40}$) can achieve 20-30% and the zero standard binding ($B_0$) has decreased to less than 0.2% using fine particles made by an acidic aqueous silanization. The new particles and an improved assay protocol gave a more precise and sensitive assay. Seven magnetic assay characteristics were summarized in Table Ⅱ[12,13].

TABLE Ⅰ. PERFORMANCE CHARACTERISTICS OF SEVEN MAGNETIC ASSAYS

| Assay | NSB% | B/T% | Intraassay | Interassay | Sensitivity | Normal value |
|---|---|---|---|---|---|---|
| $TT_3RIA$ | <3 | 30-55 | 3.8-4.1 | 7.0-12.4 | 0.33nmol/L | 1.47-2.21nmol/L |
| $TT_4RIA$ | <3 | 35-75 | 3.3-8.5 | 6.1-9.5 | 7.2nmol/L | 63.2-158.1nmol/L |
| $FT_3RIA$ | <2 | 30-40 | 7.2-18.3 | 10.9-19.1 | 0.4nmol/L | 2.5-9.5nmol/L[a] |
| $FT_4RIA$ | <2 | 30-40 | 4.9-13.6 | 7.2-16.2 | 0.3nmol/L | 9.5-25.5nmol/L[a] |
| TSH IRMA | <0.2 | 15-30 | 4.6-10.0 | 7.1-10.2 | 0.04mIU/L | 0.3-4.4mIU/L |
| B.S. $TT_4RIA$ | <15 | 35-55 | <15 | <20 | 0.7nmol/L | >25mIU/L |
| B.S. TSH IRMA | <0.5 | ~10 | <15 | <20 | 1.0mIU/L | <20mIU/L[b] |

* a. Magnetizable Cellulose was used

* b. Latex-M was used

Magnetic $T_3$, $T_4$ antibodies are stable for at least 6 months at 4℃ for use in total $T_3$, total $T_4$ and blood spot total $T_4$ RIA. While at least in the two weeks trial period at 37℃ no change in the performance of the magnetic $T_3$, $T_4$ antibodies in total $T_3$ and total $T_4$ RIA could be observed.

A magnetic $T_4$ antibody stored in HEPES buffer at 4℃ exhibits a bit poor stability for use in free $T_4$ RIA. The zero standard binding (B/T) has decreased from 36% to 29% after 20 days storage and requires to be improved further.

Magnetic TSH antibody can remain stable for at least 6 months at 4℃. A storage stability trial in three buffers with different preservatives at 37℃ was conducted. After 12 days storage, the maximum binding ($B_{40}$) in TSH IRMA only decreases to 62-81% of its original value and it still works satisfactorily.

*Coupling antibodies to magnetic particles*

Carbodiimide, glutaraldehyde, glutaric anhydride and spacer-arms methods were used for the preparation of magnetic particle $T_3$, $T_4$ and TSH antibodies, respectively. Coupling experiments showed that there are no considerable differences in total $T_3$ and total $T_4$ RIA using magnetic antibodies synthesized by above four coupling techniques except that the zero standard binding (B/T) is a little higher by use of glutaric anhydride method. The best maximum binding ($B_{40}$) in TSH IRMA was obtained by means of magnetic TSH antibody made by carbodiimide protocol. The comparison of four methods for the preparation of magnetic particle anti-TSH was shown in Table Ⅲ.

TABLE Ⅲ. COMPARISON OF FOUR METHODS FOR COUPLING ANTI-TSH IN TSH IRMA

| Particles | Amino particles | | | | | | Carboxyl particles | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Method | EDAC | | GA[a] | | GA[b] | | Spacer-arm[c] | | Spacer-arm[d] | |
| TSH mIU/L | CPM | B/T% | CPM | B/T% | CPM | B/T% | CPM | B/T% | CPM | B/T% |
| 0 | 461 | 0.26 | 473 | 0.27 | 118 | 0.06 | 249 | 0.14 | 362 | 0.21 |
| 0.3 | 875 | 0.51 | 818 | 0.47 | 323 | 0.15 | 477 | 0.28 | 619 | 0.36 |
| 0.8 | 1785 | 1.03 | 1796 | 1.04 | 1194 | 0.57 | 1796 | 1.04 | 1600 | 0.92 |
| 3.5 | 5454 | 3.14 | 5259 | 3.03 | 3843 | 1.82 | 5488 | 3.16 | 4917 | 2.85 |
| 10 | 12871 | 7.40 | 12320 | 7.10 | 9520 | 4.52 | 13169 | 7.59 | 11297 | 6.57 |
| 20 | 23707 | 13.7 | 22582 | 13.0 | 18195 | 8.64 | 23572 | 13.6 | 19532 | 11.3 |
| 40 | 48650 | 28.1 | 46998 | 27.1 | 38339 | 18.2 | 43487 | 25.1 | 36482 | 21.0 |

* a Glutaraldehyde　　* b Glutaric anhydride

* c Amino caproic acid as arm　　* d Diaminoheptane as arm

*Optimization of coupling*

*Effect of the properties of particles on couplings and assays*:

Different sizes of iron oxide particles can be prepared by changing the precipitating conditions, such as reaction time, feed rate, stirring speed and so on. Coarse and fine particles produced have semi-settling time of 80 minutes and 120 minutes. Magnetic anti-$T_3$ and anti-$T_4$ made by these particles can remain suspended in an aqueous medium for at least 1 hour during incubation, but settle down to a firm pellet in about 5 minutes with a magnetic field.

Two methods, NaOH and $NH_4OH$ precipitation, were used to produce iron oxide particles by which the similar experimental results in total $T_3$ and total $T_4$ RIA were obtained. Magnetic particles made by $NH_4OH$ method gave better maximum binding ($B_{40}$) 24.6% in TSH IRMA instead of 21.4% by the particles prepared with NaOH precipitation.

An acidic organic silanization and an aqueous one were developed for coating the iron oxide particles with trimethoxysilane or triethoxysilane. The coarse silanized magnetic particles prepared by the organic silanization procedure were suitable for coupling anti-$T_3$ and anti-$T_4$ for use in total $T_3$ and total $T_4$ RIA.

The maximum binding ($B_{40}$) in TSH IRMA using magnetic particles prepared by an improved aqueous silanization has reached 28.6% and increased by about 15% over that by the organic one and the zero standard binding ($B_0$) was a little lower.

Silanized magnetic particles can be modified by means of a bifunctional reagent to substitute carboxyl or aldehyde groups for amino ones. A comparison of four types of silanized magnetic particles in TSH IRMA was made and given in Table Ⅳ.

TABLE Ⅳ. COMPARISON OF DIFFERENT SILANIZED MAGNETIC PARTICLES IN TSH IRMA

| Particles | Amino particles | | | | Carboxyl particles | | | |
|---|---|---|---|---|---|---|---|---|
| Size | Coarse | | Fine | | Coarse | | Fine | |
| TSH mIU/L | CPM | B/T% | CPM | B/T% | CPM | B/T% | CPM | B/T% |
| 0 | 187 | 0.10 | 423 | 0.20 | 118 | 0.06 | 128 | 0.06 |
| 0.3 | 539 | 0.25 | 967 | 0.46 | 323 | 0.15 | 425 | 0.20 |
| 0.8 | 1639 | 0.77 | 2222 | 1.10 | 1194 | 0.57 | 1642 | 0.78 |
| 3.5 | 5556 | 2.62 | 6906 | 3.30 | 3843 | 1.82 | 4170 | 1.98 |
| 10 | 13210 | 6.32 | 17095 | 8.10 | 9520 | 4.51 | 11971 | 5.67 |
| 20 | 25149 | 11.9 | 30315 | 14.4 | 18195 | 8.62 | 22532 | 10.7 |
| 40 | 53180 | 25.1 | 66618 | 31.7 | 38339 | 18.2 | 41748 | 19.8 |

These results illustrated that the fine particles exhibited greater maximum binding ($B_{40}$) than that by the coarse ones due to the small particles providing high antibody binding capacity and amino particles were better than carboxyl ones for use in TSH IRMA.

*Choice of coupling conditions*:

Choice of coupling time: Routine couplings of $T_3$, $T_4$ and TSH antibodies to magnetic particles using carbodiimide method were performed at various time intervals up to 24 hours. The antibody concentration in the supernatant and the magnetic antibodies obtained were checked. The results indicated that maximum coupling of anti-$T_3$, anti-$T_4$ and anti-TSH have been achieved after about 6 hours. A overnight coupling procedure was subsequently adopted for convenience.

The coupling yield was 60-80% for coupling $T_3$, $T_4$ antibodies, 40-60% for TSH antibody.

Choice of coupling media and pH: Five coupling media, MES, PB, HEPES, NaAc-HAc(AB) buffers and distilled water, with different value of pH were used for coupling TSH antibody for use in TSH IRMA. The best effect was obtained by use of pH 5.6 of 0.05mol/L MES buffer and the similar coupling effects were found by pH 6.4, 7.2 of 0.05mol/L MES. Coupling TSH antibody using pH 5.6, 6.4 and 7.2 of 0.1mol/L PB also gave better results.

The effect of buffer pH on the couplings was also investigated when anti-TSH was immobilized on carboxyl particles by carbodiimide metbod. The best result in TSH IRMA was observed by pH 4.6 of PB. The removal of EDAC in supernatant after 2 hours of coupling reaction was adopted to avoid the possible aggregation of the particles. A pH 4.6 of PB also presented the best result by the new approach.

Coupling $T_3$, $T_4$ antibodies to magnetic particles using carbodiimide technique was carried out by pH 5.6, 6.0, 6.4 of PB and distilled water. All the results obtained by six coupling media were similar.

Choice of coupling ratio of antibody and particles: coupling TSH antibody to magnetic particles has been found to be sensitive to antibody amount used. The maximum binding ($B_{40}$) in TSH IRMA increased with the ratio of antibody and magnetic particles. Coupling ratio adopted 10ml purified TSH antibody: 1g particles.

Choice of amount of carbodiimide: Four coupling trials of TSH antibody were conducted using different amount of EDAC. It can be seen from the results that there are no obvious differences in the range between 5mg and 20mg EDAC for 100mg particles. The data also indicated that the addition of excess EDAC, more than 20mg, did not brings any further benefit and leads to not only the decrease of maximum binding but also the possible aggregation of particles.

Effect of purification of antiserum for coupling: Anti-$T_3$, anti-$T_4$ and anti-TSH were raised in sheep. A $T_3$ antibody purified by a ammonium sulfate precipitation and a plain $T_3$ antiserum were coupled to magnetic particles using the same coupling method and condition, respectively. The similar results were obtained in total $T_3$ RIA. It is clear that there is no need to purify the antisera prior the coupling $T_3$ or $T_4$ antibody.

Aggregation could be formed during coupling when a plain TSH antiserum was immobilized on magnetic particles. A TSH antibody purified by a caprylic acid precipitation method is recommended for the preparation of magnetic particle TSH antibody.

*Comparison of different types of magnetic particles for coupling anti-TSH*:

Five types of magnetic particles, amino and carboxyl silanized particles, polyacrylamide particles, polyacrolein particles and Latex-M particles were compared in TSH IRMA. A IAE polyclonal anti-TSH was immobilized on the five types of particles using suggested protocols. A commercial OMB monoclonal anti-TSH was labelled with $^{125}I$ using iodogen method as tracer. The results summarized in Table V demenstrated that all five types of magnetic particles tested were found to be suitable. Silanized magnetic particles and commercial Latex-M particles showed comparable results.

TABLE V. COMPARISON OF FIVE TYPES OF MAGNETIC PARTICLES IN TSH IRMA
Immobilized anti-TSH: polyclonal anti-TSH, IAE China
Labelled anti-TSH: monoclonal anti-TSH, OMB Finland

| Particles | Amino particles | | Carboxyl particles | | Polyacrylamide particles | | Latex-M particles | | Polyacrolein particles | |
|---|---|---|---|---|---|---|---|---|---|---|
| TSH mIU/L | CPM | B/T% | CPM | B/T% | CPM | B/T% | CPM | B/T% | CPM | B/T% |
| 0 | 220 | 0.10 | 128 | 0.06 | 108 | 0.06 | 140 | 0.06 | 302 | 0.15 |
| 0.3 | 565 | 0.27 | 425 | 0.20 | 320 | 0.17 | 459 | 0.20 | 504 | 0.25 |
| 0.8 | 1968 | 0.98 | 1642 | 0.78 | 1350 | 0.71 | 2073 | 0.96 | | |
| 3.5 | 6041 | 2.85 | 4170 | 1.98 | 4429 | 2.33 | 6789 | 2.96 | | |
| 10 | 14654 | 6.91 | 11971 | 5.67 | 10760 | 5.67 | 15899 | 6.93 | | |
| 20 | 27591 | 13.0 | 22532 | 10.7 | 15791 | 8.32 | 32598 | 14.2 | | |
| 40 | 59998 | 28.3 | 41748 | 19.8 | 39647 | 20.9 | 59634 | 26.0 | 34587 | 17.0 |

The comparison of six types of magnetic particles for coupling IAE polyclonal anti-TSH for use in blood spot TSH IRMA as shown in Table VI was included to emphasize the fact that silanized magnetic particles ofter gave rise to the higher zero standard binding ($B_0$). The best results, lower ($B_0$) and higher ($B_{160}$), were achieved by the Latex-M. Polyacrylamide and polystyrene magnetic particles presented lower maximum binding ($B_{160}$). The introduction of a spacer-arm to the surface of the particles seems helpful to reduce the $B_0$.

TABLE VI. COMPARISON OF DIFFERENT MAGNETIC PARTICLES IN BLOOD SPOT TSH IRMA
Immobilized anti-TSH: polyclonal anti-TSH, IAE China
Labelled anti-TSH: monoclonal anti-TSH, OMB Finland

| Particles | Silanized particles(c)* | Silanized particles(f)* | Silanized particles(m)* | Polyacrylamide particles | Polystyrene particles | Latex-M particles |
|---|---|---|---|---|---|---|
| TSH mIU/L | CPM | CPM | CPM | CPM | CPM | CPM |
| 0 | 489 | 658 | 281 | 281 | 287 | 281 |
| 5 | 868 | 1010 | 577 | 539 | 579 | 596 |
| 10 | 1139 | 1349 | 805 | 738 | 780 | 806 |
| 20 | 1570 | 1895 | 1155 | 1179 | 1387 | 1505 |
| 40 | 2769 | 3159 | 1836 | 1975 | 2315 | 2488 |
| 80 | 3778 | 4455 | 3237 | 3667 | 3721 | 3959 |
| 160 | 6645 | 8467 | 7325 | 6919 | 7988 | 8669 |

* (c) Coarse particles * (f) Fine particles * (m) Spacer-arm modified particles

Higher $B_0$ in this assay not only results from physical and chemical properties of the surface of the particles, but also depends on antibody system used for labelling and immobilization and assay protocol adopted. Some preliminary experiments indicated that the use of a new assay buffer and a assay-protocol with

three times of wash can help to limit the $B_0$ in blood spot TSH IRMA. Further work is clearly required fully to resolve this potential difficulty.

*Comparison of different sources of anti-TSH for coupling*:

With a view to testing different TSH antibodies used in TSH IRMA, two polyclonals (IAE China and BMS Australia) and three monoclonals (IAE China and NIH Thailand) were immobilized on silanized magnetic particles using EDAC coupling protocol under the same condition, respectively. A radioiodinated OMB monoclonal anti-TSH was used as tracer. The results as seen in Table Ⅵ showed that two polyclonal TSH antibodies and OMB monoclonal TSH antibody formed good sandwich partners, respectively. In the meantime, Thai Ⅰ, Thai Ⅱ monoclonal anti-TSH and OMB monoclonal anti-TSH also matched well and gave a high signal and were sensitive to low levels of TSH.

TABLE Ⅵ. COMPARISON OF DIFFERENT SOURCES OF ANTI-TSH FOR COUPLING

Solid phase: silanized magnetic particles, IAE China

Labelled anti-TSH: monoclonal anti-TSH, OMB Finland

| Antibody | IAE pAb | | BMS pAb | | IAE mAb | | Thai Ⅰ mAb | | Thai Ⅱ mAb* | |
|---|---|---|---|---|---|---|---|---|---|---|
| TSH mIU/L | CPM | B/T% | CPM | B/T% | CPM | B/T% | CPM | B/T% | CPM | B/T% |
| 0 | 477 | 0.24 | 471 | 0.24 | 579 | 0.33 | 481 | 0.28 | 305 | 0.18 |
| 0.3 | 1114 | 0.58 | 1074 | 0.55 | 1180 | 0.67 | 1194 | 0.68 | 978 | 0.57 |
| 0.8 | 1942 | 1.00 | 1892 | 0.98 | 2027 | 1.16 | 2171 | 1.25 | 1770 | 1.02 |
| 3.5 | 5866 | 3.03 | 5611 | 2.90 | 3483 | 2.00 | 5087 | 2.92 | 3949 | 2.27 |
| 10 | 12650 | 6.54 | 11734 | 6.06 | 6782 | 3.90 | 10575 | 6.08 | 8779 | 5.05 |
| 20 | 25876 | 13.4 | 23422 | 12.1 | 12002 | 6.90 | 20063 | 11.5 | 16036 | 9.22 |
| 40 | 54777 | 28.3 | 48194 | 24.9 | 30592 | 17.6 | 40376 | 23.2 | 32610 | 18.8 |

* a has been stored for 1 year

*Comparison of different sources of monoclonal anti-TSH for labelling*:

Matching four types of monoclonal anti-TSH, OMB anti-TSH, IAE anti-TSH, and Thai Ⅰ, Thai Ⅱ anti-TSH, which were radioiodinated with $^{125}I$ as tracer, with a IAE polyclonal anti-TSH immobilized on silanized particles was carried out in TSH IRMA. It was found that the OMB monoclonal anti-TSH was suitable for labelling and gave the best response in these selected systems.

## Conclusion

The improved methods for the preparation of silanized magnetic particles with different properties have been developed. The preparation is simple, inexpensive and reproducible. Magnetic particle antibodies prepared by carbodiimide technique under the optimal conditions have been used in total $T_3$, total $T_4$, blood spot total $T_4$ RIA and TSH IRMA and exhibited good efficiency, reproducibility and stability. Coarse particles made by an organic silanization are suitable for coupling $T_3$, $T_4$ antibodies. Fine particles prepared by an aqueous silanization gave comparable results with a commercial particles Latex-M in TSH IRMA. Some of technical problems of magnetic antibodies for use in free $T_3$, free $T_4$ RIA and blood spot TSH IRMA remained to be solved further. Matching of antibodies demonstrated that Thai Ⅰ, Thai Ⅱ monoclonal anti-TSH and a IAE polyclonal anti-TSH immobilized on silanized magnetic particles all paired well with a commercial monoclonal anti-TSH for label. They have been recommended for use in TSH IRMA.

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