Influence of Human Anti-Mouse Antibodies on Thyrotropin In-Vitro Analysis: A Comparison of 6 Thyrotropin IRMA Kits

Peter Mikosch, Hans Jürgen Gallowitsch, Ewald Kresnik, Mario Molnar, Iris Gomez and Peter Lind

Department of Nuclear Medicine and Special Endocrinology, LKH Klagenfurt, Klagenfurt, Austria

Summary: Objective: The aim of the study was to evaluate the influence of human anti-mouse antibodies on the measurement of thyrotropin.

Investigations: Samples from 11 patients with measureable human anti-mouse antibody titres (19 µg/l-3880 µg/l) after radioimmuno-scintigraphy were analysed with 6 different thyrotropin immuno-radiometric assay kits (IRMA). Each sample was analysed in the routine way (sample influenced by human anti-mouse antibodies), as well as after incubation with murine immunoglobulin to precipitate human anti-mouse antibodies (samples not influenced by human anti-mouse antibodies).

Results: Two kits showed clear deviations of measured thyrotropin levels when the human anti-mouse antibody titres were higher than 1350 µg/l. A third kit was influenced to a lesser extent by human anti-mouse antibodies. Three of the 6 investigated thyrotropin IRMA kits produced thyrotropin values that were unaffected by the presence of elevated human anti-mouse antibodies. In comparison with former studies after immunotherapy, the thyrotropin deviations were marginal. However, differences were found between the commercially available thyrotropin assays. According to the results of this study only three out of the six investigated kits were unaffected by human anti-mouse antibodies.

Conclusion: Since thyrotropin is one of the key quantities for the endocrinologist dealing with the thyroid gland, every laboratory should ensure high quality thyrotropin assays by critically analysing their method for human anti-mouse antibody.

Introduction

Immunological in-vitro methods, such as immuno-radiometric assays (IRMA), are based on antigen-antibody interactions. Murine monoclonal antibodies in the test kit can thus interact sensitively and specifically with the antigen of interest. Very small amounts of analytes can be determined. However, certain serum components may disturb the specific antigen-antibody interaction of these test-kits, leading to false results (1).

Immuno-radiometric assays for measuring thyrotropin (thyroid stimulating hormone) usually employ murine monoclonal antibodies. These antibodies are also increasingly used for therapy (e.g. therapy of tumours, immunological disorders and reduction of host versus graft reactions) and diagnostics (e.g. immuno-scintigraphy) (2). Even after a single contact with intact murine monoclonal antibodies used for immunoscintigraphy, patients may react by producing human anti-mouse antibodies (3-5). Repeated exposure to murine monoclonal antibodies leads in most cases to a booster effect (3, 5, 6). Other investigations have shown that human anti-mouse antibodies may persist for up to 160 days after the first contact with the murine antibodies (3).

The in-vitro assay is then increasingly disturbed, and the degree of interference is correlated with the extent of human anti-mouse antibody production. Human anti-mouse antibodies interfere with the antigen-antibody reaction of thyrotropin (substance of interest) with the free and radioactively marked antibody (tracer). The results are complex, tree-like antigen-antibody reactions, which lead to an increased binding of the free, radioactively labelled tracer to the solid phase (3). Binding of increased amounts of free radioactive tracer to the solid phase results in falsely high measurements of thyrotropin levels (1-3).

The manufacturers of immuno-radiometric assays have reacted to this phenomenon in the last few years by adding unspecific mouse immunoglobulin to the thyrotropin immuno-radiometric assays. By binding human anti-mouse antibodies, these non-specific mouse immuno-
globulins should exclude or, at least, minimize the interference (1).

The aim of this study was to compare the effects of human anti-mouse antibodies in different thyrotropin immuno-radiometric assays kits. Samples from 11 patients with measurable human anti-mouse antibody titres were investigated by comparing the results of six different thyrotropin immuno-radiometric assays.

**Patients and Methods**

The laboratory measurements were performed by the In-Vitro Laboratory of the Department of Nuclear Medicine and Special Endocrinology, LKH Klagenfurt, Klagenfurt, Austria.

During the period of 24 months 380 patients underwent immunoscintigraphy based on tracers with murine antigens. Sixty-eight of these patients subsequently showed a positive human anti-mouse antibody response. These patients were asked for an additional serum blood sample for the current evaluation of human anti-mouse antibody titre. Human anti-mouse antibodies were measured with a quantitative enzyme-immunoassay (HAMA-ELISA medac, Medac Diagnostica, Hamburg, Germany).

Eleven samples still showed measurable human anti-mouse antibody titres. The samples from these 11 patients (2 male, 9 female; age 29/79 a) were then analysed with 6 different thyrotropin immuno-radiometric assay kits (A: DYNOtest TSH, B. R. A. H. M. S Diagnostica GmbH; B: RIA-gnost hTSH, CIS bio international; C: TSH-IRMA magnum, Medipan Diagnostica; D: TSH-CTK-2, Sorin Biomedica; E: SimulTRAC FT4/TSH, Becton Dickinson; F: TSH IRMA, Immunotech). All of these kits were based on murine antibodies. To determine the effect of human anti-mouse antibody on each kit, all samples were analysed in duplicate in the routine way (results influenced by human anti-mouse antigen), and for a second time after 10 mg/l of murine immunoglobulin G had been added to the samples (result not influenced by human anti-mouse antigen).

**Results**

The concentrations of human anti-mouse antigen in the 11 samples ranged from a just detectable 19 μg/l to clearly elevated values of 3880 μg/l (mean value 720.5 μg/l; median 274 μg/l). Differences greater than 0.3 mU/l between thyrotropin results obtained in the presence and absence of human anti-mouse antigen are referred to be low.

Kit A showed thyrotropin deviations with the two highest human anti-mouse antigen levels (0.86 mU/l thyrotropin for human anti-mouse antibodies 1351 μg/l and 1.43 mU/l thyrotropin for human anti-mouse antibodies 3880 μg/l). The results for kit B were almost identical to those of kit A (0.89 mU/l thyrotropin for human anti-mouse antibodies 1351 μg/l and 1.53 mU/l thyrotropin for human anti-mouse antibodies 3880 μg/l). In addition, kit B also showed thyrotropin deviations of 0.32-0.42 mU/l for low levels of human anti-mouse antigen (human anti-mouse antibodies 75 μg/l, 274 μg/l and 312 μg/l). The results of kit C were better than those of kit A and kit B, but still showed thyrotropin deviations for a low human anti-mouse antigen level (0.46 mU/l thyrotropin for 75 μg/l human anti-mouse antibodies) and for the two highest levels (0.36 mU/l thyrotropin for 1351 μg/l human anti-mouse antibodies and 0.43 mU/l thyrotropin for 3880 μg/l human anti-mouse antibodies). Kits D, E and F did not show any thyrotropin elevations attributable to human anti-mouse antigens (fig. 1).

Five of the six investigated thyrotropin immuno-radiometric assay kits also showed decreased thyrotropin levels in the presence of human anti-mouse antigen (kit C none, kit A and B one, kit F two, kit D four and kit E six). These decreases ranged from 0.01 mU/l to 1.12 mU/l. A correlation with the human anti-mouse antigen titres could not be found.

In addition, thyroglobulin antibody, thyroid peroxidase antibody and thyroid receptor antibody were also investigated. In the presence of 75 μg/l human anti-mouse antibodies, thyrotropin antibody was slightly elevated (147 U/l) in only one case. All other samples showed negative results for all these antibodies.

**Discussion**

The determination of thyrotropin is one of the most important steps of the thyroid gland investigation in daily routine. This quantity is an indicator of the current thyroid function. Falsely elevated thyrotropin results caused by human anti-mouse antibodies could thus lead to incorrect diagnostic and therapeutic consequences (e. g. euthyroid patients incorrectly diagnosed as hypothyroid and therefore given L-thyroxine treatment). Exact thyrotropin measurements are required especially for patients with thyroid cancer, as these patients need full suppression of thyrotropin by a L-thyroxine drug regimen after surgery and radio-iodine ablation.

Exposure to monoclonal murine antibodies by immuno-scintigraphy, especially after repeated exposure, has been shown to cause clear elevations of human anti-mouse antibodies in response to the applied murine antigen (3,
5, 6). This leads to elevations of thyrotropin measurements of up to 9.5 mU/L, and the elevation is correlated with the human anti-mouse antigen titre (3). Comparable results have also been reported for other quantities such as CA 125 (7). These false results for in-vitro measurements caused by human anti-mouse antibodies creates a need for "human anti-mouse antibody safe assays" (7). In recent years the manufacturers of thyrotropin kits have responded to this problem and introduced assays that are supposedly "human anti-mouse antibody safe".

In comparison with another study (3) performed before the introduction of assays containing supplements to make them human anti-mouse safe, our present study did not show such high deviations in thyrotropin measurement. Our results therefore seem to confirm that the manufacturers were successful in their attempt to make the thyrotropin assays "human anti-mouse antigen safe" by adding unspecific murine immunoglobulin. However, there were still some differences between the investigated assays. Kits A and B and to a lesser degree kit C showed increasing deviations of thyrotropin measurements in correlation with concentrations of human anti-mouse antibodies greater than 1350 µg/L. Therefore the "human anti-mouse antibody safety" of these test-assays is very doubtful. In contrast, kits D, E and F showed no deviations of thyrotropin results in the presence of human anti-mouse antibodies. For the small number of 11 investigated samples, these three test-assays seem to achieve "human anti-mouse antibody safety". A possible explanation could be differences between the subclasses of murine immunoglobulin G responsible for sensitization of the patients and these used in the investigated assays. Another explanation would be that the amount of added murine immunoglobulin G differs in the test assays. Above a certain concentration of human anti-mouse antibodies some kits would then lose their capacity for inhibiting human anti-mouse interactions.

Consequences of high human anti-mouse antibody titres are diverse. The distribution of immunotherapeutic agents and tracers for immuno-scintigraphy is changed by the formation of antigen-antibody complexes, so that the immunotherapeutic agent or tracer cannot bind to the target cells. This results in a high, abnormal and unspecific uptake by the liver and spleen (5, 6). In-vitro measurements of thyrotropin can also be influenced by human anti-mouse antibodies. This study seems to confirm that the investigated thyrotropin immuno-radiometric assays show improved or good human anti-mouse safety in comparison with former assays (3). However, the question remains as to how far small deviations of thyrotropin measurements may still play a role. A precise thyrotropin measurement is especially required for patients with thyroid disease during the follow-up period. These patients need an amount of L-thyroxine that fully suppresses thyrotropin excretion from the pituitary gland. If human anti-mouse antibodies falsely increased the in-vitro measurements of thyrotropin, the prescribed amount of L-thyroxine would be incorrectly increased.

Other reasons for an inappropriate reaction to L-thyroxine, such as no-response to therapy, could be a lack of the patient's compliance; for example the taking of L-thyroxine with a meal which would lead to decreased bio-availability or intestinal malabsorption of the drug. Thus, in such cases an evaluation of human anti-mouse antibodies should be performed to exclude or confirm human anti-mouse antibodies as the source of error. As a matter of quality control and improvement, laboratories using thyrotropin assays should test these for their human anti-mouse safety. This would minimize the occurrence of false in-vitro thyrotropin measurements caused by human anti-mouse antibodies, and thereby decrease the risk of false diagnosis and its therapeutic consequences.

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TECHNICAL NOTE

The First Fully Automated Allergy Analyser UniCAP: Comparison with IMMULITE for Allergy Panel Testing

Guido M. P. J. Costongs and Bert M. Bas

Department of Clinical Chemistry, Maaslandziekenhuis, Sittard, The Netherlands

Summary: Automated immunoassay systems should be convenient to handle, flexible and give reliable results. To investigate the extent to which the UniCAP System met the above requirements, compared with the IMMULITE System, we compared the PhadiaTop (UniCAP) and AlaTOP (IMMULITE) results of 110 patients with positive clinical diagnoses for inhalant allergy. In addition, we compared food screening test results of 103 patients with a clinical positive diagnosis for food, and 110 test results of controls with negative diagnosis for allergy.

Phadiatop had a sensitivity of 96% and a specificity of 92%. AlaTOP had a sensitivity of 86% and a specificity of 94%. For food screening the results were: 75% sensitivity and 82% specificity for fx5 (UniCAP) and 63% sensitivity and 71% specificity for fp5 (IMMULITE).

Furthermore, those samples for which the test results which were not in concordance with the clinical diagnosis were tested with the follow-up panel of the different screening tests. For the AlaTOP follow-up we had to use the DPC microplate System (Milenia), because single allergen testing is not yet possible on the IMMULITE System.

With regard to sensitivity, the UniCAP specific inhalant allergen tests and the original Phadiatop results showed closer agreement with each other than did the Milenia specific allergen results with the AlaTOP. The specificity of the single inhalant allergen tests was the same for both systems.

For food allergy testing the UniCAP System shows closer agreement between the screening and the follow-up results than does the IMMULITE.

The hands on time for loading 44 samples was practically the same for both systems, but for the follow-up tests the Milenia System is used next to the IMMULITE. Therefore from a logistical point of view the UniCAP System is more convenient.

From these results we conclude that both logistically and clinically UniCAP seems to meet our requirements better than the IMMULITE.

Introduction

Last year was the introduction of the first fully automated allergy analyser, the UniCAP (Pharmacia & Upjohn), as a follow up of the Pharmacia Cap System. With this new random access analyser it is possible to perform the allergy screening tests Phadiatop® (inhalation) and fx51 (foodpanel) as well as specific allergy tests (1,2,6).

Fully automated inhalation- and food allergy screening can be performed on the IMMULITE System (DPC) (1) as well as on the UniCAP. The AlaTOP test is used for inhalation screening on IMMULITE. Last year the food allergy panel, fp5, was introduced. Table 1 shows the separate allergens screened for by the Phadiatop, AlaTOP, fx5 and fp5 tests.

Our laboratory is equipped with an IMMULITE System, as well as the Pharmacia Cap System (2). Now that the new UniCAP System has been introduced by Pharmacia, it is possible every day to perform screening tests for inhalation and food allergy as well as the follow-up tests for specific allergies. We therefore compared the handling time, sensitivity and specificity of the UniCAP and IMMULITE systems for inhalant and food allergy screening. For this comparative study 213 consecutive patients with symptoms of allergy were clinically investigated for allergy. Serum samples were taken from each patient and from 110 healthy controls with

1) List of allergens mentioned in this article:

- fx5: food mix (Pharmacia & Upjohn, Sweden) containing egg white, milk, fish, wheat, peanut, soya bean
- fp5: food mix (DPC, USA) containing egg white, milk, fish, wheat, peanut, soya bean
- gx3: Grass pollen mix, as indicated in parentheses
- g1: Anthoxanthum odoratum
- g2: Cynodon dactylon
- g5: Lolium perenne
- g6: Phleum pratense
- g12: Secale cereale
- g13: Holcus lanatus
- d1: Dermatophagoides pteronyssinus
- e1: Cat dander
- e3: Horse dander
- e5: Dog dander
- tx9: Free pollen mix, as indicated in parentheses
- t1: Acer negundo
- t3: Betula verrucosa
- t4: Corylus avellana
- t7: Quercus alba
- t12: Salix caprea
- t17: Cryptomeria japonica
- wx3: weed pollen mix, as indicated in parentheses
- w1: Ambrosia elatior
- w9: Plantago lanceolata
- w19: Parietaria officinalis
- w20: Urtica dioica
- mx1: Moulds and yeast mix, as indicated in parentheses
- m1: Penicillium notatum
- m2: Cladosporium herbarum
- m3: Aspergillus fumigatus
- m6: Alternaria alternata
- f1: Egg white
- f2: Milk
- f3: Fish (cod), Gadus morhua
- f4: Wheat, Triticum aestivum
- f13: Peanut, Arachis hypogaea
- f14: Soya bean, Glycine max
no history of allergy, to assess clinical sensitivity and specificity for AlaTOP, Phadiatop, fx5 and fp5.

Any sample whose test results differed from the clinical diagnosis was further analysed using the relevant screening test shown in table 1. The samples showing discrepancies between AlaTOP results and clinical diagnosis were further analysed for relevant specific allergens on the Milenia System (DPC), because it is not possible to perform specific allergy tests on the IMMULITE.

Patients and Methods

Patients

The following alternative criteria were used for positive diagnosis of atopy; SPT\(^2\) of 3+ (equal to wheal of histamine control, 10 g/l), SPT 2+ in combination with positive case history for the same allergen, SPT 1+ or 2+ in combination with positive RAST for the same allergen or a positive provocation test. The criteria for a negative diagnosis of atopy was; SPT negative, SPT 1+ in combination with negative case history or negative RAST or negative provocation test. Patients not fulfilling criteria for positive or negative atopy diagnosis were considered as inconclusive. Furthermore 110 healthy individuals with no history of atopy and negative diagnosis for allergy according to the criteria mentioned earlier were used as controls.

The following patient results and demographics were found:

A. 110 patients had a positive diagnosis for inhalant allergy

Age: 1—75 year, average 32 years
Sex: 68 males, 42 females
Diagnosis: Bronchial asthma, seasonal rhinitis and perennial rhinitis

B. 103 patients had a positive diagnosis for food allergy

Age: 1—70 year, average 32 years
Sex: 48 females, 55 males
Diagnosis: Atopic dermatitis, urticaria, diarrhoea

C. 110 Controls with negative diagnosis for allergy

Age: 1—75 year, average 26 years
Sex: 56 females, 54 males
Diagnosis: Phadiatop®

Methods

The UniCAP System is a fluorescent enzyme immunoassay. The allergens, covalently coupled to the ImmunoCAP, react with the specific IgE in the patient serum. Enzyme-labelled anti-IgE antibodies are added to form a complex, which is incubated with a development agent. When the reaction is finished the fluorescence is measured.

IMMULITE is a chemiluminescent enzyme immunoassay, based on the same principle as the Microplate System but utilising an alkaline phosphate-labelled anti-human IgE which reacts with a chemiluminescent substrate. Antiligand coated polystyrene beads are used to capture the ligand labelled allergens.

The DPC Microplate System is an enzyme immunometric assay based on liquid ligand-labelled allergens and separation in ligand-coated wells. The specific IgE in the patient sample forms an allergen-IgE complex which is incubated with a multivalent anti-ligand, which in turn links the allergen-IgE complexes to the ligand coated wells. Horseradish peroxidase-labelled monoclonal anti-IgE antibodies are added to the allergen IgE complex, followed by an indicator. The rate of increase in absorbance is measured.

Results

Inhalant allergy

The results for Phadiatop® (UniCAP) and AlaTOP (IMMULITE) are given in table 2.

From the 220 tested samples, 13 samples were not similar on either of the two systems:

- 11 samples were positive on the UniCAP System but negative on the IMMULITE;
- 2 samples were positive on the IMMULITE System but negative on the UniCAP;
- 12 samples gave similar results on both systems but were not in concordance with the clinical diagnosis.

From these results a calculation of the sensitivity and specificity can be made (tab. 3).

The 23 AlaTOP samples and 14 Phadiatop samples with discrepancies between test results and clinical findings were further analysed with the single inhalant allergens present in the screening tests (tab. 1). For the AlaTOP samples this was done on the Milenia System (tab. 4). For the Phadiatop samples this was done on the UniCAP; 12 samples gave similar results on both systems but were not in concordance with the clinical diagnosis. The specific allergen tests were performed on the UniCAP System (tab. 4).

Food allergy

The 103 samples from the patients with food allergy underwent the same procedure as those with inhalant allergy, but this time with fx5 and fp5. The results for screening on food allergy are presented in table 5.

Of the 213 tested samples, 24 were differently classified by UniCAP and IMMULITE. Furthermore, 43 of the samples with the same classification in both systems were not in concordance with the clinical diagnosis. Sensitivity and specificity are shown in table 6.

Tab. 1 Inhalation screening tests Phadiatop® (* summary of most important allergens); AlaTOP and food panels fx5, fp5

<table>
<thead>
<tr>
<th>Phadiatop®</th>
<th>AlaTOP</th>
<th>fx5</th>
<th>fp5</th>
</tr>
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<tbody>
<tr>
<td>gx3 (g1, g5, g6, g12, g13)</td>
<td>g6, g2</td>
<td>f1</td>
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<tr>
<td>d1</td>
<td>d1</td>
<td>f2</td>
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<td>e5</td>
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<td>f13</td>
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</tr>
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<td>b, t17</td>
<td>f14</td>
<td>f14</td>
</tr>
<tr>
<td>wx3 (w6, w9, w10, w12, w20)</td>
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<tr>
<td>mx1 (m1, m2, m3, m6)</td>
<td>m1, m6</td>
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</tr>
</tbody>
</table>

Technical note

2) Abbreviations:

SPT = skin prick test
PCDBFC = placebo controlled double-blind food challenge
S-IgE = serum immunoglobulin E
Further specific allergy testing for the samples that were not in concordance with the clinical diagnosis gave the results shown in table 7.

### Tab. 4 Milenia and UniCAP results

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>AlaTOP neg</th>
<th>AlaTOP pos</th>
<th>Phadiatop&lt;sup&gt;®&lt;/sup&gt; neg</th>
<th>Phadiatop&lt;sup&gt;®&lt;/sup&gt; pos</th>
<th>UniCAP positive</th>
<th>UniCAP negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atopic</td>
<td>16</td>
<td>4</td>
<td>20</td>
<td>7</td>
<td>56</td>
<td>44</td>
</tr>
<tr>
<td>Non-atopic</td>
<td>7</td>
<td>0</td>
<td>5</td>
<td>2</td>
<td>7</td>
<td>9</td>
</tr>
</tbody>
</table>

### Tab. 5 Results of food allergy screening with fx<sub>5</sub> and fp<sub>5</sub><sup>2)</sup>

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Number</th>
<th>fx&lt;sub&gt;5&lt;/sub&gt;</th>
<th>fp&lt;sub&gt;5&lt;/sub&gt;</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>pos.</td>
<td>neg.</td>
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<tr>
<td>Atopic</td>
<td>103</td>
<td>77</td>
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<tr>
<td>Non-atopic</td>
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<td>20</td>
<td>90</td>
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### Tab. 6 Sensitivity and specificity of food allergy screening

<table>
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<tbody>
<tr>
<td>Sensitivity (%)</td>
<td>75</td>
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<tr>
<td>Specificity (%)</td>
<td>82</td>
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### Tab. 7 Results of specific food allergy testing

<table>
<thead>
<tr>
<th>Diagnosis</th>
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<th>fp&lt;sub&gt;5&lt;/sub&gt; pos</th>
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<td>Atopic</td>
<td>16</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>2</td>
</tr>
<tr>
<td>Food</td>
<td>35</td>
<td>16</td>
</tr>
<tr>
<td>Non-atopic</td>
<td>fp&lt;sub&gt;5&lt;/sub&gt; pos</td>
<td></td>
</tr>
<tr>
<td>Food</td>
<td>32</td>
<td>12</td>
</tr>
</tbody>
</table>

Discussion

A differential diagnostic test for atopy is often the first test to be performed on a patient, followed by allergen-specific testing in the cases considered atopic, i.e. in those cases with positive Phadiatop/AlaTOP, fx<sub>5</sub>/fp<sub>5</sub>. It is obvious that high sensitivity is essential in this situation. For Phadiatop and AlaTOP the findings are in concordance with earlier results (1, 2). The sensitivity for fx<sub>5</sub> did not agree with earlier results; literature values range from 55 to 89% (3,4,5). No literature values were found for the sensitivity and specificity for fp<sub>5</sub>

It is known that positive clinical diagnosis is more difficult to define for food allergy than for inhalant allergy. The only method which is clinically used as a real standard is a PCDBFC<sup>2</sup>. In this investigation we only used SPT, but clinical symptoms and history were very clear. According to our results fx<sub>5</sub> was slightly better than fp<sub>5</sub> in meeting the clinical sensitivity. The follow-up for the discrepancies between clinical diagnosis and inhalant screening gave better results for the UniCAP System than for the Milenia System. With regard to specificity, the UniCAP S-IgE<sup>2)</sup> tests and the original Phadiatop results showed closer agreement with each other than did the Milenia System with the AlaTOP, i.e. 5 atopic patients were tested negative with Phadiatop and remained negative for the S-IgE, whereas 4 out of the 16 atopic patients which were negative for AlaTOP were tested positive on the Milenia System. There seems to be no difference between the specificities of the S-IgE tests and the inhalant screening tests.

Discrepancies between screening tests and S-IgE follow up tests are always difficult to explain. For AlaTOP it is clear that for the S-IgE tests a different method on a different instrument is used, which probably explains part of these discrepancies. On the other hand one should expect that both instruments measure the same amount of IgE. Furthermore it is known that sometimes when an allergy screening test is just above the cut-off level, i.e. just positive, the S-IgE follow-up tests may be just below their cut off point, so that there is a slightly additive effect of the total of allergens on the different screening tests. On this basis, all 7 negative S-IgE out of the 9 positive Phadiatop could be explained. Of the 7 positive AlaTOP, however, of which all 7 were negative in their S-IgE follow-up tests, only 2 could be explained. For the food allergens this explanation was valid for 3 out of the 6 negative S-IgE tests of fx<sub>5</sub>, while for fp<sub>5</sub> only 6 of the 20 negative S-IgE tests were explained.

Although the diagnosis of food allergy is very complicated, not only on the test level but very much so on the clinical level, our results from the UniCAP System seem to be in closer agreement with the clinical findings than are the results from the IMMULITE/Milenia System. We could find no explanation for the negative food screening tests and the positive S-IgE follow-up tests.

### Hands on Time

We also investigated the hands on time of both systems. For the UniCAP the hands on time for running 44 samples, which are barcode labelled and with an on-line connection with the laboratory computer, was 12 minutes.

The same number of samples on the IMMULITE System required 15 minutes hands on time. But as mentioned earlier, only the screening tests AlaTOP and fp<sub>5</sub> can be performed on the IMMULITE. Specific allergy tests must be performed on a Milenia System. The option of screening on IMMULITE and performing specific allergy testing on UniCAP is not favourable because of the low sensitivity of the IMMULITE System and the number of discrepancies between UniCAP and IMMULITE, whereas the UniCAP seems to be more in concordance with the clinical diagnosis.

Although the UniCAP seems to be an analyser which is more suitable for small to medium sized laboratories, capacity can be greatly
increased by connecting up to 5 UniCAPs. However, there is a corresponding increase in the hands on time. A disadvantage of the IMMULITE System is that at present it is not possible to perform specific allergy testing on this instrument.

Conclusions
From these results we conclude that from both a logistical and a clinical point of view UniCAP seems to meet our requirements better than the IMMULITE.

References

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Corresponding author: Dr. med. Guido M. P. J. Costongs, Maaslandziekenhuis, Department of Clinical Chemistry, Postbus 5500, NL- 6130 MB Sittard, The Netherlands
Reference Intervals for Serum Thyrotropin, Free Thyroxine and Free Triiodothyronine in Healthy Adults in Finland, Measured by an Immunoautomate Based on Time-Resolved Fluorescence (AutoDELFIA®)

Eeva Taimela, Veli Kairisto, Pertti Koskinen, Aila Leino and Kerttu Irlala

Department of Clinical Chemistry, Central Laboratory, University Central Hospital of Turku, Turku, Finland

Summary: We have established reference intervals for healthy adults of serum thyrotropin, free thyroxine and free triiodothyronine using the AutoDELFIA® (Wallac, Finland) automatic measuring device. The determination of reference intervals in a proper manner is costly, and many laboratories adopt reference ranges from the literature rather than determining them alone. This is the first report on reference values in thyroidology where this automatic system based on time-resolved fluorescence has been used.

The reference intervals for thyrotropin, free thyroxine and free triiodothyronine were 0.6–4.3 mIU/l, 9.6–17.1 pmol/l and 4.3–7.5 pmol/l, respectively.

Introduction

Since the mid-1980s, serum thyrotropin measurement has been the initial thyroid function test (1–3). In the diagnosis of thyroid disorders, additional measurements of serum free thyroxine (FT₄) and sometimes of serum free triiodothyronine (FT₃) are necessary when the concentration of thyrotropin is abnormal. Serum thyrotropin and free thyroid hormones are essential in monitoring thyroid hormone therapy.

During the late 1980s and early 1990s, the automation of immunoassays marked the trend in immunoassay development. The focus on new immunoassay strategies has improved assay sensitivity, reliability, assay simplification, and simultaneous multianalyte testing (4). Non-radioisotopic assay techniques are able to provide stable labels, signal levels, and standard curves that can be stored for future use. AutoDELFIA® is a fully automatic immunoassay system which has been developed on the basis of the conditions provided by the non-radioactive, highly sensitive Delfia method with europium labels and the time-resolved fluorescence technique (5). With this technique, the determination of reference intervals in a proper manner is costly, and many laboratories adopt reference ranges from the literature rather than determining them alone. This is the first report on reference values in thyroidology where this automatic system based on time-resolved fluorescence has been used.

The reference intervals for thyrotropin, free thyroxine and free triiodothyronine were 0.6–4.3 mIU/l, 9.6–17.1 pmol/l and 4.3–7.5 pmol/l, respectively.

Because the proper determination of reference intervals is a major and costly task, the transfer of reference intervals for comparable analytical systems probably accounts for most of the present reference interval assignments in clinical laboratories (10).

In the present study, we established the reference intervals for healthy adults of serum thyrotropin, FT₃ and FT₄ measured with the AutoDELFIA® (Wallac, Finland) measuring device. No previous reports using this system are currently available in the literature.

Materials and Methods

The serum samples for thyrotropin, FT₃ and FT₄ were obtained from healthy adults [n = 262; 155 female, ages 23–69 years (mean 44.8, SD 10.2); 107 males, ages 26–67 years (mean 45.3, SD 11.5)]. The reference subjects had no history of thyroid disorders, and they were not receiving or taking any medication (oral contraceptives were allowed). The reference values were measured in two separate AutoDELFIA® immunoautomates with three separate reagent lots, and in five different runs in order to produce realistic reference intervals.

Based on earlier results, the functional sensitivity of the thyrotropin was 0.007 mIU/l (between assay CV, 20%) (5). In free thyroxine hormone measurements, we used a three-step protocol in order to minimize the possible effects of heterophilic antibodies. In the current thyrotropin measurements, the between-assay CV was 7.0% at 0.48 mIU/l and 4.4% at 4.3 mIU/l. In FT₄ measurements, the between-assay CV was 5.6% at 5.6 pmol/l and 3.4% at 26.1 pmol/l and in FT₃ measurements, the between-assay CV was 6.8% at 3.7 pmol/l and 5.7% at 14.1 pmol/l.

Statistical Analysis

Statistical treatment of reference values was performed as recommended by NCCLS (10). Non-parametric estimates were used for each analyte, and the 0.90 confidence intervals (CI) are also presented. The program Refval was used in calculating the results (11).

Results

The reference intervals¹) are presented in table 1. The reference intervals for thyrotropin have been relatively constant since RIA methods were replaced by IRMA and methods based on non-radioactive techniques in the mid 1980s when it became possible to measure low thyrotropin concentrations reliably. Only a few reports

Tab. 1. The non-parametric central 0.95 fractions of the reference distributions for adult serum thyrotropin, free thyroxine (FT₄) and free triiodothyronine (FT₃). The confidence intervals (CI) for the 0.025 and 0.975 fractiles are also presented.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>n</th>
<th>0.90 CI of lower limit</th>
<th>Reference interval</th>
<th>0.90 CI of upper limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-Thyrotropin (mIU/l)</td>
<td>262</td>
<td>0.4–0.7</td>
<td>0.6–4.3</td>
<td>3.9–5.3</td>
</tr>
<tr>
<td>S-FT₄ (pmol/l)</td>
<td>262</td>
<td>9.4–10.3</td>
<td>9.6–17.1</td>
<td>16.6–17.7</td>
</tr>
<tr>
<td>S-FT₃ (pmol/l)</td>
<td>127</td>
<td>3.7–4.5</td>
<td>4.3–7.5</td>
<td>7.1–7.8</td>
</tr>
</tbody>
</table>

¹) The primary data used for the calculation of the reference values are available from the authors.
on reference intervals for FT₃ have been previously published (12). The upper reference limit for free thyrotropin in our laboratory was 17.1 pmol/1, which is clearly lower than the 'conventional' upper reference limit of 20–24 pmol/1 (6–8, 13). This difference may affect the interpretation of the test result. Not only analytical, but also, for example, racial and geographical differences may cause discrepancies between different reference value studies. The validation of a test should at least include analysis of some samples from healthy individuals to verify the used reference interval if the available resources do not allow the laboratory to establish its own reference interval (14).

References


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Corresponding author: Eeva Taimela, Finnish Red Cross BTS, Kivihaantie 7, FIN-00310 Helsinki, Finland
Fax: +358-0-5801 484, e-mail: eeva.taimela@bts.redcross.fi