THE PRESENCE OF INTERFERING ANTIBODIES IN SELECTED CONTROL SERA ON THE EXAMPLE OF A SET FOR DETERMINATION OF tPSA

Bogumiła Hajdrowska¹, Katarzyna Krzakiewicz¹, Zofia Czubasiewicz¹, Joanna Katarzyna Strzelczyk¹, Marek Szewczyk¹, Ryszard Brus²

¹Department and Chair of General Biology, Medical Faculty of Medicine and Medical-Dental Division, Medical University of Silesia in Katowice
²Department of Nursing, Higher School of Strategic Planning in Dąbrowa Górnicza


Summary: The human sera often contain substances which interfere in the course of immunochemical reactions. It is essential for the control sera used to determine concentrations of antigen of the prostate not to contain such ingredients. The aim of this study was to test the control sera, three producers, containing low (N) and high (P) concentrations of prostate antigen (tPSA). As a factor blocking the interfering actions, the TRU Bloc Meridian Life Science, Inc. reagent was used which contains antibodies against mouse protein and rheumatoid factor. The sera with a low concentration of tPSA demonstrated the presence of interfering substances.

Keywords: Immunoenzymatic method, control serum, PSA antigen, blocking reagent

Introduction

Marking the levels of tumor antigens is used for observation of disease or monitoring the progress of the treatment. Sometimes, as in the case of prostate antigen (PSA) testing includes a set of tests performed also prophylactically to detect prostate cancer (Zhu et al. 2003). The marking of antigen levels of PSA is carried out with the use of Immunoenzymatic method in which enzyme-labeled antibodies against antigen of the prostate, specifically bind the antigen in the serum and the specific activity of the enzyme bound to the antibody is a measure of antigen concentration. To determine concentrations of antigen the control serum containing acertain concentration of tPSA is applied (STAMAR's instructions for the set for determination of PSA No kat.91.206). Control sera routinely used for immunochemical tests are usually produced on the basis of human serum (Sztefko 2002). It is usually a mixed, so-called "poole"d serum from different individuals comprising a number of additives such as bovine serum albumin, enzymes and stabilizers. Due to the very diverse composition of sera whose donors can be persons in different states of health, one can assume that some of them contain ingredients interfering in the course of an immune response (Sztefko 2011), (Stenman UH. 2001). It is estimated that nearly 40% of the population has heterophile antibodies, including 80% of the antibodies which are HAMA (Human Anti Mouse Antibody), a major cause of interference in immunochemical assays (Park et al. 2006), (Bonetti et al., 2008), (Kricka, 1999). Of great importance in addition to anti-animal are autoantibodies which may react with both the test antigen and the antibody which blocks as internal reflection of an antigen mimicking the binding and function of the joint. They can also act as a ligand or interfere with the area of binding as a barrier to the binding of the antigen with the antibody (Sztefko 2002). The methods allowing for removal of interfering factors include: polyethylene glycol precipitation, blocking antibody fragment, precipitation with ionic detergent and a nonionic or neutralizing murine antibody of the IgG class [9]. Instructions of Meridian Life Science, Inc., TRU Block Active Blocker against Heterofilic Antibodies and Rheumatoid Factor (Turpelnen et al., 1990).

The aim of the study was to test whether commercially available control sera may contain ingredients that influence the course of immunochemical reaction.

Address for correspondence: Bogumiła Hajdrowska, NZOZ Centralne Laboratorium Sp. z o.o., Piłsudskiego 84, 41-902 Bytom, phone /fax +48 32 2811617, e-mail: bhajdrowska@wp.pl

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Material and methods

Material for the study consisted of six control sera of three manufacturers, labeled A, B and C. In case of each of the manufacturers control sera were purchased with physiological concentrations (N) and pathological ones (P). The study was conducted on a set for determining the concentration of total prostate antigen (tPSA), purchased from one of the producers of control serum (tPSA manufacturer’s instructions DiaMetra 06034 Foligno (PG)- Italy). In order to detect the presence of interfering antibodies a blocking reagent TRU Block Meridian Life Science, Inc. USA was used (TRU Block User Meridian Life Science, Inc., USA). The method for determining total PSA (tPSA) is the gradual addition to the wells of streptavidin-coated calibrator of the test sample and control. Then, biotynilized monoclonal antibodies, labeled with an enzyme, are added. The antibodies bind with PSA and streptavidin-biotin. Excess amounts of unbound antibodies are removed by aspiration and lavage. The concentration of the native PSA is proportional to the activity of the enzyme bound to the antibody (STAMAR's instructions for the set for determination of PSA No kat.91.206).

In order to verify the presence of interfering substances in sera of control, heterophile antibodies were added to the conjugate blocking reagent (broker).

Results

Table 1 shows the arithmetic mean of PSA in ng / ml, SD, standard deviation and student’s t test value.

<table>
<thead>
<tr>
<th></th>
<th>N sera without „blocker”</th>
<th>N sera with „blocker”</th>
<th>Student’s t test</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Producer</strong></td>
<td>Arithmetic mean</td>
<td>SD</td>
<td>Arithmetic mean</td>
</tr>
<tr>
<td>A</td>
<td>15,79   1,33</td>
<td>27,28</td>
<td>3,52</td>
</tr>
<tr>
<td>B</td>
<td>5,66    0,65</td>
<td>4,36</td>
<td>1,15</td>
</tr>
<tr>
<td>C</td>
<td>2,72    0,38</td>
<td>2,39</td>
<td>0,53</td>
</tr>
<tr>
<td>P sera without „blocker”</td>
<td>Arithmetic mean</td>
<td>SD</td>
<td>Arithmetic mean</td>
</tr>
<tr>
<td>A</td>
<td>39,84   4,63</td>
<td>43,13</td>
<td>10,01</td>
</tr>
<tr>
<td>B</td>
<td>33,1    1,49</td>
<td>33,7</td>
<td>2,13</td>
</tr>
<tr>
<td>C</td>
<td>25,78   1,62</td>
<td>25,67</td>
<td>2,1</td>
</tr>
</tbody>
</table>

Source: own elaboration

Table 2. The values of the variance coefficients CV % for 20 markings of tPSA concentrations in the physiological serum (N) and the pathological one (P) with and without “blocker”

<table>
<thead>
<tr>
<th></th>
<th>N sera without „blocker”</th>
<th>N sera with „blocker”</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Producer</strong></td>
<td>CV%</td>
<td>CV%</td>
</tr>
<tr>
<td>A</td>
<td>8,42</td>
<td>12,90</td>
</tr>
<tr>
<td>B</td>
<td>11,48</td>
<td>26,38</td>
</tr>
<tr>
<td>C</td>
<td>13,97</td>
<td>22,18</td>
</tr>
</tbody>
</table>

Source: own elaboration

The average concentration in physiological sera (N) differed significantly post application of the “blocker” compared to the concentration in the sera without a “blocker”.

In case of A serum the concentration of tPSA after blocking significantly increased, while in case of sera of B and C type—it decreased significantly. It should be noted that these changes are relevant despite a significant increase in the standard deviation and coefficient of variation (Table 1, Table 2). The pathological sera (P) with...
several times higher concentrations of tPSA there were no significant changes in the average concentrations after application of blocking reagent. Also apart from the A company serum, earnings volatility increased slightly (Table 1, Table 2).

Discussion

The basic conclusion from the research is that the control sera for immunochemical tests contain components interfering in the determination of tPSA. This was not observed in sera at concentrations of pathological conditions which may result from high concentrations of total PSA for which small changes caused by the blocking antibodies are within the scope of the methodological variability and do not substantially affect the concentration of the antigen. An interesting situation occurs in the sera of physiological serum in the case where serum A faces an increase in concentration, while in the case of B and C sera it is a decrease.

TRU reagent Block Meridian Life Science, Inc. actively blocks human heterophile antibodies and its main component is a purified mouse immunoglobulin G with concentration of 24.7mg/ml. (Instructions of Meridian).

Subject literature shows that interfering substances which have a significant impact on the course of the immunochemical reaction are anti-animal antibodies with a higher specificity than other heterophile antibodies (Controlling Interfering In the Diagnostic Assays Antibodies www.emdmillipore.com/diagnostics). The result is that even in low concentrations they can have an effect on the reaction and the final value for the analyte. This was very evident in physiological serum A, the already high PSA value of which nearly doubled upon addition of a blocking agent, which most likely bound anti-mouse antigens blocking the reaction with the antigen PSA. For the remaining sera, in which PSA concentration has been reduced it can be assumed that the interfering components of the sera entered into a reaction with the conjugate resulting in an apparent increase in concentration of the addition of the “blocker” caused by the binding of the blocking reagent and reducing the amount of antigens reactive with the labeled antibody. What is characteristic is the fact that control serum from A company belongs to the set manufacturer. A study shows the importance of quality of the control sera used and how it can clearly contribute to the existence of differences between the results obtained using the assemblies and control sera from different manufacturers. Lack of standardization of the production sets, control sera towards immunochemical methods is later reflected in the credibility of the results and how it contributes to their high volatility.

References:

2. Instrukcja firmy STAMAR do zestawu do oznaczania antygenu PSA nr kat.91.206.
3. Instrukcja producenta tPSA DiaMetra 06034 Foligno (PG)- Italy.
4. Instrukcja firmy Meridian Life Science, Inc. do TRU Block Active Blocker against Heterofilic Antibodies and Rheumatoid Factor.