INTRODUCTION
Tuberculosis (TB) infection is one of the major public health problems in developing country including Indonesia. The endemic of TB infection caused by Mycobacterium tuberculosis became worse because of the multidrug-resistant TB (MDR-TB). M. tuberculosis had special feature which is its cell wall or envelope that consists of complex lipids, lipoglycans and peptidoglycans (Forrelad, 2013). The cell wall consisted of two layers, the inner and the outer. The inner consisted of peptidoglycan sacculus veiled by mycolylarabinogalactan polysaccharide layer. The outer envelope consisted of trehalose 6,6′-dimycolate (TDM; cord factor) which was synthesized by fibronectin binding protein (fbp)/antigen 85 (Ag85) complex (Elamin, 2011). The Ag85 complex was a 30–32 kDa protein consisting of three proteins (Ag85A, Ag85B, and Ag85C). It had several functions, including enzymatic mycolyl-transferase activity for coupling of mycolic acids to cell wall arabinogalactan, cord factor biogenesis (trehalose-dimycolate), and had capacity to bind to fibronectin and elastin of the extracellular matrix proteins (Huygen, 2014). This protein were encoded by three paralogous genes located in different regions of the bacterial genome (D’Souza, 2003). The Ag85 complex was the major secreted protein of M. tuberculosis cell culture besides of its association with bacterial surface. It had essential role in the pathogenesis of tuberculosis. The ability of this protein to bind fibronectin promoted M. tuberculosis adhesion to the mucosal surface, thus facilitating the bacteria to enter into the host cell. However, the main role of Ag85A in the virulence of M. tuberculosis was the ability to synthesis of cell wall lipids (Forrelad, 2013).

Ag85A (fbpA) was part of the antigen 85 complex. It had several functions including as the triacylglycerol synthase and might have the key role in stability of cell wall and storage compounds biosynthetic for the survival of M. tuberculosis during the dormant state (Elamin, 2011). Belisle et al. (1997) studied the role of Fpb proteins in the virulence of mycobacteria using M. tuberculosis H37Rv fbpA mutant and suggest that the Ag85A (fbpA) would be essential in the virulence of M. tuberculosis. The study by Elamin et al (2011) had the same result that Ag85A might be the key player for formation of lipid storage bodies and thus also essential for the establishment and maintenance of a persistent tuberculosis infection. Ag85A and Ag85B are among the most promising tuberculosis vaccine candidates because their ability to induces T-cell response and production of IFN-γ (Huygen, 2014). The T-cells epitopes of the Ag85A have been identified. (Ivanyi, 2014). The B-cell epitope of Ag85A have rarely investigated. Thus, this study is aimed to predict the B-cell epitope of Ag85A. The result of this study will be useful for investigation of the immunogenicity and antigenicity of Ag85A antigen.

METHOD
Amino acid sequence of Ag85A antigen which had 338 amino acid length (form http://tuberculist.epfl.ch/) was analyzed using B-cells Tools from IEDB online prediction software http://tools.iedb.org/main/bcell/.
The software analyze amino acid scale using six methods, such as Chou & Fasman BetaTurn Prediction, Emini Surface Accessibility, Karplus & Schulz Flexibility Prediction, Kolaskar & Tongaonkar Antigenicity Prediction, Bepipred Linear Epitope Prediction, and Parker Hydrophilicity Prediction. Each method worked by its characteristic as described below according to http://tools.iedb.org/main/bcell/.

Chou & Fasman BetaTurn Prediction using Chou-Fasman algorithm predicted beta turns of a protein which is known for the immunodominant region of a protein. This area were shown by the region above threshold.

Emini Surface Accessibility Prediction is used to know the surface accessibility of a protein. The method calculated based on scale of surface accessibility on a protein. The formula for the calculation to get the profile is $Sn = (n+4+i)/(0.37)6$; $Sn$ is the surface probability, $dn$ is the fractional surface probability value, and $i$ vary from 1 to 6. The profile has $Sn$ greater than 1.0 have bigger probability for being found on the surface.

Karplus & Schulz Flexibility Prediction is used to predict the most antigenic protein based on flexibility scale. The scale calculation is based on mobility of protein segments. This method
calculation based on mobility of 31 proteins alpha-carbon on the basis of the known temperature B factors.

**Kolaskar & Tongaonkar Antigenicity Prediction** is used to predict antigenic determinants on proteins. This method used the physicochemical characterization of amino acid and their experimentally occurrence frequencies in known segmental epitopes. This method can be applied to proteins with accuracy about 75% better than the other method.

**Bepipred Linear Epitope Prediction** is used to predicts the location of linear B-cell epitopes using a combination of a hidden Markov model and a propensity scale method. Amino acid residues have score more than the threshold are predicted to be epitope.

**Parker Hydrophilicity Prediction** is used to predict linear epitope using hydrophilicity of amino acid residues. Hydrophilicity of amino acid based on retention times of a peptide on high performance liquid chromatography (HPLC) on a reversed phase column. The epitope region was determined by analyzing a window of seven residues amino acid.

**RESULT**

The Ag85A consisted of 338 amino acid as listed below

MQLVDRVRGAVTGMSRRLVVGAVGAALVSGLVGAVG
GTATAAGFSRPGLPVEYLVQPSMSMGRDKVQFQSGLG
NSPALYLDGLRAQDDFSGWIDINTPAFEWYDQSGSLV
VMPPVGGQSSFYSDVWYPACGKAGCQTYKWTFTLSEL
PGWLQANRHVKPTGSAVGSLMIAASALTALYHPQQ
FVYAGAMSGLLPSQAMTGLAMGDAHGYKASD
MWPKEPDPAWQRNPLNVGKLANNTRVWVYCGN
GKPSDLGNNLPAFKLEGFVRTSNIKFQDAYNAGGH
NGVFDGFDSGTHSWEYWGAQLNAMKPDLQRALGATP
NTGPAPOQGA

**Chou & Fasman Beta Turn Prediction**

Chou and Fasman beta turn prediction gave 10 epitopes of B cell with a minimum propensity score of 0.724, maximum score of 1.443 and threshold of 1.045.

**Emini Surface Accessibility Prediction**

Emini Surface Accessibility to predict beta turn has resulted 9 beta turns of Ag85A protein with a minimum propensity score of 0.094, maximum score of 4.112 and threshold of 1.

**Karplus & Schulz Flexibility Prediction**

Karplus & Schulz Flexibility Prediction gave 15 epitopes of Ag85A protein with a minimum propensity score of 0.891, maximum score of 1.104 and threshold of 0.999.

**Kolaskar & Tongaonkar Antigenicity Prediction**

Kolaskar & Tongaonkar Method to predict antigenicity of Ag85A protein has resulted 10 epitopes with a minimum propensity score of 0.901, maximum score of 1.186 and threshold of 1.023.

**Bepipred Linear Epitope Prediction**

Bepipred to predict linear epitope has resulted 11 antigenic determinant of Ag85A protein with a minimum propensity score of -1.143, maximum score of 2.632 and threshold of 0.38.

**Parker Hydrophilicity Prediction**

Parker Hydrophilicity Prediction gave 15 epitopes of B cell with a minimum propensity score of -3.826, maximum score of 6.100 and threshold of 1.523.

**B Cell Epitopes Analysis**

The analysis from six methods showed that Ag85A has six B-cell epitopes. These epitopes located at 83-99, 113-124, 155-167, 223-236, 252-267, 298-308 amino acid residues or nearby.

<table>
<thead>
<tr>
<th>Amino acid position</th>
<th>Length</th>
<th>Sequence</th>
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<tbody>
<tr>
<td>83-99</td>
<td>17</td>
<td>DGLRAQDDFSGWIDINTP</td>
</tr>
<tr>
<td>113-124</td>
<td>12</td>
<td>MPVGGQSSFYSD</td>
</tr>
<tr>
<td>155-167</td>
<td>13</td>
<td>NRHVKPTGSAVVG</td>
</tr>
<tr>
<td>223-236</td>
<td>14</td>
<td>WGPKEPDPAWQRNPD</td>
</tr>
<tr>
<td>252-267</td>
<td>16</td>
<td>VYCGNGKPSDLGGNNL</td>
</tr>
<tr>
<td>298-308</td>
<td>11</td>
<td>DFPDSGTHSWE</td>
</tr>
</tbody>
</table>
DISCUSSION

Ag85A is known for inducing T-cell response and production of IFN-γ (Huygen, 2014). The B cell immune epitope prediction by IEDB online software gave six potential B cell epitopes by six different methods calculation. Chou-Fasman algorithm predicted beta turns of a protein of Ag85A. Beta turns are the non-repetitive structure of a protein. The important of beta turns as it used for protein folding, stability of protein, and molecular recognition. A protein sequence with beta turns known as the antipeptide antibody and used for presentation of protein in B cell epitopes thus induced antibody production. Immunogenic determinants that can induce protein-reactive antipeptide antibodies reside mostly in those parts of the molecule that have a high tendency to form beta-turns (Krchnák et al, 1987). The Emini access found surface accessibility of Ag85A. Those surfaces could be accessed by antibody. Karplus & Schulz Flexibility Prediction and Kolaskar & Tongaonkar Antigenicity Prediction predicted the most antigenic part of protein with different approach. Bepipred Linear Epitope Prediction calculated the location of linear B-cell epitopes. Parker Hydrophilicity Prediction finding hydrophilicity part of Ag85A. The hydrophilicity of a protein was known as antigenic...
Overall, the result showed that Ag85A could act as an antigen to induce antibody production by B cell besides cellular immunity and cytokine production in human. Giri et al (2006) studied Ag85A along with Ag85B as the potential vaccine candidate using BALB-C mice given by intranasal. The result showed that Ag85A native antigen could induce the production of IFN-γ, IL-12, IL-4, IgA, and IgG in murine. This research has similar results to epitope prediction of Ag85A in human. Antibody responses were essential against mycobacterial infection, and the synergy and mutual interdependence between humoral immunity and cell-mediated immunity is needed for this action. Antibody (Abebe and Bjune, 2009). B cell major role in tuberculosis infection was to produce antibody. The antibody could do its function against pathogens such as neutralization, opsonization, antibody dependent cell cytotoxicity, and complement activation. Besides that, B lymphocytes modulate the host response in tuberculosis infection. B cells developed anti-tuberculous immunity through a variety mechanism including antigen presentation, cytokine production, and influencing leukocytes intracellular killing mechanisms (Maglione and Chan, 2009).

**CONCLUSION**

Ag85A has potential B cell epitopes to induce antibody production in human thus Ag85A could be developed as vaccine antigen of tuberculosis.

**REFERENCES**