DETECTION OF SAG1 AND BAG1 *Toxoplasma gondii* DNA PROBES LABELLED WITH DIGOXIGENIN-11-dUTP

Deteksi DNA Probe Sag1 *dan* Bag1 *Toxoplasma godii yang Dilabel Digoxigenin-11-dUTP*

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ABSTRACT

The objective of this research was to detect a minimum concentration of the probes that could be used for dot blot hybridization analysis. The method required labeled DNA probes. In this study a non-radioactive label of Digoxigenin-11-dUTP was used for labeling the *Sag1* and the *Bag1* of *Toxoplasma gondii* DNA probe. Labeling method for the probes was done according to the random primed labeling technique. The result showed that 0.67 pg/µl *Sag1* probe and 0.58 pg/µl *Bag1* probe could be detected by anti-Dig-antibody. It could be concluded that 0.67 pg/µl *Sag1* probe and 0.58 pg/µl *Bag1* probe could be used to diagnose toxoplasmosis by dot blot hybridization method.

Key words: *DNA* probe, *Toxoplasma gondii*, digoxigenin-11-dUTP, dot blot hybridization

ABSTRAK

Penelitian ini bertujuan menentukan konsentrasi minimum probe yang digunakan dalam reaksi hibridisasi dot blot. Probe terlebih dahulu dilabel. Pada penelitian ini dipergunakan label non-radioaktif yaitu Digoxigenin-11-dUTP untuk melabel probe Sag1 dan Bag1 Toxoplasma gondii. Metode pelabelan dengan random primed. Hasil menunjukan bahwa probe Sag1 0,67 pg/µl dan probe bag1 0,58 pg/µl berhasil dideteksi oleh antibodi-antiDig. Konsentrasi minimal 0,67 pg/µl dan 0,58 pg/µl Probe Sag1 dan Bag1 dapat digunakan pada metode hibridisasi dot blot untuk diagnosis toksoplasmosis.

Kata kunci: DNA pr*obe, Toxoplasma gondii*, digoxigenin-11-dUTP, hibridisasi dot blot

INTRODUCTION

Toxoplasmosis is a zoonotic disease spread world wide caused by *Toxoplasmagondii* (Tenter et al., 2000). Cases of toxoplasmosis in both animals and human in Indonesia are very high. Cases in human ranges from 40-85%, and in animals range from 50-80% (Subekti et al., 2005). Artama et al. (2009) found that the seroprevalence of *Toxoplasma gondii* (*T. gondii*) in Yogyakarta, Sleman, Bantul, Gunung Kidul, and Kulon Progo was 54.76, 60, 48.57, 20.48, and 77.14%, respectively. The epidemiological study of animal found that the prevalence of toxoplasmosis in sheep reached 95% in Brazil (Faria et al., 2007) and 77.8% in Italy (Fusco et al., 2007). In Indonesia, some researchers of *T. gondii* in animals found that prevalence in cat, goat, sheep, dairy cows, bali cattle, buffalo, chicken, duck, and pig were 50-40 , 23-61, 32-71, 21, 7.1, 27.3, 19.6-52.5, 6.1, and 20.5-32, respectively (Priyana, 2000; Subekti et al., 2005; Suratma, 2008; Artama et al., 2009). The high number of toxoplasmosis cases in both human and animal and variaty of the factor associated of the disease give the importance of the diagnosis and this prevention efforts (Subekti et al., 2005; Artama, 2009).

Molecular diagnostic for the *T. gondii* infection can be done by molecular probes. Sumartono et al. (2005) used molecular probes to diagnose coccidiosis and the results was very specific and very easy to apply. Sumartono et al. (2009) also designed probes based on repetitive genomic sequences takizoit for the diagnosis of toxoplasmosis. Jin et al. (2005) used the fast dipstick to diagnose toxoplasmosis in humans, but they used the whole takizoit. Most researchers are more focused studies on specific stages of *T. gondii* takizoit and very few researchers who focus on that specific stage, bradizoit (Cristina et al., 2000; Kazemi et al., 2007). Based on the literature, there are specific stages of gene tachyzoite and bradyzoite. The exploration of them for diagnosis has not been much done, therefore the specificity stage tachyzoite and bradyzoite needs to be explored.

Non-radioactive label is widely used, because it is more safe and more stable. Biotin, digoksigenin-dUTP, alkaline phosphatase are non-radioactive labels (Weiss, 1995; Indri, 1999; Yuwono, 2006). Digoxigenin-d UTP are digitalis plants molecules that are more natural. Also, for digoxigenin-*dUTP,* it doesn't necessary to optimize on the procedure of dot hybridization method. The label can form a dot clearly on the membrane (Ge et al., 1995; Chevalier et al., 1997; Yang et al., 1999). There are several methods of labeling, such as nick translation, random priming and end labeling or conjugation enzymes on DNA (Indri, 1999). Digoxigenin is a steroid extracted from *Digitalis purpurea* and *D. landra.* Dig oligonucleotides modified are widely used as high sensitivity probes for non-radioactive immunoassay and hibridization experiment.

The Dig probe can be detected by anti-Dig-antibodies, conyugated to an enzyme, like alkaline phosphatase (indirect detection) via enzyme-substrate reaction (Thermo Electron Corporation, 2006).

According to Keller and Manak (1989) gene can be used as a probe if the gene has several requirements, such as: the length of the gene must be 18-50 nucleotide bases. Longer probes will result in a longer hybridization time, whereas the short probe will decrease the specificity. Sumartono et al. (2007) successfully detect the complementary sample using a probe with a length of 80 bp. Similarly, Pratama (2009) using the toxo-103 bp probe failed to detect its complementary in the DNA sample. While Samuelson et al. (1989) used the recombinant *Entamoeba histolitica* sequen clone with a length of 145 bp as a probe for diagnosis of *Entamoeba* parasites. The other requirement is the percentage of the G and C should be 40-60%. A percentage outside that range will cause non-specific hybridization. Also the sequences doesn't contain four or more of the same base sequence, for example AAAA, GGGG. To avoid a false positif, a probe shouldn't homolog/similar to gene host (Yuwono, 2006).

A succesful labeling will determine the quality of a probe. Determination of the yield labeled probe is important for optimation and reproduction of hybridization. In the hybridization, high probe concentration mix causes background, while too low of concentration leads to weak signals (Roche, 2009).

The purpose of this research was to find out the successful Dig-*11-dUTP* labeling of S*ag1* and *Bag1* probe of *T. gondii* and to determine the quantity minimum concentration of labeled probe in a mixture of hybridization solution on dot blot method. These probe labeled with Dig-11-dUTP could be detected by immunologis procedure.

MATERIALS AND METHODS

Material on this research was an oligonucleotide of *Sag1 T. gondii* consisted of 136 nucleotide (CAGCGCT CACAGAGCCTCCCACTCTTGCGTACTCACCCAAC AGGCAAATCTGCCCAGCGGGTACTACAAGTAGCT GTACATCAAAGGCTGTAACATTGAGCTCCTTGAT TCCTGAAGCAGAAGATAGCTGGTGGAC) and an oligonucleotide of *Bag1 Toxoplasma gondii* probes that consisted of 98 nucleotide (GCGCGCCGGTTCCAGC TCCCGAGTAATTACAAGCCCGACGGAATCAGTGCGG CAATGGACAACGGCGTTCTACGTGTCACGATCAAGG TCGAGG) positive-charged nylon membrane; dig high prime DNA labeling and detection starter kit I, dig wash and block buffer set (Roche, 2009).

Sag1 and *Bag1 Toxoplasma gondii* probes were labeled Dig-11-dUTP by Random primed labeling technique, sing the Dig High Prime Solution. Detection of the labeled probe used an enzyme immunoassay detection method. *Sag1* and *Bag1 Toxoplasma gondii* probe Digoxigenin-11-dUTP labeled according to the instructionsin the kit (Roche, 2009), then visualized with the colorimetric substrate NBT/BCIP and compared with the DIG-Labeled Control DNA.

RESULTS AND DISCUSSION

Sag1 and *Bag1 Toxoplasma gondii* had been successfully labeled with Dig-11-*dUTP*. The labeled probes could be detected on visualization (Roche, 2006). Success detection both labeling and quantity of the label shown in Figure 1.

In the standard reaction 1 µg DNA per assay, approximal 38% of the nucleotide are incorporated into of newly synthesized Dig-labeled DNA within 20 hours (Roche, 2009). In this research, *Sag1 T. gondii* probe was 0.3539 µg/µl before labelization and *Bag1 T. gondii* probe was 0.3088 µg/µl. Prediction labeled probe for 20 hours labeling, for *Sag1* probe was 38/100 X 353.9 ng/20 µl = 6.7241 ng/µl, and for *Bag1* probe was 38/100 X 308.8 ng/20 µl = 5.8672 ng/µl.

The use of digoxigenin as a label were more secure in the labeling and in the detection process. The probes labeled with digoxigenin were more stable and it could be stored about one year (Yang et al., 1999; Herzer and Englert, 2001; Kruchen and Rueger, 2003). The process of labeling using Random Primed Labeling method would yield probes with high sensitivity levels (Roche, 2009). Random primed labeling method requires labeling of DNA template concentration of 10 ng–3 µg for all labeling reactions, and to detect single copy genes required at least 300 ng of template DNA

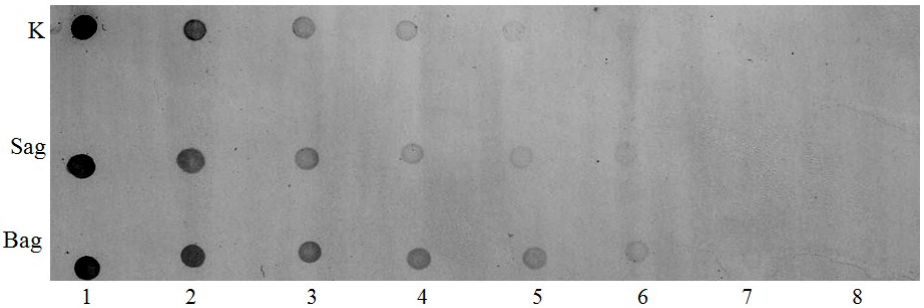

Figure 1. Visualisasi detection of labeling *Sag1* and *Bag1 Toxoplasma gondii* probe (K: Dig-labelled control DNA with concentration, 1= 5 ng/µl, 2= 50 pg/µl, 3= 15 pg/µl, 4= 5 pg/µl, 5= 1.5pg/µl, 6= 0.5 pg/µl, 7= 0.15 pg/µl, 8= 0.05 pg/µl; Sag: Dig-labeled Sag1 *Toxoplasma gondii* DNA probe with concentration, 1= 6.72 ng/µl, 2= 67.2 pg/µl, 3= 20.1 pg/µl, 4= 6,72 pg/µl, 5= 2.01, pg/µl, 6= 0.67 pg/µl 7= 0.2 pg/µl, 8= 0.06 pg/µl; Bag: Dig-labeled Bag1 *Toxoplasma gondii* DNA probe with concentration 1= 5.86 ng/µl, 2= 58.7 pg/µl, 3= 17.6 pg/µl, 4= 5.87 pg/µl, 5= 1.76 pg/µl, 6= 0.58 pg/µl, 7= 0.17 pg/µl, 8= 0.05 pg/µl)

Tabel 1. Dilution series of Dig-labeled control DNA and labeled DNA probe (Sag1 and Bag1 *Toxoplasma gondii* probe)

Dilution	Dilution factor	Concentration Dig labeled control DNA	Concentration *Sag1* *Toxoplasma gondii* labeled probe	Concentration *Bag1* *Toxoplasma gondii* labeled probe
1	1 : 1	5 ng/µl	6.72 ng/µl	5.86 ng/µl
2	1 : 10^2	50 pg/µl	67.2 pg/µl	58.6 pg/µl
3	1 : 33	15 pg/µl	20.1 pg/µl	17.6 pg/µl
4	1 : 10^3	5 pg/µl	6.72 pg/µl	5.86 pg/µl
5	1 : 330	1.5 pg/µl	2.01 pg/µl	1.76 pg/µl
6	1 : 10^4	0.5 pg/µl	0.67 pg/µl	0.58 pg/µl
7	1 : 3300	0.15 pg/µl	0.201 pg/µl	0.17 pg/µl
8	1 : 10^5	0.05 pg/µl	0.067 pg/µl	0.058 pg/µl

(Herzer and Englert, 2001). Probes that were synthesized by the method could be detect target DNA from 0.1 to 0.03 pg (Kruchen and Rueger, 2003; Sarovar and Saigopal, 2010). Its sensitivity depends both on the concentration of labeled DNA in the hybridization and on the time of color reaction (Roche, 2009).

Determination of the yield of Dig-labeled DNA is important for optimal isolation and reproduction hybridization results. Too high of probe concentration in the hybridization mix causes background, while too low concentration leads to weak signals (Kruchen and Rueger, 2003).

In the quantification of the *Sag1* and *Bag1* *Toxoplasma gondii* probe labeled digoxigenin (dig-11-dUTP) were compared with control DNA labeled DIG. Labeled DNA probes or DIG-labeled control DNA dilution series was made order to get the concentration as in Table 1.

Figure 2, showed that the Dig-labeled control DNA could be detected up to a concentration of 0.5 pg/µl, while the Sag1 *Toxoplasma gondii* probe labeled Dig could be detected up to concentrations of 0.67 pg/µl and 0.58 pg/µl for Bag1 *T. gondii* probe labeled Dig. Quantification of detection is important to determine a probe volume that must be added to the hybridization reaction, which is based on the protocol for labeling reaction using Random Primed Labeling method required the probe with a concentration no more than 25 ng/ml for one hybridization reaction (Kruchen and Rueger, 2003).

Usage non-radioactive label Digoxigenin-11-dUTP in the process of Random Primed Labeling gave a lot of benefit, such as secure in the application and the result of detection by anti-Dig-antibody provide clear that its easy reach by usage (Ge et al.,1995; Chevalier et al. 1997; Yang et al. 1999).

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