International Journal of Research in Medical Sciences
Parwanto MLE et al. Int J Res Med Sci. 2016 Oct;4(10):4319-4325
www.msjonline.org pISSN 2320-6071 | eISSN 2320-6012

DOI: http://dx.doi.org/10.18203/2320-6012.ijrms20163286

Research Article

Simple detection of *Bacillus anthracis* spores by precipitation method with goat antibody anti anthrosa

M. L. Edy Parwanto[1]*, Alfred Pakpahan[1], Hosea Jaya Edy[2]

[1]Department of Biology, Faculty of Medicine, University of Trisakti, Jakarta, Indonesia
[2]Department of Pharmacy, Faculty of Mathematics and Natural Science, University of Sam Ratulangi, Manado, Indonesia

Received: 25 July 2016
Accepted: 02 September 2016

***Correspondence:**
Dr. ML. Edy Parwanto,
E-mail: edy.parwanto@gmail.com

ABSTRACT

Background: *Bacillus anthracis* has a potential for biological weapon or bioterorism. Attack of *Bacillus anthracis* is very fatal, and the distribution is very easy and cheap through the spores. The aim of this was study to detect the spores of *Bacillus anthracis*.
Methods: *Bacillus anthracis* isolates were grown on serum agar and then sheep blood medium, to stimulate capsule formation. Spores which formed painted using the method of Schaefer and Fulton. The methods of precipitation and immuno-chromatography were used to spores detection of *Bacillus anthracis*.
Results: Painting with Schaeffer and Fulton method showed that spores of *Bacillus anthracis* are green. Precipitation reaction between spores of *Bacillus anthracis* with goat antibody anti anthrosa was resulting in a silver white color. Anthrosa of *Bacillus anthracis* spores was detected by means of immuno-chromatography using goat antibody anti anthrosa. The molecular weight of anthrosa is ±148 kDa.
Conclusions: The methods of precipitation and immuno-chromatography using goat antibody anti anthrosa can be used to detection of *Bacillus anthracis* spores. Goat antibody anti anthrosa can react positively with *Bacillus anthracis* spores.

Keywords: *Bacillus anthracis*, Bioterrorism, Biological weapon, Anthrosa, Goat antibody anti anthrosa

INTRODUCTION

Some biological agent that has been used, among others, anthrax, plaque, smallpox, tularemia and viral haemorrhagic fever.[1] anthrax disease caused by *Bacillus anthracis* that have the potential for bioterrorism.[2] *Bacillus anthracis* spores can be developed as a biological weapon.

Selection of *Bacillus anthracis* spores as a biological weapon is based on a high killing power, short incubation period, low cost and can be packaged as an aerosol form of biological agents (aerosolize bioagents). It has been proven that *Bacillus anthracis* spores then packaged in a letter sent by post, giving rise to fears that biological warfare agents (biological weapon agents = BWAs) used by terrorists to attack civilians.[3]

The United States Centers for Disease Control and Prevention (CDC) states that *Bacillus anthracis* were subjected for "biological warfare research programs" over 60 years ago in some countries. CDC classifies that anthrax including category A bioterrorism agent, because it can kill people in large numbers and the large area.[4]

Because that the mechanism of biological attacks/threats prevention should comprise: primordial, primary, secondary and tertiary levels of prevention of biological attacks/threats.[5] Anthrax disease including zoonotic disease that is potentially lethal to humans.[6,7] *Bacillus* *anthracis* found in two forms, namely vegetative and spores form.[8] The principle of anthrax virulence factor is the production of capsules and 2 exotoxin during vegetative form. Endospores is an infectious agent and are resistant to heat, drying, chemicals or irradiation, and can survive in the long term.[9]

The aerosol of *Bacillus anthracis* spores are selected as a weapon for bioterrorism. *Bacillus anthracis* spore infections affect different parts of the human body such as the intestines, skin and lungs, causing gastrointestinal, skin and lungs diseases.[7] Anthrax spores that infect into a person's body will grow tobecome deadly anthrax disease.

To counteract biological attack such as anthrax bacteria, including anthrax and biological weapons bioterorism anthrax can be done by detecting the presence of anthrax spores. Anthrax spores can be recognized in various ways such as by recognizing anthrosa compounds contained in the walls of spores. The outermost layer of *Bacillus anthracis* spores called exosporium, which contains a number of proteins. The protein constituent exosporium called Bacillus collagen-like protein of anthracis (BclA), which is a glycoprotein which contain short chain sugars with the O-glycosylation. The protein is known as anthrose.[10]

BclA arrangement can be seen using mass spectroscopy (MS) and nuclear magnetic resonance spectroscopy (NMR). Glycosyl part of BclA are oligosaccharides consisting of 2-O-methyl-4- (3-hydroxy-3-methylbutanamido)-4.6-dideoxy-D-glucose, hereafter referred anthrose, and three residues rhamnose.[11]

Exosporium of the spores of *Bacillus cereus* group (*Bacillus cereus, Bacillus anthracis and Bacillus thuringiensis*) that plays a major role in spore adhesion and virulence. BclA is a major constituent of its hair-like surface.[12,13] The BclA is known to be used for developing diagnostics of *Bacillus anthracis* spores and thus targeted therapeutic interventions.[12]

Various ways have been conducted to identify the spores of *Bacillus anthracis*, among others, with poly chain reaction (PCR),[2,14,15,16] spectrometry, flow cytometry and transmission electron microscopy.[17,18] Methods developed to detect anthrax spores contained in an envelope that is by engineering mikrospectroscopy.[19] Amperometric technique was also used for detecting *Bacillus anthracis*.[20]

Besides these ways, the technique of enzyme-linked immunosorbent assay (ELISA) was developed to detect anthrax spores.[9] The purpose of this study wanted to detect vegetative cells and spores of *Bacillus anthracis* using a microscope, biochemical tests, precipitation and immunochromatography using goat antibody anti anthrosa. Using the principle of immunochromatography, anthrosa strip can be made to detect the presence of *Bacillus anthracis* spores.

METHODS

Isolation of Bacillus anthracis

Bacteria isolated from vesicular fluid, then cultured on polymyxin-lysozyme-EDTA-thallous acetate (PLET) agar base medium (Sigma-Aldrich). Isolates were then cultured in nutrient broth (Sigma-Aldrich) was then performed Gram. Motility test used tryptic soy broth medium (Sigma-Aldrich) and incubated at 37° C for 24 hours.

Biochemistry test for vegetetive cells of Bacillus anthracis

Bacteria isolates were cultured in a nutrient broth (Sigma-Aldrich) and nutrient agar (Sigma-Aldrich). Culture in nutrient broth carried out to nutrient broth test, citrate test, and glucose test. Culture on nutrient agar carried out to 7% Na Cl test and blood agar test.

Induction and staining spores of Bacillus anthracis

Bacillus anthracis isolates were cultured on blood agar (Sigma-Aldrich) to induce sporulation. Spores are formed painted with malachite green as primary stain and the counterstain is safranin.

Anthrosa detection

Anthrosa detection is done by two ways, namely precipitation and electrophoresis. Precipitation reactions conducted on anthrosa using goat antibody anti anthrosa. Goat antibody anti anthrosa obtained by purification of the blood serum of goats previously vaccinated with anthrax vaccine. Electrophoresis was performed to detect of anthrosa using protein marker.

RESULTS

The results of Gram staining and biochemical tests on the *Bacillus anthracis* is presented in Figure 1.

The results of Gram staining and biochemical tests on the *Bacillus anthracis*. A=the results of Gram staining; B=broth test (B1 and B2=*Bacillus anthracis* in the brooth medium, B3=*Bacillus cereus* in the brooth medium, B4=*Bacillus thuringiensis* in the brooth medium); C=nutrient broth were shaken test (C1=*Bacillus anthracis*, C2=*Bacillus cereus*, C3=*Bacillus thuringiensis*); D=citrate test (D1 and D2=*Bacillus anthracis*); E=glucose test (E1-8=*Bacillus anthracis*); F=blood agar test (F1=*Bacillus anthracis* on agar medium with 7% Na Cl, F2=*Bacillus anthracis* on blood agar, F3=*Bacillus subtilis* on blood agar medium) (Figure 1).

The results of *Bacillus anthracis* painting with paint Gram, showed a purple color that react positively. *Bacillus anthracis* including Gram positive, bacilli shaped and non motile bacteria.

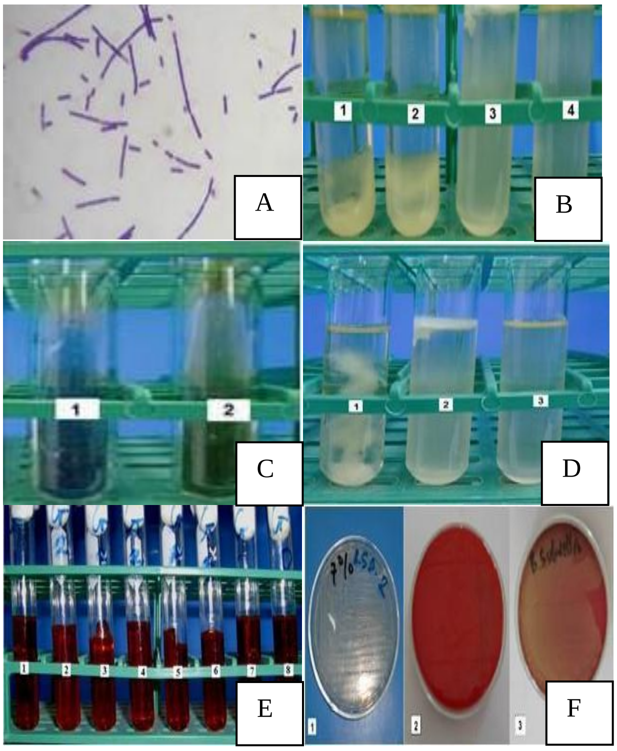

Figure 1: The results of gram staining and biochemical tests on the *Bacillus anthracis*.

Bacillus anthracis cultured in nutrient broth is not made turbid (Figure 1 (B1 and B2)), while *Bacillus cereus* (Figure 1 (B3)) and *Bacilluc thuringiensis* (Figure 1 (B4)) were cultured in a nutrient broth becomes cloudy.

Bacillus anthracis culture in nutrient broth when shaken showed the typical image that is like tendrils of silkworms (Figure 1 (C1)), while *Bacillus cereus* (Figure 1 (C2)) and *Bacillus thuringiensis* (Figure 1 (C3)) in nutrient broth when shaken remain murky. *Bacillus anthracis* cultured in medium enriched citrate to show negative (Figure 1 (D1)) and positive reaction (Figure 1 (D2)).

Bacillus anthracis cultured in medium enriched glucose will not form acid and gas (Figure 1E). *Bacillus anthracis* cultured on agar medium with 7% NaCl showed good growth (Figure 1 (F1)). Culture of *Bacillus anthracis* did not hemolysis on blood agar medium (Figure 1 (F2)), while the *Bacillus subtilis* cultures do hemolysis on blood agar medium (Figure 1 (F3)).

Results of painting vegetative cells and spores of *Bacillus anthracis* presented in Figure 2. Figure 2. Results of painting vegetative cells and spores of *Bacillus anthracis*. A=vegetative cells of *Bacillus anthracis* seem rod-shaped chain; B, C and D=spores of *Bacillus anthracis* seem solitary and green. Gram staining on cells of *Bacillus anthracis* showed purple. Results of precipitation reactions on the anthrose using goat antibody anti anthrosa and electrophoresis is presented in Figure 3.

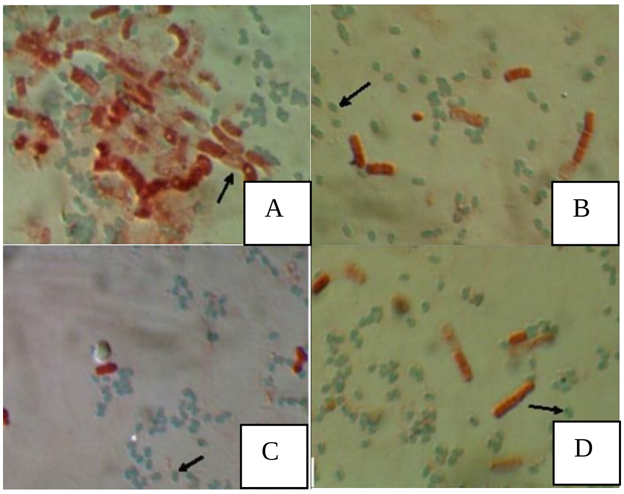

Figure 2: Results of painting vegetative cells and spores of *Bacillus anthracis*.

Figure 3 Anthrosa precipitation of *Bacillus anthracis* with goat antibody anti anthrosa and electrophoresis. A=anthrosa precipitation of *Bacillus anthracis* with goat antibody anti anthrose; B=precipitation reaction of *Bacillus cereus* with goat antibody anti anthrose. C=Electrophoresis of anthrose that reaction with goat antibody anti anthrose (1=protein marker, 2 and 3=*Bacillus anthracis*, 4=*Bacillus cereus*, 5 and 6=*Bacillus subtilis*, 7=*Bacillus thuringiensis*, 8 and 9=buffer sample); µM=micro Mol; kDa=kilo Dalton.

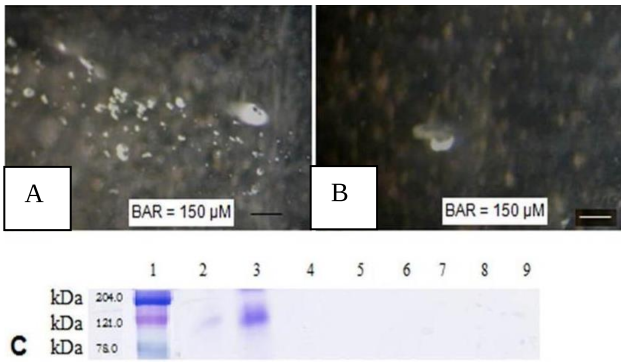

Figure 3: Anthrosa precipitation of *Bacillus anthracis* with goat antibody anti anthrosa and electrophoresis.

Precipitation reaction between anthrosa of *Bacillus anthracis* with goat antobody anti anthrose showed a lot of whitish, while the *Bacillus cereus* are not many shows a whitish color (Figure 3A and B).

The result meant that the precipitation test on the *Bacillus anthracis* reacted positively, whereas on the *Bacillus cereus* showed negatively. Electrophoresis results on

Bacillus spore suspension shows that anthrosa in *Bacillus anthracis* is detected with a molecular weight ± 148 kDa (Figure 3. C).

DISCUSSION

In this study, isolates of *Bacillus anthracis* cultured on PLET agar medium. The medium is used as a selective growth medium.[21] In many countries, the use of PLET agar medium that contains highly toxic thallium acetate at a high concentration, excludes its use due to work-safety regulations.[22] Gram staining on cells of *Bacillus anthracis* showed purple so reacted positively. *Bacillus anthracis* including Gram positive bacteria, bacillus shaped according to research confirming that anthracis, the causative agent of anthrax disease, is a Gram-positive spore-forming bacterium.[23,24] Previous research showed that the composition of the cell wall of *Bacillus anthracis* is dominated by carbohydrates and very specific.[25]

Bacillus anthracis found in two forms, namely vegetative and spores form. However, vegetative cell of *Bacillus anthracis* have very specific nutrient and physiological requirements survive outside a host.[26] Subsequent research showed that *Bacillus anthracis* the causative agent of anthrax secretes a tri-partite exotoxin that exerts pleiotropic effects on the host. The purification of the exotoxin components, protective antigen, lethal factor, edema factor and allowed the rapid characterization of reviews their physiologic effects on the host.[27]

The other result of study show that poly-gamma-D-glutamic acid capsule antiphagocytic properties confers on *Bacillus anthracis* and is essential for virulence.[8] Another study show that sporulation was induced by growth on nutrient agar plates at 30°C with appropriate antibiotic selection. Sporulation was essentially complete (>95%) after 72 hours.[28] Some spore proteins play roles in assembly of both the coat and exosporium. CotE of *Bacillus anthracis* directs assembly of at least one coat protein other than itself.[29]

Results of previous studies show that gene expression in *Bacillus anthracis* during growth and sporulation. Large portion (~36%) of the *Bacillus anthracis* genome is regulated in a growth phase-dependent manner, and this regulation is marked by five distinct waves of gene expression as cells proceed from exponential growth through sporulation. More than 750 proteins present in the spore. Also reported that the genes responsible for the assembly and maturation of the spore are tightly regulated in discrete stages. The spore also contains an assortment of specialized, but not obviously related, metabolic and protective proteins.[30]

Bacillus anthracis in a nutrient broth medium does not make turbid (Figure 1 (B1 and B2)), this means that *Bacillus anthracis* does not ferment glucose, peptone and yeast extract that are components of the medium. Instead, *Bacillus cereus* (Figure 1 (B3)) and *Bacillus thuringiensis* (Figure 1 (B4)) were cultured in a nutrient broth becomes cloudy, it means that the bacteria are able to ferment glucose, peptone and yeast extract in a medium so as to produce metabolites. Results of research have shown that transcription of the major virulence factors, namely: toxin and capsule, triggered by bicarbonate which is becoming a major compound in the mammalian body. Further also been shown that glucose is an additional signaling molecule recognized by *Bacillus anthracis* for toxin synthesis.[31]

Bacillus anthracis cultured in nutrient broth when shaken showed the typical image that is like tendrils of silkworms (Figure 1 (C1)), while *Bacillus cereus* (Figure 1 (C2)) and *Bacillus thuringiensis* (Figure 1 (C3)) remain murky. The fact is the hallmark of *Bacillus anthracis*. *Bacillus anthracis* cultured in medium enriched citrate react negatively (Figure 1 (D1)) and positively (Figure 1 (D2)). The varied reactions may be due to genetic variation of *Bacillus anthracis* causes the difference in response to the formation of siderophore citrate.

The results showed that asbABCDEF gen of *Bacillus anthracis* responsive to petrobactin (catecholate siderophore that functions in both iron acquisition and virulence in a murine models of anthrax) biosynthesis. Results of in vitro analysis showed that each asb gen mutant grew to a very limited extent as vegetative cells in iron-depleted medium. In contrast, none of the *Bacillus anthracis* asb mutant strains were able to outgrow from spores under the same culture conditions. Further data showed that asbA gen also play role in the step of petrobactin biosynthesis, while asbB role in catalyzes condensation of a second molecule of 3,4-dihydroxybenzoyl spermidine with 3,4-dihydroxybenzoyl spermidinyl citrate to form the mature siderophore.[32] Based on the facts in this study that there are variations in results of citrate test to *Bacillus anthracis*, it needs further research.

Bacillus anthracis cultured in medium enriched glucose did not form acid and gas (figure 1. E). The results of another study showed the presence of glucose increased the expression of the protective antigen toxin component-encoding gene (pagA) by stimulating induction of transcription of the AtxA virulence transcription factor. We know that glucose is a critical element in human and animal cells used as a primary source of energy. It has been shown that induction of *Bacillus anthracis* toxin gene expression by glucose and determined that CcpA transcription factor plays a positive role by indirectly regulating the transcription of the gene encoding AtxA.[31]

Bacillus anthracis cultured on agar with 7% NaCl showed good growth (Figure 1 (F1)). We suspect, the nature of which is caused by the structure of the cell membrane that are typical of *Bacillus anthracis*. The assumption is based on the fact that the study results showed that the lipoprotein cell membrane function and regulate multiple cellular processes in Gram-positive bacteria. Has been observed that *Bacillus anthracis* has BA0330 and BA0331 lipoproteins that interact with peptidoglycan. BA0330 lipoprotein plays an important role in the adaptation of bacteria to grow in high salinity, whereas BA0331 contribute arranged to form uniform cells. The data show that the BA0330 and BA0331 play a role in the regulation of *Bacillus anthracis* cell wall structure to be stable.[33]

Bacillus anthracis did not do hemolysis on blood agar medium (Figure 1 (F2)), while the *Bacillus subtilis* cultures do hemolysis on blood agar medium (Figure 1 (F3)). However, the ability of *Bacillus anthracis* to express β-hemolysis was reported.[34,35] The production of strong β–hemolysis on human blood agar plates by the *Bacillus anthracis* strains was unexpected, as this organism has been considered traditionally non hemolytic.[36]

Bacillus anthracis vegetative cells seem rod-shaped chain (Figure 2A). *Bacillus anthracis* spores seem solitary and green (Figure 2 (B, C and D)). Each *Bacillus anthracis* vegetative cells produce a spore.[37] Nutrient-poor medium that is known to promote sporulation.[38,39] The other study show that *Bacillus anthracis* sporulation is triggered under growth-limiting conditions and enables the organism to remain dormant and highly resistant to degradation.[40] Rapid germination of *Bacillus anthracis* spores is induced by serum that is commonly supplemented in cell culture growth formulations; however, some serum-free growth media formulations can also induce *Bacillus anthracis* germination.[41,42]

Anthrosa precipitation of *Bacillus anthracis* with goat antibody anti anthrosa showed a lot of whitish, while the *Bacillus cereus* are not many shows a whitish color (Figure 3). The result meant that test positive precipitation occurs in *Bacillus anthracis*, while the *Bacillus cereus* precipitation test negative. Study result previously showed that to obtain protein samples for immunoprecipitation, BclA-eGFP fusion-expressing sporulating cultures in Tiger broth at T_5 were harvested by centrifugation, and the cells lysed by bead-beating using 0.1 milli meter glass beads (Biospec Products).[28]

Other studies show that there are similarities endospores surface antigen between *Bacillus cereus* and *Bacillus anthracis*. Result of analysis showe that anthrose is monosaccharide, who compiled tetrasaccharide on endospre of *Bacillus anthracis*. Anti tetrasaccharide monoclonal antibodies and anti-anthrose-rhamnose disaccharide monoclonal antibodies were tested for their fine specificities in a direct spore ELISA. The tested with inactivated spores of a broad spectrum of *Bacillus anthracis* strains and related species of the *Bacillus* genus.[9]

Electrophoresis results of *Bacillus anthracis* spore suspension in this study shows that BclA detected with molecular weight about 148 kDa (Figure 3C), whereas other bacteria, namely *Bacillus cereus*, *Bacillus subtilis* and *Bacillus thuringiensis* was not found. Other investigators have also been demonstrated BclA. BclA protein is located on exosporium shown by monoclonal antibody labeling.

The peptide backbone has a predicted size of approximately 39 kDa, but the intact protein migrates with an apparent mass of 250 kDa for the Sterne strain, which is consistent with its being heavily glycosylated.[43] Glycoprotein purified from *Bacillus thuringiensis* exosporium it was identical for both 205-kDa and 70-kDa monomeric forms. At *Bacillus cereus*, the protein is shown in band 205-kDa stains heavily as a glycoprotein. The protein does not correspond to the BclA glycoprotein, although independent evidence of the presence in *Bacillus cereus* exosporium.[44]

CONCLUSION

Data from our study shows that isolates is *Bacillus anthracis*. Isolates were characterized as Gram positive, bacilli and non motile bacteria. Bacteria that cultured in nutrient broth is not made turbid, when shaken showing typical images such as tendrils of silkworms, react negatively or positively towards the medium-enriched citrate, did not form acid and gas in the medium-enriched glucose, grow well on agar enriched 7% NaCl, did not do hemolysis on blood agar medium. Painting to spores of *Bacillus anthracis* are green.

Precipitation reaction between spores of *Bacillus anthracis* with goat antibody anti anthrosa showed in a silver white color. Anthrosa of *Bacillus anthracis* spores detected by means of immunochromatography using goat antibody anti anthrosa. We therefore conclude that the methods of precipitation and immunochromatography using goat antibody anti anthrosa can be used to detection of *Bacillus anthracis* spores. Goat antibody anti anthrosa react positively with *Bacillus anthracis* spores. Anthrosa on the spores of *Bacillus anthracis* size of ± 148 kDa.

Funding: Directorate General of Higher Education, Ministry of National Education, Republic of Indonesia number: 645 / SP2H / PP / DP2M / VII / 2009.
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. Yuen ECP. Biological warfare: the facts. Hong Kong J Emerg Med. 2001;8:232-40.
2. Ahmod NZ, Gupta RS, Shah HN. Identification of a Bacillus anthracis specific indel in the yea C gene and development of a rapid pyrosequencing assay for distinguishing B. anthracis from the B. cereus group. J Microbiol Methods. 2011;87:278-85.
3. Hess G. Biosecurity: an evolving challenge. Chem & Engin News. 2012;90(7):30-2.

4. Centers for Disease Control and Prevention of The United State of America (CDC). Use of anthrax vaccine in the United States: recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR. 2000;49:1-20.
5. Radosavljević V. Biodefense System-Communicable Diseases and Public Health. J Bioterror Biodef. 2016;7:1.
6. Spencer RC. Bacillus anthracis. J Clin Pathol. 2003;56:182-7.
7. Saraswathi SV, Padmavathy J, Mamatha B, Bindu HSN, Vijayalakshmi S. A Sin of Biotechnology, Bioterrorism–Anthrax. Int J PharmTech Res. 2010;2:2044-7.
8. Scorpio A, Chabot DJ, Day WA, O'brien DK, Vietri NJ, Itoh Y, et al. Poly-gamma-glutamate capsule-degrading enzyme treatment enhances phagocytosis and killing of encapsulated Bacillus anthracis. Antimicrob Agents Chemother. 2007;51(1):215-22.
9. Tamborrini M, Holzer M, Seeberger PH, Schu¨rch N, Pluschke G. Anthrax spore detection by a luminex assay based on monoclonal antibodies that recognize anthrose-containing oligosaccharides. Clin Vaccine Immunol. 2010;17:1446-51.
10. Daubenspeck JM, Zeng H, Chen P, Dong S, Steichen CT, Krishna NR, et al. Novel oligosaccharide side–chains of the collagen–like region of BclA, the major glycoprotein of Bacillus anthracis. J Biol Chem. 2004;279:30945-53.
11. Kubler-Kielb J, Vinogradov E, Hu H, Leppla SH, Robbins JB, Schneerson R. Saccharides cross-reactive with Bacillus anthracis spore glycoprotein as an anthrax vaccine component. Proc Natl Acad Sci USA. 2008;105(25):8709-12.
12. Maes E, Krzewinski F, Garenaux E, Lequette Y, Coddeville B, Trivelli X, et al. Glycosylation of BclA glycoprotein from Bacillus cereus and Bacillus anthracis exosporium is domain specific. J Biol Chem. 2016;291(18):9666-77.
13. Peng Q, Kao G, Qu N, Zhang J, Li J, Song F. The Regulation of Exosporium Related Genes in Bacillus thuringiensis. SciRep. 2016;6(19005):1-12.
14. Bell CA, Uhl JR, Hadfield TL. Detection of Bacillus anthracis DNA by light cycler PCR. J Clin Microbiol. 2002;40(8):2897-902.
15. Ryu C, Lee K, Yoo C, Seong WK, Oh HB. Sensitive and rapid quantitative detection of anthrax spore isolated from soil samples by real-time PCR. Microbiol Immunol. 2003;47(10):693-9.
16. Vahedi F, Moazeni Jula G, Kianizadeh M, Mahmoudi M. Characterization of Bacillus anthracis spores isolates from soil by biochemical and multiplex PCR analysis. Epppkiiast Mediterr Health J. 2009;15(1):149-56.
17. Hathout Y, Setlow B, Cabrera-Martinez RM, Fenselau C, Setlow P. Small, acid-soluble proteins as biomarkers in mass spectrometry analysis of bacillus spores. Appl & Environtl Microbiol. 2003;69(2):1100-7.
18. Thompson BM, Stewart GC. Targeting of the BclA and BclB proteins to the Bacillus anthracis spore surface. Mol Microbiol. 2008;70(2):421-34.
19. Aroraa R, Petrova GI, Yakovleva VV, Scullyc MO. Detecting anthrax in the mail by coherent Raman microspectroscopy. PNAS. 2012;109(4):1151-3.
20. Peckham GD, Hew BE, Waller DF, Holdaway C, Jen M. Amperometric Detection of Bacillus anthracis Spores: A Portable, Low-Cost Approach to the ELISA. Int J Electrochem. 2013;2013(803485):1-6.
21. Knisely RF. Selective medium for Bacillus anthracis. J Bacteriol. 1966;92:784-6.
22. Tomaso H, Bartling C, Dahouk SA, Hagen RM, Scholz HC, Beyer W, et al. Growth characteristics of Bacillus anthracis compared to other Bacillus spp. on the selective nutrient media Anthrax Blood Agar® and Cereus Ident Agar®. Syst & Appl Microbiol. 2006;29:24-8.
23. Wang DB, Yang R, Zhang ZP, Bi LJ, You XY, Wei HP, et al. Detection of Bacillus anthracis Spores and Vegetative Cells with the Same Monoclonal Antibodies. PLoS ONE. 2009;4(11):e7810.
24. Akbulut A, Akbulut H, Özgüler M, Inci N, Yalçin S. Gastrointestinal Anthrax: A Case and Review of Literature. Adv in Infect Dis. 2012:2:67-71.
25. Choudhury B, Leoff C, Saile E, Wilkins P, Quinn CP, Kannenberg EL, et al. The Structure of the Major Cell Wall Polysaccharide of Bacillus anthracis Is Species-specific. J Biol Chem. 2006;281(38):27932-41.
26. Jula GM, Jabbari A, Darmian FV. Determination of anthrax foci through isolation of Bacillus anthracis form soil samples of different regions of Iran. Arch of Razi Inst. 2007;62(1):23-30.
27. Lowe DE, Glomski IJ. Cellular and physiological effects of anthrax exotoxin and its relevance to disease. Front Cell Infect Microbiol. 2012;2(76):1-13.
28. Thompson BM, Hsieh H, Spreng KA, Stewart GC. The Co-Dependency of BxpB/ExsFA and BclA for Proper Incorporation into the Exosporium of Bacillus anthracis. Mol Microbiol. 2011;79(3):799-813.
29. Giorno R, Bozue J, Cote C, Wenzel T, Moody KS, Mallozzi M, et al. Morphogenesis of the Bacillus anthracis spore coat. J Bacteriol. 2007;189:691-705.
30. Liu H, Bergman NH, Thomason B, Shallom S, Hazen A, Crossno J, et al. Formation and composition of the Bacillus anthracis endospore. J Bacteriol. 2004;186(1):164-78.
31. Chiang C, Bongiorni C, Perego M. Glucose-Dependent Activation of Bacillus anthracis Toxin Gene Expression and Virulence Requires the Carbon Catabolite Protein CcpA. J Bacteriol. 2011;193(1): 52-62.
32. Lee JY, Janes BK, Passalacqua KD, Pfleger BF, Bergman NH, Liu H, et al. Biosynthetic analysis of the petrobactin siderophore pathway from Bacillus anthracis. J Bacteriol. 2007;189(5):1698-710.

33. Arnaouteli, Giastas P, Andreou A, Tzanodaskalaki M, Aldridge C, Tzartos SJ, et al. Two Putative Polysaccharide Deacetylases Are Required for Osmotic Stability and Cell Shape Maintenance in Bacillus anthracis. J Biol Chem. 2015;290(21):13465-78.
34. Shannon JG, Ross CL, Koehler M, Rest RF. Characterization of anthrolysin O, the Bacillus anthracis cholesterol-dependent cytolysin. Infect Immun. 2003;71:3183-9.
35. Klichko VI, Miller J, Wu A, Popov S, Alibek K. Anaerobic induction of Bacillus anthracis hemolytic activity. Biochem and Biophys Res Commun. 2003;303:855-62.
36. Papaparaskevas J, Houhoula DP, Papadimitriou M, Saroglou G, Legakis NJ, Zerva L. Ruling Out Bacillus anthracis. Emerg Infect Dis. 2004;10(4):732-5.
37. Cybulski RJ, Sanz P, McDaniel D, Darnell S, Bull RL, O'Brien AD. Recombinant Bacillus anthracis spore proteins enhance protection of mice primed with suboptimal amounts of protective antigen. Vaccine. 2008;26:4927-39.
38. Dixon T, Fahd A, Koehler T, Swanson J, Hanna P. Early events in anthrax pathogenesis: intracellular survival of B. anthracis and its escape from RAW264.7 macrophages. Cell Microbiol. 2000,2:453-63.
39. Ireland JA, Hanna PC. Amino acid–and purine ribonucleoside-induced germination of Bacillus anthracis Delta-Sterne endospores: gerS mediates responses to aromatic ring structures. J Bacteriol. 2002;184:1296-303.
40. Whitney SEA, Beatty ME, Taylor TH Jr, Weyant R, Sobel J, Arduino MJ, et al. Inactivation of Bacillus anthracis spores. Emerg Infect Dis. 2003;9:623-7.
41. Gut IM, Tamilselvam B, Prouty AM, Stojkovic B, Czeschin S, van der Donk WA et al. Bacillus anthracis spore interactions with mammalian cells: relationship between germination state and the outcome of in vitro. BMC Microbiol. 2011,11(46):1-12.
42. Bensman MD, Mackie RS, Minter ZA, Gutting BW. Effect of animal sera on Bacillus anthracis Sterne spore germination and vegetative cell growth. J Appl Microbiol. 2012;113:276-83.
43. Sylvestre P, Couture-Tosi E, Mock M. A collagen-like surface glycoprotein is a structural component of the Bacillus anthracis exosporium. Mol Microbiol. 2002;45(1):5240-7.
44. Todd SJ, Moir AJG, Johnson MJ, Moir A. Genes of Bacillus cereus and Bacillus anthracis encoding proteins of the exosporium. J Bacteriol. 2003;185(11):3373-8.

Cite this article as: Parwanto MLE, Pakpahan A, Edy HJ. Simple detection of *Bacillus anthracis* spores by precipitation method with goat antibody anti anthrosa. Int J Res Med Sci 2016;4:4319-25.