Applications of Recombinant Monoclonal Antibodies against Filarial Antigen Proteins

Anizah Rahumatullah, Muhammad Hafiznur Yunus, Gee Jun Tye, and Rahmah Noordin*
Institute for Research in Molecular Medicine (INFORMM), Universiti Sains Malaysia, Pulau Pinang, Malaysia

Abstract. This study investigated the applications of recombinant monoclonal antibodies (rmAbs) produced against two recombinant filarial proteins of diagnostic value. Ab5B and Ab3A were produced against recombinant BmSXP, and Ab4 and Ab4-fragment crystallizable (Fc) against recombinant BmR1. Ab5B and Ab4-Fc were found to be useful as quality control (QC) reagents for two commercial rapid test kits, such as Brugia RapidTM and BLF Rapid[®] (Reszon Diagnostics International Sdn. Bhd., 47600 Subang Jaya, Selangor, Malaysia), respectively. The two rmAbs reacted positively with the corresponding recombinant proteins lined on the nitrocellulose strips of the cassette tests, thus may replace or reduce the need for patient serum samples as positive controls for QC of the commercial kits. They were also successfully conjugated to gold nanoparticles and reacted positively with the test lines containing the corresponding recombinant proteins when directly applied to the cassette tests. The gold-conjugated reagents can be used to confirm the antigenicity of test lines after the storage of the rapid tests for a prolonged period or under unfavorable conditions. Furthermore, Ab5B and Ab3A were shown to be able to capture the target recombinant proteins through immunoaffinity purification, enabling their use for applications that need very highly purified proteins. In conclusion, this study demonstrated several potential uses of rmAb proteins produced against recombinant filarial proteins.

INTRODUCTION

Lymphatic filariasis (LF) is a mosquito-borne parasitic disease that is estimated to affect 67.88 million people in 73 countries, causing morbidity and disability.¹ *Brugia malayi* and *Brugia timori* cause brugian filariasis, whereas *Wuchereria bancrofti* causes bancroftian filariasis. The WHO has initiated a global program to eliminate LF as a public health problem (GPELF), and the target year to achieve this objective is 2020.²

Brugia Rapid is a lateral flow rapid test that is being used in the transmission assessment surveys of the elimination program in brugian filariasis-endemic areas. It uses BmR1 recombinant protein as the test line. Another highly sensitive and specific rapid test reported for detection of bancroftian filariasis is the BLF Rapid test comprising BmSXP recombinant protein as the test line.³ Reszon Diagnostics International Sdn. Bhd. (Selangor, Malaysia) manufactures both rapid tests. Although many endemic countries would have eliminated LF as a public health problem by year 2020, surveillance for transmission of the infection must still be performed for several years after that to ensure no re-emergence of the disease. The surveillance is especially crucial for brugian filariasis because *B. malayi* is a zoonotic parasite in some settings. Thus, the commercial production of the two kits needs to continue beyond the year 2020.

As part of the quality control (QC) process during manufacturing of the rapid tests, positive and negative serum samples are used to determine the diagnostic sensitivity and specificity. However, it is getting more challenging to obtain serum samples from LF patients to confirm reactivity/sensitivity of the tests. This difficulty is because of significant reduction in LF cases due to success of the GPELF.^{2,4} Thus, there is a need to address this challenge, which may affect the sustainability of the test production. One way is by using a recombinant monoclonal antibody (rmAb) protein as a QC reagent.

Previously, our group had reported the isolation of highly specific and sensitive novel rmAb proteins using phage display technology.^{5,6} The present study aimed to develop potential applications of the rmAb proteins. Two of the rmAb proteins were tested as direct QC reagents by replacing the filarial patient serum samples when testing the diagnostic sensitivity of the kits. Subsequently, the rmAb proteins were conjugated with gold nanoparticles and tested with the rapid tests to confirm their binding with the corresponding recombinant proteins on the test lines. Another application of the rmAb proteins was their use in immunoaffinity purification to produce highly purified recombinant proteins.

MATERIALS AND METHODS

Production, purification, and verification of rmAb proteins. The monoclonal antibodies against BmSXP and BmR1 proteins were isolated using phage display technology, as previously described.⁵ The monoclonal antibody protein expression and purification were performed as previously described.⁶ The preparation of Ab4-Fc is described elsewhere (article submitted for publication). In brief, Ab4-Fc plasmid was first constructed, then transfected into HEK293F cell lines (Thermo Fisher Scientific, Waltham, MA). The cells were harvested, and the supernatant containing the protein was purified using Protein A affinity chromatography.

Colloidal gold conjugation to rmAb protein. Conjugation of the antibody to colloidal gold nanoparticles (40 nm) was performed by the nanobiotechnology laboratory of our institute using a previously described method.⁷

Evaluation of rmAb and gold-conjugated rmAb proteins as the QC reagent. An initial evaluation of the rmAb proteins (Ab5B, Ab4, and Ab4-Fc) as the QC reagent was performed using the commercially available Brugia Rapid and BLF Rapid according to the manufacturer's instructions. As positive controls that the test was working well, a serum sample from a *W. bancrofti* patient was tested with BLF Rapid, and a serum sample from a *B. malayi* patient was tested with Brugia Rapid. On the other hand, the evaluation of the gold-conjugated rmAb proteins (Ab5B, Ab4, and Ab4-Fc) was performed using

* Address correspondence to Rahmah Noordin, Institute for Research in Molecular Medicine (INFORMM), Universiti Sains Malaysia (USM), 11800 Gelugor, Penang, Malaysia. E-mails: rahmah8485@gmail.com or rahmah@usm.my

a lateral flow dipstick lined separately with 1 mg/mL of BmSXP or BmR1 recombinant antigens. The lateral flow dipstick was prepared and tested, as previously reported.⁸ The volume of the gold-conjugated rAb proteins (optical density 10) used was 20 μ L. For both tests, the appearance of a red-purple colored line at the test line signified a positive result, whereas no line observed signified a negative result. The testing took approximately 15 minutes to complete; and if no line was visible after 30 minutes, the test result was confirmed as negative.

Immunoaffinity purification. The immunoaffinity purification was performed as previously described.⁶ Ab5B and Ab3A were coupled to cyanogen bromide-activated sepharose resin based on the previously published protocol.⁹ The coupling efficiency was calculated by dividing the supernatant of antibody protein before and after coupling and analyzed using sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). Then, the rAb protein-coupled resin was packed into individual and mixture columns. The binding efficiency of the columns were evaluated using His-tag affinity-purified protein and analyzed using SDS PAGE.

RESULTS

The rAb proteins were successfully expressed and purified and were observed on SDS-PAGE at the expected molecular masses of approximately 35 kDa for Ab5B, Ab3A, and Ab4 and 70 kDa for Ab4-Fc. Western blot using the respective recombinant antibodies showed bands at the same approximate molecular masses.

Then, the rAb proteins were directly tested as QC reagents (Figure 1). Brugia Rapid and BLF Rapid contain recombinant BmR1 and BmSXP, respectively, lined at position C (test line). The Ab5B protein showed a positive reaction with BLF Rapid and a negative reaction with Brugia Rapid, thus showing the specific binding of Ab5B to BmSXP recombinant protein. Meanwhile, Ab4-Fc showed positive reaction with Brugia Rapid and negative reaction with BLF

Rapid, thus showing the specific binding of Ab4-Fc to BmR1 recombinant protein. However, Ab4 did not show reactivity with Brugia Rapid. When filarial patients' serum samples (positive controls) were tested with the diagnostic kits, they showed the expected positive reaction. Subsequently, the rAb proteins were gold-conjugated, and binding with the lined BmSXP or BmR1 on the cassette tests was determined (Figure 2). Ab5B and Ab4-Fc showed positive reactions against BmSXP and BmR1, respectively. Meanwhile, Ab4 was not reactive with the BmR1 line on Brugia Rapid.

The immunoaffinity purification was performed using Ab5B and Ab3A. A similar assay using Ab4 had previously been published.⁶ Both rAb proteins (Ab5B and Ab3A) were successfully coupled and packaged to produce the affinity columns. The coupling efficiency was satisfactory, at 80% for Ab5B and 69% for Ab3A. Single and mixture columns were evaluated using His-tag affinity-purified BmSXP recombinant protein. Both columns showed functional specificity in capturing the target antigen; however, the mixture rAb protein column showed better target antigen recovery than the single rAb protein column (Figure 3).

DISCUSSION

In the present study, three of the four rAb proteins (Ab4, Ab5B, and Ab3A) were in the form of single chain fragment variables (scFv). Meanwhile, Ab4-Fc is Ab4 that has been fused with the Fc region of IgG4. The rAb proteins were tested for use as QC reagents and in immunoaffinity assay.

Ab5B, which is a BmSXP-specific antibody protein, showed a positive reaction with BLF Rapid containing BmSXP as the test line. On the other hand, Ab4, which is a BmR1-specific antibody protein, did not show reactivity with Brugia Rapid (lined with BmR1), whereas Ab4-Fc showed positive reaction. The difference in the reactivity of Ab4 and Ab4-Fc when tested with Brugia Rapid was notable. From our previous work, Ab4 showed high specificity and sensitivity to BmR1 when tested in an ELISA.⁶ The different antibody formats between the two

FIGURE 1. Application of recombinant monoclonal antibody proteins as a direct quality control reagent. The left group of tests shows that Ab5B protein gave positive reaction with the BLF Rapid test and negative reaction with Brugia Rapid at test line C. Positive control comprised the serum sample from a *Wuchereria bancrofti* patient tested with BLF Rapid. The right hand group of tests shows that Ab4 did not react with Brugia Rapid; however, Ab4-Fc gave positive reaction with Brugia Rapid (at test line C) and negative reaction with BLF Rapid. Positive control comprised the serum sample from a *Brugia malayi* patient tested with Brugia Rapid. This figure appears in color at www.ajtmh.org.

FIGURE 2. Application of recombinant monoclonal antibody proteins as gold-conjugated antibodies to check antigenicity of the lined proteins. Gold-conjugated Ab5B protein showed positive reaction with BmSXP-lined dipstick and no reaction on BmR1-lined dipstick. Gold-conjugated Ab4-Fc protein showed positive reaction on BmR1-lined dipstick and no reaction on BmSXP-lined dipstick. Gold-conjugated Ab4 showed no reaction on both dipsticks. T indicates where the recombinant antibody protein was lined (test line). This figure appears in color at www.ajtmh.org.

rmAb proteins (i.e., scFV versus scFv-Fc) may account for this difference. Theoretically, the addition of Fc increases the protein stability, and binding affinity decreases the propensity of protein aggregation and recovers the Fc function.^{10,11} Certainly, in this study, the addition of the Fc region seemed to improve the diagnostic sensitivity of Ab4 when tested with *Brugia Rapid*. However, it is difficult to formulate a specific explanation on why Ab4 did not bind the BmR1 line on the

rapid test. Further investigations need to be performed to elucidate the possible reasons, such as by analyzing the antigen and antibody interactions using surface plasmon resonance. This method allows real-time monitoring of antigen–antibody complex interactions by providing the equilibrium dissociation constant (KD) value. Methods such as fluorescence ELISA¹² and kinetic exclusion¹³ assays are also other technologies that can be used to determine the KD value. Furthermore,

FIGURE 3. SDS PAGE analysis of the outcome of the immunoaffinity purification, and evaluation of binding of Ab5B and Ab3A using purified BmSXP antigen. (A) shows a prominent band of purified BmSXP band at ~22 kDa, the additional bands above it are probably aggregated BmSXP. (B) shows that both antibody columns (Ab3A and Ab5B) demonstrated good specificity in capturing the target BmSXP antigen; however, the mixture protein column (Ab3A5B) showed better target antigen recovery than the single protein column. This figure appears in color at www.ajtmh.org.

structural bioinformatic studies, such as molecular docking and antibody structure modeling, may give insights into the biophysical properties of an antibody molecule and the binding epitope involved during antibody–antigen interaction.^{14,15}

When gold-conjugated rMab proteins were tested with the commercial kits, Ab5B and Ab4-Fc showed positive reactions against BLF Rapid and Brugia Rapid, respectively. Rapid tests that have been stored for some time under non-optimal conditions can be tested with the gold-conjugated antibodies to confirm whether the recombinant proteins lined on the nitrocellulose strips still retain their antigenicity.

The strong binding to their targets makes antibodies suited as immobilized ligands.^{16–18} In this regard, the two BmSXP-specific rMAbs (5B and 3A) were used in immunoaffinity purification. The rMab proteins were able to specifically capture and release the target antigens from a pool of proteins, and the best yield was obtained when the two antibody proteins were combined. The eluted protein can be used for applications that require highly purified proteins such as for crystallography analysis and biosensor development. The immunoaffinity column may also be used to characterize the circulating filarial antigens captured by the recently reported *W. bancrofti* antigen-detection 5B-ELISA.¹⁹

In summary, this study demonstrated three applications of rMab proteins. Ab5B and Ab4-Fc successfully showed good performance as direct QC reagents for Brugia Rapid and BLF Rapid test, respectively. Gold-conjugated Ab5B and Ab4-Fc showed excellent sensitivity toward their respective target antigens and, thus, may be used to check the antigenicity of test lines after being stored for prolonged periods or under unfavorable conditions. The test users may acquire the rMab reagents for batch-testing of the rapid tests to confirm that they are working before using them in the field. Also, the immunoaffinity purification showed that Ab5B and Ab3A were able to capture BmSXP recombinant protein from a pool of proteins.

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Disclosure: R. N., A. R., and M. H. Y. reports a sale of Ab5B (against recombinant BmSXP protein) was made via the university commercial arm to the Reson Diagnostics International. Sdn. Bhd. received a small fraction of the sale value.

Authors' addresses: Anizah Rahumatullah, Muhammad Hafiznur Yunus, Gee Jun Tye, and Rahmah Noordin, Institute for Research in Molecular Medicine (INFORMM), Universiti Sains Malaysia, Pulau Pinang, Malaysia, E-mails: anirah82@gmail.com, mhn1016@yahoo.com, geejun@usm.my, and rahmah8485@gmail.com.

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