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ORIGINAL PAPER

Production, purification and diagnostic application of filarial recombinant protein WbSXP-1 expressed in salt inducible *Escherichia coli*

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Abstract *Wuchereria bancrofti* protein WbSXP-1 was identified and established as a potential candidate for the diagnosis of lymphatic filariasis. For the economic production of rWbSXP-1, osmotically (salt) inducible *Escherichia coli* GJ1158 was preferred. Cultivation and expression was optimized in 3 L airlift bioreactor (ALB) and was successfully extended to 30 L ALB. Purification of rWbSXP-1 his-tag protein was optimized in technical scale using FPLC and the maximal recovery of rWbSXP-1 with significant level of purity was achieved using the combination of IMAC and gel filtration. Quality criteria for immuno-reactivity of purified rWbSXP-1 were established for diagnostic applications. Enhancement of sensitivity in rapid diagnostic format was optimized to effectively detect weak to strong antibody reactivity in individuals exposed to lymphatic filariasis. Performance of the rapid format during field evaluation was successful. The accelerated stability assessment of the rapid format satisfied the requirements of WHO-cGMP norms. This investigation presents a successful technical scale production and purification of rWbSXP-1 considering the future industrial application and an enhanced rapid flow through antibody assay for the diagnosis of human lymphatic filariasis.

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Introduction

Human lymphatic filariasis caused by *Brugia malayi* and *Wuchereria bancrofti* represents an important public health problem with morbidity and disability. It puts at risk more than a billion people across 80 countries. In the global program to eliminate lymphatic filariasis initiated by WHO, early and specific diagnosis was emphasized [23] for surveillance of the disease. Therefore, it is imperative to develop rapid immunodiagnostic kits for diagnosis, monitoring and evaluation of disease control programme. Contrary to the traditional clinical diagnosis, which requires night blood, various commercial diagnostics are currently available to detect *brugian* and *bancroftian* antigens or antibodies. For the detection of bancroftian antigens, the Og4C3 ELISA kit [30] or the use of PCR based techniques cannot be considered as rapid methods. Moreover, they are expensive for field application in endemic area and require skilled manpower and equipments. The other Wb-ICT rapid card test is sensitive than Og4C3, faces instability problem [24]. Bm-rapid also pose problem with stability and specificity. Thus, improvement factor in formats to provide stable diagnostic kits, specificity, simplicity, time and cost of the tests are to be considered before its large-scale use for surveillance in developing and under developed endemic countries [24]. Therefore, none of the assays can be termed a gold standard. In this scenario, we have developed recombinant *Wuchereria bancrofti* SXP-1 (rWbSXP-1) protein antigen based immunoassay, which can detect circulating antibodies in both bancroftian and brugian infections

[16, 25]. We have further improved the rWbSXP-1 antigen based immunoassay into a specific, single, rapid (3 min) field applicable test for diagnosis and surveillance of filarial elimination programmes [6, 17]. Considering the endemic population, there is a need for economic production of rWbSXP-1 and diagnostic kits on a large scale. *Escherichia coli* is the most preferred host for expressing recombinant proteins due to several advantages like media, faster growth and easy fermentation [12]. *E. coli* BL21 (DE3) is widely used for recombinant protein over-production [7, 8, 27], however, the limitation includes formation of inclusion body, high cost and toxicity of IPTG [21]. To overcome these limitations, osmotically inducible *E. coli* GJ1158 host with salt (NaCl) as inducer was developed for over production of recombinant proteins cloned in T7 vectors [9]. The recombinant protein was expressed more in soluble form in GJ1158 compared to BL21 [9].

For *E. coli* fermentation in any scale, stirred-tank reactor (STR) and airlift bioreactor (ALB) are used extensively. However, the STR's require much energy for agitation and aeration compared to ALB's, which is more economical, energy efficient and simple in structure [29]. Efficient downstream processing and protein purification steps are essential part of production process for achieving maximal recovery, quality and purity of the target recombinant protein. Our work, focus on the feasibility of economic production of diagnostically important rWbSXP-1 protein using GJ1158 in 30 L ALB, and purification in technical scale. The enhancement of assay in rapid diagnostic format based on field requirements for better test spot and antibody titre related spot intensity and its performance was investigated. Accelerated stability testing as per European pharmacopoeia norms with a follow-up for a period of 1 year was studied. The work has been carried out considering the future industrial applications to suit the conditions in developing and under developed endemic countries where the human lymphatic filariasis is an important concern.

Materials and methods

Microorganism and plasmid

The salt inducible *E. coli* GJ1158 (Courtesy Dr. J. Gowrishankar, US Patent #5830690) was used as host. The regions corresponding to the open reading frame of *W. bancrofti sxp*-1 gene, (Accn. No. AFO98861), were PCR amplified from *Wb*L3 stage cDNA library for cloning in the expression vector pRSETB (Invitrogen). The forward primer (5′-CCTTAAGAATTCGTCACTTCATCACTCAAT-3′) and the reverse primer (5′-AACCAAGAATTCCTATTTATTACTTTTTGTCG-3′) included *Eco*RI cloning sites [25]. WbSXP-1 was expressed as his-tag fusion protein. The gene was sequenced before use in our work.

Media. Luria Bertani medium without NaCl (LB/ON) pH 7.2 was used for all inoculum preparation. M9 medium [11] was modified and optimized for all GJ1158 fermentation [13] comprising of: Na_2HPO_4 6 g/L, KH_2PO_4 3 g/L, NH_4Cl 2 g/L. Additionally, yeast extract 4 g/L, glucose 10 g/L, 1 M $CaCl_2$ 0.1 mL/L, 1 M $MgSO_4$ 1 mL/L, sterile trace metal mix [3] 1 mL/L were added to the sterilized medium. pH 7.2 was set with ammonia (aq). Filter sterilized ampicillin was used as 100 µg/mL [8]. Sterile NaCl, at a final concentration of 250 mM was used for induction.

Cultivation. A standard 5% of inoculum with freshly transformed recombinant *E. coli*, 0.6 OD_{600}, was used for seed culture for 3 and 30 L airlift bioreactor (Bioengineering, Switzerland). Growth parameters 37 °C; pH 7.0 ± 0.2; dissolved oxygen (pO_2%) above 40% saturation [1, 14]; antifoaming agent 0.02% poly propylene glycol. kLa was determined by dynamic gassing method in 3 and 30 L ALB [29], briefly, actively growing culture at 1.5 OD and pO_2 at 80% saturation was used. Air flow (0.5 or 1.0 VVM) was closed till pO_2 saturation decreased to 30% and was resumed until pO_2 reached near 80% value, the decrease and increase in pO_2% was plotted with respect to time.

Analytical techniques

Biomass was measured as optical density (OD) at 600 nm. *Glucose* was estimated using YSI analyzer. *Protein* estimation was done by Bradford method (BIO-RAD, CA). *Protein profile* of all samples was resolved using 12% SDS-PAGE [15] and analyzed after silver staining [10]. Quantitation of total rWbSXP-1 in samples was done by densitometry using MultiImager (AlphaInnotech, USA). Soluble rWbSXP-1 in culture was estimated by sandwich ELISA, developed from methods previously described for serum [16, 25]. Briefly, rabbit polyclonal anti-WbSXP-1 was used for capture, followed by wbsxp-1 antigen and murine polyclonal anti-wbSXP-1 antibodies. After a wash, probing was done with anti-mouse IgG-HRP conjugate. TMB was used as substrate. Known concentrations of highly purified r*Wb*-SXP-1 were used as standard. *Western blotting* of rWbSXP-1 protein was done with MF serum [25] and anti-His monoclonal antibodies (Sigma) as described by manufacturer.

Centrifugation and cell disruption

The culture from reactor was centrifuged and the cell pellet was re-suspended at 2.5 g wet weight per mL of lysis buffer comprising 50 mM Na_2HPO_4–NaH_2PO_4, 50 mM Tris, 400 mM NaCl, 10 mM Imidazole (pH 8.0) and 0.3 mg/mL lysozyme. The suspension was incubated for 1 h at 4 °C and

subjected to french pressure (1,000 psi), 5 cycles. The cell lysates were added with 1 mM PMSF, 1 mM β-mercaptoethanol and centrifuged at 12,000*g* for 10 min prior to purification.

Purification

AKTA-prime FPLC system (Amersham Pharmacia Biotech, Sweden) was used in all purification experiments. His-tagged rWbSXP-1 was purified by immobilized metal affinity chromatography (*IMAC)* on chelating sepharose fast flow (Ni^{2+}) matrix (Amersham Pharmacia Biotech). Column were packed and equilibrated as recommended. The optimized binding and wash buffer compositions for IMAC consisted of 50 mM Na_2HPO_4–NaH_2PO_4, 50 mM Tris, 400 mM NaCl and 10 mM Imidazole. The optimal pH for binding was 8.0 and the wash at 6.2. The elution buffer comprised of the wash buffer with 0.5 M imidazole, pH 6.2. The flow rates include 0.5 mL/min for binding, 1 mL/min for wash and elution. For the inclusion body solublization, pellet was washed with 100 mM Tris–Cl buffer with 500 mM NaCl, pH 8.0 and centrifuged at 8,000*g* for 30 min. The inclusion bodies were solubilized in 100 mM Tris buffer containing 8 M urea [5, 20]. After the complete solubilization, the fraction was diluted five times for optimal re-folding with dilution buffer 100 mM Tris, 500 mM NaCl, pH 8.0 and was loaded on IMAC PD-10 columns. The buffers for *anion exchange chromatography* (Q-Sepharose, 7.5 mL bed volume) consisted of 10 mM Tris, pH 9.0 for binding and 10 mM Tris–Cl with 1 M NaCl pH 9.0 for elution. *Gel filtration* (Superdex-100, 50 mL bed volume) was performed with combination of 10 mM Tris–NaH_2PO_4, pH 7.8 containing 1 mM PMSF. For all FPLC experiments, protein elution was measured at absorbance 280 nm with an online UV monitor and fractions were analyzed in SDS-PAGE. Protein fractions were membrane dialyzed (3.5 kDa, PEIRCE, LA) against 10 mM Tris–NaH_2PO_4 pH 7.8 buffer and concentrated by freeze-drying.

The patients and individuals were separated in the following clinical groups: endemic normal (EN; individuals residing in an endemic area who are free of infection), microfilaremics (MF; individuals with *B. malayi* or *W. bancrofti* microfilaria in the peripheral circulation), chronic pathology patients (CP; individuals with filariasis mediated chronic pathology) and non-endemic normal (NEN; are populations outside the endemic area). Sera used were obtained from authorities of Department of Public Health, Chennai and Mahatma Gandhi Institute of Medical Sciences, Sevagram, India. The parasitological status of patients and individuals was assessed by blood smear and membrane filtration of 1.0 mL of heparinized venous night blood collected [22]. Serum of healthy individuals collected from approved blood banks services (Surat and Chennai, India) were used for evaluation.

Rapid flow through filarial diagnostic kits and reagents were manufactured and assayed as described [6] at the research facility, SPAN Diagnostics Ltd. Briefly, the kit contains a test spot with rWbSXP-1 and a control spot with goat-anti human IgG on nitro cellulose paper. The anti-SXP-1 antibodies if present in test serum binds to rWbSXP-1, whereas any IgG binds to control spot. Following a wash, the protein-A-colloidal gold signal reagent was used to detect bound IgG. Optimization of assay, evaluation and stability kinetics of diagnostic kit was performed using MF, EN, CP and NEN sera. ELISA was performed as described [6, 16, 25]. Briefly, rWbSXP-1 protein coated at different concentration in 50 mM phosphate buffer pH 9.5. Blocking was done by 1% BSA, washed and incubated human sera at appropriate dilutions. After a wash probing was done with anti-human IgG-HRP conjugate and TMB as substrate.

ICT filariasis (Brookevale, NSW, Australia) and *brugia-Rapid* (Bio-diagnostics research, Malaysia) were used in comparative evaluation study.

Results

Batch cultivation of *E. coli* GJ1158 pRSETB-WbSXP in 30 L Airlift reactor

Recombinant *E. coli* GJ1158 was cultivated in modified M9 medium. The medium and cultivation conditions were optimized in the 3 L ALB [13] and were extended to 30 L ALB considering industrial applications. Growth kinetics of induced and uninduced batches and rWbSXP-1 protein expression in 30 L ALB was consistent in trial batches (Fig. 1). The estimated mass transfer coefficient (kLa) in 3

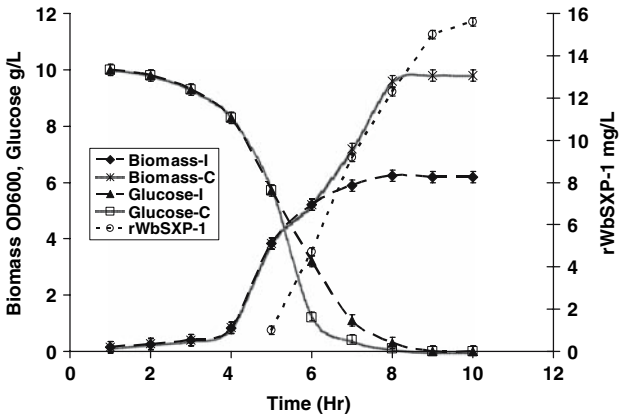

Fig. 1 Batch cultivation of *E. coli* GJ1158 in 30 L airlift bioreactor with modified M9 medium, under induced condition and uninduced condition. The glucose utilization, biomass (OD_{600}) profiles and rWbSXP-1 concentrations after induction are shown. *C* and *I* denote control and induced conditions. rWbSXP-1 was estimated using sandwich ELISA with polyclonal rabbit and polyclonal mice anti-WbSXP-1 specific antibodies

Table 1 Comparison of maximal biomass (dry cell weight) and specific protein yield in airlift bioreactor

Capacity	Maximal DCW (g/L)	Specific protein yield (mg / g DCW)
ALB 3 L	3.72 ± 0.2	8.39 ± 0.1
ALB 30 L	36.8 ± 0.3	8.18 ± 0.1

Averages arrived from batches = 3 and estimated in triplicates

and 30 L ALB was comparable at varied aeration and agitation (data not shown). The maximal biomass achieved and specific rWbSXP-1 yields in 3 and 30 L ALB's shown in Table 1, was reasonable to comprehend the efficiency.

Optimization of rWbSXP-1 purification

Escherichia coli lysis was carried out to recover rWbSXP-1 protein. Experiments on protein purification from GJ1158 were initially tried by IMAC in 2.5 mL bed volume using both urea solublization (8 M) and soluble fractions (native) or clarified lysates obtained after french pressure. Though the recovery of rWbSXP-1 is more in the urea solubilized purification, the contaminating proteins in purified product was more as evident from the lower specific activity and lesser fold purification. Whereas the purification from the soluble fraction had slightly lesser recovery but highly purified product was obtained (Table 2).

Further, urea solublized rWbSXP-1 has other problems such as much diluted product, removal of urea, refolding the protein, etc. for diagnostic application. On the other hand, purification from soluble fractions at higher scales with FPLC columns (15 mL bed volumes) showed identical levels of purity and recovery of rWbSXP-1. The rWbSXP-1 protein eluted at 250 mM imidazole. The concentrated IMAC purified samples had few contaminating host proteins as evident from Fig. 3, which has to be removed in the subsequent steps.

A purification strategy involving anion exchange chromatography and gel filtration to effectively remove the contaminating proteins was investigated. The IMAC purified rWbSXP-1 was dialyzed, freeze dried (10 mg/mL) and purified by either anion exchange chromatography or gel filtration. WbSXP-1 protein eluted at 400 mM NaCl in anion exchange. The purity of WbSXP-1 protein obtained after IMAC step followed by gel filtration and anion exchange chromatography was analyzed by SDS-PAGE (Fig. 2, lanes 3 and 4). A higher level of purity was apparent in IMAC followed by gel filtration procedure. The protein was assessed for immuno-reactivity by western blot (Fig. 3).

The rWbSXP-1 protein recovery was estimated after each purification step compared to total rWbSXP-1 protein concentration present in culture during harvest. Fold purification was derived from the total protein and specific rWbSXP-1 concentration at each purification step. The results showed that the fold purification was maximal in IMAC followed by gel filtration (Table 2). The recovery of rWbSXP-1 was 75% considering the initial concentration at harvest, whereas, it was 95% from the soluble fraction alone. The total protein were estimated by Bradford method and correlated by densitometry, whereas the rWbSXP-1 protein was quantified by sandwich ELISA.

Enhancement of sensitivity of rWbSXP-1 antigen based diagnostic assay

On the basis of field and clinical requirement to enhance sensitivity and spot size on the performance of already existing rapid flow through assay format [6], we obtained different serum groups comprising of MF positives, CP, NEN and EN. MF positive sera were analyzed for the antibody titre levels by ELISA coated (Fig. 4) and further classified them into weak to very strong reactive patient groups exposed to lymphatic filariasis. The other cases of CP, EN and NEN sera were used as control. Of the various serum dilutions experimented for ELISA, 1:20 was found to be optimal for any sera group. The enhancement of rapid format was optimized with this panel of known cases of human lymphatic filariasis with weak, moderate, strong and very strong exposure to the disease. For protein the maximum concentration at which the undiluted control serum might just show a slight background is the optimal

Table 2 Comparison of rWbSXP-1 recovered after purification using different chromatographic steps

Purification steps	Total protein, mg	rWbSXP, mg	Specific activity	Fold purification	Recovery, %
Initial broth sample	940	15.6	0.016	1	100
IMAC- soluble fraction (native)	16.3	12.02	0.741	44.7	77
IMAC (urea solubilization)	23.2	12.8	0.551	33.2	82
IMAC (native) and gel filtration	12.2	11.78	0.965	58.1	75.5
IMAC (native) and anion exchange	12.1	10.8	0.892	53.7	69.2

The final rWbSXP-1 concentration in broth (harvest) and in soluble fractions after lysis was estimated to be 15.6 mg/L (desitometry) and 12.4 mg/L (sandwich ELISA), respectively. Purification runs = 5 and estimated in triplicates

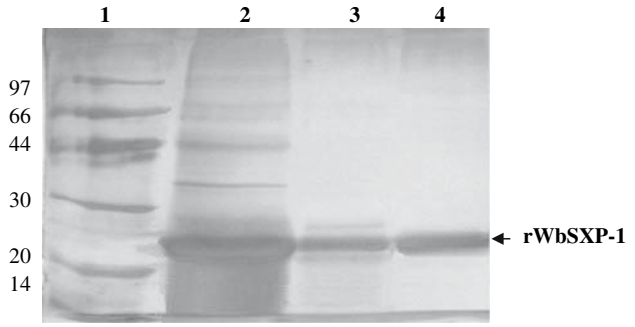

Fig. 2 Silver stained SDS-PAGE showing quality of purified rWbSXP-1 after each step. *Lane 1* protein molecular weight maker, *lane 2* IMAC, *lane 3* IMAC followed by anion exchange, *lane 4* IMAC followed by gel filtration

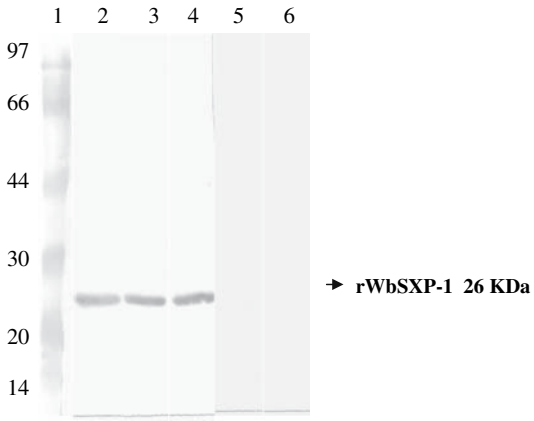

Fig. 3 Western blot analysis showing the immuno-reactivity of purified rWbSXP-1 treated separately. *Lane 1* molecular weight marker, *lane 2* human microfilareamic positive serum, *lane 3*: MAb-anti-His antibody, *lane 4* murine anti-wbSXP-1 polyclonal antibodies, *lanes 5, 6* the human endemic normal serum, normal mouse serum used controls, shows no reactivity

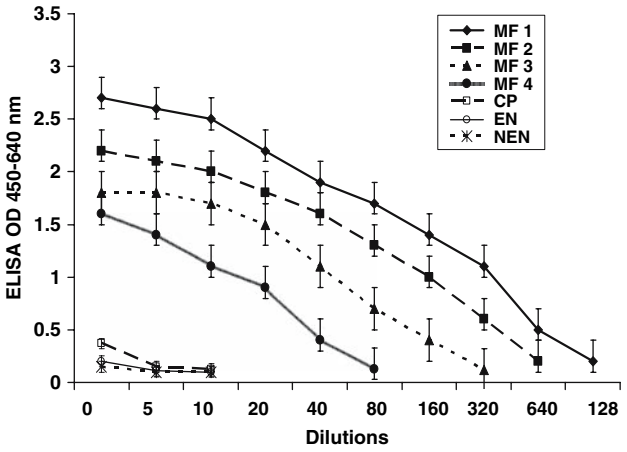

Fig. 4 ELISA (coated 0.5 µg rWbSXP-1) showing microfilareamic positive serum titre of MF1 ($n = 4$); very strong reactivity, MF2 ($n = 3$); strong reactivity MF3 ($n = 4$); moderate reactivity MF4 ($n = 4$); weak reactivity and control groups chronic pathology (CP; $n = 15$), endemic normal (EN; $n = 15$) and non-endemic normal (NEN; $n = 15$) groups. The mean values and deviation bars are represented. Each sample was tested in triplicates. Undiluted serum (0) in control groups did not show reactivity

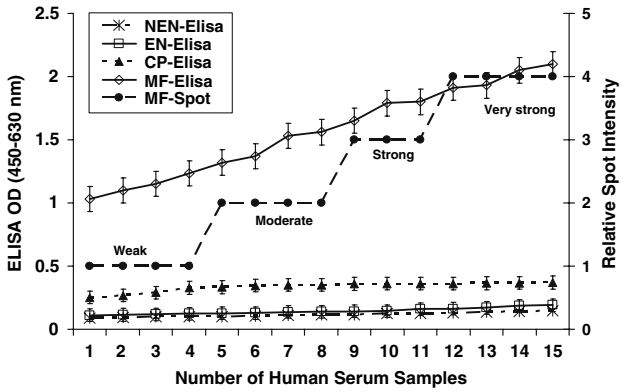

Fig. 5 ELISA (rWbSXP 125 ng, sera dilution of 1:20) and rapid kit (rwbsxp-1 500 ng and 1:5 sera dilution) based analysis of the MF groups classified based on degree of exposure. The ELISA values of chronic pathology (CP), non-endemic normal (NEN) and endemic normal (EN) control groups are reasonable and the cutoff value for a weak positive reaction was fixed at >0.9 OD after 10 min incubation. The CP, NEN and EN sera applied at 1:5 dilutions on kit did not show any colour spot. In ELISA mean values (triplicates) are with error bars are shown. The spot intensity is a relative value indicated as per industrial standards done in triplicates

cutoff value. The maximum protein concentration thus coated, the dilution of control group serum at which it does not produce a slight background is the optimal cut off for serum. However, background did not appear even in undiluted control serum. The range shows, weak serum with a value of 1 and very strong serum with a value of 4 in the relative spot intensity. The rWbSXP-1 protein requirement or cutoff values on enhanced rapid format was optimized to 0.5 µg in 0.7 µL per spot, which is 4 times of that used in ELISA format. The serum dilution for rapid format was optimized and 1:5 dilutions was effective for any MF serum group. The detection pattern of ELISA and rapid formats were compared, the ELISA and rapid formats showed similar trend in antibody reactivity levels and was clearly distinguished into weak to very strong (Fig. 5). The antibody titre levels for MF positive test groups based on degree of antibody reactivity were analyzed in both ELISA and rapid formats and the curves were compared (Fig. 6). Thus, sensitivity to capture the circulating antibodies in serum using rapid format was similar to the response in ELISA format and shows spot intensity was proportional to titre. The correlation of ELISA and rapid kit formats is shown in Figs. 5 and 6 with 100% concordance in all MF and control groups tested during the optimization.

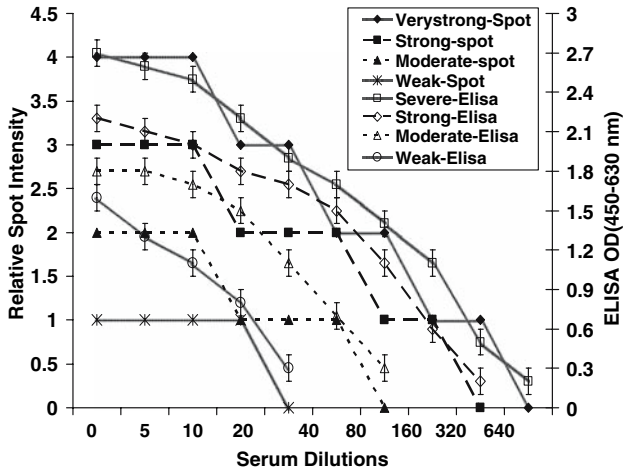

Fig. 6 Performance analysis of the rWbSXP-1 antigen (0.5 μg) in ELISA and rapid formats based on serum antibody titre in different microfilareamic positive (MF) groups showing weak, moderate, strong and very strong reactivity. The trend in detection of antibodies by ELISA and rapid kit is similar and gives a titre-based prediction to the exposure or antibody levels

Evaluation of enhanced diagnostic kit format

The performance and efficacy of improvised rapid kit was studied by screening with human serum collected from healthy individuals (blood banks). The enhanced kit could detect cases to be positively exposed to the disease. We also tested the efficacy of the enhanced rapid diagnostic kit by screening with known MF positive serum samples obtained from health departments and medical institutes (Table 3).

Performance of rapid diagnostic kit was further evaluated using the positive cases identified from normal or healthy test group showing weak, moderate, strong and very strong antibody reaction. The rWbSXP-1 ELISA showed 100% concordance; Bm-rapid and Wb-ICT kits had 75% concordance (43.75 + 31.25) with rWbSXP-1 rapid format, whereas 25% cases in weak reaction group could not be detected by either of the commercial kits (Table 3). In strong reaction group 12.5% (2/16) were positive in both Bm-dipstick and Wb-ICT kits showing some cross reactivity, while, it was 12% (3/25) in the known MF cases tested. Thus, slightest antibody levels were enough to be positive in rWbSXP-1 kit. However, antibody detection cannot be taken into account to ascertain active filarial infections or it rather indicates presence of antibodies due to an exposure to disease.

Stability kinetics of enhanced diagnostic kit

Performance of the improvised diagnostic kit was subjected to accelerated stability test as per the guidelines of European pharmacopoeia, at 4, 37 and 45 °C. The kits were tested against dilutions of high titre MF serum and compared. The maximum dilution where there occurred a positive spot and the final dilution at which no positive spot occurred was identified in rapid kit format. The maximal dilution (1:320 in this study) there occurred a positive spot was tested for stable performance and consistency of the results, (Figs. 7–9). Endemic normal serum at 1:5 dilutions was used as control and did not develop any positive spot. The kits were stored at 4 °C and its stability was regularly

Table 3 Performance of rapid diagnostic kit

Degree of antibody reactivity in rapid format	Known MF cases (rWbSXP-1 kit)	Serum of healthy individuals screened		Bm-rapid		Wb-ICT	
		(rWbsxp-1 kit)	ELISA	IP	MF	IP	MF
Total tested	25	320	16	16	25	16	25
Weak reaction	2	5	5	1	2	1	2
Moderate reaction	3	3	3	3	2		2
Strong reaction	12	5	5	2	4	2	5
Very strong reaction	8	3	3	1	3	2	5
Total Exposed or positive	25	16	16	7	11	5	14
Percent (%) exposed	100	5	100	43.75	44	31.25	56
Specificity%	100	–[a]	100		100		100
Sensitivity%	100	–[a]	100		100		100

The confirmed MF clinical cases were identified by night blood smears, whereas the serum of individuals tested during evaluation are donors in blood banks and do not represent night blood samples. The groups were categorized by titre based reactivity in the rWbSXP-1 kit and the commercial kits were used only to distinguish the specific causative infections. IP indicates identified positives

Bm dipstick and Wb-ICT are antigen detection kits and do not indicate titre

[a] Rapid WbSXP-1 kit shows 100% concordance in rWbSXP-1-ELISA, the dilution pattern correlated with spot intensity values. Also, it shows 75% (43.75 + 31.25) concordance with Bm-rapid and Wb-ICT kits

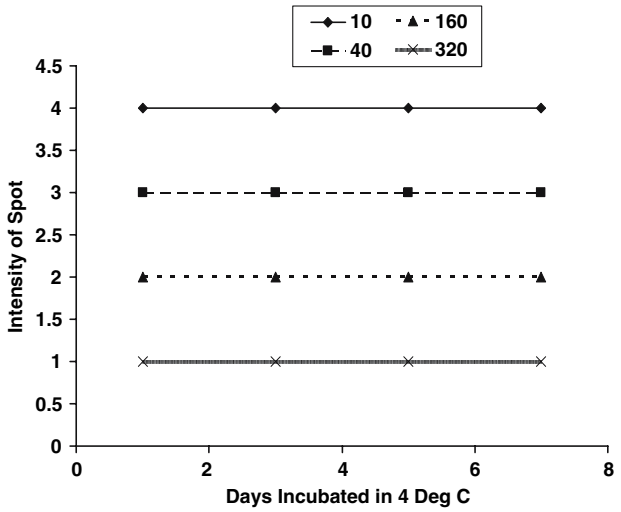

Fig. 7 Stability kinetics at 4 °C with high tire microfilareamic positive (MF) serum at different dilutions. Endemic normal (EN) serum (1:5) was used as control showed no spot

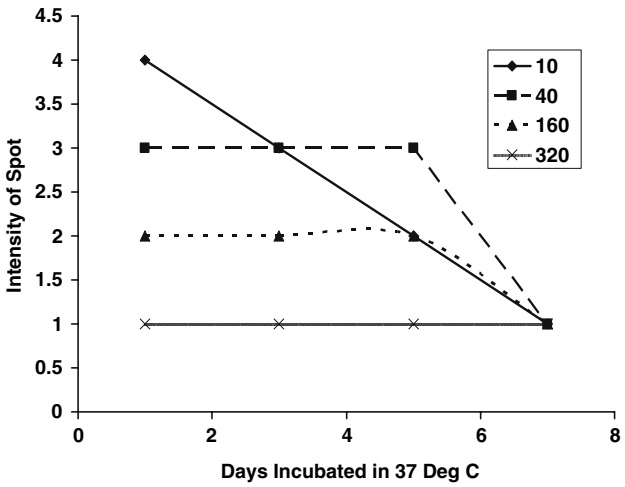

Fig. 8 Stability kinetics at 37 °C with high tire microfilareamic positive (MF) serum at different dilutions. Endemic normal (EN) serum (1:5) was used as control

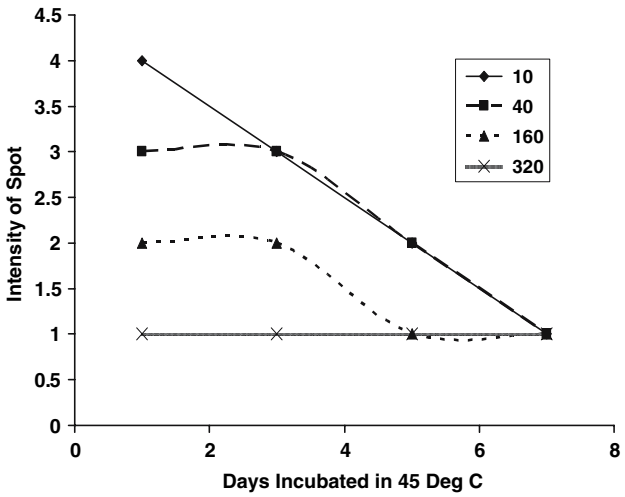

Fig. 9 Stability kinetics at 45 °C with high tire microfilareamic positive (MF) serum at different dilutions. Endemic normal (EN) serum (1:5) was used as control

monitored over a period of 1, 3, 6 and 12 months and assessed to be satisfactory as per the industrial norms.

Discussion

For the early diagnosis of human lymphatic filariasis, rWbSXP-1 was identified to be a prospective candidate [16, 25] and evaluation results of national and global surveillance were highly encouraging [6, 17]. However, the sensitivity was needed to be improved further to detect weakly reactive serum antibodies. To cover large endemic areas for surveillance, developing an economical production of rWbSXP-1 based diagnostic kits was critical for extending the technology to industrial application. The advantage with protein over-expression in osmotically inducible GJ1158 was that inclusion body formation was three times less [9]. Moreover, in this work airlift bioreactors (ALB) were opted. ALB's are economical and energy efficient compared to CSTR's [29]. In glucose based media oscillations in growth rate affects productivity, thus it is important to design media depending on the *E. coli* strain for the avoidance of acetate accumulation [1, 8, 26]. The recombinant protein production in GJ1158 with modified M9 medium using 30 L ALB was consistent. For downstream processing, many cell disruption methods such as sonication, french pressure and enzyme treatment have been reported for *E. coli* [2, 4, 31]. The purification of any protein by chromatography and number of steps required varies and depends on the nature of protein itself [18, 19]. Multi-step purification strategies reduce the recovery of the target protein though the purity may improve tremendously [28]. The purity and quality of rWbSXP-1 was observed to be very critical in performance of the diagnostic kit. The purification of rWbSXP-1 protein, although was successful in 2.5 mL bed volume, posed problem in achieving purity at higher bed volumes. In addition during IMAC, the recovery of rWbSXP-1 from soluble fraction was significantly high compared to urea solubilization. The loss in recovery during urea solubilization, purification and refolding has been reported to be greater than 10% [2]. Thus, the rWbSXP-1 largely available in soluble fraction was considered for further purification experiments in this work. The inclusion of anion exchange chromatography step after the IMAC considerably enhanced the purity of the rWbSXP-1, but an increased loss was also observed. Remarkably, the purity and recovery of the rWbSXP-1 greatly improved

with the inclusion of gel filtration after IMAC. The estimated loss of rWbSXP-1 recovered after IMAC followed by gel filtration was 25% as compared to 31% in anion exchange. Considering the maximum rWbSXP-1 released into soluble fraction, the loss after IMAC and gel filtration was 5%. The quality of purified rWbSXP-1 protein tremendously improved to meet the manufacturing requirements in diagnostic industry. Further, the sensitivity of rapid diagnostic kit was considerably improved to detect even weak exposure to lymphatic filarial infections. The enhanced rapid assay was particularly sensitive in identifying the degree of exposure by virtue of the antibody reactivity and titre related spot intensity. The enhancement of spot size and sensitivity based spot intensity was a key development in assessing the antibody reactivity and exposure in individuals or MF positive cases, compared to a less sufficient results of mere positive or negative tests as described previously [6]. Thus, the rapid test will be useful in providing status of endemic population for mass drug administrative programs and its withdrawal. During evaluation studies the immuno-characteristics and response of purified rWbSXP-1 in detecting anti-SXP antibodies in specific cases of microfilareamic positive serum and from healthy test groups were satisfactory. Stability of rWbSXP-1 protein based rapid flow through antibody assay kit was incompliance with European pharmacopoeia and WHO-cGMP regulations for manufacturing. This investigation presents a successful and consistent economic rWbSXP-1 protein production in 30 L airlift bioreactor using a salt inducible *E. coli* GJ1158. We have successfully optimized downstream processing and protein purification in technical scale considering the future industrial applications. The performance of improved rapid flow through antibody assay for diagnosis of human lymphatic filariasis was more sensitive and satisfactory. This work is expected to suit the conditions in developing and under developed endemic countries where diagnosis, surveillance and eradication of the disease is an important concern.

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