INTRODUCTION

Sarcoptes scabiei var. hominis (S. scabiei var. hominis) causes a common human skin infestation known as scabies.1–4 Scabies occurs worldwide among people of all ages, races, genders, and social classes and has been identified as a neglected tropical infectious disease.5,6 It has been estimated that about 1–10% of the global population is infected with scabies with an infection rate as high as 50–80% in certain populations.7 Scabies ranges from common ordinary scabies (OS) – infection of 10–15 mites per person8 to rare crusty scabies (CS) with infestation of up to millions of mites per person.9 Clinical manifestations of OS are wide ranging, depending on degree and activation of the inflammatory response, burrows, and itching. CS is a severe form of the disease and often found in patients with human immunodeficiency virus infection, human T lymphotropic virus 1 (HTLV-1) infection, organ transplant patients, the mentally impaired, and physically incapacitated. It has also been recognised in overtly immunocompetent patients.10 These patients have been recognized as core transmitters of scabies,11 due to hyperproliferation of mites.

Scabies is a challenging disease to diagnose due to difficulties in isolation of Sarcoptes mite on the human host in OS4 and physical signs confounding with other skin diseases. There is no in vitro propagation system of S. scabiei for antigen preparation. Routinely used diagnostic methods are direct observation of clinical signs including the typical history of pruritus, presence of inflammatory papules, and visible lesions on the host body. The microscopic identification of scabies mites or eggs in infected skin scrapings is rarely carried out but shows less than 50% sensitivity.12,13 Clinical diagnosis is often complicated because visible lesions on scabies patients are sometimes masked by eczema or impetigo or are atypical.4 Alternative diagnostic options include polymerase chain reaction (PCR)14 and dermatoscopy,15 both of which have been demonstrated as informative in patients where mites or mite DNA can be located. This practice is uncommon worldwide and not easily applied to clinical health settings.

Because of the lack of sensitivity in the present diagnostic methods for human scabies, there is a great need for the development of a reliable serodiagnostic test such as enzyme-linked immunosorbent assay (ELISA) to control scabies at both the individual and community level. There is no commercially available ELISA for the diagnosis of scabies in humans but an ELISA has been developed for detection of mange in animals utilizing whole mite antigen (WMA) extracts from S. scabiei var. suis.16,17 However, the reported specificity and sensitivity of these tests are variable.18,19

Expressed sequence tag (EST) libraries from S. scabiei var. hominis and var. vulpis are available, and include homologues of multiple house dust mite (HDM) allergens, opening new possibilities in scabies research.20–23 As a result, several S. scabiei homologues to multiple HDM allergens have been sequenced, cloned, and expressed including S. scabiei serine proteases, cysteine proteases, glutathione S-transferase, and apolipoprotein.21,22,24,25 The S. scabiei var. hominis cysteine protease and apolipoprotein have been shown to bind scabies specific antibodies in IgE ELISA tests.26 The S. scabiei apolipoprotein is considered a potentially valuable serodiagnostic agent as it has been proved to be highly specific for scabies and does not show cross reactivity with HDM allergens.12,26 Recombinant S. scabiei cofilin also showed potential value as a diagnostic antigen and could be used to develop an ELISA-based serological test for the diagnosis of scabies in animals.27

Trompomosin, a muscle protein, is classified as a group 10 allergen.28 It is reported as a major allergen of shellfish that is
cross-reactive with Der p 10, the HDM tropomyosin and an important component of immune and allergic reactions. Tropomyosin has a conserved sequence and is a pan-allergen in invertebrates including shrimps, molluscs, mites, and cockroaches with 80% amino acid sequence homology between different species, that is, S. scabiei, Dermatophagoides pteronyssinus, Dermatophagoides farinae, Blomia tropicalis, and Psoroptes ovis. Several isoforms of the protein exist within a species. Sarcopes scabiei recombinant tropomyosin was evaluated as a vaccine candidate, but showed no significant protection against sarcoplastic mange in rabbits.

Paramyosin is a major HDM allergen and designated as group 11 allergen. It has a molecular weight of approximately 98 kDa and is conserved among a variety of species. Tropomyosin has a conserved sequence and is a pan-allergen in invertebrates. In general, invertebrate paramyosin is cross-reactive in many invertebrates, including shrimps, molluscs, mites, and cockroaches with 80% amino acid sequence homology between different species, that is, D. farinae, D. pteronyssinus, S. scabiei, P. ovis, and S. scabiei. Several isoforms of the protein exist within a species. S. scabiei var. vulpus sequence (Table 1). The nucleotide positions of these overlapping Sar s 11 fragments were Sar s 11 = 1–1,612 bp, Sar s 2 = 1,300–2,100 bp, and Sar s 3 = 1,750–2,652 bp. Cycling conditions for Sar s 11 and Sar s 12 were initial denaturation 95°C, 2 minutes, followed by 35 cycles of 95°C, 30 seconds; 50°C, 30 seconds; 72°C, 2 minutes; with final extension at 72°C, 8 minutes.

For the amplification of Sar s 11 cDNA, three sets of primers were designed to amplify these three overlapping paramyosin fragments (Sspara1, Sspara2, and Sspara3) based on S. scabiei var. vulpus sequence (Table 1). The nucleotide sequence43 (Table 1). The nucleotide sequence was translated and analyzed by BLASTx (https://blast.ncbi.nlm.nih.gov/Blast.cgi) for homology in GenBank protein database of Sar s 10 and Sar s 11. The resulting top hit amino acid sequences of Sar s 10 and Sar s 11 were aligned using ClustalW (http://www.genome.jp/tools-bin/clustalw) and aligned sequences were further used for phylogenetic tree construction using the software Molecular Evolutionary Genetics Analysis (MEGA 6).

Expression and purification of recombinant Sar s 10 and Sspara1, Sspara2, and Sspara3. The cDNAs encoding Sar s 10 and Sspara1, Sspara2, and Sspara3 were subcloned into pET-15b and pET-28a expression vectors, respectively, in frame with the 6-His tag and then expressed in the Escherichia coli strain BL21 (DE3) (Bioline). Recombinant protein expression was induced by addition of 0.4 mM isopropyl-β-D-thiogalacto-pyranoside to the culture at 37°C for 4 hours. For purification of the recombinant Sar s 10 and Sar s 11 protein fragments, the pellet was resuspended in native lysis buffer (50 mM NaH2PO4, 300 mM NaCl, pH 8.0), treated with lysozyme (1 mg/mL) and one complete ethylenediaminetetraacetic acid-free protease inhibitor cocktail tablet (Roche, Castlehill, New South Wales, Australia), incubated for 30 min following sonication. The lysate was centrifuged for 30 minutes (10,000 × g at 4°C) and the supernatant collected for purification. The recombinant proteins were purified using Nickel-nitroacetic-acid (Ni-NTA) agarose column as per the manufacturer’s standard protocol (Qiagen) under native conditions. The purified proteins (Sar s 10, Sspara1, Sspara2, and Sspara3) were quantified using the Qubit™ Protein Assay Kits provided with the Qubit™ 2.0 Fluorometer following the manufacturers’ protocol (Life Technologies). Proteins were column purified by PureLink RNA mini Kit (Life Technologies, Mulgrave, Victoria, Australia) following manufacturers protocol. The concentration was checked on the NanoDrop 2000 Spectrophotometer (NanoDrop Technologies, Scoresby, Victoria, Australia). One microgram of total RNA was transcribed to cDNA using the QuantiTect Reverse Transcription Kit (Qiagen, Chadstone, Victoria, Australia) as per manufacturer protocol. For the amplification of Sar s 10 cDNA, primers were designed based on Der p 10 sequence using the Primer3 software (http://frodo.wi.mit.edu/) (Table 1). Cycling conditions for Sar s 10 primer were initial denaturation 95°C 2 minutes, followed by 35 cycles of 95°C, 30 seconds; 50°C, 30 seconds; 72°C, 2 minutes; with final extension at 72°C, 8 minutes.

For the amplification of Sar s 11 cDNA, three sets of primers were designed to amplify these three overlapping paramyosin fragments (Sspara1, Sspara2, and Sspara3) based on S. scabiei var. vulpus sequence (Table 1). The nucleotide sequence was translated and analyzed by BLASTx (https://blast.ncbi.nlm.nih.gov/Blast.cgi) for homology in GenBank protein database of Sar s 10 and Sar s 11. The resulting top hit amino acid sequences of Sar s 10 and Sar s 11 were aligned using ClustalW (http://www.genome.jp/tools-bin/clustalw) and aligned sequences were further used for phylogenetic tree construction using the software Molecular Evolutionary Genetics Analysis (MEGA 6).
analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).

**SDS-PAGE and immunoblotting.** Recombinant proteins (2 μg/lane) were separated by 12% SDS-PAGE and stained with Coomassie Brilliant Blue R-250, or by immunoblotting. For immunoblotting, purified proteins (2 μg), Sar s 14.325 (2 μg) as a positive control, and bovine serum albumin (0.5 μg) as a negative control were run on SDS-PAGE and proteins transferred to nitrocellulose membranes using the Biorad Trans-Blot Turbo Transfer System (Biorad, Gladesville, New South Wales, Australia). Membranes were blocked with 5% skim-milk powder (SMP) in phosphate-buffered saline with 0.5% Tween 20, overnight at 4°C. Membranes were incubated with 1:50 dilutions of pooled sera from 1) three OS subjects; 2) three HDM subjects; and 3) three scabies naive subjects, followed by anti-human IgE alkaline phosphatase (AP)-conjugated H&L (Abcam ab7426, Sapphire Bioscience, Melbourne, Victoria, Australia). Between each step, membranes were washed three times with 0.05% PBS-T. Membranes were developed and visualized with AP substrate (BCIP/NBT) (Sigma-Aldrich, Castle Hill, New South Wales, Australia).

**Enzyme-linked immunosorbent assay.** A three-stage ELISA was performed for both tropomyosin and for fragments of paramyosin (individually and then cocktail of Sspara2 and Sspara3 fragments). Briefly, 2 μg/well Sar s 10 and Sspara2 and Sspara3 fragments was bound to High Protein 96 well plates (Corning) (Sigma-Aldrich) in carbonate coating buffer (15 mM/L Na2CO3, 35 mM/L NaHCO3, 3 mM/L NaN3, pH 9.6) overnight at 4°C. Plates were blocked with 1% SMP-PBS-T (0.01%) at 37°C for 2 hours, then serum applied at a 1:10 (IgE assay) or 1:200 (IgG assay) diluted in 1% SMP-PBS-T (0.01%) at 37°C for 2 hours. Between each step, plates were washed three times with 0.05% PBS-T. Secondary antibody, either 1/1,000 anti-human IgE (Abcam ab91559, Sapphire Bioscience) (IgE assay) or AP-conjugated anti-human IgG dilution (Sigma-Aldrich, A9544) diluted 1/2,500 in 1% SMP-PBS-T at 37°C for 2 hours. After washing, AP-conjugated polyclonal rabbit anti-goat IgG H&L (Abcam ab6742, Sapphire Bioscience) (1/1,000 dilution) (IgE assay) was incubated at 37°C for 2 hours. Color was developed using phosphatase substrate (Sigma-Aldrich) dissolved in glycine buffer (0.1 M/L glycine, 1 mM/L MgCl2, 1 mM/L ZnCl2, pH 10.4) and developed for 30 minutes at 37°C and absorbance was measured at 405 nm with the Perkin-Elmer Enspire 2300 Multimode Reader and Enspire software 4.13 (Perkin-Elmer, Waltham, MA).

**Statistical analysis.** Prism v. 5.01 (GraphPad Software, Inc., San Diego, CA) software was used to carry out statistical analysis. Differences between groups were assessed by nonparametric Kruskal–Wallis analysis of variance. To check the distribution of two unmatched groups, nonparametric Mann–Whitney U tests were used. Comparisons were considered to be significant at P values of < 0.05. The ELISA cutoff was calculated by taking the average of the negative control group (naive) + 2 standard deviations. To calculate sensitivity percentages following formula was used: true positives/(true positives + false negatives) × 100% whereas specificity percentages were calculated by: (true negatives/ [true negatives + false positives]) × 100%. The receiver operator characteristic (ROC) curves and the area under the curve were calculated using Prism for subjects with a current scabies...
infestation (OS) against subjects with HDM allergy, or no infestation (naive).

RESULTS

Amplification of cDNA-encoding Sar s 10 and Sar s 11. The PCR product obtained by amplification with Sar s 10 primers (F/R) contained an open reading frame (ORF) of 855 bp and encoded a predicted protein of 284 amino acids. The Sar s 10 sequence was deposited in the European Nucleotide Archive database under accession number LT669813. The top hits when the nucleotide sequence for Sar s 10 was searched with D. pteronyssinus showed 99% identity with \( D. \) pteronyssinus (Q9NFZ4.1). The next two species with closest homologues were Chortoglyphus arcuatus and Lepidoglyphus destructor with 95% identity. With Sar s 10 Glycyphagus domesticus and B. tropicalis, tropomyosin showed 91% and 94% identification (Figure 1).

The sequence of the three partial overlapping fragments of paramyosin Sar s 11 (Sspara1, Sspara2, and Sspara3) was assembled into contigs using DNA Dragon-DNA Sequence Contig Assembler Software (http://www.dna-dragon.com/) and encoded into contigs using DNA Dragon-DNA Sequence Assembly Software (http://www.dna-dragon.com/assembly). The sequence of the three partial overlapping fragments of paramyosin Sar s 11 was assembled into contigs using DNA Dragon-DNA Sequence Contig Assembler Software (http://www.dna-dragon.com/download.php), and the complete Sar s 11 ORF was 2,652 bp in length and encoded 884 amino acids and is available in the European Nucleotide Archive under accession number LT669814. The sequences showed the highest homology to Sar s 11 from S. scabiei var. suis, var. canis, var. cuniculi with amino acid identity of 99%. Chorioptes panda and P. ovis had amino acid identity of 83% and amino acid identity of 82% was shown by Chorioptes texanus, D. farinae, and D. pteronyssinus, whereas the amino acid identities of Sar s 11 was lower at 79% for B. tropicalis (Figure 2).

Expression and purification of recombinant Sar s 10 and Sar s 11 fragments. The full coding sequence of the S. scabiei Sar s 10 and Sar s 11 fragments was cloned into the vectors pET-15b and pET-28a, respectively. The expression of the analyzed proteins was checked by indirect ELISA for binding of IgG to Sspara2 and Sspara3.

Figure 1. Relationships of identified Sar s 10 sequence with selected tropomyosin sequences. The amino acid sequences of the identified molecules were aligned using ClustalW with phylogenetic tree was constructed using MEGA 6. Abbreviations and accession numbers are as follows: S. scabiei var. suis (AF311760.1), D. pteronyssinus (DQ131648.1), D. farinae (K010008.1), D. pteronyssinus (AY189697.1), P. ovis (AM114275.1), C. panda (EU543652.1), C. taxanus (EF053124.1), B. tropicalis (AF525465.1).

Figure 2. Relationships of identified Sar s 11 sequence with selected paramyosin sequences. The amino acid sequences of the identified molecules were aligned using ClustalW with phylogenetic tree was constructed using MEGA 6. Abbreviations and accession numbers are as follows: S. scabiei var. canis (AF311760.1), S. scabiei var. suis (EU606301.1), S. scabiei var. hominis 1 (LT669813), S. scabiei var. suis (AK484744.1), C. arcuatus (AF031649.1), B. tropicalis (AF031649.1), G. domesticus (AF031649.1), L. destructor (Q9NFZ4.1).
There was a significant difference in mean IgG binding to Sspara2 between the OS group and for the subjects naive to scabies group (P < 0.0001). Interestingly, however, there is no significant difference in mean IgG binding between OS and HDM allergy subjects (P = 0.3907) (Figure 6, left panel).

IgG binding to Sspara3 was significantly different between the OS group and for the subjects naive to scabies group (P = 0.0130), as determined by Mann–Whitney U test. There was no significant difference between OS and HDM subjects (P = 0.7969) (Figure 6, right panel).

The IgG binding to Sspara3 was significantly different between the OS group and for the subjects naive to scabies group (P = 0.0130), as determined by Mann–Whitney U test. There was no significant difference between OS and HDM subjects (P = 0.7969) (Figure 6, right panel).

The IgG binding to the antigen cocktail (mixture of Sspara2 and Sspara3) of individual OS sera (N = 48) was high. The mean value of the OS group was significantly higher compared with HDM and naive groups (P < 0.0001). The HDM allergy subjects mean IgE response to the cocktail antigen was significantly different than the mean observed for subjects in the naive to scabies group (P = 0.7969) (Figure 6, right panel).

The mean IgG binding to the antigen cocktail for the OS group was significantly greater compared with HDM (P = 0.0007) and naive groups (P = 0.0001). There was also significant difference in IgG binding between HDM and naive subjects (P = 0.0024) (Figure 7, right panel).

Comparison of diagnostic sensitivity and specificity of recombinant Sar s 10 and Sar s 11 fragments. The diagnostic sensitivity of the Sar s 10 for detection of active scabies infestation was very low. The assay had 30% and 67% sensitivity and specificity, respectively, with a positive likelihood ratio of 0.95 and a negative likelihood ratio of 1.11. For Sspara2, 98% sensitivity and 90% specificity were observed with a positive likelihood ratio of 5.48 and a negative likelihood ratio of 0.03. The area under curve was 0.9706. In regard to IgG binding, Sspara2 has 70% sensitivity and 96% specificity and area under curve was 0.82. The diagnostic sensitivity of IgG was low compared with IgE with this antigenic fragment. For Sspara3, 84% sensitivity and 100% specificity were observed, with a positive likelihood ratio of 4.00 and a negative likelihood ratio of 0 and area under curve was 0.9600. With regard to IgG binding, Sspara3 has 40% sensitivity and 87.5% specificity and area under curve was 0.70. The diagnostic competence of the antigen cocktail was calculated at 94% sensitivity and 100% specificity for IgE binding and 62% sensitivity and 92% specificity for IgG binding. In comparing both antigens, that is, Sspara2 and Sspara3, Sspara2 gave excellent diagnostic specificity and sensitivity (Table 2).

DISCUSSION

Scabies and Sar s 10. The tropomyosin (Sar s 10) and paramyosin (Sar s 11) encoding genes of S. scabiei var. hominis were characterized and inferred as immuno-reactive allergens in scabies. Sar s 10 from S. scabiei var. hominis has an open reading frame of 855 bp and 285 amino acids, coding for an allergenic 31 kDa protein. Tropomyosin is considered a pan-allergen in invertebrates31 and reported as a cross reactive allergen among invertebrates42 because of the high degree of amino acid sequence identity.34 Sar s 10 amino acid sequence revealed a 99% sequence homology with S. scabiei var. suis.33 This protein also revealed 97% homology to the group 10 allergens of D. pteronyssinus, D. farinae, and P. ovis.45 The S. scabiei tropomyosin also displayed 90–96%
homology with storage mites, that is, L. destructor, G. domesticus, B. tropicalis, C. arcuatus. This result was not unexpected and in support of previous data reporting the group 10 HDM allergen as highly conserved and cross-reactive with tropomyosin of species such as shellfish and other arthropods.

Among insects, S. scabiei tropomyosin had 80% amino acid identity with Periplaneta americana, Blattella germanica. It has been demonstrated that tropomyosin protein from cockroaches shares 80% homology with other arthropod tropomyosin. Tropomyosin was also found to be a major allergenic component accounting for the cross-reactivity between cockroaches and dust mites. In several studies tropomyosin is reported as major shellfish allergen and commonly used as a biomarker for detection of shellfish allergens. The tropomyosin protein from cockroaches also shares homology of 58%, 64%, 74% with other invertebrates, that is, Ascaris lumbricoides, Sinonovacula constricta, Haliotis diversicolor, respectively. Because of the high sequence identity of Sar s 10 with other mite and invertebrate tropomyosins, it is unlikely to be a specific allergen for Sarcoptic mites and may have high cross-reactivity with tropomyosin of other mites.

**Immunoaalysis of Sar s 10.** Western blot analysis showed IgE binding by pooled OS patient sera and additionally with pooled HDM positive and naive sera. Previous studies reported that naïve and HDM patient sera exhibited strong binding to variety of S. scabiei antigens. Western blot and dissociation-enhanced lanthanide fluorescence immunoassay (DELFIA) has also demonstrated that individuals with scabies and HDM allergy showed strong IgE binding to both scabies mites and HDM antigens.

The sera from OS subjects, HDM-positive subjects, and naïve subjects showed no clear difference in specific IgE
binding to Sar s 10. Strong IgE antibody binding to tropomyosin from *A. lumbricoides* and *P. americana* has been reported in sera from patients with cockroach allergy. From this study, high homology of tropomyosin within invertebrates has been established and studies have shown that tropomyosin from phylogenetically related organisms have IgE reactivity from allergenic individuals. Thus, the observed homology in the multiple sequence alignment and subsequent binding of IgE antibodies in all groups to Sar s 10 are consistent with other studies.

**Scabies and Sar s 11.** The cDNA for *S. scabiei var. hominis* Sar s 11 is 2,652-bp long encoding for 884-kDa protein. The three oligonucleotide fragments of Sar s 11 from *S. scabiei var. hominis* have open reading frames of 1,612-, 800-, and 900-bp nucleotides. These three overlapping fragments have a deduced amino acid sequence of 537, 267, and 300 amino acids encoding for 55-, 29-, and 33-kDa allergenic protein fragments designated as Sspara1, Sspara2, and Sspara3 respectively. Paramyosin is found only in invertebrates and the molecule is evolutionary somewhat conserved among a variety of species. Paramyosin has been identified as a major immunogen in parasites and has shown potential as a vaccine candidate for parasitic diseases such as filariasis and schistosomiasis. It is a major allergen associated with HDM allergy. The BLAST analysis of Sar s 11 amino acid sequences revealed an 99% sequence homology with *S. scabiei var. canis*, *S. scabiei var. hominis*, *S. scabiei var. cuniculi*. A sequence homology of 100% was shown to a partial paramyosin amino acid sequence of *S. scabiei var. hominis*.

**Immunooanalysis of Sar s 11.** Immunooanalysis of Sspara1, Sspara2, and Sspara3 fragments of *S. scabiei var. hominis* was performed via Western blot and a three-stage ELISA. Western blot analysis showed strong IgE binding by pooled OS sera to Sspara2 and Sspara3 as compared with Sspara1 that showed no IgE binding. This suggests the 5'-end of the protein is non-immunogenic compared with middle fragment and 3'-end and that paramyosin is indeed recognized by sera from individuals that have generated an immune response to scabies mites. It has previously been reported that C- and N-terminal of paramyosin are much less conserved when compared with paramyosin from other species.

Paramyosin appears to be an important immunogen in scabies. ELISA detection indicated that the IgE binding of sera from the OS group was significantly higher to both Sspara2 and Sspara3 as compared with HDM and naive sera. There is also a clear distinction between specific IgE binding in the OS sera compared with naive sera when the antigen cocktail ELISA was performed. This is a similar result to that observed for other scabies antigens, namely, Sar s 3 (serine protease), Sar s 8 (glutathione S-transferases [GST]), and Sar s 14 (apolipoprotein).

Both Sspara2 and Sspara3 also bound IgG from OS subjects and additionally from subjects with HDM allergy. Both fragments do not seem to be strongly recognized by sera from individuals in the naive to scabies group as reported in previous studies. *Sarcopes scabiei* has been demonstrated to be antigenically cross-reactive with HDMs, that is, *D. pteronyssinus* and *D. farinae*.
molluscs are ultimate targets of immunological attacks. Recombinant paramyosin *Boophilus microplus* (Bm PRM) is reported to be functionally related to host immune system evasion and considered as a new concealed antigen. Immunization with the selected *S. scabiei* recombinant proteins as a vaccine candidates induced high levels of humoral responses (IgGs and IgE) but had no protective efficacy against *S. scabiei* infestation.

The sensitivity and specificity are important in the performance of diagnostic tests. A previous study using DELFIA reported that rSar s 14 has excellent diagnostic capability, with 100% sensitivity and 93.75% specificity and proved to be a highly sensitive method for diagnosis of scabies infestation in clinical practice. ELISA-based diagnosis using Sar s 11 fragments is also highly sensitive and specific for diagnosis of patients with OS. The Sspara2/Sspara3 cocktail ELISA showed IgE sensitivity at 94%. The Sspara2 ELISA with area under the ROC curve of 0.9706 provides a highly discriminatory assay for OS infestation. Several human studies also reported increase in total and antigen-specific IgE in scabies, and the development of ELISA for human scabies has focused on detection of IgE, rather than the more commonly used IgG, which was considered a less specific marker of infection.

In conclusion, our results demonstrate that the applicability of tropomyosin (Sar s 10) to immunodiagnosis of scabies infestation is limited likely due to the high sequence similarity between Sar s 10 and the tropomyosin of other invertebrate species, in agreement with previous studies. Results suggest that *S. scabiei* paramyosin is predominantly recognized by OS-infested individuals and not HDM allergic subjects, and subjects with OS have a specific IgE response to Sar s 11. The IgE binding observed in subjects with OS was also significantly higher than that of controls and no cross-reactivity with the dust mite homolog was observed.

Any serological test for scabies is going to be limited in its utility to diagnose scabies early. When compared, WMA extract and Sar s 14.3 showed high sensitivity and specificity for diagnosis of sarcoptic mange from weeks 8–16 postinfection. Despite this, serology has a significant advantage over clinical

| Table 2: Comparison of sensitivity and specificity of ELISA for Sspara2, Sspara3, and cocktail antigens |
|-------------------------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Sar s 10 | IgE | IgG | IgE | IgG | IgE | IgG |
| Cutoff | 0.24 | 0.2 | 0.22 | 0.34 | 0.2 | 0.15 |
| Sensitivity | 98% | 70% | 84% | 40% | 94% | 62% |
| Specificity | 90% | 96% | 100% | 87.5% | 100% | 92% |
| AUC | 0.97 | 0.82 | 0.96 | 0.70 | 0.96 | 0.91 |

* AUC = area under curve; ELISA = enzyme-linked immunosorbent assay.
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diagnosis and/or skin scrapings that have poor specificity and sensitivity, respectively. Overall, it appears that paramyosin is an important immunogen in scabies and a potential allergenic protein in mite infestations. A recombinant S. scabiei paramyosin (Sar s 11) could, therefore, potentially be used as an infection marker in immunodiagnosis of OS and in better control of the infection in communities where scabies is endemic.

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