**Supplementary information**

**Results from the epitope definition stage**

**Section 1. Further sample information**

All ME patients were from the Gottfries Clinic in Gothenburg. The Test (n=69; during 2008-2009) and Evaluation (n=61; during 2010-2011) sets were diagnosed according to the same criteria, but the patients were not the same. The main diagnosis of all 61 in the Evaluation set was ME. The Test set included both samples from ME and non-ME patients,where 69 had ME with of without FM and IBS, 4 had FM and 3 had FM+IBS.

**Section 2. Further information regarding antigens for the suspension array**

*Synthetic peptides*

Human HSP60 peptides were chosen from the published sequences of human HSP60 (GenBank id NP\_955472), *Chlamydia pneumoniae* HSP60 (Genbank Id NP\_224342) and *Mycoplasma penetrans* HSP60, also named GroL (GenBank ID NP\_757486). All peptides were 30-mers and had a three-carbon polyethylene glycol spacer coupled at the amino end. The spacer started with a primary amino group. Two overlapping series of *Chlamydia* peptides were tested, one with a three-carbon and one with a six-carbon polyethylene glycol spacer. The peptides overlapped by 15 amino acids. The peptides were synthesized by the coauthor dr Rüdiger Pipkorn. Lyophilized peptides were dissolved in sterile phosphate-buffered saline (PBS), pH 7. The dissolution sometimes had to be facilitated by warming the solution to 37°C overnight on a shaker or by sonication for 5 min. Highly hydrophobic peptides were dissolved in 10 to 50% (vol/vol) dimethyl sulfoxide (DMSO) (Sigma D2650). Initially, 38 overlapping peptides of HSP60 were synthesized, (Supplementary table ST1).

In initial experiments, two human HSP60 peptides G15 and G20, gave significant IgG reactions. To better localize the epitopes, peptides overlapping G15 (G15a, G15b, G15c, G15d, G15e, and G15f) and G20 (G20a, G20b, G20c, G20d, G20e, and G20f), systematically shifted three amino acids before and after G15 and G20, respectively, were synthesized (Supplementary table ST2).

A human HSP60 peptide (G20c) showed a higher antibody binding activity than the human HSP60 G20 peptide. Twentysix new peptides were therefore designed after an alignment of Human HSP60 G20c analogs with HSP60 of many microbes, spanning much of the HSP60 variation. The prokaryotes *Orientia tsutsugamushi, Neoehrlichia mikurensis, Wolbachia, Legionella pneumophila, Borrelia garinii, Bartonella henselae, Tropheryma whipplei, Rickettsia bellii, Anaplasma phagocytophilium, Burkholderia multivorans, Escherichia coli, Chlamydophila pneumoniae , Staphylococcus aureus, Mycobacterium tuberculosis, Mycoplasma penetrans, Listeria monocytogenes, Streptococcus pneumoniae, Treponema pallidum,*and the eukaryotes *Leishmania (strain Friedlin), Schistosoma mansoni , Plasmodium falciparum, Leptospira interrogans, Giardia lamblia, Cryptosporidium parvum,* and *Entamoeba histolytica* were included (cf. Supplementary table ST3).

Further, 36 overlapping peptides from *Mycoplasma* penetrans HSP60 (Supplementary table ST4), and two sets of 36 overlapping *Chlamydia pneumoniae* HSP60 peptides, with a three and six carbon polyethylene glycol spacer, respectively, were synthesized (Supplementary table ST5).

*E. coli* HSP60 (GroEL) selectively binds tryptophan-rich synthetic peptides (here termed “Strongly Binding Peptide, SBP”, defined from panning with *E. coli* HSP60 of a peptide library ([*1*](#_ENREF_1)). The binding of such a peptide (here called SBP1), defined by crystallography, was close to the apical domain helix I. Binding of SBPs is therefore a way of demonstrating chaperonin activity. We synthesized five SBP dodecamers defined in (Supplementary table ST6). They were biotinylated at the amino terminus. The biotin was joined to the SBP via a 6 carbon polyethylene glycol spacer, the same arrangement as in the overlapping long spacer chlamydia HSP60 30-mer peptides.

**Table ST1.** The amino acid sequences of overlapping peptides of intact human HSP60 (GenBank id NP\_955472).

Turqoise background: Cross reactive epitopes (between Mycobacteria, *Chlamydia*, *E.coli* and Human HSP60) defined by Perschinka et al ([*2*](#_ENREF_2)). Gray background: Sequence homologous with the *Porphyromonas gingivalis* peptide of Jeong et al ([*3*](#_ENREF_3)), antibodies to which were more frequent in chronic periodontitis, atherosclerosis, type 2 diabetes mellitus and rheumatoid arthritis. Another peptide sequence, antibodies to which correlated with cardiovascular disease, Okada et al ([*4*](#_ENREF_4)) is shown with overstrike. Dark green background shows the peptide selectively recognized by patients with unstable angina or myocardial infarction, Wysocki et alc([*5*](#_ENREF_5)). The bold V in position 73 is mutated in Hereditary Spastic Paraplegia SPG13, labelled as position 72 in Hansen et al ([*6*](#_ENREF_6)). The underlined letters shows the autoepitopes to which antibodies were boosted by Coxsackievirus A9 infection, Härkönen et al. ([*7*](#_ENREF_7)).

A detailed discussion of peptidic HSP60 epitopes is given below (“Further details on HSP60 epitopes”).

|  |  |  |
| --- | --- | --- |
| **Peptide name** | | **Peptide sequence** |
| G1 | Human HSP60 1-30 | MLRLPTVFRQMRPVSRVLAPHLTRAYAKDV |
| G2 | Human HSP60 16-45 | RVLAPHLTRAYAKDVKFGADARALMLQGVD |
| G3 | Human HSP60 31-60 | KFGADARALMLQGVDLLADAVAVTMGPKGR |
| G4 | Human HSP60 46-75 | LLADAVAVTMGPKGRTVIIEQSWGSPKVTK |
| G5 | Human HSP60 61-90 | TVIIEQSWGSPK**V**TKDGVTVAKSIDLKDKY |
| G6 | Human HSP60 76-105 | DGVTVAKSIDLKDKYKNIGAKLVQDVANNT |
| G7 | Human HSP60 91-120 | KNIGAKLVQDVANNTNEEAGDGTTTATVLA |
| G8 | Human HSP60106-135 | NEEAGDGTTTATVLARSIAKEGFEKISKGA |
| G9 | Human HSP60121-150 | RSIAKEGFEKISKGANPVEIRRGVMLAVDA |
| G10 | Human HSP60136-165 | NPVEIRRGVMLAVDAVIAELKKQSKPVTTP |
| G11 | Human HSP60151-180 | VIAELKKQSKPVTTP~~EEIAQVATISANGDK~~ |
| G12 | Human HSP60166-195 | ~~EEIAQVATISANGDKEIGNII~~SDAMKKVGR |
| G13 | Human HSP60181-210 | EIGNIISDAMKKVGRKGVITVKDGKTLNDE |
| G14 | Human HSP60196-225 | KGVITVKDGKTLNDELEIIEGMKFDRGYIS |
| G15 | Human HSP60211-240 | LEIIEGMKFDRGYISPYFINTSKGQKCEFQ |
| G16 | Human HSP60226-255 | PYFINTSKGQKCEFQDAYVLLSEKKISSIQ |
| G17 | Human HSP60241-270 | DAYVLLSEKKISSIQSIVPALEIANAHRKP |
| G18 | Human GroEL256-285 | SIVPALEIANAHRKPLVIIAEDVDGEALST |
| G19 | Human GroEL271-300 | LVIIAEDVDGEALSTLVLNRLKVGLQVVAV |
| G20 | Human GroEL286-315 | LVLNRLKVGLQVVAVKAPGFGDNRKNQLKD |
| G21 | Human GroEL301-330 | KAPGFGDNRKNQLKDMAIATGGAVFGEEGL |
| G22 | Human GroEL316-345 | MAIATGGAVFGEEGLTLNLEDVQPHDLGKV |
| G23 | Human GroEL331-360 | TLNLEDVQPHDLGKVGEVIVTKDDAMLLKG |
| G24 | Human GroEL346-375 | GEVIVTKDDAMLLKGKGDKAQIEKRIQEII |
| G25 | Human GroEL361-390 | KGDKAQIEKRIQEIIEQLDVTTSEYEKEKL |
| G26 | Human GroEL376-405 | EQLDVTTSEYEKEKLNERLAKLSDGVAVLK |
| G27 | Human GroEL391-420 | NERLAKLSDGVAVLKVGGTSDVEVNEKKDR |
| G28 | Human GroEL406-435 | VGGTSDVEVNEKKDRVTDALNATRAAVEEG |
| G29 | Human GroEL421-450 | VTDALNATRAAVEEGIVLGGGCALLRCIPA |
| G30 | Human GroEL436-465 | IVLGGGCALLRCIPALDSLTPANEDQKIGI |
| G31 | Human GroEL451-480 | LDSLTPANEDQKIGIEIIKRTLKIPAMTIA |
| G32 | Human GroEL466-495 | EIIKRTLKIPAMTIAKNAGVEGSLIVEKIM |
| G33 | Human GroEL481-510 | KNAGVEGSLIVEKIMQSSSEVGYDAMAGDF |
| G34 | Human GroEL496-525 | QSSSEVGYDAMAGDFVNMVEKGIIDPTKVV |
| G35 | Human GroEL511-540 | VNMVEKGIIDPTKVVRTALLDAAGVASLLT |
| G36 | Human GroEL526-555 | RTALLDAAGVASLLTTAEVVVTEIPKEEKD |
| G37 | Human GroEL541-570 | TAEVVVTEIPKEEKDPGMGAMGGMGGGMGG |
| G38 | Human GroEL556-573 | PGMGAMGGMGGGMGGGMF |

**Table ST2.** The amino acid sequences of overlapping peptides of fragments around human HSP60 G15 (aa211-240) and human HSP60 G20 (aa286-320), shown in red.

|  |  |  |
| --- | --- | --- |
| **Peptide name** | | **Peptide sequence** |
| G15a | Human GroEL202-231 | KDGKTLNDELEIIEGMKFDRGYISPYFINT |
| G15b | Human GroEL205-234 | KTLNDELEIIEGMKFDRGYISPYFINTSKG |
| G15c | Human GroEL208-237 | NDELEIIEGMKFDRGYISPYFINTSKGQKC |
| G15 | Human GroEL211-240 | LEIIEGMKFDRGYISPYFINTSKGQKCEFQ |
| G15d | Human GroEL214-243 | IEGMKFDRGYISPYFINTSKGQKCEFQDAY |
| G15e | Human GroEL217-246 | MKFDRGYISPYFINTSKGQKCEFQDAYVLL |
| G15f | Human GroEL220-249 | DRGYISPYFINTSKGQKCEFQDAYVLLSEK |
| G20a | Human GroEL277-306 | DVDGEALSTLVLNRLKVGLQVVAVKAPGFG |
| G20b | Human GroEL280-309 | GEALSTLVLNRLKVGLQVVAVKAPGFGDNR |
| G20c | Human GroEL283-312 | LSTLVLNRLKVGLQVVAVKAPGFGDNRKNQ |
| G20 | Human GroEL286-320 | LVLNRLKVGLQVVAVKAPGFGDNRKNQLKD |
| G20d | Human GroEL289-318 | NRLKVGLQVVAVKAPGFGDNRKNQLKDMAI |
| G20e | Human GroEL292-321 | KVGLQVVAVKAPGFGDNRKNQLKDMAIATG |
| G20f | Human GroEL295-324 | LQVVAVKAPGFGDNRKNQLKDMAIATGGAV |

**Table ST3.** The amino acid sequences of GroEL of many microbes which are homologs of Human HSP60 (283-312) (Here referred to as “G20c homologs”); Peptide names contain the respective GenBank identity numbers)

|  |  |
| --- | --- |
| **Peptide name** | **Peptide sequence** |
| GroEL\_orientia\_tsutsugamushi\_CH60\_ORITS 258-287 | LTALILNNLKGSIKVVAVKAPGFGDRKKEM |
| GroEL5\_neoehrlichia\_mikurensis\_FJ966359 259-288 | LSTLVLNKLRGGLHVAAVKAPGFGDRRKDM |
| GroEL\_Wolbachia\_YP\_198181 261-290 | LSTLVINKLRGGLKVTAVKAPGFGDRRKEM |
| GroEL\_Legionella\_YP\_126086 258-287 | LATLVVNNMRGIVKVCAVKAPGFGDRRKAM |
| GroEL\_Borrelia\_garinii\_YP\_073092 258-287 | LAALVLNSVRGALKVCAIKSPGFGDRRKAM |
| GroEL\_Bartonella\_henselae\_YP\_034075 259-288 | LATLVVNKLRGGLKIAAVKAPGFGDRRKAM |
| GroEL\_tropheryma\_whipplei\_NP\_789261 257-286 | LATLVVNKIRGIFKSVAVKAPGFGDRRKMM |
| GroEL\_rickettsia\_bellii\_YP\_537760 259-288 | LATLVVNRLRGGLKVAAVKAPGFGDRRKAM |
| GroEL\_anaplasma\_phagocytophilium\_HZ\_YP\_504857 258-287 | LSTLVLNKLRGGLQVAAVKAPGFGDRRKDM |
| GroEL\_Burkholderia\_multivorans\_YP\_001947677 259-288 | LATLVVNAMRGILKVAAVKAPGFGDRRKAM |
| GroEL\_E\_coli\_K12\_AAC77103 259-288 | LATLVVNTMRGIVKVAAVKAPGFGDRRKAM |
| GroEL\_CH60\_CHLPN\_chlamydia\_pneumoniae 259-288 | LATLVVNRLRAGFRVCAVKAPGFGDRRKAM |
| GroEL\_Staph\_aureus\_MRSA\_YP\_041479 257-286 | LTNIVLNRMRGTFTAVAVKAPGFGDRRKAM |
| GroEL\_Mycobact\_tuberc\_CAA17397 257-286 | LSTLVVNKIRGTFKSVAVKAPGFGDRRKAM |
| GroEL\_mycoplasma\_penetrans\_NP\_757486 258-287 | LTTLVVNKMRGVFNVVAVKAPEFGDKRKQV |
| HSP60\_Leishmania\_Strain\_Friedlin\_XP\_001685504 272-301 | MHTFLYNKIQGRISGCAVKAPGFGDMRINQ |
| HSP60\_Schistosoma\_mansoni\_XP\_002572332 276-305 | LTALVLNRLKLGLQVCAVKAPGFGDNRKNT |
| HSP60\_Plasmodium\_falciparum\_XP\_001347438 288-317 | LATLIVNKLRLGLKICAVKAPGFGEHRKAL |
| GroEL\_Leptospira\_interrogans\_YP\_001299 258-287 | LATIVVNTLRKTISCVAVKAPGFGDRRKSM |
| GroEL\_Listeria\_monocytogenes\_AF335323\_2 257-286 | QATLVLNKLRGTFNVVAVKAPGFGDRRKAM |
| GroEL\_streptococcus\_pneumoniae\_AF325449\_1 257-286 | LPTLVLNKIRGTFNVVAVKAPGFGDRRKAM |
| GroEL\_treponema\_pallidum\_YP\_001933036 258-287 | LATLVVNSLRGTLKTCAVKAPGFGDRRKEM |
| Chaperonin60\_Giardia\_lamblia\_XP\_001705532 261-290 | LSTLAINTLKGTVRCCAVRAPGYGDVKKGV |
| chaperonin60\_Cryptosporidium\_parvum\_XP\_627821 291-320 | LTALILNKLQLNLKVCAVKAPGFGDHRKQI |
| Chaperonin60\_Entamoeba\_histolytica\_XP\_656268 265-294 | LTTLVLNKLRGLPIAAVRAPGFGETRKGILH |

Results with the above peptides are shown in figure 2 with the following abbreviations:

The G20c homologs were 5. *Escherichia coli* (ESCHCOLI), 6. *Legionella pneumophila* (LEGIONELL)*,* 7. *Burkholderia multivorans* (BURKHMULT), 8. *Bartonella henselae* (BARTHENS), 9. *Borrelia garinii* (BORRGAR), 10. *Treponema pallidum* (TREPPALL),11. *Leptospira interrogans* (LEPTINTERR), 12*. Chlamydia pneumoniae* (CHLAMPNEUM), 13. *Mycobacterium tuberculosis* (MYCTUB), 14. *Tropheryma whipplei* (TROPHWHIP), 15. *Staphylococcus aureus* (STAPAUR), 16. *Listeria monocytogenes* (LISTMONO), 17. *Streptococcus pneumoniae* (STREPTPNEU), 18. *Mycoplasma penetrans* (MYCPEN), 19. *Neoehrlichia mikurensis* (NEOMIK), 20. *Anaplasma phagocytophilium* (ANAPPHAG), 21. *Wolbachia* (WOLBAC)*,* 22*. Orientia tutsugamishi* (ORITSU)*,* 23. *Rickettsia bellii* (RICKBELL)*,* 24*.* *Leishmania major* (LEISHMAN),25*. Giardia lamblia* (GIARLAMB), 26. *Entamoeba histolytica* (ENTHIS), 27*. Plasmodium falciparum* (PLASFALC), 28. *Cryptosporidium parvum* (CRYPTPARV), 29. *Schistosoma mansoni* (SCHISMANS) (cf. Supplementary Table ST3).

All G20c homologs gave high NTC values (100-900 MFI). The values presented in Figure 2 were partially subtracted with the NTC value as described in Materials and methods. The subtraction was uniform, because equal numbers of patient and control samples were always analyzed together. It could therefore not create artificial statistical differences between the groups. Rather, occasional oversubtraction where negative values were set to 0 could reduce, not exaggerate, differences and significances. G20c homolog peptides with the highest positive predictive (100%) and negative predictive values (62-68%) for ME in the IgM test were *Staphylococcus* at cutoff 66 MFI, *Plasmodium* at cutoff 321 MFI, *Listeria* at cutoff 198 MFI, *Burkholderia* at cutoff 278 MFI, *Chlamydia* at cutoff 244 MFI and *Legionella* at cutoff 271 MFI.

**Table ST4.** The amino acid sequences of *Mycoplasma* penetrans GroEl peptides (GenBank id NP\_757486).

|  |  |  |
| --- | --- | --- |
| **Peptide name** | | **Peptide sequence** |
| D1 | GroEL\_Mycopl\_pen 1-30 | MAKEIKFSDSARNKLFNGVQQLFDAVKVTM |
| D2 | GroEL\_Mycopl\_pen 16-45 | FNGVQQLFDAVKVTMGPRGRNVLIQKSYGA |
| D3 | GroEL\_Mycopl\_pen 31-60 | GPRGRNVLIQKSYGAPVITKDGVSVAKEVD |
| D4 | GroEL\_Mycopl\_pen 46-75 | PVITKDGVSVAKEVDLTNPIENMGAQLVKD |
| D5 | GroEL\_Mycopl\_pen 61-90 | LTNPIENMGAQLVKDVASKTADEAGDGTTT |
| D6 | GroEL\_Mycopl\_pen 76-105 | VASKTADEAGDGTTTATVLAYGVFKEGLRN |
| D7 | GroEL\_Mycopl\_pen 91-120 | ATVLAYGVFKEGLRNVISGANPIEIKRGMD |
| D8 | GroEL\_Mycopl\_pen 106-135 | VISGANPIEIKRGMDKTVNAIVNELNKSSK |
| D9 | GroEL\_Mycopl\_pen 121-200 | KTVNAIVNELNKSSKKIARKDEIIQVATIS |
| D10 | GroEL\_Mycopl\_pen 136-165 | KIARKDEIIQVATISANSDKKIGELIANAM |
| D11 | GroEL\_Mycopl\_pen 201-180 | ANSDKKIGELIANAMEKVGSDGVITVEEAK |
| D12 | GroEL\_Mycopl\_pen 166-195 | EKVGSDGVITVEEAKGINDELTVVEGMQFD |
| D13 | GroEL\_Mycopl\_pen 181-210 | GINDELTVVEGMQFDRGYISPYFVTDTNKM |
| D14 | GroEL\_Mycopl\_pen 196-225 | RGYISPYFVTDTNKMIAKLENPYILITDKK |
| D15 | GroEL\_Mycopl\_pen 211-240 | IAKLENPYILITDKKVSSIKDILPILEEIM |
| D16 | GroEL\_Mycopl\_pen 226-255 | VSSIKDILPILEEIMKTGRPLLIIADDVDG |
| D17 | GroEL\_Mycopl\_pen 241-270 | KTGRPLLIIADDVDGEALTTLVVNKMRGVF |
| D18 | GroEL\_Mycopl\_pen 256-285 | EALTTLVVNKMRGVFNVVAVKAPEFGDKRK |
| D19 | GroEL\_Mycopl\_pen 271-300 | NVVAVKAPEFGDKRKQVLEDIAILTGGSFV |
| D20 | GroEL\_Mycopl\_pen 286-320 | QVLEDIAILTGGSFVTDDLGISFDKVTLQD |
| D21 | GroEL\_Mycopl\_pen 301-330 | TDDLGISFDKVTLQDLGQAESVVIDKDNST |
| D22 | GroEL\_Mycopl\_pen 316-345 | LGQAESVVIDKDNSTIVKGKGLESQIKERI |
| D23 | GroEL\_Mycopl\_pen 331-360 | IVKGKGLESQIKERISKIKTAIEMTDSDYD |
| D24 | GroEL\_Mycopl\_pen 346-375 | SKIKTAIEMTDSDYDKDSLRNRLAKLNKGV |
| D25 | GroEL\_Mycopl\_pen 361-390 | KDSLRNRLAKLNKGVAVIKVGAVSEVELKE |
| D26 | GroEL\_Mycopl\_pen 376-405 | AVIKVGAVSEVELKEKKDRVDDALSATKAA |
| D27 | GroEL\_Mycopl\_pen 391-420 | KKDRVDDALSATKAAIEEGIVIGGGAALVH |
| D28 | GroEL\_Mycopl\_pen 406-435 | IEEGIVIGGGAALVHVSKRINVNTLNLIGD |
| D29 | GroEL\_Mycopl\_pen 421-450 | VSKRINVNTLNLIGDEKIGYQIVMSAIMSP |
| D30 | GroEL\_Mycopl\_pen 436-465 | EKIGYQIVMSAIMSPISQIVSNAGFDKGVV |
| D31 | GroEL\_Mycopl\_pen 451-480 | ISQIVSNAGFDKGVVINEILKATNPHLGFN |
| D32 | GroEL\_Mycopl\_pen 466-495 | INEILKATNPHLGFNAATGKYVDMFQTGII |
| D33 | GroEL\_Mycopl\_pen 481-510 | AATGKYVDMFQTGIIDPVKVTRIALQNAVS |
| D34 | GroEL\_Mycopl\_pen 496-525 | DPVKVTRIALQNAVSVSSMLLTTEAVIYDV |
| D35 | GroEL\_Mycopl\_pen 511-540 | VSSMLLTTEAVIYDVKDDKEDSVPAMPNMG |
| D36 | GroEL\_Mycopl\_pen 526-555 | KDDKEDSVPAMPNMGMGGMM |

**Table ST5.**  The amino acid sequences of overlapping *Chlamydia pneumoniae* GroEL (GenBank id NP\_224342) peptides. The peptides were synthesized with short (tri-ethylene glycol, C series) and long (hexa-ethylene glycol, E series) spacers.

Yellow background: Peptides recognized by IgG in sera from women with *Chlamydia trachomatis* associated ectopic pregnancy, Yi et al ([*8*](#_ENREF_8)). Most *Chlamydia pneumoniae* peptides had an identical sequence. Positions which differed were underlined. The overlapping epitopes of peptides C30-C32 and E30-E32 defined by Yi et al were indicated by additional overstrike and italic marks.

|  |  |  |  |
| --- | --- | --- | --- |
| **Short**  **Spacer** | **Long**  **Spacer** | **Peptide name** | **Peptide sequence** |
| C1 | E1 | GroEL\_Chlamyd\_pneum 1-30 | MVAKNIKYNEEARKKIQKGVKTLAEAVKVT |
| C2 | E2 | GroEL\_Chlamyd\_pneum 16-45 | IQKGVKTLAEAVKVTLGPKGRHVVIDKSFG |
| C3 | E3 | GroEL\_Chlamyd\_pneum 31-60 | LGPKGRHVVIDKSFGSPQVTKDGVTVAKEV |
| C4 | E4 | GroEL\_Chlamyd\_pneum 46-75 | SPQVTKDGVTVAKEVELADKHENMGAQMVK |
| C5 | E5 | GroEL\_Chlamyd\_pneum 61-90 | ELADKHENMGAQMVKEVASKTADKAGDGTT |
| C6 | E6 | GroEL\_Chlamyd\_pneum 76-105 | EVASKTADKAGDGTTTATVLAEAIYTEGLR |
| C7 | E7 | GroEL\_Chlamyd\_pneum 91-120 | TATVLAEAIYTEGLRNVTAGANPMDLKRGI |
| C8 | E8 | GroEL\_Chlamyd\_pneum 106-135 | NVTAGANPMDLKRGIDKAVKVVVDQIKKIS |
| C9 | E9 | GroEL\_Chlamyd\_pneum 121-200 | DKAVKVVVDQIKKISKPVQHHKEIAQVATI |
| C10 | E10 | GroEL\_Chlamyd\_pneum 136-165 | KPVQHHKEIAQVATISANNDAEIGNLIAEA |
| C11 | E11 | GroEL\_Chlamyd\_pneum 201-180 | SANNDAEIGNLIAEAMEKVGKNGSITVEEA |
| C12 | E12 | GroEL\_Chlamyd\_pneum 166-195 | MEKVGKNGSITVEEAKGFETVLDVVEGMNF |
| C13 | E13 | GroEL\_Chlamyd\_pneum 181-210 | KGFETVLDVVEGMNFNRGYLSSYFATNPET |
| C14 | E14 | GroEL\_Chlamyd\_pneum 196-225 | NRGYLSSYFATNPETQECVLEDALVLIYDK |
| C15 | E15 | GroEL\_Chlamyd\_pneum 211-240 | QECVLEDALVLIYDKKISGIKDFLPILQQV |
| C16 | E16 | GroEL\_Chlamyd\_pneum 226-255 | KISGIKDFLPILQQVAESGRPLLIIAEDIE |
| C17 | E17 | GroEL\_Chlamyd\_pneum 241-270 | AESGRPLLIIAEDIEGEALATLVVNRIRGG |
| C18 | E18 | GroEL\_Chlamyd\_pneum 256-285 | GEALATLVVNRIRGGFRVCAVKAPGFGDRR |
| C19 | E19 | GroEL\_Chlamyd\_pneum 271-300 | FRVCAVKAPGFGDRRKAMLEDIAILTGGQL |
| C20 | E20 | GroEL\_Chlamyd\_pneum 286-320 | KAMLEDIAILTGGQLISEELGMKLENANLA |
| C21 | E21 | GroEL\_Chlamyd\_pneum 301-330 | ISEELGMKLENANLAMLGKAKKVIVSKEDT |
| C22 | E22 | GroEL\_Chlamyd\_pneum 316-345 | MLGKAKKVIVSKEDTTIVEGMGEKEALEAR |
| C23 | E23 | GroEL\_Chlamyd\_pneum 331-360 | TIVEGMGEKEALEARCESIKKQIEDSSSDY |
| C24 | E24 | GroEL\_Chlamyd\_pneum 346-375 | CESIKKQIEDSSSDYDKEKLQERLAKLSGG |
| C25 | E25 | GroEL\_Chlamyd\_pneum 361-390 | DKEKLQERLAKLSGGVAVIRVGAATEIEMK |
| C26 | E26 | GroEL\_Chlamyd\_pneum 376-405 | VAVIRVGAATEIEMKEKKDRVDDAQHATIA |
| C27 | E27 | GroEL\_Chlamyd\_pneum 391-420 | EKKDRVDDAQHATIAAVEEGILPGGGTALI |
| C28 | E28 | GroEL\_Chlamyd\_pneum 406-435 | AVEEGILPGGGTALIRCIPTLEAFLPMLTN |
| C29 | E29 | GroEL\_Chlamyd\_pneum 421-450 | RCIPTLEAFLPMLTNEDEQIGARIVLKALS |
| C30 | E30 | GroEL\_Chlamyd\_pneum 436-465 | EDEQIGARIVLKALSAPLKQIAA~~NAGKEGA~~ |
| C31 | E31 | GroEL\_Chlamyd\_pneum 451-480 | APLKQIAA~~NAGKE~~*~~GAIIFQQ~~VMSRS*ANEGY |
| C32 | E32 | GroEL\_Chlamyd\_pneum 466-495 | *IIFQQVMSRS*ANEGYDALRDAYTDMLEAGI |
| C33 | E33 | GroEL\_Chlamyd\_pneum 481-510 | DALRDAYTDMLEAGILDPAKVTRSALESAA |
| C34 | E34 | GroEL\_Chlamyd\_pneum 496-525 | LDPAKVTRSALESAASVAGLLLTTEALIAE |
| C35**(m)** | E35**g(m)** | GroEL\_Chlamyd\_pneum 511-540 | SVAGLLLTTEALIAEIPEEKPAAAPAMPGA |
| C36 | E36 | GroEL\_Chlamyd\_pneum 526-555 | IPEEKPAAAPAMPGAGMDY |

Polyethylene glycol of varying length has earlier been used as an inert spacer to display peptides with minimal spatial constraint on surfaces ([*9*](#_ENREF_9)). The spacer can restrict the availability of a peptide to antibodies. Potentially it could lead to false negative results in SMIA, and we wanted to investigate this variable. It turned out that an IgG epitope that was not visible in the scan with Chlamydia peptides with a short spacer was prominent in the scan with a longer spacer (E35). On the other hand, an epitope which was relatively strongly recognized with a short spacer (C25) was weaker with a long spacer. All peptides were anchored at the amino terminal end. It is possible that further epitopes could have been discovered if peptides anchored at the carboxy terminus also were tested.

**Supplementary table ST6.** Sequences of ”strongly binding peptides” (SBPs), taken from Chen and Sigler ([*1*](#_ENREF_1)). In that publication, the peptides were selected by panning with E. coli HSP60 from a peptide library. The biotinylated hexaethylene glycol spacer is written as Bio-(EG)6.

|  |  |
| --- | --- |
| **Peptide name** | **Peptide sequence** |
| SBP1 | Bio-(EG)6- SWMTTPWGFLHP |
| SBP2 | Bio-(EG)6- FHYEIWIPPHRG |
| SBP3 | Bio-(EG)6- SSPWWLVSFTST |
| SBP4 | Bio-(EG)6- SHSLIWRIPLLH |
| SBP5 | Bio-(EG)6- IYVPWYYAENLP |

**Section 3. Details regarding the SMIA procedure**

*Coupling of proteins and peptides to carboxylated microspheres*

The synthetic peptides or protein were coupled to Luminex Carboxylated Microspheres, as described in the “Sample protocol for two step carbodiimide coupling of protein to carboxylated microspheres” provided by the Luminex Corporation (Austin, TX). Briefly, the stock uncoupled beads (xMAP Technology, Austin, TX) were vortexed for 20 s, then sonicated for 20 s. An appropriate amount (i.e. 100 - 400 µl) of the stock microspheres, containing 1.25 × 107 beads per ml, was transferred to a 1.5 ml Eppendorf tube which then was centrifuged at 13,000 × *g* for 3 min. The supernatant was carefully removed and the beads were resuspended in 100 μl of distilled water, followed by vortexing and sonication for 20 s, and then centrifuged at 13,000 ×*g* for 3 min. Supernatants were subsequently removed and resuspended in 80 µl of 100 mM monobasic sodium phosphate (MSP, Sigma, cat. nr S3139) pH 6.2 , then vortexed and sonicated for 20 s. Ten microliters of freshly made *N*-hydroxysuccinimide (NHS)(Pierce, cat. nr 24510) and 10 μl of 50-mg/ml 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC; water-soluble carbodiimide; Pierce, cat. nr 25952-53-8 ) in H2O were added to the beads. The suspension was then incubated in the dark for 20 min at room temperature. The beads were then centrifuged as described above. The supernatant was carefully removed, and the pellet was washed twice in 250 μl of 50 mM 2-(*N*-morpholino) ethanesulfonic acid (MES) sodium salt (Sigma, cat. nr 71119-23-8), pH 5. The supernatant was removed and the beads resuspended in an appropriate volume of the MES buffer (i.e. 125 µl MES to 100µl of beads), vortexed and sonicated. Fifty micrograms of the peptide or 1- 100 µg protein was added. Beads were mixed by careful vortexing and then incubated in the dark for 2 hours at room temperature on a plate shaker (100 rpm). The coupled microspheres were pelleted by centrifugation at 13,000 × *g* for 3 min and resuspended in 500 μl of StabilGuard buffer (SurModics, Eden Prairie, MN, cat. nr SG01-1000 ). The coupled microspheres were again pelleted by centrifugation as described above and resuspended in 1 ml of StabilGuard buffer twice. The final pellet was resuspended in an appropriate amount of StabilGuard buffer (1000 µl of StabilGuard buffer if 100µl of beads were taken). This created a bead mixture consisting of 1250 beads/μl. The coupled beads were stored at 4°C in the dark.

Ten µg of *Haemophilus influenzae* type b (Hib) vaccine (Act-HIB®; Sanofi Pasteur MSD) was diluted in 100µL sterile water and coupled to the Luminex bead following the coupling protocol. An unprocessed (“naked”) bead, without any bound antigen, was used to control for nonspecific binding to the Luminex beads. The stock uncoupled beads were vortexed for 20 s, then sonicated for 20 s. An appropriate amount of the stock microspheres was transferred to a 1.5 ml Eppendorf tube which then was centrifuged at 13,000 × *g* for 3 min. The supernatant was carefully removed and the beads were resuspended in 100 μl of distilled water, followed by vortexing and sonication for 20 s, and then centrifuged at 13,000 ×*g* for 3 min. Supernatants were subsequently removed and resuspended in 500 µl of StabilGuard. The uncoupled beads were again pelleted by centrifugation as described above and resuspended in 1 ml of StabilGuard buffer twice. The final pellet was resuspended in StabilGuard buffer to 1250 beads/μl. The coupled beads in this stock suspension were stored at 4°C in the dark.

*Preanalytical procedures*

Samples were immediately frozen in small aliquots, to avoid repetitive freezing and thawing.

*Suspension Multiplex Immunoassay (SMIA)*

During the development of SMIA for many of the pathogens previously associated with ME we noted that the sensitivity of the SMIA was the same as, or better than, EIA, with a wide dynamic range and possibility to simultaneously read 100 analytes in the Luminex® 200™ analyzer (Luminex Corporation, Austin, Texas) (J Blomberg, unpublished, and ([*10*](#_ENREF_10)*,* [*11*](#_ENREF_11)). The assay system required only 5-10 µl of serum or plasma. Antigen (10-50 µg of whole microbe lysate, recombinant protein and synthetic peptide) was coupled covalently to carboxylated color-coded beads as described above. IgG was detected using biotinylated protein G (BPG) ([*12*](#_ENREF_12)*,* [*13*](#_ENREF_13)) as secondary antibody. For IgM antibody detection biotinylated anti-human IgM (affinity purified, μ-chain specific, Sigma-Aldrich cat. Nr. B1140) was used as secondary antibody.

A few control experiments with 4 µg/ml biotinylated monoclonal anti-IgG (BioLegend inc, San Diego, CA, cat. nr. 409307) and 4 µg/ml biotinylated monoclonal anti-IgM (BioLegend, cat nr. 314504) are reported in the Supplementary Figure SF3.

*Preparations prior to filter plate loading*

Samples were diluted prior to loading the filter plate using StabilGuard as a diluent. Each ME patient plasma sample was diluted 1:10 (this dilution included the dilution during plasma preparation). BD serum samples were also diluted 1:10. After a concluded coupling the bead stock solution contained 1250 beads/ µl. For a typical experiment, a bead mixture consisting of 25 beads/μl was made using StabilGuard as a diluent. All the beads with coupled proteins or peptides as well as the naked bead and *Hemophilus influenzae* B bead were sonicated and vortexed for 20 s before being added to the bead mixture. A fetuin bead (see below) was included in IgM experiments as an additional positive control.

*Filter plate loading and washing*

For an IgG run, the standard Luminex protocol for the filter plate, namely “Sample protocol for indirect antibody capture immunoassay” was largely followed ([*10*](#_ENREF_10)*,* [*11*](#_ENREF_11)), with the exception that coupling conditions were different. Briefly, the multiplex assay was carried out in a 96 well Multiscreen® filter plates (Millipore, UK, cat. nr MSHVN4B10) with 0.45 μm pore size. Initially, the filter plate was pre-wetted twice with 100 µl PBS (pH7.4).The PBS was removed from the wells by aspiration through the MultiScreenHTS vacuum manifold (Millipore, USA, cat. nr. MSVMHTS0D). Fifty microliters of serum diluted 1:10 in StabilGuard buffer were added to each well, except one, a well containing only StabilGuard and beads, as a non-template control (NTC). The bead mixture was sonicated and vortexed for 20 s. Fifty microliters of the bead mix was then added to each well. After this, the wells were incubated in the dark for 30 min at room temperature on a plate shaker. After incubation, the plate was aspirated and washed with 100 µl PBS twice. Fifty µl StabilGuard were added and followed by 50µl of a 4µg/ml BPG solution (Pierce, cat. nr 29988) diluted in StabilGuard. The beads were resuspended by pipetting up and down five times. The plate was incubated in the dark for 30 min at room temperature on a plate shaker (100 rpm). After incubation, the wells were aspirated and washed twice with 100 µl PBS. 50µl StabilGuard were added to each well and followed by 50 µl of a 4 μg/ml streptavidin-phycoerythrin (SA-PE) solution (Life Technologies Europe, cat. nr SA1004-4) diluted in StabilGuard, and the beads were resuspended by pipetting up and down five times. The plate was then incubated in the dark for 15 min at room temperature on a plate shaker. After incubation, the wells were aspirated and washed twice with 100 μl PBS. The filter plate well was resuspended in 150 μl of PBS. One hundred μl were then analyzed on the Luminex® 200™ system analyzer following the manufacturer's instructions. The StarStation (Applied Cytometry, Sheffield, UK) or xPONENT® (Luminex corp. Austin, Tx) softwares were used to analyze the data.

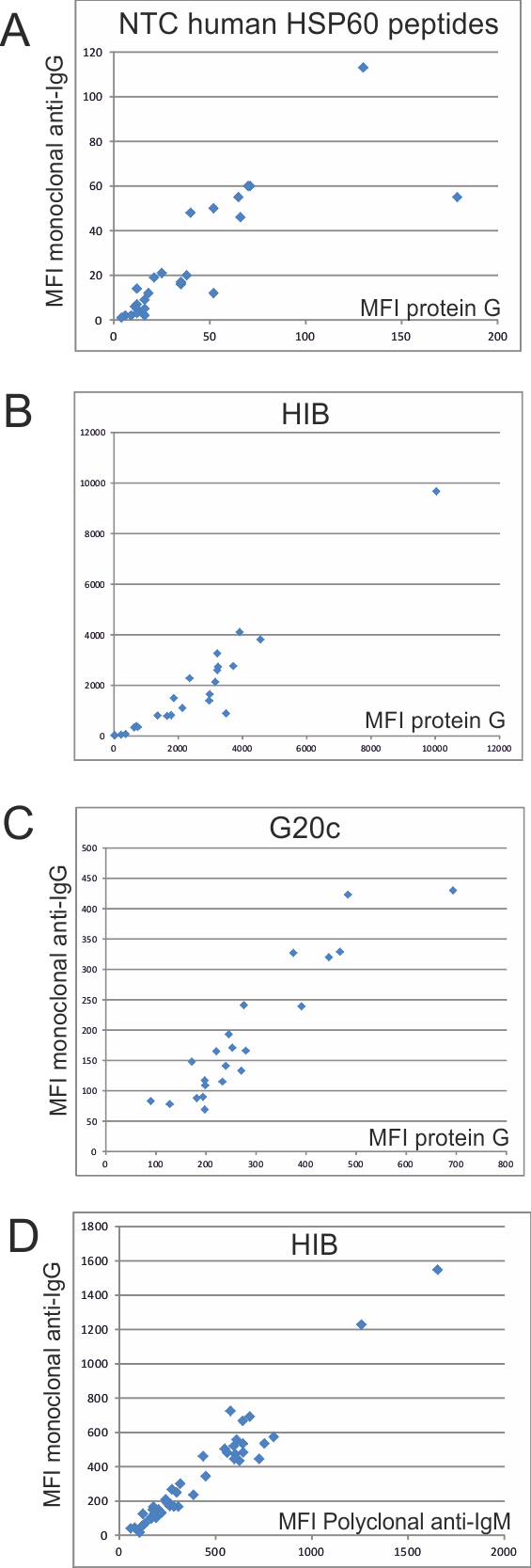
For an IgM run, filter plate loading was done as described for IgG, with the exception that 0.4 µg/ml of anti-human IgM (Biotin conjugated, μ-chain specific, Sigma-Aldrich, cat. nr. B1265) was used as a secondary antibody instead of Protein G. The HIB vaccine bead, intended as a positive control for IgG detection, proved to work also as a positive control for IgM detection. The HIB vaccine is a polysaccharide conjugated to tetanus toxoid, and it is not surprising that it also detects IgM, because anti-carbohydrate antibodies tend to be of IgM type. As a further positive control for presence of IgM (Katona and Blomberg, unpublished), we used a bead coupled with 100 μg of bovine fetuin (Sigma-Aldrich, cat. nr F2379), also called a2HS-Glycoprotein. Fetuin is a heavily glycosylated glycoprotein which reacts with anti-betagalactoside antibodies. The majority of humans have such IgM antibodies.

*Specificity of the MFI signals*

**Supplementary figure SF1.**

1. NTC Protein G vs NTC monoclonal anti IgG for 24 human HSP60 peptides (G1-G24).
2. Correlation between protein G and monoclonal anti-IgG values for a HIB-coupled bead, and 24 ME samples.
3. Correlation between protein G and monoclonal anti-IgG for a G20c-coupled bead, and 22 ME samples.

1. Correlation between poly- and monoclonal anti-IgM for a HIB-coupled bead and 39 ME samples.

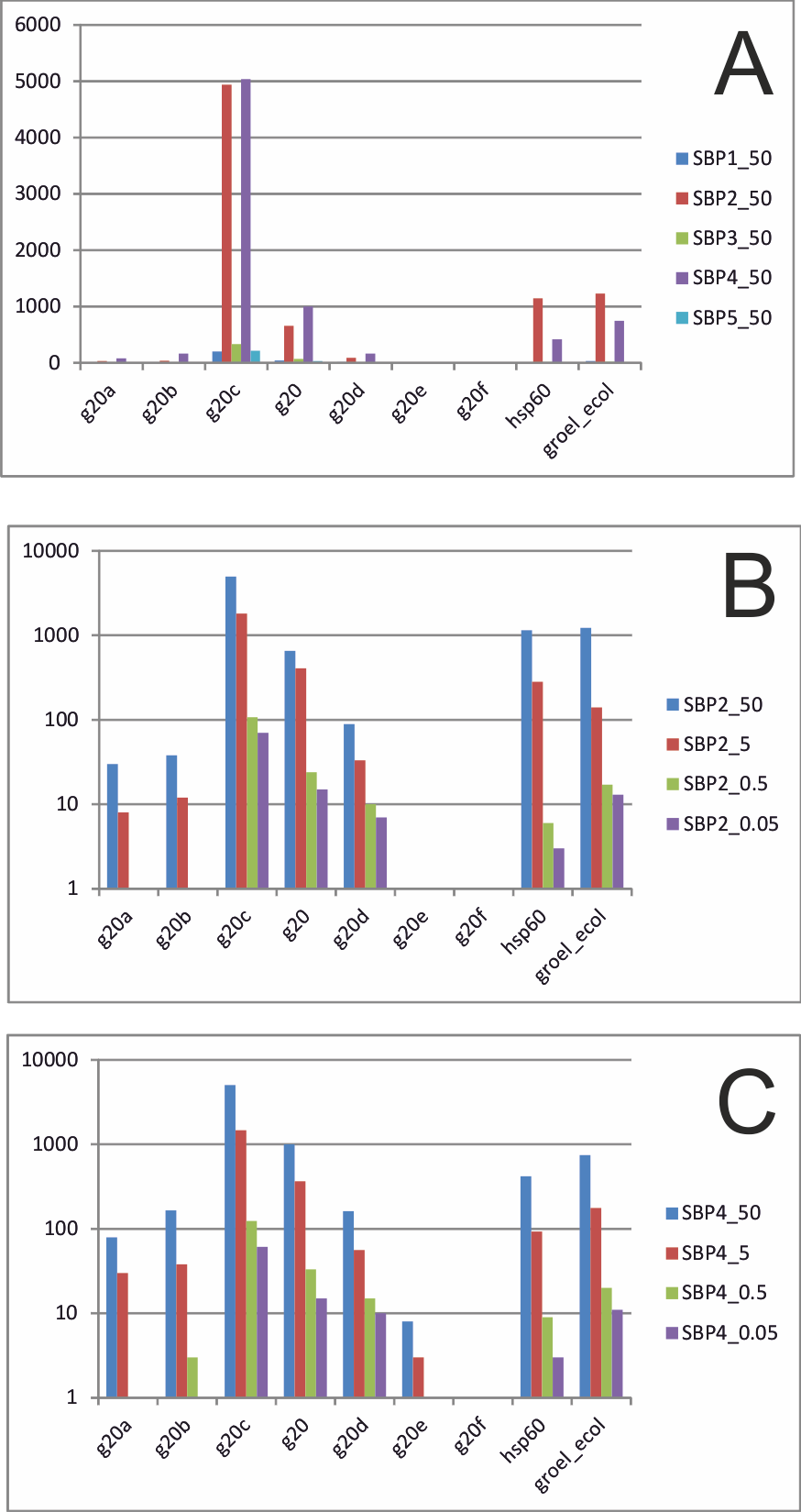
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In experiments using Strongly Binding Peptides, SBPs, the five biotinylated SBPs (SBP1-SBP5, see Supplementary Table ST6) were dissolved in sterile phosphate-buffered saline (PBS), pH 7 and 10 to 50% DMSO. For a Strongly Binding Peptide assay, filter plate loading was done as described for IgG, with the exception that 500, 50, 5 and 0.5 µg/ml of each five biotinylated SBP (SBP1-SBP5) was used instead of Protein G. Fifty microliters of sample (IgG diluted 1:10, ME pool diluted 1:5, and ME pool diluted 1:10) diluted in StabilGuard buffer was added to each well. One well containing only StabilGuard was used as a non-template control “NTC” well. Fifty microliters of the bead mix (containing beads with human HSP60 G20, G20a, G20b, G20c, G20d, G20e, G20f, recombinant human HSP60, GroEL recombinant protein, *Hemophilus influenzae* B, and naked beads) was then added to each well. Four different concentrations for each of the five biotinylated SBPs (500, 50, 5 and 0.5 µg/ml) were incubated for 30 minutes. The beads were then washed, and incubated with SA-PE like in the experiments with biotinylated protein G and anti human IgM, and read in the Luminex bead counter. The results are described in Supplementary Figure SF2.

**Supplementary figure SF2.** Binding of “strongly binding peptides” (SBPs) to HSP60 recombinant proteins and human HSP60 G20 and G20c peptides.

1. Out of the five SBPs, SBP2 and SBP4, but not the other SBPs, bound to recombinant human HSP60, E coli GroEL, G20c and G20c homolog peptides, suggesting a chaperonin-like peptide binding activity of G20c homolog peptides. Signals of ME sera with high MFI values to these antigens were not much diminished.

B and C. SBP2 and SBP4 bound without saturation effects, with a concentration of 5 µg/ml being optimal for further experiments.



*Absorption of IgG with protein A.*

A source of falsely positive IgM values is IgG anti-IgM (“rheuma factor”), which occurs in autoimmune diseases like rheumatoid arthritis. Ten strongly IgM positive and 6 weakly IgM positive ME samples and 6 blood donor sera were incubated with recombinant Protein A Sepharose™ Fast Flow and Sepharose™ Fast Flow beads alone (17-1279-01, 17-0149-01, GE Healthcare, Bio-Sciences AB Uppsala, Sweden) at 37°C on a shaker for 1 h. The samples were centrifugated for 3 minutes at 13000 rpm. The supernatant was subsequently tested in the IgG and IgM SMIA tests, as described in Methods. In the IgG test, 0-30% of the MFI were recovered, while 60-150% of the MFI were recovered in the IgM test (data not shown). The result makes it unlikely that rheuma factor was a major cause of the IgM values in the ME samples.

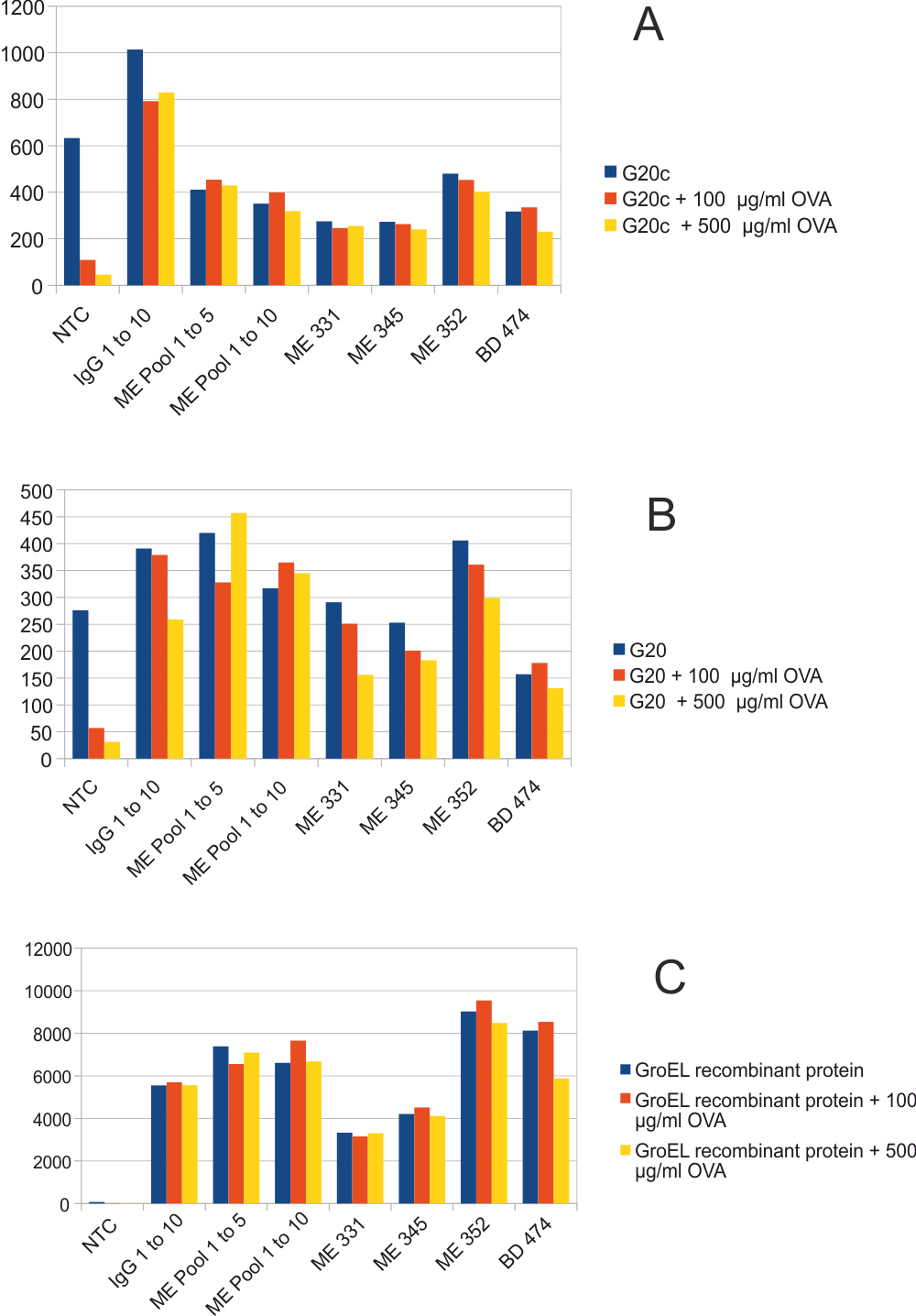
*Competition experiments using blocking protein and SBPs.*

During the course of this investigation we found that including ovalbumin together with the secondary antibody drastically reduced the NTC values with a marginal effect on the antibody specific signals from the samples (Supplementary Figure SF2). Obviously, the human blood samples contained enough non-antibody protein (a typical serum or plasma contains 70 mg/ml) to block the low affinity detected by the NTC. The StabilGuard diluent does not contain protein. We did not routinely include highly concentrated blocking protein in the tests because of occasional clogging of the probe in the Luminex machine in the presence of blocking protein. Instead, we opted for a partial subtraction of NTC values, as described in Materials and Methods (Supplementary Figure SF3).

# A few peptides, mostly those containing the helix I of human and microbial HSP60s gave high NTC values, making this control less useful. The binding in the presence of patient sample could be lower than that of the NTC (binding of the signal generating system to the bead without presence of patient sample). We therefore tried to reduce the NTC values by addition of 0.1, 1, 10 and 100 µg/ml Ovalbumin (Albumin from chicken egg white), (Sigma-Aldrich, cat. nr A 5503) and Bovine Serum Albumin (Bovine Plasma Albumin; BSA), (Sigma-Aldrich, cat. nr A3913) during incubation with BPG or SA-PE. A tenfold reduction of the NTC value of bead-bound G20c, without affecting the MFI of wells containing patient samples, was obtained by inclusion of 100 µg/mL of ovalbumin during the incubation with BPG while BSA was less effective. However, the ovalbumin was not routinely added because of occasional clogging of the Luminex machine probe. NTC MFI were therefore partially subtracted from the MFI of patient and BD samples as described above.

# Competition experiments with blocking proteins were conducted using 0.1, 1, 10 and 100 µg/ml ovalbumin and the same concentrations of BSA (not shown) during incubation with BPG or SA-PE during the incubations with protein G and SA-PE. The results are described in Supplementary Figure SF3.

**Supplementary Figure SF3.** Competition experiments with high concentrations of blocking protein. Ovalbumin (and BSA, not shown) was added at the protein G and SAPE incubation steps. The IgG preparation (5 mg/ml), the ME pool and ME patient and a BD sample with high IgG binding to the three antigens were run without and with different concentrations of ovalbumin at the protein G/anti-IgM incubation stage. The effect of ovalbumin was stronger than that of BSA at the same weight percentages. Results for peptides G20 (A), G20c (B) and *E. coli* GroEL (HSP60) (C) are shown.

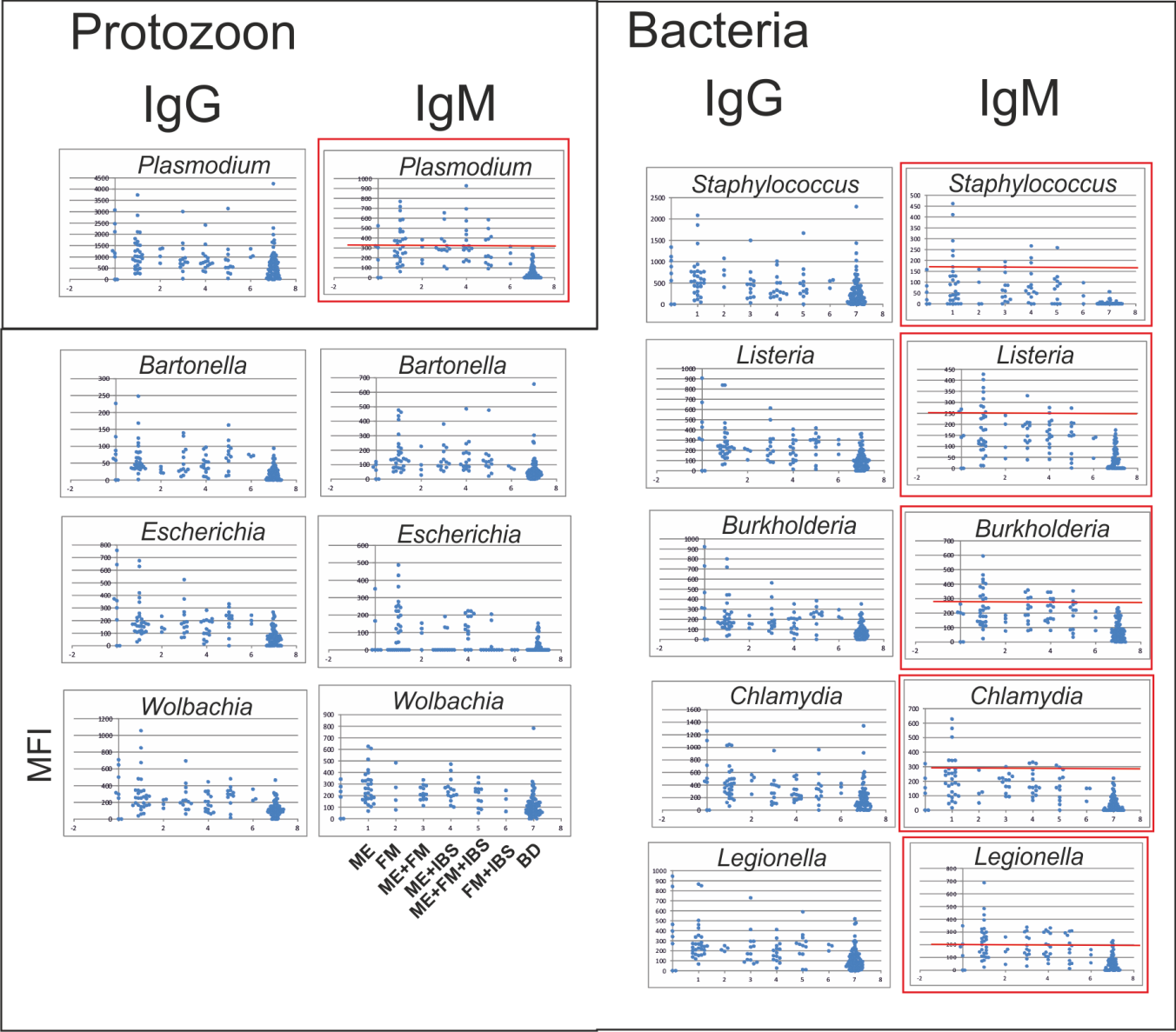
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*Data management and Statistics*

A computer program (MultiPlus™) which subtracts the NTC and naked bead values, and checks the results of the control sera for each peptide and protein, and stores them in a relational database, was written in Visual Foxpro by J. Blomberg. The subtraction was trivial for nearly all naked bead values, which were 5-10 MFI, but nontrivial for a few peptides, where the NTC values could reach several hundred MFI. In these cases the lowest MFI obtained with some samples were often lower than the NTC value. To avoid oversubtraction, the subtraction was made with 10% of the NTC and 90% of the lowest sample value for the bead. Negative values after subtraction were set to 0 MFI. The experiments always contained an equal number of patient and control samples, so the two groups were always treated equally. The validity of the subtraction was ascertained in later experiments, when we found that the NTC values could be reduced by inclusion of ovalbumin during the incubation with BPG or biotinylated anti-IgM. Subtracted results with and without the ovalbumin inclusion were highly similar (Supplementary Figure SF1). Significant differences in antigen reactivity between ME patient and BD samples were automatically evaluated using the two-tailed Fisher exact test (FET) and two-tailed Wilcoxon rank sum tests (WRST) with computer procedures written by J. Blomberg. A cutoff of 200 MFI was used in the FET unless stated otherwise. To reduce the influence of weak binding values in the WRST, all MFI values were subtracted with 50, with resulting negative values listed as 0, before WRST analysis. At this initial stage, no (“Bonferroni”) correction of p values for repeated comparisons was made. Cross-correlation of variables to each other, and principal component analysis (PCA), was performed using the Unscrambler statistical package (version X, Camo AS, Bergen, Norway).

**Section 4. Further results from the phyloscanning with G20c homologs**

**Supplementary Figure SF5.** Needle plots of reactivities with Test set samples with G20c homolog peptides in A. IgG and B. IgM assays. MFI values subtracted with NTC MFI of selected G20c homologs from the phyloscanning. Results from G20c homologs of a protozoon and bacteria are presented separately. Although the ME preference in most IgG tests was inferior to that of the IgM tests it is shown for comparison. The patient group was here split into ME (lane 1), FM (lane 2), ME+FM (lane 3), ME+IBS (lane 4), ME+FM+IBS (lane 5) and FM+IBS (lane 6) groups. BD samples were in lane 7 and control samples in lane 0. The most strongly discriminating peptides are framed in red. A cutoff which may be useful if the peptide is to be used as a biomarker,based on the Test set alone, is also shown in red. For abbreviations, see the legend of Figure 5.

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**Supplementary table ST7.** Correlation coefficients of MFI of G20c homolog peptides and recombinant human and *E. coli* HSP60 proteins in a serological assay with patient and BD samples. Correlation coefficients and sequence similarity according to the BLOSUM62 scoring system (“blosum”) are included.

**Table ST7A.** The 20 highest correlations between the IgG binding (MFI) of G20c homologs.

Five of the combinations were discordant, between a prokaryotic and a eukaryotic HSP60 sequence (\*).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Sequence 1 | Sequence 2 | Discordant  Combination | blosum | Iggcorr | Igmcorr |
| *Escherichia coli* | *Burkholderia multivorans* |  | 135 | 0,99228 | 0,64098 |
| *Treponema pallidum* | *Mycobacterium tuberculosis* |  | 116 | 0,96978 | 0,39078 |
| *Leptospira interrogans* | *Legionella pneumophila* |  | 99 | 0,96167 | 0,78119 |
| *Burkholderia multivorans* | *Bartonella henselae* |  | 126 | 0,93848 | 0,55282 |
| *Escherichia coli* | *Bartonella henselae* |  | 124 | 0,93809 | 0,27021 |
| *Escherichia coli* | *Legionella pneumophila* |  | 135 | 0,93669 | 0,5946 |
| *Leptospira interrogans* | *Chlamydia pneumoniae* |  | 102 | 0,93266 | 0,84728 |
| *Anaplasma phagocytophilium* | *Wolbachia* |  | 132 | 0,93076 | 0,42341 |
| *Staphylococcus aureus* | *Homo sapiens* | \* | 90 | 0,92763 | 0,46618 |
| *Schistosoma mansonii* | *Homo sapiens* |  | 127 | 0,91305 | 0,37872 |
| *Rickettsia bellii* | *Candidatus neoehrlichia mikurensis* |  | 124 | 0,91003 | 0,51955 |
| *Leptospira interrogans* | *Escherichia coli* |  | 104 | 0,90108 | 0,66396 |
| *Chlamydia pneumoniae* | *Homo sapiens* | \* | 100 | 0,89957 | 0,52151 |
| *Leptospira interrogans* | *Burkholderia multivorans* |  | 100 | 0,89639 | 0,87349 |
| *Leptospira interrogans* | *Plasmodium falciparum* | \* | 92 | 0,89507 | 0,46447 |
| *Leptospira interrogans* | *Homo sapiens* | \* | 89 | 0,89501 | 0,45683 |
| *Anaplasma phagocytophilium* | *Bartonella henselae* |  | 130 | 0,887 | 0,69313 |
| *Leptospira interrogans* | *Staphylococcus aureus* |  | 100 | 0,882 | 0,81089 |
| *Leptospira interrogans* | *Schistosoma mansonii* | \* | 77 | 0,87907 | 0,66144 |
| *Borrelia\_garinii* | *Homo sapiens* | \* | 83 | 0,86963 | 0,38378 |

**Table ST7B.** The 20 highest correlations between the IgM binding (MFI) of G20c homologs. One of the combinations was discordant, between a prokaryotic and a eukaryotic HSP60 sequence (\*).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Sequence 1 | Sequence 2 | Discordant  combination | blosum | Iggcorr | Igmcorr |
| *Borrelia garinii* | *Legionella pneumophila* |  | 123 | 0,86684 | 0,93864 |
| *Rickettsia bellii* | *Bartonella henselae* |  | 141 | 0,72258 | 0,87917 |
| *Leptospira interrogans* | *Burkholderia multivorans* |  | 100 | 0,89639 | 0,87349 |
| *Rickettsia bellii* | *Tropheryma whipplei* |  | 109 | 0,7994 | 0,86233 |
| *Chlamydia pneumoniae* | *Burkholderia multivorans* |  | 110 | 0,71103 | 0,85463 |
| *Leptospira interrogans* | *Chlamydia pneumoniae* |  | 102 | 0,93266 | 0,84728 |
| *Treponema pallidum* | *Legionella pneumophila* |  | 124 | 0,741 | 0,84472 |
| *Leptospira interrogans* | *Staphylococcus aureus* |  | 100 | 0,882 | 0,81089 |
| *Leptospira interrogans* | *Legionella pneumophila* |  | 99 | 0,96167 | 0,78119 |
| *Mycobacterium tuberculosis* | *Tropheryma whipplei* |  | 131 | 0,81951 | 0,77923 |
| *Chlamydia pneumoniae* | *Legionella pneumophila* |  | 119 | 0,85473 | 0,76039 |
| *Treponema pallidum* | *Staphylococcus aureus* |  | 97 | 0,43771 | 0,75873 |
| *Treponema pallidum* | *Leptospira interrogans* |  | 108 | 0,63775 | 0,74766 |
| *Treponema pallidum* | *Burkholderia\_multivorans* |  | 116 | 0,67714 | 0,74684 |
| *Leishmania* | *Homo sapiens* |  | 76 | 0,16706 | 0,74027 |
| *Chlamydia pneumoniae* | *Escherichia coli* |  | 109 | 0,72024 | 0,73912 |
| *Treponema pallidum* | *Schistosoma mansonii* | \* | 88 | 0,46295 | 0,71362 |
| *Anaplasma phagocytophilium* | *Tropheryma whipplei* |  | 102 | 0,81087 | 0,71279 |
| *Leptospira\_interrogans* | *Mycobacterium\_tuberculosis* |  | 106 | 0,71761 | 0,70157 |

**Section 5. Alignment of HSP60 sequences.**

**Supplementary Figure SF4.** An alignment of 34 HSP60 sequences.

Turqoise background: Epitopes crossreactive with HSP60 peptides of Mycobacterial, Chlamydial, *E. coli* and human origin, defined by Perschinka et al ([*2*](#_ENREF_2)).

Gray background: *Porphyromonas gingivalis* peptide related to autoimmunity Jeong et al ([*3*](#_ENREF_3)).

Underlined are the i. nonapeptide from the leader of human HSP60 which binds HLA-E and promotes NK cell killing, Michaelsson et al ([*14*](#_ENREF_14)),

ii. Overstrike: *E. coli* Peptide frequently antigenic in cardiovascular disease (Okada et al, ([*4*](#_ENREF_4)).

Red text indicates homologs of human HSP60 G20c.

Serologically reactive octameric peptides of Orientia tsutsugamushi Sta58, an HSP60 protein, (Lachumanan et al, ([*15*](#_ENREF_15)) are shown. Further details on HSP60 epitopes are given in section 6.

The positions of the apical loops and helices, as defined in *E. coli* GroEL, Braig et al ([*16*](#_ENREF_16)) are also indicated. Alignment positions, referring to the numbers in Figure 7, are shown to the right.

CLUSTAL W (1.83) multiple sequence alignment

50

GroEL\_orientia\_tsutsugamushi\_C --------------------------------MSKQIVHGDQCRKKIIEG

GroEL\_rickettsia\_bellii\_YP\_537 -------------------------------MATKLIKHGSKAREQMLEG

GroEL5\_neoehrlichia\_mikurensis --------------------------------MANVVVTGETLDKSIRDI

GroEL\_anaplasma\_phagocytophili --------------------------------MSNTVVTGEVLDKSIREV

GroEL\_ehrlichia\_deer\_AB454077 --------------------------------MANVVVTGEQLDKSIREV

GroEL\_Wolbachia\_YP\_198181 --------------------------------MTNVVVSGEQLQEAFREV

GroEL\_Borrelia\_garinii\_YP\_0730 --------------------------------MAKDIYFNEDARKSLLSG

GroEL\_treponema\_pallidum\_YP\_00 --------------------------------MAKQLLFNEEARKKLLSG

GroEL\_Leptospira\_interrogans\_Y --------------------------------MAKDIEYNETARRKLLEG

GroEL\_CH60\_CHLPN\_chlamydophila -------------------------------MAAKNIKYNEEARKKIHKG

GroEL3\_chlamydia\_psittaci\_AEG8 -------------------------------MAAKNIKYNEDARKKIHKG

GroEL2\_salmonella\_enterica\_typ -------------------------------MAAKDVKFGNDARVKMLRG

GroEL\_salmonella\_typhi\_TY2\_U01 -------------------------------MAAKDVKFGNDARVKMLRG

GroEL\_E\_coli\_K12\_AAC77103 -------------------------------MAAKDVKFGNDARVKMLRG

GroEL\_Yersinia\_pestis\_NP\_99189 -------------------------------MAAKDVKFGNDARIKMLRG

GroEL\_haemophilus\_influenzae\_Y -------------------------------MAAKDVKFGNDARVKMLKG

GroEL\_Legionella\_YP\_126086 --------------------------------MAKELRFGDDARLQMLAG

GroEL\_Burkholderia\_multivorans -------------------------------MAAKDVKFHDGARSRIVKG

GroEL\_Bartonella\_henselae\_YP\_0 -------------------------------MAAKEVKFGREARERLLRG

GroEL\_brucella\_abortus\_ZP\_0587 -------------------------------MAAKDVKFGRTAREKMLRG

GroEL\_Mycobact\_tuberc\_CAA17397 --------------------------------MAKTIAYDEEARRGLERG

GroEL\_Mycobacterium\_leprae\_TN\_ --------------------------------MAKTIAYDEEARRGLERG

GroEL\_tropheryma\_whipplei\_NP\_7 --------------------------------MAKKITFNEDARRGLERG

GroEL\_Staph\_aureus\_MRSA\_YP\_041 --------------------------------MVKQLKFSEDARQAMLRG

GroEL\_Listeria\_monocytogenes\_A --------------------------------MAKDIKFSEDARRAMLRG

GroEL\_streptococcus\_pneumoniae --------------------------------MSKEIKFSSDARSAMVRG

GroEL\_mycoplasma\_penetrans\_NP\_ --------------------------------MAKEIKFSDSARNKLFNG

HSP60\_Leishmania\_Strain\_Friedl ------------------MLSRTVPRCVKYGSTPKDIRYGMEARNALLAG

HSP60\_Schistosoma\_mansoni\_XP\_0 -------MLRAFATLRGT--LAPVRHRVIQRSYAKEVKFGADARSAMLVG

Hsp60\_human\_mitochondria\_NP\_95 -------MLRLPTVFRQMRPVSRVLAPHLTRAYAKDVKFGADARALMLQG

HSP60\_Plasmodium\_falciparum\_XP MISTLRGKIFN--NGSNRNKCVSILSNIQKRNISKDIRFGSDARTAMLTG

chaperonin60\_Cryptosporidium\_p MLLRSGINLYKSVEGSIGLRSAAIRFGMRYISSGKELSFGGKARKEMLKG

Chaperonin60\_Entamoeba\_histoly ------------------------MLSSSSHYNGKLLSLNIDCRENVLSG

Chaperonin60\_Giardia\_lamblia\_X -----------------------------MLQHYTSVISGEDARSGLLRG

. . .

100

GroEL\_orientia\_tsutsugamushi\_C INVVANAVGITLGPKGRCVAIEQSYG--PPKITKDGVSVAKAIQLKDKSL

GroEL\_rickettsia\_bellii\_YP\_537 IDILADAVKVTLGPKGRNVLIEQSFG--APKITKDGVTVAKSIELKDKIR

GroEL5\_neoehrlichia\_mikurensis IRILEDAVGCTAGPKGLTIAISKPYG--TPEITKDGYKVIKSIKPEEPLA

GroEL\_anaplasma\_phagocytophili VRILEDAVGCTAGPKGLTVAISKPYG--SPEITKDGYKVMKSIKPEEPLA

GroEL\_ehrlichia\_deer\_AB454077 VRILEDAVGCTAGPKGLTVAIGKSYG--APEITKDGYKVIKSIKPEDPLA

GroEL\_Wolbachia\_YP\_198181 AVMVDSTVAITAGPRGKTVGINKPYG--APEITKDGYKVMKGIKPEKPLH

GroEL\_Borrelia\_garinii\_YP\_0730 VEKLSNAVKVTLGPKGRNVLIDKKFG--SPTVTKDGVSVAREIELENPFE

GroEL\_treponema\_pallidum\_YP\_00 VEQISSAVKVTLGPKGRNVLLEKGYG--APTVTKDGVSVAKEVELEDPFE

GroEL\_Leptospira\_interrogans\_Y VNKLANAVKVTLGPKGRNVVIDKKFG--APTITKDGVTVAKEIELEDPLE

GroEL\_CH60\_CHLPN\_chlamydophila VKTLAEAVKVTLGPKGRHVVIDKSFG--SPQVTKDGVTVAKEIELEDKHE

GroEL3\_chlamydia\_psittaci\_AEG8 VKTLAEAVKVTLGPKGRHVVIDKSFG--SPQVTKDGVTVAKEIELEDKHE

GroEL2\_salmonella\_enterica\_typ VNVLADAVKVTLGPKGRNVVLDKSFG--APTITKDGVSVAREIELEDKFE

GroEL\_salmonella\_typhi\_TY2\_U01 VNVLADAVKVTLGPKGRNVVLDKSFG--APTITKDGVSVAREIELEDKFE

GroEL\_E\_coli\_K12\_AAC77103 VNVLADAVKVTLGPKGRNVVLDKSFG--APTITKDGVSVAREIELEDKFE

GroEL\_Yersinia\_pestis\_NP\_99189 VNILADAVKVTLGPKGRNVVLDKSFG--SPTITKDGVSVAREIELEDKFE

GroEL\_haemophilus\_influenzae\_Y VNVLADAVKVTLGPKGRNVILDKSFG--APTITKDGVSVAREIELEDKFE

GroEL\_Legionella\_YP\_126086 VNALADAVQVTMGPRGRNVVLEKSYG--APTVTKDGVSVAKEIEFEHRFM

GroEL\_Burkholderia\_multivorans VNVLADAVKVTLGPKGRNVLIERSFG--APTITKDGVSVAKEIELKDRFE

GroEL\_Bartonella\_henselae\_YP\_0 VDILANAVKVTLGPKGRNVVIDKSFG--APRITKDGVSVAKEIELEDKFE

GroEL\_brucella\_abortus\_ZP\_0587 VDILADAVKVTLGPKGRNVVIEKSFG--APRITKDGVSVAKEVELEDKFE

GroEL\_Mycobact\_tuberc\_CAA17397 LNALADAVKVTLGPKGRNVVLEKKWG--APTITNDGVSIAKEIELEDPYE

GroEL\_Mycobacterium\_leprae\_TN\_ LNSLADAVKVTLGPKGRNVVLEKKWG--APTITNDGVSIAKEIELEDPYE

GroEL\_tropheryma\_whipplei\_NP\_7 LNTLADTVKVTLGPRGRNVVLEKKWG--APVITNDGVTIAKEIELDDPYE

GroEL\_Staph\_aureus\_MRSA\_YP\_041 VDQLANAVKVTIGPKGRNVVLDKEFT--APLITNDGVTIAKEIELEDPYE

GroEL\_Listeria\_monocytogenes\_A VDQLANAVKVTLGPKGRNVVLEKKFG--SPLITNDGVTIAKEIELEDPFE

GroEL\_streptococcus\_pneumoniae VDILADTVKVTLGPKGRNVVLEKSFG--SPLITNDGVTIAKEIELEDHFE

GroEL\_mycoplasma\_penetrans\_NP\_ VQQLFDAVKVTMGPRGRNVLIQKSYG--APVITKDGVSVAKEVDLTNPIE

HSP60\_Leishmania\_Strain\_Friedl VENLVKAVGVTLGPKGRNVILEMPYA--CPKITKDGVTVAKSIEFEDSFE

HSP60\_Schistosoma\_mansoni\_XP\_0 VDILADAVAVTMGPKGRNVIIESSWK--SPKITKDGVTVAKGIELKDKFQ

Hsp60\_human\_mitochondria\_NP\_95 VDLLADAVAVTMGPKGRTVIIEQSWG--SPKVTKDGVTVAKSIDLKDKYK

HSP60\_Plasmodium\_falciparum\_XP CNKLADAVSVTLGPKGRNVIIEQSFG--SPKITKDGVTVAKSIEFNNKLA

chaperonin60\_Cryptosporidium\_p ANDLADAVGVTLGPRGRNVVIEQGFGE-APKITKDGVTVAKAIQFGKGSV

Chaperonin60\_Entamoeba\_histoly IKKVADAVSVTLGPKGRTVIIDQPYG--NARVTKDGVSVAKALTFSDNTL

Chaperonin60\_Giardia\_lamblia\_X IKTIADVVATTLGPRGRAVILADGSASGTTKVTKDGVSVARAINLSG-LE

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GroEL\_orientia\_tsutsugamushi\_C NVGAQFVISVASKTADVAGDGTTTATVIADAAVRELNKAEVAGIDIQEVR

GroEL\_rickettsia\_bellii\_YP\_537 NAGAQLLKSAATKAAEVAGDGTTTATVLARALAREGNKLVAAGYNPMDLK

GroEL5\_neoehrlichia\_mikurensis QAIANIIAQSASQCNDKVGDGTTTCSILTAKVIEEVSKAKAAGADIISIK

GroEL\_anaplasma\_phagocytophili AAIASIITQSASQCNDKVGDGTTTCSILTAKVIEEVSKAKAAGSDIVSIK

GroEL\_ehrlichia\_deer\_AB454077 LAIANIITQSASQCNDKVGDGTTTCSILTAKVIEEVSKAKAAGADIVCIK

GroEL\_Wolbachia\_YP\_198181 AAITSIFAQSCFQCNDKVGDGTTTCSILTSNMIMEALKSIAAGNDRVSIK

GroEL\_Borrelia\_garinii\_YP\_0730 NMGAQLLKEVAIKTNDVAGDGTTTATVLAYAIAREGLKNVSSGINPIGIK

GroEL\_treponema\_pallidum\_YP\_00 NMGAQLLKEVATKTNDVAGDGTTTATVLAYSMVREGLKAVAAGMTPLELK

GroEL\_Leptospira\_interrogans\_Y NMGAQMVKEVSTKTNDVAGDGTTTATILAQSIINEGLKNVTAGANPMSLK

GroEL\_CH60\_CHLPN\_chlamydophila NMGAQMVKEVASKTADKAGDGTTTATVLAEAIYSEGLRNVTAGANPMDLK

GroEL3\_chlamydia\_psittaci\_AEG8 NMGAQMVKEVASKTADKAGDGTTTATVLAEAIYSEGLRNVTAGANPMDLK

GroEL2\_salmonella\_enterica\_typ NMGAQMVKEVASKANDAAGDGTTTATVLAQSIITEGLKAVAAGMNPMDLK

GroEL\_salmonella\_typhi\_TY2\_U01 NMGAQMVKEVASKANDAAGDGTTTATVLAQSIITEGLKAVAAGMNPMDLK

GroEL\_E\_coli\_K12\_AAC77103 NMGAQMVKEVASKANDAAGDGTTTATVLAQAIITEGLKAVAAGMNPMDLK

GroEL\_Yersinia\_pestis\_NP\_99189 NMGAQMVKEVASKANDAAGDGTTTATVLAQSIITEGLKAVAAGMNPMDLK

GroEL\_haemophilus\_influenzae\_Y NMGAQMVKEVASKANDAAGDGTTTATVLAQAIVNEGLKAVAAGMNPMDLK

GroEL\_Legionella\_YP\_126086 NMGAQMVKEVASKTSDTAGDGTTTATVLARSILVEGHKAVAAGMNPMDLK

GroEL\_Burkholderia\_multivorans NMGAQVVKQVASKTADVAGDGTTTATVLAQAIVQEGMKHVAAGINPMDLK

GroEL\_Bartonella\_henselae\_YP\_0 NMGAQMLREVASKTNDIAGDGTTTATVLGQAIVQEGVKAVAAGMNPMDLK

GroEL\_brucella\_abortus\_ZP\_0587 NMGAQMLREVASKTNDTAGDGTTTATVLGQAIVQEGAKAVAAGMNPMDLK

GroEL\_Mycobact\_tuberc\_CAA17397 KIGAELVKEVAKKTDDVAGDGTTTATVLAQALVREGLRNVAAGANPLGLK

GroEL\_Mycobacterium\_leprae\_TN\_ KIGAELVKEVAKKTDDVAGDGTTTATVLAQALVKEGLRNVAAGANPLGLK

GroEL\_tropheryma\_whipplei\_NP\_7 KIGAELVKEVAKKTDDVAGDGTTTSVVLAQAMVREGLKNVAAGADPISLR

GroEL\_Staph\_aureus\_MRSA\_YP\_041 NMGAKLVQEVANKTNEIAGDGTTTATVLAQAMIQEGLKNVTSGANPVGLR

GroEL\_Listeria\_monocytogenes\_A NMGAKLVSEVASKTNDVAGDGTTTATVLAQAMIQEGLKNVTAGANPVGVR

GroEL\_streptococcus\_pneumoniae NMGAKLVSEVASKTNDIAGDGTTTATVLTQAIVREGIKNVTAGANPIGIR

GroEL\_mycoplasma\_penetrans\_NP\_ NMGAQLVKDVASKTADEAGDGTTTATVLAYGVFKEGLRNVISGANPIEIK

HSP60\_Leishmania\_Strain\_Friedl NLGANLVRQVAGLTNDNAGDGTTTATVLSGAIFKEGFRSVASGTNPMDLK

HSP60\_Schistosoma\_mansoni\_XP\_0 NIGAKLVQDVANNTNEEAGDGTTTATVLARAIAKEGFEKISKGANPIEFR

Hsp60\_human\_mitochondria\_NP\_95 NIGAKLVQDVANNTNEEAGDGTTTATVLARSIAKEGFEKISKGANPVEIR

HSP60\_Plasmodium\_falciparum\_XP NLGAQMVKQVAANTNDKAGDGTTTATILARSIFQQGCKAVDSGMNPMDLL

chaperonin60\_Cryptosporidium\_p NLGAQLLKNVAISTNEEAGDGTTTATVLARAIFKSGCEKVDAGLNPMDLL

Chaperonin60\_Entamoeba\_histoly NVGGKIAKEVASKVNDRSGDGTTTATCLLRKVACEGVQAINTGLSGTDLL

Chaperonin60\_Giardia\_lamblia\_X GVGADLIKDASLRTNTMAGDGTTTSLILSGKLVNEMNKYALSGLGNLQLL

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GroEL\_orientia\_tsutsugamushi\_C KGAEKAVEAVIADVRKNSS--PVKNEEEIAQVATVSSNGDREIGEKIANA

GroEL\_rickettsia\_bellii\_YP\_537 RGMDLAVNTVLEEVKKASK--KIDSQEEIAQVGTISSNGDKEIGEKIAKA

GroEL5\_neoehrlichia\_mikurensis NGILKAKELVLESLLSMKR--DVSSEDEIAQVATISANGDKNIGSKIAQC

GroEL\_anaplasma\_phagocytophili NGILKAKEAVLTALMSMRR--EVE-EDEIAQVATLSANGDKNIGSKIAQC

GroEL\_ehrlichia\_deer\_AB454077 EGVLKAKEAVLEALMSMKR--EVLSEEEIAQVATISANGDKNIGSKIAQC

GroEL\_Wolbachia\_YP\_198181 NGMQKAKDAVLEGITSMSRTIPLEKMDEVAQVAIISANGDKDIGNSIADA

GroEL\_Borrelia\_garinii\_YP\_0730 KGIDHAVNLAAEKIRQSAK--KITTKEEIAQVASISANNDSYIGEKIAEA

GroEL\_treponema\_pallidum\_YP\_00 RGMDKAVAIAVDDIKQNSK--GIKSNEEVAHVASVSANNDKEIGRILASA

GroEL\_Leptospira\_interrogans\_Y KGIDKAVTAAVESIQKRAV--KIENKKDIANVASISANNDNTIGNLIADA

GroEL\_CH60\_CHLPN\_chlamydophila RGIDKAVKVVVDELKKISK--PVQHHKEIAQVATISANNDSEIGNLIAEA

GroEL3\_chlamydia\_psittaci\_AEG8 RGIDKAVKVVVDQIKKISK--PVQHHKEIAQVATISANNDSEIGNLIAEA

GroEL2\_salmonella\_enterica\_typ RGIDKAVAAAVEELKALSV--PCSDSKAIAQVGTISANSDETVGKLIAEA

GroEL\_salmonella\_typhi\_TY2\_U01 RGIDKAVAAAVEELKALSV--PCSDSKAIAQVGTISANSDETVGKLIAEA

GroEL\_E\_coli\_K12\_AAC77103 RGIDKAVTAAVEELKALSV--PCSDSKAIAQVGTISANSDETVGKLIAEA

GroEL\_Yersinia\_pestis\_NP\_99189 RGIDKAVIAAVEELKKLSV--PCSDSKAIAQVGTISANSDSTVGELIAQA

GroEL\_haemophilus\_influenzae\_Y RGIDKAVSAVVSELKNLSK--PCETAKEIEQVGTISANSDSIVGQLISQA

GroEL\_Legionella\_YP\_126086 RGIDKAVLAVTKKLQAMSK--PCKDSKAIAQVGTISANSDEAIGAIIAEA

GroEL\_Burkholderia\_multivorans RGIDKAVGAVLDELRKLSR--PIATNKEIAQVGAISANSDEAIGKIIADA

GroEL\_Bartonella\_henselae\_YP\_0 RGIDAAVDEVVANLFKKAK--KIQTSAEIAQVGTISANGAAEIGKMIADA

GroEL\_brucella\_abortus\_ZP\_0587 RGIDLAVNEVVAELLKKAK--KINTSEEVAQVGTISANGEAEIGKMIAEA

GroEL\_Mycobact\_tuberc\_CAA17397 RGIEKAVEKVTETLLKGAK--EVETKEQIAATAAISA-GDQSIGDLIAEA

GroEL\_Mycobacterium\_leprae\_TN\_ RGIEKAVDKVTETLLKDAK--EVETKEQIAATAAISA-GDQSIGDLIAEA

GroEL\_tropheryma\_whipplei\_NP\_7 RGIEKSVAAVSKALLTSAK--EVETEAEIAACASISA-GDPQIGDIIAQA

GroEL\_Staph\_aureus\_MRSA\_YP\_041 QGIDKAVKVAVEALHENSQ--KVENKNEIAQVGAISA-ADEEIGRYISEA

GroEL\_Listeria\_monocytogenes\_A RGIEKAVATAIEELKAISK--PIESKESIAQVAAISS-GDEEVGKLIAEA

GroEL\_streptococcus\_pneumoniae RGIETAVAAAVEALKNNAI--PVANKEAIAQVAAVSS-RSEKVGEYISEA

GroEL\_mycoplasma\_penetrans\_NP\_ RGMDKTVNAIVNELNKSSK--KIARKDEIIQVATISANSDKKIGELIANA

HSP60\_Leishmania\_Strain\_Friedl RGIDLACREVLISLAEQSR--PVTSKSEITQVAMISANMDQEIGSLIGDA

HSP60\_Schistosoma\_mansoni\_XP\_0 RGVMSAVDAVVKELKSLSK--PISTPEEIAKSQQYQPTVTKRLA-----I

Hsp60\_human\_mitochondria\_NP\_95 RGVMLAVDAVIAELKKQSK--PVTTP~~EEIAQVATISANGDKEIGNII~~SDA

HSP60\_Plasmodium\_falciparum\_XP RGINKGVEKVLEYLNSIKK--DVTTTEEIFNVASISANGDKNIGQLIADT

chaperonin60\_Cryptosporidium\_p RGIKLGVEHVVNELDLLSQ--PVKSHDDILNVATISANGDSIVGSLIAQA

Chaperonin60\_Entamoeba\_histoly KGISIAKDIVLKEITKQSK---PTLKEDIISVARVSANNDEKIGEMVGDI

Chaperonin60\_Giardia\_lamblia\_X QALNSAGVDCLQSLRKQSR--AIESNKMLYSVATIAANNDPKIGKVVSDA

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1st apical loop 250

GroEL\_orientia\_tsutsugamushi\_C MKQVGQEGVITVEDSKNFN-FEVEVVKGMRFDRGYISQYFATNREKMITE

GroEL\_rickettsia\_bellii\_YP\_537 MEEVGKEGVITVEEAKNFS-FDVEVVKGMMFDRGYLSPYFVTNSEKMVAE

GroEL5\_neoehrlichia\_mikurensis VKEVGKDGVITVEESKGFKELEVEKTDGMQFDRGYLSPYFVTNAEKMLIE

GroEL\_anaplasma\_phagocytophili VKEVGKDGVITVEESKGFKDLEVEKTDGMQFDRGYLSPYFVTNAEKMLVE

GroEL\_ehrlichia\_deer\_AB454077 VQEVGKDGVITVEESKGFKELDVEKTDGMQFDRGYLSPYFVTNSEKMLVE

GroEL\_Wolbachia\_YP\_198181 VKKVGKEGVITVEESKGSKELEVELTTGMQFDRGYLSPYFITSNEKMIVE

GroEL\_Borrelia\_garinii\_YP\_0730 MDKVGKDGVITVEESKTFD-TTISYVEGMQFDRGYLSPYFSTNKENMSVS

GroEL\_treponema\_pallidum\_YP\_00 IEKVGNDGVIDVDEAQTME-TVTEFVEGMQFDRGYISSYFVTDRDRMETV

GroEL\_Leptospira\_interrogans\_Y MDKVGKDGVITVEEAKSIE-TTLDVVEGMQFDRGYISPYMVTDAESMVAT

GroEL\_CH60\_CHLPN\_chlamydophila MEKVGKNGSITVEEAKGFE-TVLDVVEGMNFNRGYLSSYFSTNPETQECV

GroEL3\_chlamydia\_psittaci\_AEG8 MEKVGKNGSITVEEAKGFE-TVLDVVEGMNFNRGYLSSYFSTNPETQECV

GroEL2\_salmonella\_enterica\_typ MDKVGKEGVITVEDGTGLQ-DELDVVEGMQFDRGYLSPYFINKPETGAVE

GroEL\_salmonella\_typhi\_TY2\_U01 MDKVGKEGVITVEDGTGLQ-DELDVVEGMQFDRGYLSPYFINKPETGAVE

GroEL\_E\_coli\_K12\_AAC77103 MDKVGKEGVITVEDGTGLQ-DELDVVEGMQFDRGYLSPYFINKPETGAVE

GroEL\_Yersinia\_pestis\_NP\_99189 MEKVGKEGVITVEEGSGLQ-DELDVVEGMQFDRGYLSPYFINKPETGSIE

GroEL\_haemophilus\_influenzae\_Y MEKVGKEGVITVEDGTGLE-DELDVVEGMQFDRGYLSPYFINKPETATVE

GroEL\_Legionella\_YP\_126086 MEKVGKEGVITVEDGNGLE-NELSVVEGMQFDRGYISPYFINNQQNMSCE

GroEL\_Burkholderia\_multivorans MERVGKEGVITVEDGKSLE-NELEVVEGMQFDRGYVSPYFINDPEKQAAY

GroEL\_Bartonella\_henselae\_YP\_0 MEKVGNEGVITVEEAKTAE-TELEVVEGMQFDRGYLSPYFVTNAEKMVAD

GroEL\_brucella\_abortus\_ZP\_0587 MQKVGNEGVITVEEAKTAE-TELEVVEGMQFDRGYLSPYFVTNPEKMVAD

GroEL\_Mycobact\_tuberc\_CAA17397 MDKVGNEGVITVEESNTFG-LQLELTEGMRFDKGYISGYFVTDPERQEAV

GroEL\_Mycobacterium\_leprae\_TN\_ MDKVGNEGVITVEESNTFG-LQLELTEGMRFDKGYISGYFVTDAERQEAV

GroEL\_tropheryma\_whipplei\_NP\_7 LEKVGKEGVVTVEESNTFG-TELEITEGMRFDKGYLSAYFVTDAERQETV

GroEL\_Staph\_aureus\_MRSA\_YP\_041 MEKVGNDGVITIEESNGLN-TELEVVEGMQFDRGYQSPYMVTDSDKMVAE

GroEL\_Listeria\_monocytogenes\_A MERVGNDGVITIEESKGFA-TELDVVEGMQFDRGYTSPYMVTDSDKMEAV

GroEL\_streptococcus\_pneumoniae MEKVGKDGVITIEESRGME-TELEVVEGMQFDRGYLSQYMVTDSEKMVAD

GroEL\_mycoplasma\_penetrans\_NP\_ MEKVGSDGVITVEEAKGIN-DELTVVEGMQFDRGYISPYFVTDTNKMIAK

HSP60\_Leishmania\_Strain\_Friedl MQQVGKDGVITTQEGRSLN-TELELVEGMSFERGYTSPYFVTNTKAQRCE

HSP60\_Schistosoma\_mansoni\_XP\_0 MKKVGNDGTITVKDGKTLH-DELEFIEGMKFDRGYISPYFLNTEKGARCE

Hsp60\_human\_mitochondria\_NP\_95 MKKVGRKGVITVKDGKTLN-DELEIIEGMKFDRGYISPYFINTSKGQKCE

HSP60\_Plasmodium\_falciparum\_XP MKKVGKEGTITVTEGKTLQ-HELEIVEGIKFDRGYISPYFINNSKDQKVE

chaperonin60\_Cryptosporidium\_p YSKVGRHGTINIEEGNTTQ-SELEIVEGLKLDKGYISPYFITNQKYQKVE

Chaperonin60\_Entamoeba\_histoly FGKIGRDGAVDIETGKGTK-DIVNIVEGMVLDQGFLSRYFTTDEKNTKVD

Chaperonin60\_Giardia\_lamblia\_X FAAVGREGTITVEDG-YTDIDTLNVTDGCSIPSGFLSPYFALGGSR-YLE

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apical H helix, apical I helix 300

HHHHHHHHHH IIIIII

GroEL\_orientia\_tsutsugamushi\_C FENPYILLLDQKVSTV-QPLVPVLEAVAHTGK-PLVLIADDVDGEALTAL

GroEL\_rickettsia\_bellii\_YP\_537 LENPYILLFEKKLSNL-QPMLPILEAVVQSQR-PLLIIAEDVEGEALATL

GroEL5\_neoehrlichia\_mikurensis FENPYILLTEKKLNII-QPILPILENIARSGR-PLLIIAEDVEGEALSTL

GroEL\_anaplasma\_phagocytophili FENPYIFLTEKKINLV-QSILPILENVARSGR-PLLIIAEDVEGEALSTL

GroEL\_ehrlichia\_deer\_AB454077 FENPYILLTEKKLNII-QPILPILENVARSGR-PLLIIAEDVEGEALSTL

GroEL\_Wolbachia\_YP\_198181 FDDPYLLITEKKLSII-QPLLPILEAVVKSGK-PLLIIAEDIEGEALSTL

GroEL\_Borrelia\_garinii\_YP\_0730 FDDAFILIYEKKISSI-KELLPVLEKVLGTNK-PLLIIAEDIEGDALAAL

GroEL\_treponema\_pallidum\_YP\_00 YENPYILIYDKSISTM-KDLLPLLEKIAQTGR-PLLIIAEDVEGEALATL

GroEL\_Leptospira\_interrogans\_Y LNDPFILIYDKKISSM-KDLIHILEKVAQAGK-PLVIISEEVEGEALATI

GroEL\_CH60\_CHLPN\_chlamydophila LEDALILIYDKKISGI-KDFLPVLQQVAESGR-PLLIIAEEIEGEALATL

GroEL3\_chlamydia\_psittaci\_AEG8 LEEALVLIYDKKISGI-KDFLPVLQQVAESGR-PLLIIAEDIEGEALATL

GroEL2\_salmonella\_enterica\_typ LESPFILLADKKI*SNI-REMLPVLEAVAKAG*K-PLLIIAEDVEG*EALATL*

GroEL\_salmonella\_typhi\_TY2\_U01 LESPFILLADKKISNI-REMLPVLEAVAKAGK-PLLIIAEDVEGEALATL

GroEL\_E\_coli\_K12\_AAC77103 LESPFILLADKKISNI-REMLPVLEAVAKAGK-PLLIIAEDVEGEALATL

GroEL\_Yersinia\_pestis\_NP\_99189 LESPFILLADKKISNI-REMLPVLEAVAKAGK-PLLIIAEDVEGEALATL

GroEL\_haemophilus\_influenzae\_Y LDNPYLLLVDKKISNI-RELLPVLEGVAKAGK-PLLIIAEDVEGEALATL

GroEL\_Legionella\_YP\_126086 LEHPFILLVDKKVSSI-REMLSVLEGVAKSGR-PLLIIAEDVEGEALATL

GroEL\_Burkholderia\_multivorans LDDPLILLHDKKISSI-RDLLPILEAASKAGK-PLLIVAEDVDGEALATL

GroEL\_Bartonella\_henselae\_YP\_0 LDDPYILIHEKKLSNL-QSLLPVLEAVVQSGK-PLLIIAEDVEGEALATL

GroEL\_brucella\_abortus\_ZP\_0587 LEDAYILLHEKKLSNL-QALLPVLEAVVQTSK-PLLIIAEDVEGEALATL

GroEL\_Mycobact\_tuberc\_CAA17397 LEDPYILLVSSKVSTV-KDLLPLLEKVIGAGK-PLLIIAEDVEGEALSTL

GroEL\_Mycobacterium\_leprae\_TN\_ LEEPYILLVSSKVSTV-KDLLPLLEKVIQAGK-SLLIIAEDVEGEALSTL

GroEL\_tropheryma\_whipplei\_NP\_7 FENPYILICDSKISSV-KDLLPVVDKVIQSGK-QLLIIAEDVDGEALATL

GroEL\_Staph\_aureus\_MRSA\_YP\_041 LERPYILVTDKKISSF-QDILPLLEQVVQSNR-PILIVADEVEGDALTNI

GroEL\_Listeria\_monocytogenes\_A LEKPYILITDKKINNI-QEILPVLEQVVQQGR-PMLIIAEDVEGEAQATL

GroEL\_streptococcus\_pneumoniae LENPYILITDKKISNI-QEILPLLESILQSNR-PLLIIADDVDGEALPTL

GroEL\_mycoplasma\_penetrans\_NP\_ LENPYILITDKKVSSI-KDILPILEEIMKTGR-PLLIIADDVDGEALTTL

HSP60\_Leishmania\_Strain\_Friedl LENALVYVANRKLTSV-AHILPALNYAIQQKR-PLLVIAEDVEGEAMHTF

HSP60\_Schistosoma\_mansoni\_XP\_0 FQDAFVLFSEKKINSI-QTLLPALELCHQQKR-PLLIIAEDVEGEALTAL

Hsp60\_human\_mitochondria\_NP\_95 FQDAYVLLSEKKISSI-QSIVPALEIANAHRK-PLVIIAEDVDGEALSTL

HSP60\_Plasmodium\_falciparum\_XP LDKPYILIHEKKISTV-KSLLPVLEHVLQNQS-SLLVIAEDVDSDALATL

chaperonin60\_Cryptosporidium\_p LENPYILISQGKISSL-KSILPILEFCISSRS-PLLIIAEEIEGEALTAL

Chaperonin60\_Entamoeba\_histoly IRNTDVIVCDYKLSSS-QSVVPLLELCLKRKR-PLVVISDTIDGDALTTL

Chaperonin60\_Giardia\_lamblia\_X LTNPLVVITDTVLSSA-APLVSILERCVKEKR-PLLIIASDVTGDALSTL

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2nd apical loop 350

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GroEL\_orientia\_tsutsugamushi\_C ILNNLKGSIKVVAVKAPGFGDRKKEMLEDIAILTNG-EVITEQLGIKLEK

GroEL\_rickettsia\_bellii\_YP\_537 VVNRLRGGLKVAAVKAPGFGDRRKAMMEDIAILTNG-ELITEDLGMKLEN

GroEL5\_neoehrlichia\_mikurensis VLNKLRGGLHVAAVKAPGFGDRRKDMLGDIAILTGAKYVINDELAVKMED

GroEL\_anaplasma\_phagocytophili VLNKLRGGLQVAAVKAPGFGDRRKDMLGDIAVIVGAKYVVNDELAVKMED

GroEL\_ehrlichia\_deer\_AB454077 VLNKLRGGLHVAAVKAPGFGDRRKDMLGDIAILTGAKHVISDDLAIKMED

GroEL\_Wolbachia\_YP\_198181 VINKLRGGLKVTAVKAPGFGDRRKEMLEDIAALTGAKYVIKDELGIKMED

GroEL\_Borrelia\_garinii\_YP\_0730 VLNSVRGALKVCAIKSPGFGDRRKAMLEDIAVLTGG-VLISEELGITLET

GroEL\_treponema\_pallidum\_YP\_00 VVNSLRGTLKTCAVKAPGFGDRRKEMLEDIAILSGG-QVISEDLGLKLES

GroEL\_Leptospira\_interrogans\_Y VVNTLRKTISCVAVKAPGFGDRRKSMLEDIAILTGG-QVISEDLGMKLEN

GroEL\_CH60\_CHLPN\_chlamydophila VVNRLRAGFRVCAVKAPGFGDRRKAMLEDIAILTGG-QLVSEELGMKLEN

GroEL3\_chlamydia\_psittaci\_AEG8 VVNRLRAGFRVCAVKAPGFGDRRKAMLEDIAILTGG-QLISEELGMKLEN

GroEL2\_salmonella\_enterica\_typ VVNTMRGIVKVAAVKAPGFGDRRKAMLQDIATLTGG-TVISEEIGMELEK

GroEL\_salmonella\_typhi\_TY2\_U01 VVNTMRGIVKVAAVKAPGFGDRRKAMLQDIATLTGG-TVISEEIGMELEK

GroEL\_E\_coli\_K12\_AAC77103 *VVNTMRGIVKVAA*VKAPGFGDRRKAMLQDIATLTGG-TVISEEIGMELEK

GroEL\_Yersinia\_pestis\_NP\_99189 VVNTMRGIVKVAAVKAPGFGDRRKAMLQDIATLTAG-TVISEEIGLELEK

GroEL\_haemophilus\_influenzae\_Y VVNTMRGIVKVAAVKAPGFGDRRKAMLQDIAILTAG-TVISEEIGMELEK

GroEL\_Legionella\_YP\_126086 VVNNMRGIVKVCAVKAPGFGDRRKAMLQDIAILTKG-QVISEEIGKSLEG

GroEL\_Burkholderia\_multivorans VVNAMRGILKVAAVKAPGFGDRRKAMLEDIAILTGA-TVISEETGKQLEK

GroEL\_Bartonella\_henselae\_YP\_0 VVNKLRGGLKIAAVKAPGFGDRRKAMLEDIAILTSG-QVISEDVGIKLEN

GroEL\_brucella\_abortus\_ZP\_0587 VVNKLRGGLKIAAVKAPGFGDRRKAMLEDIAILTGG-QVISEDLGIKLES

GroEL\_Mycobact\_tuberc\_CAA17397 VVNKIRGTFKSVAVKAPGFGDRRKAMLQDMAILTGG-QVISEEVGLTLEN

GroEL\_Mycobacterium\_leprae\_TN\_ VVNKIRGTFKSVAVKAPGFGDRRKAMLQDMAILTGA-QVISEEVGLTLEN

GroEL\_tropheryma\_whipplei\_NP\_7 VVNKIRGIFKSVAVKAPGFGDRRKMMLQDIAVLTGG-QVISEEVGLKLEN

GroEL\_Staph\_aureus\_MRSA\_YP\_041 VLNRMRGTFTAVAVKAPGFGDRRKAMLEDLAILTGA-QVITDDLGLDLKD

GroEL\_Listeria\_monocytogenes\_A VLNKLRGTFNVVAVKAPGFGDRRKAMLEDIAILTGG-QVITEDLGLELKT

GroEL\_streptococcus\_pneumoniae VLNKIRGTFNVVAVKAPGFGDRRKAMLEDIAILTGG-TVITEDLGLELKD

GroEL\_mycoplasma\_penetrans\_NP\_ VVNKMRGVFNVVAVKAPEFGDKRKQVLEDIAILTGG-SFVTDDLGISFDK

HSP60\_Leishmania\_Strain\_Friedl LYNKIQGRISGCAVKAPGFGDMRINQLQDIAVFTGS-QMISEDLGLSLDQ

HSP60\_Schistosoma\_mansoni\_XP\_0 VLNRLKLGLQVCAVKAPGFGDNRKNTLKDMAVATGGIVFGDEADMYKLED

Hsp60\_human\_mitochondria\_NP\_95 VLNRLKVGLQVVAVKAPGFGDNRKNQLKDMAIATGGAVFGEEGLTLNLED

HSP60\_Plasmodium\_falciparum\_XP IVNKLRLGLKICAVKAPGFGEHRKALIHDIAVMTGAKVITEETG-LKLDD

chaperonin60\_Cryptosporidium\_p ILNKLQLNLKVCAVKAPGFGDHRKQILEDISVSVGAKIIQEEFSNAKLDQ

Chaperonin60\_Entamoeba\_histoly VLNKLRG-LPIAAVRAPGFGETRKGILHDIGIITGA-TVISNEAGKKIEE

Chaperonin60\_Giardia\_lamblia\_X AINTLKGTVRCCAVRAPGYGDVKKGVLEDLAAVVGIPTYISDELHTASAP

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GroEL\_orientia\_tsutsugamushi\_C VN--DTSK-LGTANRVIVTKDHTTIVHDKNNSDIEKKVNSRCEQIREAIK

GroEL\_rickettsia\_bellii\_YP\_537 VS--LKS--LGHAKRVTISKENTVIVDGSGD---KKNIEERVLQIKSHIA

GroEL5\_neoehrlichia\_mikurensis LT--LDD--LGTAKNIRITKDTTTLIGSVDSNS--SNVQSRINQIKMQID

GroEL\_anaplasma\_phagocytophili IA--LSD--LGTAKSVRITKDATTIIGSVDSSS--ESIASRTNQIKAQIE

GroEL\_ehrlichia\_deer\_AB454077 LT--LAE--LGTAKNIRITKDTTTIIGSVDNSS--ANVQNRINQIKMQIE

GroEL\_Wolbachia\_YP\_198181 LT--LED--LGTAKNVKVTKDNTTIVSGS-SDS--DRVKARVEQIKSQIE

GroEL\_Borrelia\_garinii\_YP\_0730 VE--IEQ--LGQAKTIKVDKDNTTIIN-TGNKE---QIKERSELIKKQIE

GroEL\_treponema\_pallidum\_YP\_00 AD--IAL--LGQAKSVKVDKENTTIIDGSGKSK---DIKDRIEQIKKQIE

GroEL\_Leptospira\_interrogans\_Y TT--LQM--LGRANKVTVDKENTTIIEGKGQTK---EIQGRIGQIKKQIE

GroEL\_CH60\_CHLPN\_chlamydophila TT--LAM--LGKAKKVIVTKEDTTIVEGLGNKP---DIQARCDNIKKQIE

GroEL3\_chlamydia\_psittaci\_AEG8 TT--LSM--LGKAKKVIVSKEDTTIVEGLGNKE---DIEARCENIKKQIE

GroEL2\_salmonella\_enterica\_typ AT--LED--LGQAKRVVINKDTTTIIDGVGEEA---AIQGRVAQIRQQIE

GroEL\_salmonella\_typhi\_TY2\_U01 AT--LED--LGQAKRVVINKDTTTIIDGVGEEA---AIQGRVAQIRQQIE

GroEL\_E\_coli\_K12\_AAC77103 AT--LED--LGQAKRVVINKDTTTIIDGVGEEA---AIQGRVAQIRQQIE

GroEL\_Yersinia\_pestis\_NP\_99189 TT--LED--LGQAKRVVINKDTTIIIDGVGDEA---AIQGRVAQIRQQIE

GroEL\_haemophilus\_influenzae\_Y AT--LED--LGQAKRVVINKDNTTIIDGIGDEA---QIKGRVAQIRQQIE

GroEL\_Legionella\_YP\_126086 AT--LED--LGSAKRIVVTKENTTIIDGEGKAT---EINARITQIRAQME

GroEL\_Burkholderia\_multivorans AT--LED--LGRAKRVEVRKDDTIIIDGAGDPA---RIDARVKAIRVQID

GroEL\_Bartonella\_henselae\_YP\_0 VT--LDM--LGRAKKVNISKENTTIIDGAGQKS---EINARVNQIKVQIE

GroEL\_brucella\_abortus\_ZP\_0587 VT--LDM--LGRAKKVSISKENTTIVDGAGQKA---EIDARVGQIKQQIE

GroEL\_Mycobact\_tuberc\_CAA17397 AD--LSL--LGKARKVVVTKDETTIVEGAGDTD---AIAGRVAQIRQEIE

GroEL\_Mycobacterium\_leprae\_TN\_ TD--LSL--LGKARKVVMTKDETTIVEGAGDTD---AIAGRVAQIRTEIE

GroEL\_tropheryma\_whipplei\_NP\_7 AT--LDL--LGCARKVVVSKDETTIVDGAGSSD---QIAGRVSQIRKELE

GroEL\_Staph\_aureus\_MRSA\_YP\_041 AT--IDM--LGTASKVEVTKDNTTVVDGDGDEN---SIDARVSQLKSQIE

GroEL\_Listeria\_monocytogenes\_A AT--VDQ--LGTANKVVVTKDDTTIVEGAGDST---QISARVNQIRAQME

GroEL\_streptococcus\_pneumoniae AT--IEA--LGQAARVTVDKDSTVIVEGAGNPE---AISHRVAVIKSQIE

GroEL\_mycoplasma\_penetrans\_NP\_ VT--LQD--LGQAESVVIDKDNSTIVKGKGLES---QIKERISKIKTAIE

HSP60\_Leishmania\_Strain\_Friedl ND--FSERFLGTCRKVTVSRDECILMEGGGSAI---AVEERVQMIKDMIS

HSP60\_Schistosoma\_mansoni\_XP\_0 VQ--LQD--LGRVAEVVVTKDDCLLMRGRGSKT---DVDKRIAQIKEEME

Hsp60\_human\_mitochondria\_NP\_95 VQ--PHD--LGKVGEVIVTKDDAMLLKGKGDKA---QIEKRIQEIIEQLD

HSP60\_Plasmodium\_falciparum\_XP P---QVVSYLGKAKSINVTKDSTLIMEGEGKKE---EINERCESIRNAIK

chaperonin60\_Cryptosporidium\_p MNSNQIQEFLGKCKSISVSKDETIITQGQGSPK---DVKDTISLLKSQIE

Chaperonin60\_Entamoeba\_histoly VT--EKD--LGKIGHFVSTKDETIITGGAGSKA---EVLARINELKNAKE

Chaperonin60\_Giardia\_lamblia\_X GSAVLSN--IGSCHKAIITPANTVLHFNDDKNCN-SLIRGRVAGLRSLLE

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GroEL\_orientia\_tsutsugamushi\_C DT--TSDYEKEKLQERLAKLRNGVAVLKVGGATEVEQKERKDRVEDALHA

GroEL\_rickettsia\_bellii\_YP\_537 ET--TSDYDKEKLQERLAKLSGGVAVLKVGGATEVEVKERKDRVEDALAA

GroEL5\_neoehrlichia\_mikurensis TS--TSDYDKEKLKERLAKLSGGVAVLKVGGSSEVEVKERKDRVE-----

GroEL\_anaplasma\_phagocytophili NS--SSDYDKEKLRERLAKLSGGVAVLKVGGSSEVEVKERKDRVEDALHA

GroEL\_ehrlichia\_deer\_AB454077 AS--TSDYDKEKLRERLAKLSGGVAVLKVGGSSEVEVKERKDRVEDALHA

GroEL\_Wolbachia\_YP\_198181 TS--TSDYDKEKLRERLAKLSGGVAVLKVGGVTEVEVKERRDRVEDALHA

GroEL\_Borrelia\_garinii\_YP\_0730 DS--TSEYDKEKLQERLAKLVGGVAVINVGAVTEVELKEKKHRVEDALSA

GroEL\_treponema\_pallidum\_YP\_00 AS--TSDYDSEKLKERLAKLSGGVAVIKIGAVTEVEMKEKKHRVEDALNA

GroEL\_Leptospira\_interrogans\_Y DT--TSEYDREKLQERLAKLAGGVAVIHVGAATEVEMKEKKARVEDALSA

GroEL\_CH60\_CHLPN\_chlamydophila DS--TSDYDKEKLQERLAKLSGGVAVIRVGAATEIEMKEKKDRVDDAQHA

GroEL3\_chlamydia\_psittaci\_AEG8 DS--TSDYDKEKLQERLAKLSGGVAVIRVGAATEIEMKEKKDRVDDAQHA

GroEL2\_salmonella\_enterica\_typ EA--TSDYDREKLQERVAKLAGGVAVIKVGAATEVEMKEKKARVEDALHA

GroEL\_salmonella\_typhi\_TY2\_U01 EA--TSDYDREKLQERVAKLAGGVAVIKVGAATEVEMKEKKARVEDALHA

GroEL\_E\_coli\_K12\_AAC77103 EA--TSDYDREKLQERVAKLAGGVAVIKVGAATEVEMKEKKARVEDALHA

GroEL\_Yersinia\_pestis\_NP\_99189 DA--TSDYDKEKLQERVAKLAGGVAVIKVGAATEVEMKEKKARVEDALHA

GroEL\_haemophilus\_influenzae\_Y ES--TSDYDKEKLQERVAKLAGGVAVIKVGAATEVEMKEKKDRVDDALHA

GroEL\_Legionella\_YP\_126086 ET--TSDYDREKLQERVAKLAGGVAVIKVGAATEVEMKEKKARVEDALHA

GroEL\_Burkholderia\_multivorans EA--TSDYDREKLQERVAKLAGGVAVIKVGAATEVEMKEKKDRVDDALHA

GroEL\_Bartonella\_henselae\_YP\_0 ET--TSDYDREKLQERLAKLAGGVAVIRVGGATEVEVKEKKDRVDDALNA

GroEL\_brucella\_abortus\_ZP\_0587 ET--TSDYDREKLQERLAKLAGGVAVIRVGGATEVEVKEKKDRVDDALNA

GroEL\_Mycobact\_tuberc\_CAA17397 NS--DSDYDREKLQERLAKLAGGVAVIKAGAATEVELKERKHRIEDAVRN

GroEL\_Mycobacterium\_leprae\_TN\_ NS--DSDYDREKLQERLAKLAGGVAVIKAGAATEVELKERKHRIEDAVRN

GroEL\_tropheryma\_whipplei\_NP\_7 NS--DSDYDREKLQERLAKLSGGVAVIRSGAATEVELKERKHRIEDAVRN

GroEL\_Staph\_aureus\_MRSA\_YP\_041 ET--ESDFDREKLQERLAKLAGGVAVIKVGAASETELKERKLRIEDALNS

GroEL\_Listeria\_monocytogenes\_A ET--TSEFDREKLQERLAKLAGGVAVVKVGAATETELKERKLRIEDALNS

GroEL\_streptococcus\_pneumoniae TT--TSEFDREKLQERLAKLSGGVAVIKVGAATETELKEMKLRIEDALNA

GroEL\_mycoplasma\_penetrans\_NP\_ MT--DSDYDKDSLRNRLAKLNKGVAVIKVGAVSEVELKEKKDRVDDALSA

HSP60\_Leishmania\_Strain\_Friedl AE--DHEYNRERLVERLAKLSGGVAVIKVGGASEVEINEKKDRIIDALNA

HSP60\_Schistosoma\_mansoni\_XP\_0 AS--NSEYEKEKMHERLAKLSNGVAVIKVGGSSEVEVSEKKDRYTDALNA

Hsp60\_human\_mitochondria\_NP\_95 VT--TSEYEKEKLNERLAKLSDGVAVLKVGGTSDVEVNEKKDRVTDALNA

HSP60\_Plasmodium\_falciparum\_XP MN--TSDYEKEKLQERLAKITGGVALIKVGGISEVEVNEIKDRIQDALCA

chaperonin60\_Cryptosporidium\_p ENQKLTDYDKEKLRERLARLTGRVALIKIGGYSDTEISELKDRFIDALNA

Chaperonin60\_Entamoeba\_histoly VS--DSSYEKEKLEGRIARLTGGVAVISVGGSSEAEVGERKDRIEDAVCA

Chaperonin60\_Giardia\_lamblia\_X SNN-LTNYQRSKLNERIGRLLGKVCTIRIGAKTELEAEEKKDRYIDSLSA

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GroEL\_orientia\_tsutsugamushi\_C TRAAVEEGIVPGGGVALFYASR--VLDS--LKFD----------------

GroEL\_rickettsia\_bellii\_YP\_537 TRAAVEEGVVAGGGVTLLHASQ--ALKN--LKVD----------------

GroEL5\_neoehrlichia\_mikurensis --------------------------------------------------

GroEL\_anaplasma\_phagocytophili TRAAVEEGVVPGGGAALLYALS--SLDG--LKGK----------------

GroEL\_ehrlichia\_deer\_AB454077 TRACC---------------------------------------------

GroEL\_Wolbachia\_YP\_198181 TRAAIEEGIVPGGGVALLYASS--ALDK--LKGG----------------

GroEL\_Borrelia\_garinii\_YP\_0730 TRAAVEEGVVPGGGSTLIEVAM--YLDTIDTSKL----------------

GroEL\_treponema\_pallidum\_YP\_00 TRAAIEEGIVAGGGLALIQAAA--ALEKADLSGL----------------

GroEL\_Leptospira\_interrogans\_Y TRAAVEEGIVPGGGLTLLKAQE--AVGSLKLDG-----------------

GroEL\_CH60\_CHLPN\_chlamydophila TIAAVEEGILPGGGTALVRCIP--TLEAFLPMLA----------------

GroEL3\_chlamydia\_psittaci\_AEG8 TLAAVEEGILPGGGTALVRCIP--TLEAFIPVLT----------------

GroEL2\_salmonella\_enterica\_typ TRAAVEEGVVAGGGVALIRVAS--KIAD--LKGQ----------------

GroEL\_salmonella\_typhi\_TY2\_U01 TRAAVEEGVVAGGGVALIRVAS--KIAD--LKGQ----------------

GroEL\_E\_coli\_K12\_AAC77103 TRAAVEEGVVAGGGVALIRVAS--KLAD--LRGQ----------------

GroEL\_Yersinia\_pestis\_NP\_99189 TRAAVEEGVVAGGGVALIRAAH--AIAG--LKGD----------------

GroEL\_haemophilus\_influenzae\_Y TRAAVEEGIVAGGGVALVRAAA--KVAAS-LKGD----------------

GroEL\_Legionella\_YP\_126086 TRAAVEEGIVAGGGVALIRAQK--ALDS--LKGD----------------

GroEL\_Burkholderia\_multivorans TRAAVEEGIVPGGGVALLRARA--ALAD--IKGA----------------

GroEL\_Bartonella\_henselae\_YP\_0 TRAAVEEGIVAGGGTALLRAAN--ALTV---KGS----------------

GroEL\_brucella\_abortus\_ZP\_0587 TRAAVEEGIVAGGGTALLRAST--KITA---KGV----------------

GroEL\_Mycobact\_tuberc\_CAA17397 AKAAVEEGIVAGGGVTLLQAAP--TLDELKLEG-----------------

GroEL\_Mycobacterium\_leprae\_TN\_ AKAAVEEGIVAGGGVTLLQAAP--ALDKLKLTG-----------------

GroEL\_tropheryma\_whipplei\_NP\_7 AKAAVEEGIVAGGGAALLQSGTS-ALKDLQLTS-----------------

GroEL\_Staph\_aureus\_MRSA\_YP\_041 TRAAVEEGIVAGGGTALVNVYQ--KVSEIEAEG-----------------

GroEL\_Listeria\_monocytogenes\_A TRAAVEEGIVAGGGTALVSIYN--KVAALEAEG-----------------

GroEL\_streptococcus\_pneumoniae TRAAVEEGIVAGGGTALANVIP--AVATLELTG-----------------

GroEL\_mycoplasma\_penetrans\_NP\_ TKAAIEEGIVIGGGAALVHVSKRINVNTLNLIG-----------------

HSP60\_Leishmania\_Strain\_Friedl TRAAVSEGILAGGGTGLLMASLR-LESISKDRRL----------------

HSP60\_Schistosoma\_mansoni\_XP\_0 TRAAIEEGIVPGGGTALLRCIP--ILKS--LESK----------------

Hsp60\_human\_mitochondria\_NP\_95 TRAAVEEGIVLGGGCALLRCIP--ALDS--LTPA----------------

HSP60\_Plasmodium\_falciparum\_XP TKAAVEEGIVPGGGSALLFASK--ELDSVQTD------------------

chaperonin60\_Cryptosporidium\_p TKCAIEQGIVPGGGSALLWASR--NLGKLYSQSPPPGKTLTPSQSSSNES

Chaperonin60\_Entamoeba\_histoly VKAALAEGIVPGGGVALIRAGS--SLDKIRSQNW----------------

Chaperonin60\_Giardia\_lamblia\_X ARAALEGGLLPGGGVAFLRAAQVMERKLAEGKVADP--------------

550

GroEL\_orientia\_tsutsugamushi\_C ----NEDQRVGINIIKKVLEAPVRQIVKNAGGKEDVVVN-ELSK--STDK

GroEL\_rickettsia\_bellii\_YP\_537 ----NKDQQAGIELVIEALKDPIKQIVENAGENGGVVVG-KLLE--HKDK

GroEL5\_neoehrlichia\_mikurensis --------------------------------------------------

GroEL\_anaplasma\_phagocytophili ----NDDEQWGIDIIRRAACAPIKRIIKNSGSEEAPCVIQHLLK--QNDK

GroEL\_ehrlichia\_deer\_AB454077 --------------------------------------------------

GroEL\_Wolbachia\_YP\_198181 ----SDEEQIGINIIKKVLSAPIKRLVKNAGLES-AVIIDHLTK--QNDK

GroEL\_Borrelia\_garinii\_YP\_0730 ----SYEEKQGFEIVKRSLEEPMRQIISNAGFEGSIYIHQIKT----EKK

GroEL\_treponema\_pallidum\_YP\_00 ----TPDEAVGFKIVRRALEEPIRQISENAGIDGAVVAEKAK-----EKR

GroEL\_Leptospira\_interrogans\_Y ------DEATGAKIIFRALEEPIRMITSNAGLEGSVIVEHAKA----KKG

GroEL\_CH60\_CHLPN\_chlamydophila ----NEDEAIGTRIILKALTAPLKQIASNAGKEGAIICQQVLA----RSA

GroEL3\_chlamydia\_psittaci\_AEG8 ----NEDEQIGARIVLKALSAPLKQIAANAGKEGAIICQQVLS----RSS

GroEL2\_salmonella\_enterica\_typ ----NEDQNVGIKVALRAMEAPLRQIVLNCGEEPSVVANTVKG----GDG

GroEL\_salmonella\_typhi\_TY2\_U01 ----NEDQNVGIKVALRAMEAPLRQIVLNCGEEPSVVANTVKG----GDG

GroEL\_E\_coli\_K12\_AAC77103 ----NEDQNVGIKVALRAMEAPLRQIVLNCGEEPSVVANTVKG----GDG

GroEL\_Yersinia\_pestis\_NP\_99189 ----NEDQNVGIKVALRAMESPLRQIVVNAGEEASVIANKVKA----GEG

GroEL\_haemophilus\_influenzae\_Y ----NEEQNVGIKLALRAMEAPLRQIVTNAGEEASVVASAVKN----GEG

GroEL\_Legionella\_YP\_126086 ----NDDQNMGINILRRAIESPMRQIVTNAGYEASVVVNKVAE----HKD

GroEL\_Burkholderia\_multivorans ----NADQDAGIRIVLRALEAPLRVIVSNAGEEPSVVIAKVLE----GKG

GroEL\_Bartonella\_henselae\_YP\_0 ----NPDQEAGINIVRRALQAPARQIATNAGEEAAIIVGKVLEN---NAD

GroEL\_brucella\_abortus\_ZP\_0587 ----NADQEAGINIVRRAIQAPARQITTNAGEEASVIVGKILEN---TSE

GroEL\_Mycobact\_tuberc\_CAA17397 ------DEATGANIVKVALEAPLKQIAFNSGLEPGVVAEKVRN----LPA

GroEL\_Mycobacterium\_leprae\_TN\_ ------DEATGANIVKVALEAPLKQIAFNSGMEPGVVAEKVRN----LSV

GroEL\_tropheryma\_whipplei\_NP\_7 ------EEAVGRNIVRSAIEAPLRQISLNAGLEPGVVVGKVSS----LPQ

GroEL\_Staph\_aureus\_MRSA\_YP\_041 ------DIETGVNIVLKALTAPVRQIAENAGLEGSVIVERLKN----AEP

GroEL\_Listeria\_monocytogenes\_A ------DVETGINIVLRSLEEPVRQIAHNAGLEGSVIVERLKH----EAV

GroEL\_streptococcus\_pneumoniae ------DEATGRNIVLRALEEPVRQIAHNAGFEGSIVIDRLKN----AEL

GroEL\_mycoplasma\_penetrans\_NP\_ ------DEKIGYQIVMSAIMSPISQIVSNAGFDKGVVINEILKA---TNP

HSP60\_Leishmania\_Strain\_Friedl ----PPDIRTGVNIVKKAIGLPARYIANNAGVEGSVVAGKVLAR---KDP

HSP60\_Schistosoma\_mansoni\_XP\_0 ----NEDQRTGVQIVLRALSTPCYTIAHNAGVNASVVVEKVMG----MGQ

Hsp60\_human\_mitochondria\_NP\_95 ----NEDQKIGIEIIKRTLKIPAMTIAKNAGVEGSLIVEKIMQ----SSS

HSP60\_Plasmodium\_falciparum\_XP ----NYDQRVGVNIIKDACKAPIKQIAENAGHEGSVVAGNILK--EKNS-

chaperonin60\_Cryptosporidium\_p NPIRNYDMAMGVKIVQDACKVPCHLISSNAGFDGSVIVGELVKVFSKGSK

Chaperonin60\_Entamoeba\_histoly ------AEKVGIDIVRKVTEEPTRIIARNAGIDGGIVIQKIKEG----TG

Chaperonin60\_Giardia\_lamblia\_X ------VTIAAHKALIAALHEPARIIAESAGASGHVVAEAIKNS---PDN

600

GroEL\_orientia\_tsutsugamushi\_C NRGFDARTMQYVDMIKAGIVDPTKVVRTALQDAFSVASLVIATSAMITD-

GroEL\_rickettsia\_bellii\_YP\_537 NFGFNAQDMQYVDMIKAGIIDPAKVVRTALQDAASVASLIITTETLIVD-

GroEL5\_neoehrlichia\_mikurensis --------------------------------------------------

GroEL\_anaplasma\_phagocytophili ELIYNVDTMNYANAFTSGVMDPLKVVRIAFDLAVSLAAVFMTLNAVVVD-

GroEL\_ehrlichia\_deer\_AB454077 --------------------------------------------------

GroEL\_Wolbachia\_YP\_198181 ELIYNVEAMNYANAFTAGVIDPAKVVRIAFETAISVASVLITTESMIVD-

GroEL\_Borrelia\_garinii\_YP\_0730 GLGFDASSFKWVNMIESGIIDPAKVTRSALQNAASIAGLLLTTECAITD-

GroEL\_treponema\_pallidum\_YP\_00 GIGFDASKMEWVDMIKVGIIDPAKVTRSALQNAASVSGLLLTTECAIAA-

GroEL\_Leptospira\_interrogans\_Y NEGFNALTMVWEDMIQAGVVDPAKVVRSALQNAASIGSMILTTEVTITD-

GroEL\_CH60\_CHLPN\_chlamydophila NEGYDALRDAYTDMIDAGILDPTKVTRSALESAASIAGLLLTTEALIAD-

GroEL3\_chlamydia\_psittaci\_AEG8 NEGYDALRDAYTDMIEAGILDPTKVTRCALESAASVAGLLLTTEALIAD-

GroEL2\_salmonella\_enterica\_typ NYGYNAATEEYGNMIDMGILDPTKVTRSALQYAASVAGLMITTECMVTD-

GroEL\_salmonella\_typhi\_TY2\_U01 NYGYNAATEEYGNMIDMGILDPTKVTRSALQYAASVAGLMITTECMVTD-

GroEL\_E\_coli\_K12\_AAC77103 NYGYNAATEEYGNMIDMGILDPTKVTRSALQYAASVAGLMITTECMVTD-

GroEL\_Yersinia\_pestis\_NP\_99189 SFGYNAYTEEYGDMIAMGILDPTKVTRSALQYAASIAGLMITTECMVTD-

GroEL\_haemophilus\_influenzae\_Y NFGYNAGTEQYGDMIEMGILDPTKVTRSALQFAASVAGLMITTECMVTD-

GroEL\_Legionella\_YP\_126086 NYGFNAATGEYGDMVEMGILDPTKVTRMALQNAASVASLMLTTECMVAD-

GroEL\_Burkholderia\_multivorans NFGYNAATGEYGDLVEAGVVDPTKVTRTALQNAASIAGLILTTDATVAD-

GroEL\_Bartonella\_henselae\_YP\_0 TFGYNTATGEFGDLIALGIVDPVKVVRSALQNAASIASLLITTEAMVAE-

GroEL\_brucella\_abortus\_ZP\_0587 TFGYNTANGEYGDLISLGIVDPVKVVRTALQNAASVAGLLITTEAMIAE-

GroEL\_Mycobact\_tuberc\_CAA17397 GHGLNAQTGVYEDLLAAGVADPVKVTRSALQNAASIAGLFLTTEAVVAD-

GroEL\_Mycobacterium\_leprae\_TN\_ GHGLNAATGEYEDLLKAGVADPVKVTRSALQNAASIAGLFLTTEAVVAD-

GroEL\_tropheryma\_whipplei\_NP\_7 GHGLDASTGEYVDMLSRGISDPVKVTRSALENAASIAGLFLTTEAVVAE-

GroEL\_Staph\_aureus\_MRSA\_YP\_041 GVGFNAATNEWVNMLEVGIVDPTKVTRSALQHAASVAAMFLTTEAVVAS-

GroEL\_Listeria\_monocytogenes\_A GVGFNAANGEWVNMIDAGIVDPTKVTRSALQNASSVAALLLTTEAVVAD-

GroEL\_streptococcus\_pneumoniae GIGFNAATGEWVNMIDQGIIDPVKVSRSALQNAASVASLILTTEAVVAN-

GroEL\_mycoplasma\_penetrans\_NP\_ HLGFNAATGKYVDMFQTGIIDPVKVTRIALQNAVSVSSMLLTTEAVIYD-

HSP60\_Leishmania\_Strain\_Friedl SFGYNAQTGEYVNMFEAGIIDPMKVVKSAVVNACSVAGMMITTEAAVVEK

HSP60\_Schistosoma\_mansoni\_XP\_0 NMGYDAQNDAYVDMIEAGIIDPTKVVRTALVDAAGVASLLTTAETVVTD-

Hsp60\_human\_mitochondria\_NP\_95 EVGYDAMAGDFVNMVEKGIIDPTKVVRTALLDAAGVASLLTTAEVVVTE-

HSP60\_Plasmodium\_falciparum\_XP NIGFNAQEGKYVDMIESGIIDPTKVVKTAISDAASIASLMTTTEVAIVD-

chaperonin60\_Cryptosporidium\_p HFGFDAQTGQFVDMIESGILDPTKVVKSGLRDAASIASLMTTTQVSVFE-

Chaperonin60\_Entamoeba\_histoly SFGYDVRKNVYCDLMKVGIVDPTKVVRNAFNEAISVGSLIATSEALITD-

Chaperonin60\_Giardia\_lamblia\_X FYGFDALNGQFVNMEKAGILDATKVVTTALDSALGVSSVLLNTDAVVQP-

650

GroEL\_orientia\_tsutsugamushi\_C ----------------------HEEDNNTGNRSGGGVGGGHHGGMGGMDF

GroEL\_rickettsia\_bellii\_YP\_537 ----------------------EPEDKENPMPMRGGMGG--MGGMGGMDF

GroEL5\_neoehrlichia\_mikurensis --------------------------------------------------

GroEL\_anaplasma\_phagocytophili ----------------------VPSKNDAAGAGAGGMGG--MGGMGGF--

GroEL\_ehrlichia\_deer\_AB454077 --------------------------------------------------

GroEL\_Wolbachia\_YP\_198181 ----------------------VPNKEENASSSMG-AGG--MGGMNGF--

GroEL\_Borrelia\_garinii\_YP\_0730 ----------------------IKEE---KNTSGGGGYPMDPGMGMM---

GroEL\_treponema\_pallidum\_YP\_00 ----------------------IPE----KSSSTPPAPDMG-GMGGMY--

GroEL\_Leptospira\_interrogans\_Y ----------------------KPDKDAPNPMAGMGGGGMG-GMGGMM--

GroEL\_CH60\_CHLPN\_chlamydophila ----------------------IPEE---KSSSAPAMPSAG--MDY----

GroEL3\_chlamydia\_psittaci\_AEG8 ----------------------IPEE---KSSSVPAMPGAG--MDY----

GroEL2\_salmonella\_enterica\_typ ----------------------LPK-SDAPDLGAAGGMGGMGGMGGMM--

GroEL\_salmonella\_typhi\_TY2\_U01 ----------------------LPK-SDAPDLGAAGGMGGMGGMGGMM--

GroEL\_E\_coli\_K12\_AAC77103 ----------------------LPK-NDAADLGAAGGMGGMGGMGGMM--

GroEL\_Yersinia\_pestis\_NP\_99189 ----------------------LPR-DDKGADMGAGGMGGMGGMGGMM--

GroEL\_haemophilus\_influenzae\_Y ----------------------LPK-DDKADLGAAG-MGGMGGMGGMM--

GroEL\_Legionella\_YP\_126086 ----------------------LPKKEEGVGAGDMGGMGGMGGMGGMM--

GroEL\_Burkholderia\_multivorans ----------------------APK-DESAAPAPSPALDY----------

GroEL\_Bartonella\_henselae\_YP\_0 ----------------------VPKKDTPVPPMPGGGMGGMGGMDF----

GroEL\_brucella\_abortus\_ZP\_0587 ----------------------LPKKDAAPAGMPGG-MGGMGGMDF----

GroEL\_Mycobact\_tuberc\_CAA17397 ----------------------KPEKEKA--SVPG-GGDMG-GMDF----

GroEL\_Mycobacterium\_leprae\_TN\_ ----------------------KPEKTAA--PASDPTGGMG-GMDF----

GroEL\_tropheryma\_whipplei\_NP\_7 ----------------------KPEPK----PAPGPADPGA-GMDF----

GroEL\_Staph\_aureus\_MRSA\_YP\_041 ----------------------IPEKNN----DQPNMGGMP-GM------

GroEL\_Listeria\_monocytogenes\_A ----------------------KPDENGPAAVPDMGMGGMG-GMM-----

GroEL\_streptococcus\_pneumoniae ----------------------KPEPVAP--APAMDPSMMG-GMM-----

GroEL\_mycoplasma\_penetrans\_NP\_ ----------------------VKD-DKEDSVPAMPNMGMG-GMM-----

HSP60\_Leishmania\_Strain\_Friedl DLLGREKRIEDEGMEDKEKKRSVDKLRKQVNERDAPMPKMAPPMKFDMKG

HSP60\_Schistosoma\_mansoni\_XP\_0 ----------------------LPKEETGANAAGMGGMGGMGGMGGMM--

Hsp60\_human\_mitochondria\_NP\_95 ----------------------IPKEEKDPGMGAMGGMG--GGMGGGMF-

HSP60\_Plasmodium\_falciparum\_XP ----------------------FKDSKNEESSQHMNSVNSMGDMGGMY--

chaperonin60\_Cryptosporidium\_p ----------------------PSNQSEKNNSSGSNSSESSSSFGSLPGD

Chaperonin60\_Entamoeba\_histoly ----------------------EPIKKEIN--------------------

Chaperonin60\_Giardia\_lamblia\_X ----------------------IPTDTNLFKNK-----------------

GroEL\_orientia\_tsutsugamushi\_C --

GroEL\_rickettsia\_bellii\_YP\_537 --

GroEL5\_neoehrlichia\_mikurensis --

GroEL\_anaplasma\_phagocytophili --

GroEL\_ehrlichia\_deer\_AB454077 --

GroEL\_Wolbachia\_YP\_198181 --

GroEL\_Borrelia\_garinii\_YP\_0730 --

GroEL\_treponema\_pallidum\_YP\_00 --

GroEL\_Leptospira\_interrogans\_Y --

GroEL\_CH60\_CHLPN\_chlamydophila --

GroEL3\_chlamydia\_psittaci\_AEG8 --

GroEL2\_salmonella\_enterica\_typ --

GroEL\_salmonella\_typhi\_TY2\_U01 --

GroEL\_E\_coli\_K12\_AAC77103 --

GroEL\_Yersinia\_pestis\_NP\_99189 --

GroEL\_haemophilus\_influenzae\_Y --

GroEL\_Legionella\_YP\_126086 --

GroEL\_Burkholderia\_multivorans --

GroEL\_Bartonella\_henselae\_YP\_0 --

GroEL\_brucella\_abortus\_ZP\_0587 --

GroEL\_Mycobact\_tuberc\_CAA17397 --

GroEL\_Mycobacterium\_leprae\_TN\_ --

GroEL\_tropheryma\_whipplei\_NP\_7 --

GroEL\_Staph\_aureus\_MRSA\_YP\_041 --

GroEL\_Listeria\_monocytogenes\_A --

GroEL\_streptococcus\_pneumoniae --

GroEL\_mycoplasma\_penetrans\_NP\_ --

HSP60\_Leishmania\_Strain\_Friedl L-

HSP60\_Schistosoma\_mansoni\_XP\_0 --

Hsp60\_human\_mitochondria\_NP\_95 --

HSP60\_Plasmodium\_falciparum\_XP --

chaperonin60\_Cryptosporidium\_p FY

Chaperonin60\_Entamoeba\_histoly --

Chaperonin60\_Giardia\_lamblia\_X --

**Section 6. Further discussion on HSP60 epitopes**

Antibodies to certain peptides from human HSP60 (not overlapping the ones preferentially recognized by antibodies in ME samples described here) are also more common in diabetes type 1 compared to controls ([*17*](#_ENREF_17)).

As shown in Figure 5, several of the chlamydia HSP60 epitopes defined with shorter synthetic peptides ([*8*](#_ENREF_8)*,* [*18-20*](#_ENREF_18)) were also seen in our epitope survey. Compared to controls, secretory IgA reactive with a chlamydia peptide (ATLVGNRIRGGF) was more common in women with infertility ([*20*](#_ENREF_20)) and was more commonly recognized by IgG from women with pelvic inflammatory disease ([*18*](#_ENREF_18)). It overlapped our human HSP60 peptides G20 and G20c and their microbial homologs. However it contained only two amino acids (underlined) of the consensus antigenicity profile defined here. Although unlikely, its cross-reactions with G20c homologs and ME samples should be evaluated. Women with *Chlamydia trachomatis*-associated ectopic pregnancy recognized 13 peptides from *Chlamydia trachomatis* HSP60 ([*8*](#_ENREF_8)*,* [*19*](#_ENREF_19)) (marked in the alignment of Supplementary Figure SF2). Our longer peptides detected some, but not all, of these epitopes, using BD and ME patient samples. It remains to investigate if hidden in these reactions lie diagnostically useful epitopes, and whether there exist disease-specific *Chlamydia pneumoniae* HSP60 antibody patterns with our set of peptides, aside from the selective ME reactions reported here.

Knowing that HSP60 is a highly conserved and cross-reactive protein, the ability of some *Chlamydia pneumoniae*, and *Mycoplasma penetrans* HSP60 peptides to selectively detect IgM antibodies in blood samples from ME patients cannot be taken as definite evidence for *Chlamydia* or *Mycoplasma* as etiologic agents in ME. The matter requires an investigation with more HSP60 antigens than was possible in this report.

*Other disease associations of antibodies reacting with HSP60 peptides, and their crossreactivities.*

In the mycobacterially induced adjuvant arthritis of rats, the arthritis starts a T cell response to a centrally placed HSP60 epitope which subsides when a response to the C terminal portion of HSP65 occurs ([*21*](#_ENREF_21)). The humoral anti-HSP60 response appears to protect against the arthritis ([*22*](#_ENREF_22)). The antibody response initially involves several epitopes, but later narrows down to fewer epitopes. This is reminiscent of the epitope differences in the IgM and IgG tests seen in this work, where IgM epitopes were more numerous than the IgG ones. The *Porphyromonas gingivalis* peptide 19 (*TLVVNRLRG*SLKICAVKAPG) was frequently antigenic in patients suffering from cardiovascular disease ([*5*](#_ENREF_5)*,* [*23*](#_ENREF_23)). It contains nine (underlined) of the 16 consensus amino acids critical for detection of antibodies in some ME patients defined here. It contains a part of apical helix I (italics). The possibility that peptide p19 and the G20/G20c homologs share some antigenicity should be tested experimentally. Antibodies to certain peptides from human HSP60 are also more common in T1D compared to controls ([*17*](#_ENREF_17)). There exists a correlation between enterovirus infection and T1D, see e.g. ([*24*](#_ENREF_24)*,* [*25*](#_ENREF_25)). Of note, antibodies to HSP60 peptides were also induced after enterovirus infection ([*25*](#_ENREF_25)).

**Section 7. Further details regarding neurological autoimmune diseases**

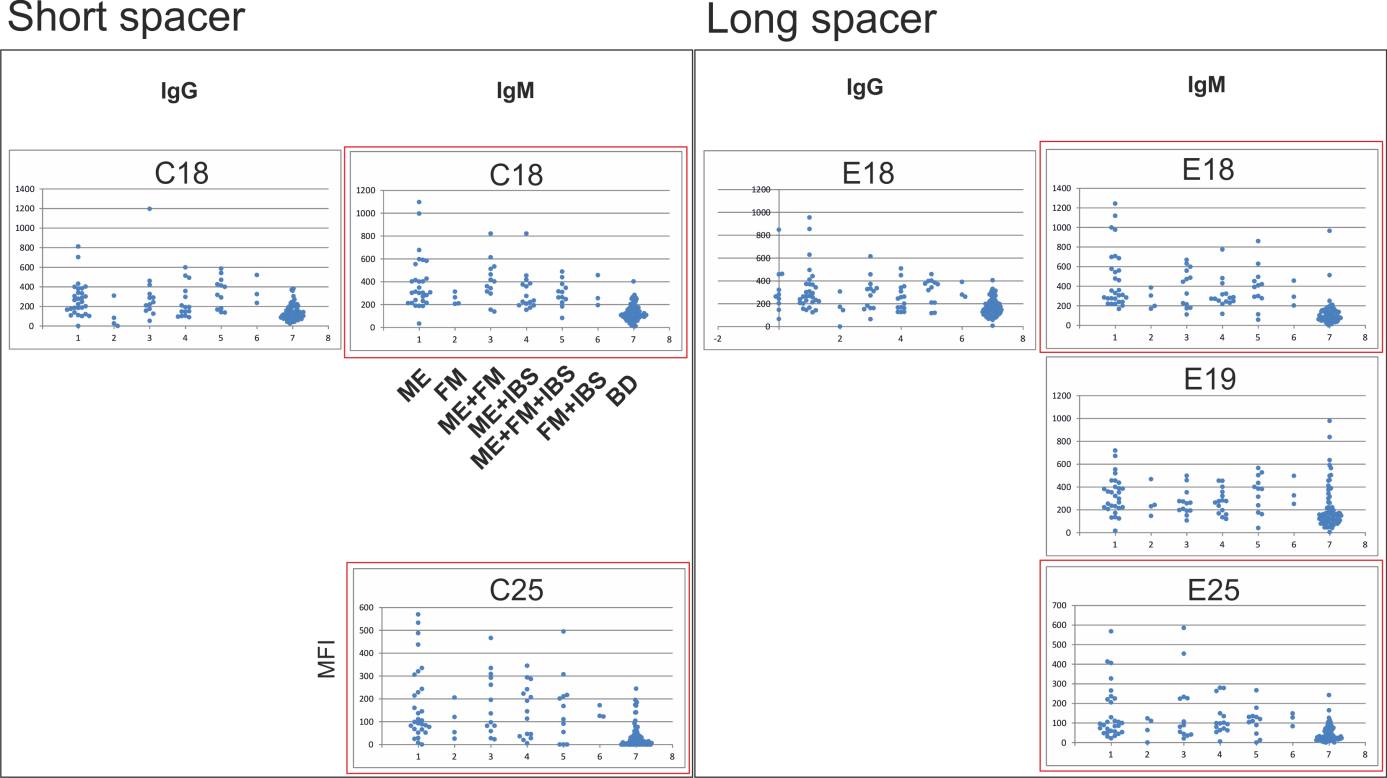
A large number of neurological diseases, like narcolepsy ([*26-29*](#_ENREF_26)), postinfectious encephalomyelitis, including the Guillain-Barré syndrome ([*30*](#_ENREF_30)), and pediatric neuropsychiatric disorder with tics and Sydenham´s chorea after streptococcal infection ([*31-33*](#_ENREF_31)) are now known. Other diseases with autoimmunity to specific brain tissues are multiple sclerosis ([*34*](#_ENREF_34)) and anti-NMDA receptor encephalitis ([*35*](#_ENREF_35)). It is relevant for this paper that IgM autoepitopes of human HSP60 and human HSP70 proteins are commonly recognized in MS ([*36*](#_ENREF_36)*,* [*37*](#_ENREF_37)).

**Section 8. Further details regarding the association of Chlamydia and Mycoplasma with ME.**

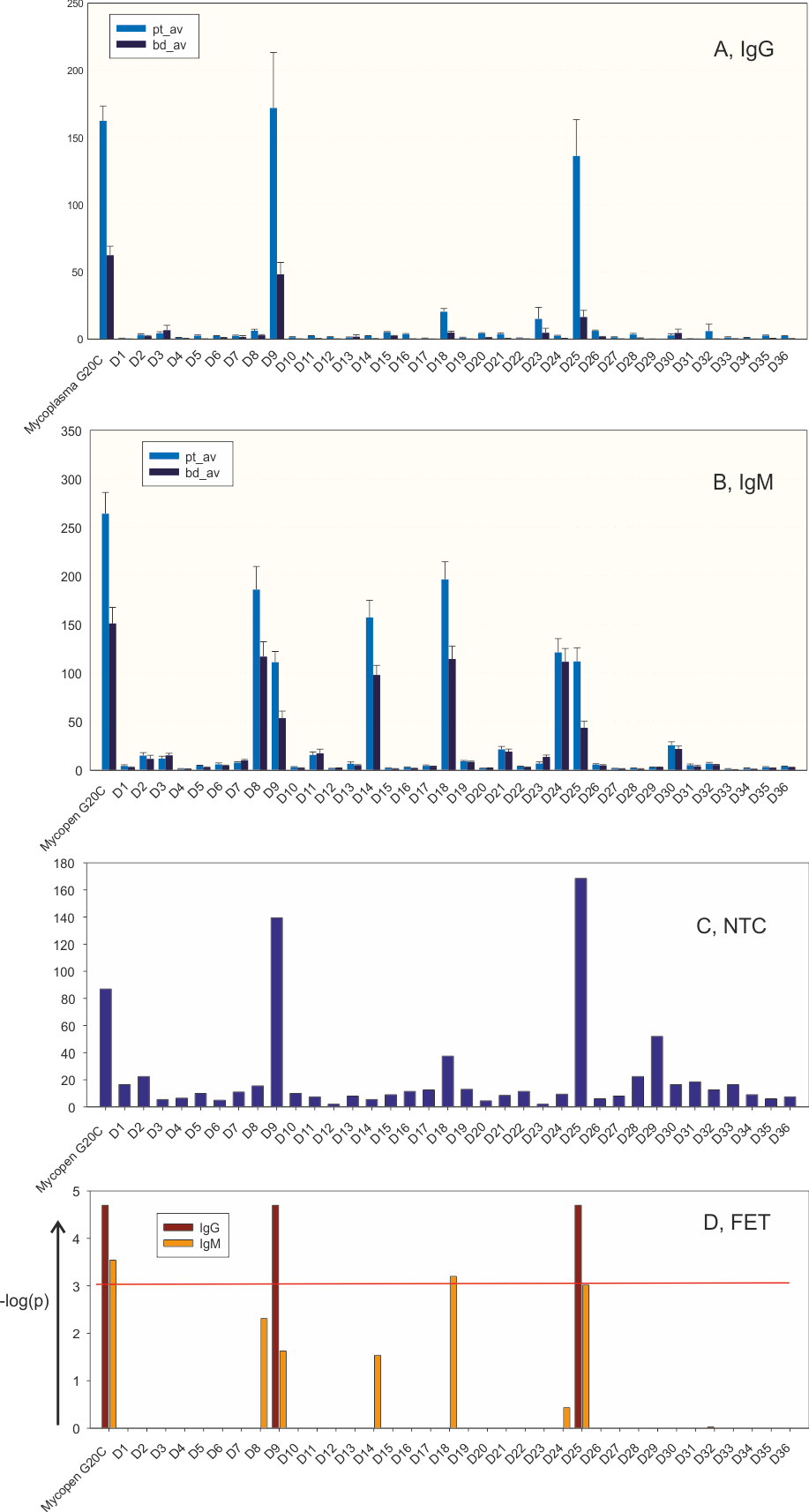
The literature contains conflicting results regarding *Chlamydia* and *Mycoplasma* as contributing factors in the etiology of ME ([*38-48*](#_ENREF_38)).

**Section 9. Other supplementary information**

**Supplementary Figure SF6.** Needle plots of *Chlamydia pneumoniae* HSP60 peptides with significant reactivity and selectivity for ME. Results with peptides coupled with short and long spacer, IgG and IgM are shown. Abscissas: Patient categories as detailed in the legend of Figure 1. Ordinates: MFI.

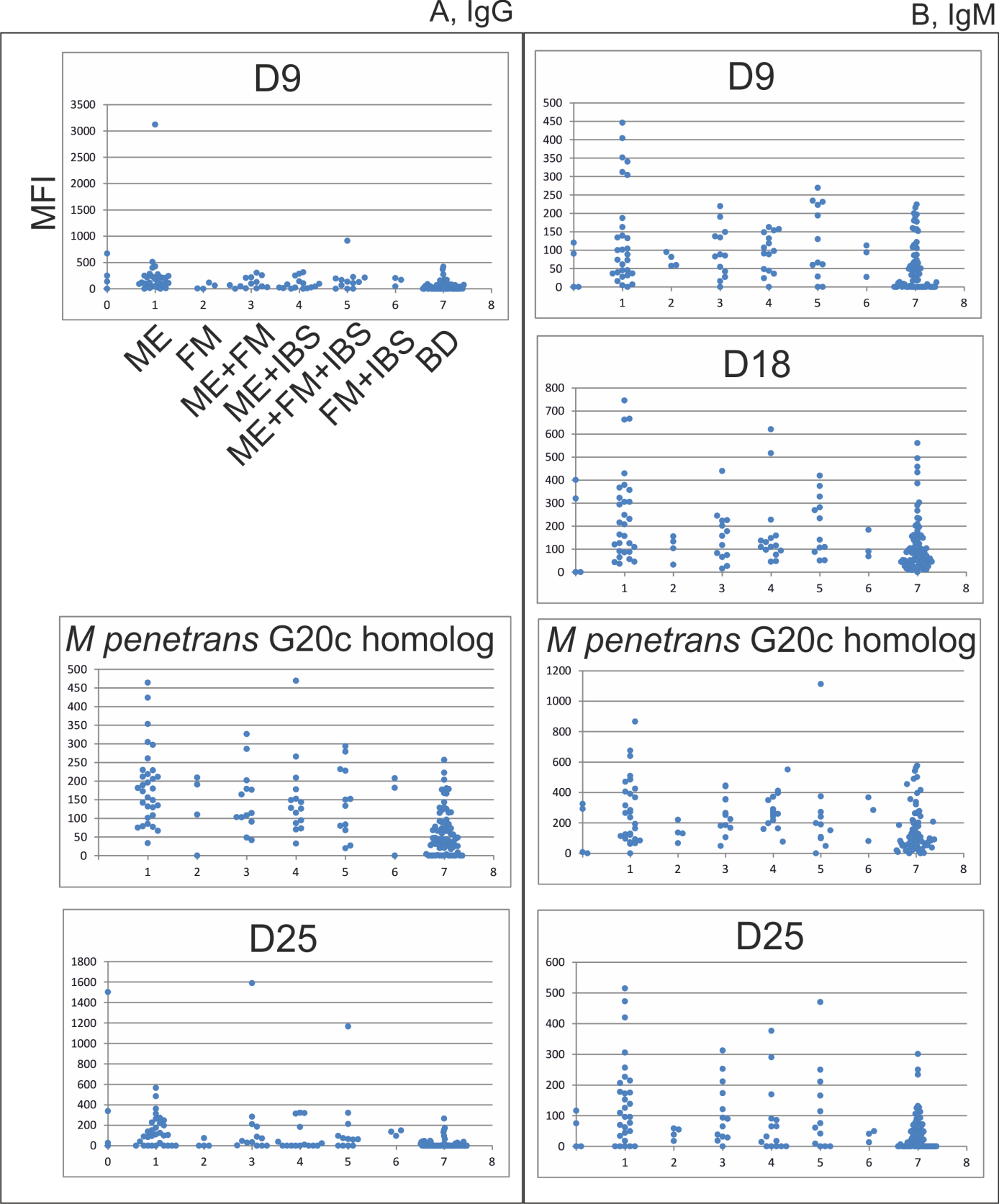
****

**Supplementary figure SF7.** Epitope scanning with overlapping HSP60 peptides from *Mycoplasma penetrans.*



**Supplementary figure SF8.**

Needle plots of antigenicity of the most discriminatory *Mycoplasma penetrans* peptides separated according to Test set patient category and BD status (cf Figure SF3). Ordinates: MFI. Abscissae: Categories. A. IgG data. B. IgM data.



The pattern for IgG reactivity of the *Mycoplasma* HSP60 peptides was somewhat different from that of the *Chlamydia* HSP60 peptides, with D18 (EALTTLVVNKMRGVFNVVAVKAPEFGDKRK, Supplementary Table ST4) being largely non-reactive. However, the separately synthesized *Mycoplasma penetrans* G20c homolog (LTTLVVNKMRGVFNVVAVKAPEFGDKRKQV, Supplementary Table ST3) reacted with a strong ME sample preference. The D18 peptide contained the whole apical helix I sequence (underlined) and the G20c homolog contained most of it.

**Supplementary table ST8.** Panel of antigen candidates.

|  |  |  |
| --- | --- | --- |
| **Short name** | **Antigen type** | **Origin of HSP60** |
| P5 | G20c homolog peptide | *Borrelia gariini* |
| P7 | “ | *Tropheryma whipplei* |
| P11 | “ | *Escherichia coli* |
| P12 | “ | *Chlamydia pneumoniae* |
| P13 | “ | *Staphylococcus aureus* |
| P15 | “ | *Mycoplasma penetrans* |
| P16 | “ | *Leishmania major* |
| P17 | “ | *Schistosoma mansonii* |
| P18 | “ | *Plasmodium falciparum* |
| P19 | “ | *Leptospira interrogans* |
| P20 | “ | *Listeria monocytogenes* |
| P22 | “ | *Treponema pallidum* |
| P24 | “ | *Cryptosporidium parvum* |
| P25 | “ | *Entamoeba histolytica* |
| D9 | Peptide from N terminal half | *Mycoplasma penetrans* |
| D25 | Peptide from C terminal half | *Mycoplasma penetrans* |
| C18 | Peptide overlapping G20c, short spacer | *Chlamydia pneumoniae* |
| C25 | Peptide from C terminal half, short spacer | *Chlamydia pneumoniae* |
| C29 | Peptide from C terminal half, short spacer | *Chlamydia pneumoniae* |
| E18 | Peptide overlapping g20c, long spacer | *Chlamydia pneumoniae* |
| E25 | Peptide from C terminal half, long spacer | *Chlamydia pneumoniae* |
| G20 | Peptide overlapping g20c | *Homo sapiens* |
| G20c | g20c homolog peptide | *Homo sapiens* |
| Human HSP60 | Recombinant human HSP60 | *Homo sapiens* |
| E coli HSP60 | Recombinant *E coli* HSP60 (“GroEL”) | *Escherichia coli* |

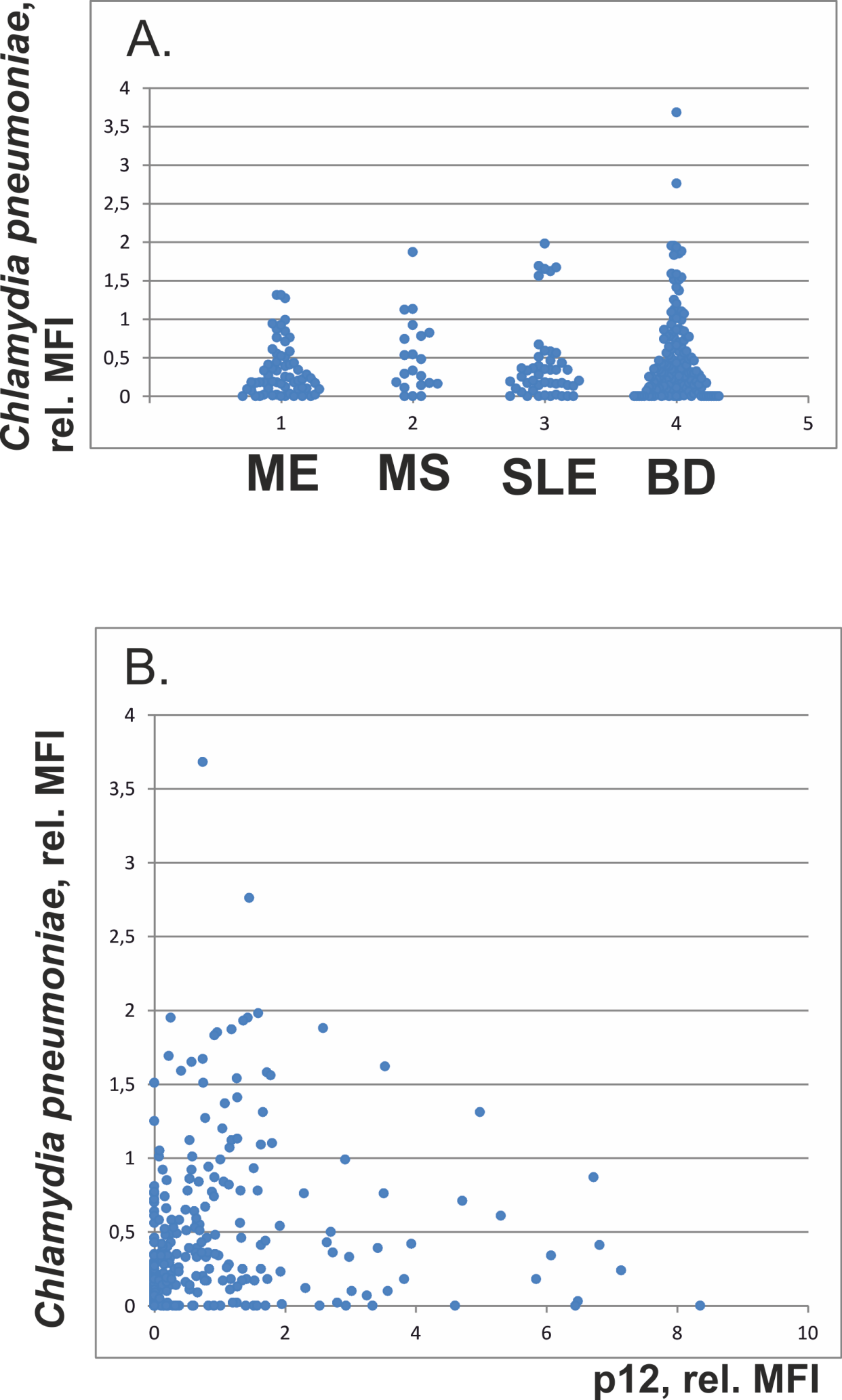
**Results from the evaluation stage**

**Figure SF9.** Overview of the results from the evaluation stage

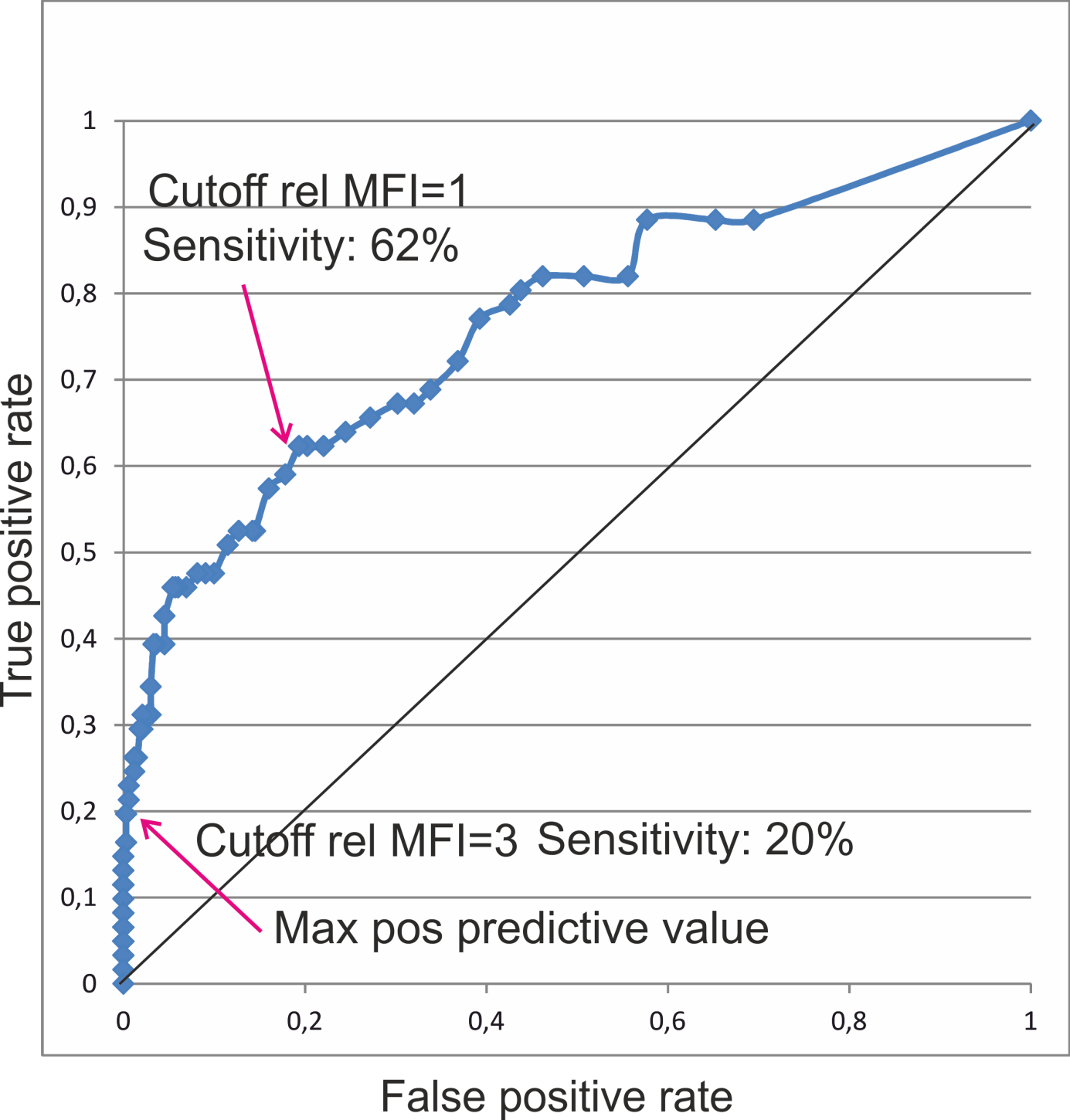
Figure SF9 depicts the result of the IgM test of the selected antigens with the Evaluation sample set. Blood donors from 2010 (n=91) gave lower values than the other blood donor groups (BD2005, n=50, and BD2013, n=161) for all antigens. ME (n=61), MS (n=20) and SLE (n=48) samples were included. The differences between ME and BD observed in the Test set, where a different set of blood donors from 2010 was used, could not be reproduced in the Evaluation set, with the exception of the P12 (*Chlamydia pneumoniae* G20c homolog) antigen. Thus, the date of blood donation influenced the Test set results.

We wanted to test the hypothesis that the IgM reactivity of the p12 peptide simply reflected the presence of IgM reactive with whole *Chlamydia pneumoniae* antigen. As seen in supplementary figure SF10 A the reactivity of this antigen was not higher in ME patients than in MS and SLE patients and blood donors. The whole antigen relative MFI values did not correlate with p12 relative MFI. Supplementary figure SF10 B shows that there is no evidence for a correlation of these two serological variables.

**Figure SF10.** Results with elementary body *Chlamydia pneumoniae* antigen. A. Needle plot of MFI for the categories ME, MS, SLE and BD. B. Scatter plot of MFI of Chlamydia antigen versus that of peptide p12 (*Chlamydia* homolog of G20c).



**Supplementary figure SF11**. ROC curve for discrimination of ME from BD by p12 and IgM in the Evaluation set. Two breakpoints were calculated, either with a relative MFI of 1, optimizing for sensitivity, or a relative MFI of 3, optimizing for positive predictive value.



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