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(54) **ANTI-TRYPANOSOMIASIS VACCINES AND
DIAGNOSTICS**

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435/252.3; 435/7.4

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(57) **ABSTRACT**

The present invention has as an object a novel genetic material coding for trans-sialidase-like proteins of African trypanosomes, and relates to the use of said genes and proteins in vaccines, therapeutics and diagnostics. The present invention also relates to the immunization of human and/or nonhuman animals against trypanosomosis.

FIGURE 1

第11章~113*1

FIGURE 2

Tc07S-like2

AACGTTTAACTGGCATGCCATGTTGCCACTCACGGTACITTTTCGACTCAATTACATCCTTGAAAGCCCGAGTT
GGTGTCAACCCCTAATGCCAGGCCATGCCAATTTCCATTAAATGAAATTACCTCTCTTGCAGAACGGAGCAT
ACCCATAACCTTCCCCTGTTGACTCCGCTCACATACATTCTCTGAAACCTTCCGAGCTTCCCTGCTTCCTATC
GGAGAACCCAGGTATCGTTGCCAGCTGAATGCCACTCAACACTTTCTCCCTGCTCCAGGCTAGATGGTGGTAAG
ACATGGACAAAGGGATGGTATGGCTGGGTATGGGAGTGGAGTACCCACTCTAACCTTAATCTGTAGAGGCA
ATTTGAAAGAGAAATAGTATTTCACCTTTCGCGAGGATACCCATAGATACTCTTGGAGACGGTAAATTTAAAT
ATAAGCTAGCCGAGGGTGGGACCCCTTCTCTTATCGGAAAGCTGAGTATGGCTTCCAGGTTTATTTCCAGCTA
GCCAAGTAACTTGGGGTACACGTTGGCTTAAAGGGGRCATTCCTGAGGGCTCGAGATGGACCTGTGAGC
AACGTTTATCCGGCAATTAACGGTTCAGCTTCTGAACTGAGCTGGGGTCACTTGTATTTCTTGTGAGCTTGACAAAT
AGCCACRAATCAAGACGTTCTGTTGTGAAITTCACCTGAGAAAGCAGGGGAGGATGGGACCTAGGGCTCTGGAT
CTTGGGTGTTGTAAGGGTATTTCTCACTTTCTGAAATGGGAGCTCATGCTGGTGTGAGCTTACCCATGGAGTLAG
GGACATCAAATTGTATACGGAGTCATTAAACTTGGAAAGGGAGTGGGTAGAGCTTGTGAGCTTAACTTGTGTTG
TGGGGAGTGGAAAGCAGAAACGGCAATTAACAAATTGGTTACACACGTTGAAAGGAAAGGGTGTGGTGGTGGTGG
TTTCCCACCCAAACCATCAATGATAATTCCUACGGGCTTCCGGATTTCCTCTGATGTTGACCAATTGGT
GAAATAGATCATTTCTGATGATGATGATGATGG
TACCTTACACAAAAAAATAGGTTATTGGGCGAGTGAATTTAACGTTCACTTCCGGTATGCAATTGGTAACTTGG
CACTTGGGAGTGGTGGGAGTGGTGGGAGTGGTGGGAGTGGTGGGAGTGGTGGGAGTGGTGGGAGTGGTGG
ACTCACTGGAAAGGAGTGGTACGGGAGTGGCAGACATTAAACGTTAACAGGGGGTACACAAAGGGGAGTGGTGGGAGTGG
TTTACGGGGACGGACACGGGCTTGGATGTTGAGGGGGGAGAAAGTGAAGGAGGAGGAGTGGGAGTGGTGGGAGTGG
TACGAAATTCAACATTGGTGTGATGAGGG
GGTCAACGAGGTGATGGCAACAAATTCTGGGGGTTTACGTTGGGAGTGGGAGTGGGAGTGGGAGTGGGAGTGG
GGTAAAGTGTGGCTTACGTTGGCTTGGCTTGGGAGTGGGAGTGGGAGTGGGAGTGGGAGTGGGAGTGGGAGTGG
TCTTTTGGCTTAAAGGTTGATGGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAG
GGCATTGATATTGGTACATTGGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAG
GTCAGGAG
CCTGGGAAATGGTGTGGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAG
TTTCTTACCTTGTGTGGCTTAATATCTTACGGTGTGGCAGGGAG

FIGURE 3

Toots-like3

FIGURE 4

Toots-like 1

MCERKSVTAFPEVVENGRSVDOGHQGTACTPLLLISAIFLPLTOCSESTIDSTWLEKRRVELTFRPWKEGNPNVPGASYS
SSDGRIVTEGNSLLEVNDQIVTLAGARYNSWVDGYAGMMKTIRLSEGHQGPGRADYMQEKHNKGZAVIVNEK
VE6HRYALMCFRAAVVGDKIFFLSITSNKSKDALSSPSDESNLDVRLYIGTVOKSFVGDASVHWNGPRSLLVTFM
KELKKKNWKFDFVCGSGKSVVMCDIIFTFLVALTHNQGRSCVIARYRANDEWMTITRVALDIDDCCTHTFTLLUMHED
LMIVVVAHNLKNKVYRSVDNGLTWTDASKTERRYALTINFQRHADODVDRGDIILSVRVGETDLILLFAYRMMFFSSATAGH
RPLLLLWMTDNKRTTHCLGPPISTQHLPFGFALLYTRESKLYSLHQESFESLSSLFPTNLTCRRLRTMRPVVLUTWKTIA
DKRVMGLYORSAAGTTNFKAEPGSEDETGLVGVWSTASHNQDEYLQMDGVLHCPPLKRVTTCYTMEECAAH
VVWPFVGGESENKVYHLISMGLTVVMSVAVHTAPKVRIPLLGVTVRNGSNWATDVGIMWYDNKTRWQMGCGEVGRVL
AMEVGKTYQLVFTVEGGVARTYVDGCRVGAERCIIIVPOSGEMEVDEMYIGNRDKAMTECSADALNVTVFHMLYN
YELSPFADVRTLLTMKGRSAFETIGMSGDDDEGEARESGGGSMILNTLAVLIIPAIVLIFGAAAFPLVKGERRAGTTMPP
ATVHHNPYFMDNATDQTLLEVSK

FIGURE 5

TcoTS-like 2

MCTTGMRVALTVLCLTHYILEQARVGVTPNAGHEPNSVNEFTLFAEGEEHTYRLAAVDSVHIBSLVKVGDVLVAI
GERRYRLAGEMRLNTFSILCSVDDGKTTWTKDVIAVGMGSTSYHSYPILYEAIVKENSITLFAGGYDIDTVGTGNIN
TSSRGWDPLLIIVGKVEVSRLGLPSQSAKVTWGTQVPLKGSIPDGLRMPVSKFYRGVKGAVVTEVGSLVFLVELTN
SHMQDVPVVIYSTNDGENWWNLEPLDPGVCKGYCHIFVWNGRLMLGNQSSKGHQIVYESINFGREWVEAVTSYSRV
WAIEAENGKLYNFVTATVEGRRVLVFQRSINDKLREVLRIWLSODGDHFAEIIDRIHLDDIVGEGTLLFDENTLL
YFYRKIGYLDEFSSSVPYDIGNIAQLDDALAKIKSVLRMWKIESSTGAVEGGGVVKNLRCIDVSPVVLLSNDVNA
THWKDVYGTANINVGTGATKADGGVLFRGTNRGAAWYVGERSGTQMYTFVNYEFTLVMTVVISEGVKENIPVLA
VNEGDSNKILEVSYNADGRWHLTPGGKVPTVGFHLHNSTHQVAVTMGGSFPSVKVDGTALSSARNSIKVLKQPS
RISYFYIGGYGNPRTTPNGELMVRNVALYKRELSSLELDVHFQSYWARCPAKSLLAAQEKPTEGDGVEAPGRMGL
FLYLLLAIISYAVQA*

FIGURE 6

Toots-like 3

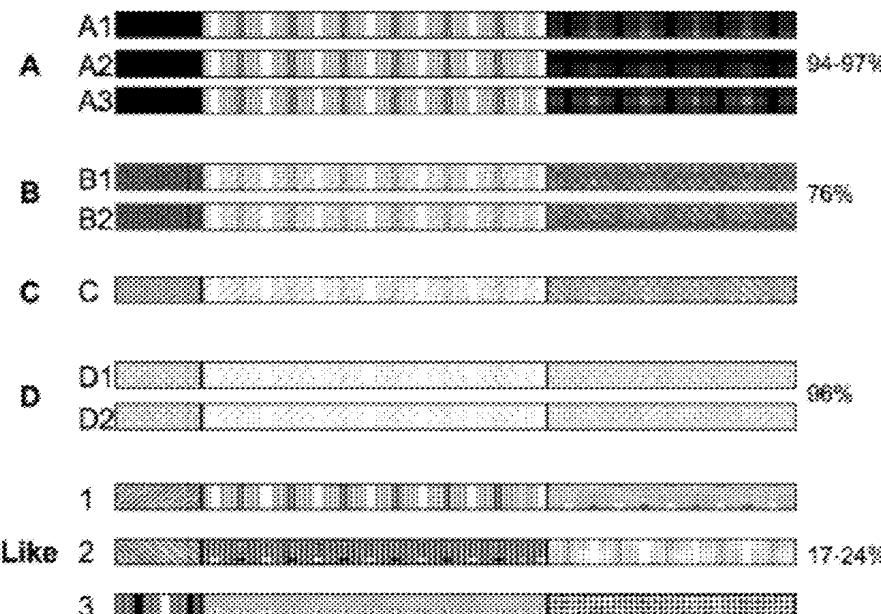
MEPTRMANVHALAHAGMRLVSEAFVVVLIRVSHMPSEGGLARYVDAGDKGNHEVGVMRTGPYSYRSPPELLAVQGSLLIT
VSETWDITTDKRYVDDVITEYGRDYGTSLLTQVAIRSDRAOFHAYVTHQEDRESTILHPTAVASGGDKVIVYLUFCKNI
GANDSLTGDCQVIMPIVATGTVLPLGAICETWVVDMTALNPIRALLPGFVACKRASRFPRKGCGNGIAITFOGTIIIPV
QVVRTDUGYFASIILYSTINGGSSNALAKCVTDRCGRESSVLEWKCKLILVSRGNDGFTKVYESCDMCTKNTZALCT
ISRAVFQNSPNRTCPGNCQGSAVVANIDNIVFMITCITTVLRCCCDGDDGCRIREIHNQRIWLSDCNKIVVKVGHLYWD
DHLOSSHHNNLLYDKGKLPCAYRAGAEKTCAVLVRSLLDELSKVEAALEANKRQDSYLSIVCAGSBDTAPCESGVP
IDGLVCLLSTTLSERQWIDAYLSVSAEVVGCARSIPQGVLFEGPIRGGRMFVAHQGQHQRYHFVSKHFTLVITVSI
HERTTDKAPLLVLRPQEDAGADLELSYTADHKMHVREHGMENGSTSGAMVKDREHQLVLYCEAGDASLYLDGKRMF
TIGRRLVESGAFLGVSHPSIGGYGLEKRSPNGKLTVRNVMLYNRPLNKTIEDTVFHVRDKITAATTIVKAPEQKN
EVNVQMVNSRQUHTAPNNEEACGALSTSLLALLLALLTLI*

FIGURE 7

Alignment TcTS-like 3 et T. cruzi TS

TcTS-like 3	T. cruzi TS	Length
1	-	79
2	-	47
3	-	183
4	-	186
5	140	239
6	137	183
7	210	279
8	186	268
9	283	384
10	256	323
11	349	434
12	336	323
13	513	681
14	394	463
15	452	581
16	444	583
17	552	683
18	534	683
19	622	683
20	604	683

FIGURE 8

A**B**

	Nov78-86c3	Nov78-86c2	Nov78-86c1	Nov78-82	Nov78-81	Nov78-C	Nov78-B1	Nov78-B2	Nov78-A3	Nov78-A2	Nov78-A1
Nov78-86c3	98.9	99.7	99.8	99.3	99.4	99.5	99.3	99.3	99.8	99.4	99.4
Nov78-86c2		99.9	99.4	99.7	99.7	99.8	99.8	99.5	99.8	99.8	99.8
Nov78-86c1			99.8	99.4	99.4	99.2	99.1	98.8	99.2	99.5	99.5
Nov78-82				99.9	99.4	99.3	99.3	99.3	99.3	99.8	99.3
Nov78-C					99.9	99.9	99.5	99.9	99.9	99.2	99.9
Nov78-B1						99.9	99.7	99.5	99.9	99.2	99.9
Nov78-B2							99.1	99.1	99.5	99.1	99.2
Nov78-A3								99.6	99.8	99.5	99.4
Nov78-A2									99.8	99.8	99.8
Nov78-A1										99.3	99.5

FIGURE 9

Tcots-A1

ATCTGGCGGTGAATTCTTACGCCGCTGCTGGCGCTTTCTGGCGGCTCACTGCTGGGGCCCCCATCCATGCCACTGCCGCTGTGGAACGGACCCACC
AGCGCGCTGCATATGGGCTCCAATGGCGCTCTGAGGAAAGACGACCCCAGAAGATGCGGAGGCTGTGGAGCAACCCCCAGGCCGCGCTGGAACGA
CGCTAACGGACCATGACTGCCAACGACTGCTTTTGCGACGCCAGGCCGCGCCAGCGCTGTGGATGCGGAGCTGGCTACCCGATGAGGAT
GGCTATAACTCTTGGCGGACCCAAAGCTGAACTCACCGACATGAGAACGCCCGCATGAGCGGAGCTGTGGCTACCGACCGCCATACAGCAC
TGGTGGAGTTGGCGGTGTGGTAATGCTGTGGGATGGCGGCTACATGAGCGGATTATTTCACGGACACCCGCTGGCGCATACAGCAC
TGAGCGTGGGAGAACGCTGGAGAGCGGAGTTATAATCCGGATGCTGCTGTGGATGCCACTACTCCGCGCTGCTCCACTGCGAC
GGTATAACATTIAATGTTCTCGTGGGGCGTACAATGTCACGCCGGCTACTGGCACAATCGAGACGGAGCTTGATAGCCATTGGAGCCCT
TGGTGTACGAGGGCACCGTGAGCTGGGAGCGAACATGACTGATGTTGCTGATGAGCTGGAGAGGACTGCAACTGAAAGTGGCTGATACAAATT
CCCGGTTTGGGAAGGCCCTGGCACCGAACCTTCCCTGGGGGGCTGGCGGTGTGGTGTAAACATCCAGGGAGCGATTGCTGCCACTGGCAGGGAAAG
AACAAAGCCAAACCGTGTGGAGCATGATCCCTGACTTGCGCTGACCGATGCAAGTCATGCCACTTTGGGAGGGCTGAGCCGGCTGAGCTCC
AGCGCTCCCTCACTGACTGGGACGGCAAGCTGGCTGATTACTGCAAGGATCCTGGCTGAGACGGGCTACCGTATGATATTGAAATGAGCTGACCTTG
TGCGACCTGGAAAGAGAGCTGCAACAGCATCTCCCGGCTGATGCTCCGGAGCTGAGCTGGCAATTGGAGCTTCATCAGGCTG
ACATGGAGGGCTGUCCTGTGATGCTTCATCCGAAACCTTAAGCGCTGCTATTATGATGCGATGCGCTGAGATGTGAGATGAGCGACGGCA
ATCTTATGTCGCATGTCGGCGAGCGCTCTTGAGGGCGMCGAACAGCTCCGACACCTCCCTGCTGATGCTGACTTCGCGACCGCGCTCC
TGAGCAGAACATGTGAGGCTGTACAGGCTCCACCTTGCGGGCTGCGAGGAGCTGAAAGGATTAATCAACGGCTGTGGTGTGGAAAGGGACAG
GACCGCTTCTCTCGCCAACTTCCTCCCGGCTGATTAATGATCCCGGCTGATGAGGGCATGCCACCGGGCTGCGGGCTGCGCTGCG
CCCGGACGGAGAACGCTGGGGGAGCTGGCTGAGCTGGGAGCTGAGGCTGAAAGGAGCTGAGGAGCTGAGGAGCTGAGGAGCTGAGGAGCTGAG
TGCGACCTGGGAGCTGTTGCTGAGCTGGGGAGCTGAGGAGCTGAGGAGCTGAGGAGCTGAGGAGCTGAGGAGCTGAGGAGCTGAGGAGCTGAG
TTTGAGGAGATGCCACGGGGGAGCTGGCTGAGCTGGGTTTGCTGAGGAGCTGAGGAGCTGAGGAGCTGAGGAGCTGAGGAGCTGAGGAGCTGAG
AGTGGCTGCTGGCTGAGCCGAGCTGAGGAGCTGAGGAGCTGAGGAGCTGAGGAGCTGAGGAGCTGAGGAGCTGAGGAGCTGAGGAGCTGAG
GAGCTGCTGCTGAGGAGCTGAGGAGCTGAGGAGCTGAGGAGCTGAGGAGCTGAGGAGCTGAGGAGCTGAGGAGCTGAGGAGCTGAGGAGCTGAG
CTTGGGAGCCGAGCTGAGGAGCTGAGGAGCTGAGGAGCTGAGGAGCTGAGGAGCTGAGGAGCTGAGGAGCTGAGGAGCTGAGGAGCTGAG
TTGGCTGAGGAGCTGAGGAGCTGAGGAGCTGAGGAGCTGAGGAGCTGAGGAGCTGAGGAGCTGAGGAGCTGAGGAGCTGAGGAGCTGAG

FIGURE 10

TcoTS-A2

ATGTCGGCCCGCTGAATTCGTACGCCCTCTCTCCCGCTTGTTCGCGCCGCTCACTGCCTGCGCCCGCCATGGCATGCCACTGCCCTGCGAAGCCAC
AGCCCGCTGCTGCGCCTCTCAAATGCCCTCTGACGAAACAGACGCCCGGAAAGACGCCGAGCTGCTGCGCAACCCCCAGGCCGGCTCGAAAGGA
CGCTGTACGACCATGACTGGCACCGACTGCTTATGACCCAGGAGCCGACCCAGCTGAACTCACCCGACATGAAACACCCGACCGATGCCGAT
CGGTAAATACTCTTGGCCGACCCAGCTGAACTCACCCGACATGAAACACCCGACCGATGCCGACTCTGCGACTCTAACCCGATACCCCTCAA
TTGCTTGAGCTTGCGCCGTGTGCTTAATCTCTCTGGCGATGCCGCGTACATCACCGTGGATTATTTCTCACCGACACCCCTGCCGATACAGCAC
TGACCGCTGGAGAACCTGGAAGACCCGACCTTATATCCCMAATGCTGCTGCGATGCCGACTACTCCCCGCTGCTGATCCACTCTTGTGCCAG
CGTAATAACATTATGCTTCGTTGGCGGTACACATGTCACCCCGCCGCTACTGCCMATAAGAACGACAGCGCTGCCATACCCGATGGCGAAGCC
TCCGTACAAAGGGCACCGTGAACCGTGGCACGAAAGGCACTGCCACTGATTTCTGATCACGCTGGAGAGGACTGACTGAMGTGCCGTACAGCTT
CCCCCTTCGGGAAGCCCTGCCACCGCAGTTCCCTGGAGGGCTGCCGCTGGCTGCTTCTACATCAGCCGACGGGATTCCTGCCACTGCAAGCCAG
AACAAAGCCCAACCCCTGTTCTGACCGATGATCCTGTAATCTCCGCTCACCCGATGCCAAACTCATGGCACTTTGGAAAGCCGCTGCCGCTGAGGCGCC
AGGCTGCCCTCACTGACTGGACCCGCAAGCTGCTGATTACTACACGGATCCGATGGCGACAGGCTACCCGATGATTTGCAATCGAATCGACTGACCTTGC
TGCCGACTGCGAGGAGCTCAACACGATCTCCCGGCGTGAATTGGCAACTTCCCGGCGTGGCACTGCTGATGCCGAGCTGCACTGGCTTCACTCACCGTG
ACAGTGAGGGTGTGCCCTGTGATGCCCTCTCACCCGATCCGAAGAACCTTAAAGGGCGTGTACTCTCTGATGCCGAGCTGCACTGGATGCCGAGGCGCA
ATCCGTATGTGCCATGTCGGCCAGGCTCTGTMGGGGAGACGACAACAGTGCCCTACAGCTCCCTGCTGTACACTCCGGACCGGGCTGCCCTGTACTGCGA
TGACCGAGAACATTGATGAGGTGTACAGCCCTCCACCTTGTGCCGCTTGTGCCGAGCTTAAAGCATTAATCAACGGCTCTGCTGTGCCGAGGCGACAG
GACCGAGCTCTGCTGCGCTGCGCAACTGCCCTGCCGGGATAAAATATGATGCCGGGCTGTGACGGGATCCTCCACCGCTGCCGCTGCCGCTGCCGACGGCTGG
CCCTGACGGAGACGACGCTGCCGGGACGCCCTATCCCTGCCCTGAAACGCTGCCAACCGGGGGCTGCGACGGCTGCCGCTGCCGACGGCTGG
TGGGGCTGCCGATGTTGTGCCGCGTCACTGACGAAACGGGGAGCCGACGGCTATTACTTTTACCAACGGGAGCTGCAACGGCTGCCGCTGCCGCTGCC
TTTGACGAGATGCCACGGGGAGCTGCCGCTGGGCTTTGTGAAACCCAAAGGGAGGCTAAAGAAGATACTGAAAGCTGATGCGGCTGCCGCTGCCGCTGCC
AGTGGCTGCCATACGGCAATGACTACACACACGCGGCCGCTGACCCGCTGAAACGCTGAAATGAGAGCCGACCCGCTGCCGACGGCTGCCGCTGCCGCTGCC
GATGGCTGCCCTGCCACGGCTGACGGGGCTAACATGACGGGAGCTGACCCGCTGCCGCTGCCGACGGCTGCCGCTGAAACATTCACTCATCTCTTC
CTTGGCCACCCGACGGCTGATGCCACGGGCCAACATCACACTGACCAATCTCCCTGCTGTACATGCCACGGCTGCCGCTGCCGCTGCCGCTGCC
TTGGCGCTATTCGGAACGGGATTCGCTGCCGAGCCGACACTGCTGCCGCTGCCGCTGCCGCTGCCGCTGCCGCTGCCGCTGCCGCTGCCGCTGCC

FIGURE 11

TcoTS-A3

AAGCCGGGCGGTGAATTCTTACCCGGCTGCCTGGCGCTTGCTTGCCCCGTCAGTCGCTGCCGACATCGATGCCACGCCAACGCCACC
AAGCCGGCTGCTCTGGCGCTCCAAATGGCGCTCTGCGGAAACAGACGGGAAAGATGGCAAGGCTGCTGGCTGAGCAGCCACCCCGATGGAAAGGA
CTGTGTAAGGACCATGACTGGGAGGACTGGTTATGGACCCAGGGACCAACGGGCTGTAATGGCTGGCGCTGAGCTGCTGGCTACCGCTGGCATGAAAGGAT
GGCTATATACTGCTGCGGGACCCGAAAGCTGAACTCACCCGACATGAAACGCCACCCCGACGGGACTGTGGCACTCGTACCCCATACCCCTCAA
TTGTTCAAGGCTTGGCGCTCTGCTAAATGCTGCTGCGGCTACATCNCCTGCGGCAACCTTATTTCTTCACCCGACACCCGTTGGCGGATACAGCAG
TGACGGGTGGAAAAACCTGGAAGACGGGAGGTTATAATGCGGAAATGGCTGGTGTGGATGCCACTACTCGGGGCTGGTGTGGATGCCACTGTGGTTGGGAAG
GCTAAATAACATTTATGTTCTCTTGGCGCTACAAATGTCAGCCGGGCTACTGGCACAAATCAGAACGACGAGGCTGCCATAGCCGATTTGGAGGCCCT
TCCGTGACGAGGCAACCGCTGAACTGGCCACGGAAACCCGACTGCCACTGATGCTGCGATCACCTGGGAGGACTGCAACTGCCCTGTAACAACTT
CCGGGTCTGGGMAAGCCCTGCGACGGCTGGAGGGCTGGGGGTGGTGTGACATCCACGGGAGGATGGTGTGCCAGTGCGAGGCAAGG
AACAAAGCCAACCGCTGCTGACCATGATCCCTGACTCGGCTGACCGATGCCACTTGGCGAGGCTGACGCCGCTGTAAGCCACCTGG
AGGCTGGCGCTGACTGAGCTGGGACCGCAGCTGCTGGCGCTGATGTTGCGACGGCTGGCGCTGAGCTGGCGCTGAGCTGGCGCTGGCG
TCCGAGGTGGAAAGAGATGCTCAACACCATCTCCCGGCGTGATTGGCAACTGCGCCGAAACCTAAATGCTCTGCGAGCTGCGACTGCTTCACTACGGCTG
ACACTGGAGGGCTGCTGCTGATGCTTCTCACCCGAAAGACCTTAAGGGCTGCTATTATGCTGATGCGCTGCAACATGCGGATGACGGACGGCA
ACCGTATGCGCTGCTCCGCTGCGCTGCTGCGAGGCTCTACAGCTCCGCTGCTACACTCCGGACGGGCTGCCCTGACTGCTTCA
TGACCGAGAACATTGATGAGGCTGACCGCTGACMCCCTGACCGCTGTTGGCGCTGACGGCTGAAAGGCTTAAGTCAGCGGCTCTGGTGTGGAAAGGACAG
GACCGAGCTTCTCCCTGGGACMCTGCCCTCCCCTGGGATTAATGATCCCGGCTGTGACCGCATCCCCMCCCTGGCGCTGCCGGCTGCTGGTAGGNC
CCCTGACCGAGAACCTGGGCGACCGCTGACCGCTGACCGCTGACCGCTGACCGCTGACCGCTGACCGCTGACCGCTGACCGCTGACCGCTG
TGGCGCTGCCGATGTTCTGCGCCCGTGACTGAGGCAAGGGCGAGGACCGCTGGCGTATTACTTTACCAACAGGCAAGCTGACCGCTGCCGCTACCGCTGCG
TTTGCGAGGATGCCACGGGGGAGGCTCCCTGCTGGCGTTGTGACCCACAAAGGCAAGCTGACCAAGCTACTGAAACGTTGCGCTGACCCGGCTGCC
AGTGTGCTGCGCTACGGGAAATGACTTCACAGGCAACGGCAACGGGACTGTGACCGCTGCCGCTGACCGCTGACCGCTGACCGCTGACCGCTGCG
CATCGCTGCCCTGACCGCTGACGGGGGCTAACAGGCAACGGGACTGTGACCGCTGCCGCTGACCGCTGACCGCTGACCGCTGACCGCTGACCGCTGCG
CTGGCGACCCGACTTGATGGAGGCGCCAGGAGCACCGAACATCACAGTGACCGAAATGCTGGCTGACAAATGACCGGCTGCCGCTGACCGCTG
TGGCGCTGATTCGGGAAACGGCAACGGGATTCGCTGCGCTGGAGGCAACCGCTGACCGCTGACCGCTGACCGCTGACCGCTGACCGCTGCG

FIGURE 12

TcOTS-B1

FIGURE 13

TcoTS-B2

ATGAGCCCATGCTTCGTGCCAGTATGGTCCMAGGCGTTGTGCGTTCTGCGCTTGCTACTGCCTTTACTGCCCTTGCGAACGGCGCTCGGAAACGGAGGA
CGACACCGTCACTTCTTTCTCGGAGGGCGCCATTGGCTGCGTGGCTAAGGACTGCTTGCTGTTAACAGGAAAGCTTCCGTCCCCAGACTETGGAAATC
CAATGGAAACTCGAGCCCCGATGAAAGACTCACCGATAACGAACTGGCGGATCACAAATGATGGCTTACAGGAGAACCCATTAAATTGCCCAACTAGAGCCC
AGATCGAGGAAACTTGTGCTTGTGCGAAAGATAATGAAAGGAAAGCATATGTTGACTCATTTGATGATCTTGAAASTGCACTGTGATATAA
TTACCGTATCTGACGTCCCTTACCTCAACTCTAACGACCTTCTTATGACACGCTTGCCTAGATAACACTCCGGATGCTGGAGGACUTGGAGAC
TGAACTTAATCATCAAGAAACGCCAGAGTCAAGAGAACGACTCTCTGCTGCTGATCTTGTGAAAGAACGACCCATTATTGTTCTGCTT
GGGAGGTAACANAAAGAGTGTGCGTACTGGACCGTGGCGATTGGGATATTCTCATGACAAAGGGCACTGTGACGGAACTGCGCTGA
GGGGGGGAAGAACATCTGTAACATTTGACTGGCATGAGCCACAAAACCTTGAAACTATTGCTGAGCACAGTTGTAAGGTAGAGTGCTGACTGT
TCAGTATAATTGGGGAGTTGGAAACTTGCTGCTGTAACGCCGAACGGTACCCATCTACTCCCTGTTCAAGTCTGAAACACGAAACAGATTGTGATGGCC
ATGATTAATTACTCAACAGATGAAAGGAAATCTGCGACTTCAACGCCGAACGGTACCCATCTACTCCCTGTTCAAGTCTGAAACACGAAACAGATTGTGATGGCC
ACAAACTGCTCCCTGAAATGATGAGCAACAAACGAAATTGCGCTTACCCCAACGGTACCCATCTACTCCCTGTTCAAGTCTGAAACACGAAACAGATTGTGATGGCC
GACGATTTCAACCCGTGATCGGAACACTCGCCCGCGCCCGCAATCAACCGGGAAGTTGCTATTGCGCATAACATTTGAGGGATGCGCTGCTGATG
CTAACTAACCTCAACCGGAAGAAATATAAAAGTCTTGTGACCGCTTGTGACCTTGTGACCGACHTAACTGCGCTGCTGATGCGCTGATG
TTTCCGAAGCTGATGATGACCGAACCTACACCTTGCTCTGCTATACCTGAAATGGACCCCTCTACTCTTGTGACCGACGGACAARTCCGGCTTTT
GACTTAATTACCTTATAAAAGCTGAGAACGAGGCTTGAAAGCATTAACCTGAGCTTGTGATGACGCTTGTGAAAGGATGCGCTGCGCTTGTGATGCG
TGTGCGCTGATGCTGATTATACTTGACGGCTGCGCTGCGCATTTCCACCTGCTGCGCTGCGCTGCGCTGCGACCTTGCGATGGGATGCGCTGCG
ACGACGCGTACCGCTTGCTGATGCGACTGTGCGATAATGTGCGTCAATGTTGCGAGATGGGCTGCGCAACTGAGTGTGCGCTGCGCTTGG
GCCCGTCAAGCACTGAGGGCGACGGACCAAATTACGCTTGTGCGATGCGCTGCGCTGCGCTGCGCTGCGCTGCGCTGCGCTGCGCTGCG
GACTTGTCACTGCTGCGCTTGTGCGCTTGTGCGCTGCGCTGCGCTGCGCTGCGCTGCGCTGCGCTGCGCTGCGCTGCGCTGCGCTGCG
CCGATTTAACCGACGGGGGATCCACGGGCTCTCCCTGCGCATGCGACTTGTGCGCTGCGCTGCGCTGCGCTGCGCTGCGCTGCGCTGCG
CAACGGAAAGACACCTATCACTCTGCGATACGAAAGATTGGCGGGCGCAATGCGCTTGTGAAACATAACTAAATTGTTGCGATCCCGAGTGGCC
GACCGCTTGCGCTGGCGACGGCACTGCGTACGGAGATGTGCGCTGCTATAACGCCCGCGCTGCGATGAGACTGAGACTGACTTGTGCGCTGCG
ATGTAATAAAAGCTGAGAACGACCGCGACGCTTGCGATATGCCCTGCTGCTGAGACTGAGACTGACTTGTGCGATGCGCTGCGCTGCG
TGTGCGCTGCGATGAGCTAGCAATAATTACGGATGACCGCTTGCGGGCGTTGAGACGGCTTACCAAATGCGCTGCGATGCGCTGCG

FIGURE 14

Tcots-C

ATGCCCTCATACCTCCTGCCACCCGTACTGCTGACTGCTTTGCCGGACTCGCTTCCGTGCCAGTCATCTAACGACCCGGAGGCATTCA
TGGAGCCCCGTCAGTCGCTGCCATTAATTAAGATTCGTCATCAGTCGCTGCCAGTCACGCTTCAAGGCTCGGGCTCTTACGAAAGGGTACCC
TTCCGTCGAAGAATACAAACAAAAGATGTCAGAACACTCGAGGTGCTGTCAGACAAAGCCAGGGCTGCACTGTTGAGAAAGACGGGCGCTTATCGGAGACATTGCAATACAGCTT
TCAGCTGATCGGAAGGAAGACATTGATTCACTCCCTCGAATTCCCTCACTTGTTGAGAAAGACGGGCGCTTATCGGAGACATTGCAATACAGCTT
ATCTCACCGCTTCCGACAGCAGTCAGTCAGACAGCTATGAAATAACAGTCGCGATCAGGGAAAGACGGTGGAAAGACTGAAATCATATAAATGCA
TAGACTAACTGATAACTTTCCCCTGCTGATCCAAACGGCTGCTTAACGGCTGATAACTTTTATTTCTGGAGCTACAAACACCTCAGCTT
ACCCCATGGCTCTGGCAGAAAAACCCAAAGACTGGATGTAACTCTTACAAGGCCAAGCTAACGGAGCATCAGGGCTGGGGTACCATGAGTGA
GCTTACATGCCACCAACCCCTACACCTGAGCATCTGCTCACCTCTGCTCCGTAAAATAGAGCCCAGGCTCCCTCATACAAATACATTGGTGGGGTTGG
AAATGGTATTUTRAACACCGAAAGCTACTATGGTGGTCCAGGTTTAAACACCAACAAATCCGTCATGAAACATGCTTCTGTATTCAAGTRAC
GACGGAAAAGCTGGAGTTGAGCAAAGCTTCCACACCCGGGGCACAGCTGAGGCGCTCCCTTGTTGGTGGAGGACAGACTACTTCTCACAGGCA
GAACRACTCCCGATGTCGGCAGCCAAAGTATAATTAAACAGGCTCGGAACTTCATGAAATGAGGCAATCCGAAACTATCTCTGCTGTAATTGG
TAACTCCGGCTACCGCTAACGATCCTGGGGGCTCACGGTAGCTCAATTCCATAAACTGTOGAGGGACTACCGCTGATGCTGTTACTCAACCCGGAAAT
GGGAGGCTACGGCTGGAATGGGACCCGAATGCGACTGTGGCTCACGGACCCGAATCGGAGCTGTCGCTTGTGAGCTTCTGAAAGGGACCCGAACAAACA
GTCCTACACCTTACCTGTTATACACTAAAAATGGGAGCTTATTGGTGTCTTACGGACCCGAACATTCGCTGAGGATATTCAGGCTTATCTAGCCGGCT
GGGAGGATGAGATGGAGGATATAAGCTAACCTGAGGCTATGAAAGCCCATGAGGAGCTCTGTOGGGAGACTGTCAGCTGAAACAAAGGGGGCG
ACCGGCTGCAAGCCATTCCTATCACGGGCTTCTGGACTTTCTGGGGGCTTCTGGAGGAAAAGTGTGTTGGCCAGATGCAATAATTGTCGATG
CCAGTATATCCAGAAATACACGCAAGCTCCACGATCCGCACTGAGGAGGACCCATGAGGCGCTGTCGCTGAGGCTATGAGCTGCAAGGCTCA
AGACCAACGATACCAACTTTGTCACACACCCACTTTACCTTCTGGCAACCCGCTTCTGAGGCTTCTGAGGCTATGAGGCTTCACTGAGGCTTGGAA
AACAAATGAAAGAAACTAAACTCTGACAGTTTCCATCGGGAAACGGCTTCTGAGGCTATGAGGCTTCTGGAGGAAATCCCTATGAGGCT
GGCTGGAGCTGAGCTGAGGCTAACACATCAGGTGGCCCTGACACTTCAATGGGGAGGCTGGCCAGCTGAGGCTTCACTGAGGCTTGGAGGAACTGAG
ACTGAAAGCTCCACCGAGGCTGACAGGCTACTAAACATCTGACAGTTTACTTCACCCGGGCCCCGCTGAAAGGTAGGCAACGGTACCG
GTGAATAATGTCATCTGTACAAACGGTATGCTAACGAAACCGAACTTGCACGTTTATTCACAGCAAGGGAGGCTCATGAGTGAAGTGGGAGGATGTGC
ATCCCTGTCGGAGGCTGGAGCTGGAGCTGGCTTCCACGCTGAGGCTACTACTGAGGCTGATGCTGAGGCTTCACTGAGGCTTGGAGGATGTGC

FIGURE 15

TcoTS-D1

ATCGGATATTCTAAATCGGTTGGCAGACCTTGATATGCCCTTGTGCTCCGATTGACACCGTATCACTGCACACGCCGATGGATCCGGAAATCA
GAGGGAGGAGGAAGAATCGAAGCTTCTCCAGGGGGCTTGTACAAGAAGATGATGGAAAGGATGGCAATTGGCTCCAATCTAAAGA
CTGCAAMCCCCCTATGCCCTGCCCTGCCGCTCTGCTCCAATAAAACTATGCCCTCCCTCCCCAAAGAACACTTTECCCACAGGAATGCCAC
TCCTCAGAGGGAAAAGGATACACACTAACGCCAAGGGAAAGCCGCTCTTACGGAGACTTGCCACAAAGAGAATGCCGGGGTTCACTCTCTTC
CCATACCTTCCATGGTTGACGCCAACCGUTGTGTATTTGGCATTTCCAGACCCCCGATATTGAGCTCCCCGGACTTCACCTTTCATCCACACCGTTGC
CAAGTACAGTCTGACGGCCGCTGAGCGCTGCCAAACTGAAGTCATCAATGAAAATGGCTGTCGATTTCACCTCCCGCTGGCGATCCCA
GTTGCCGTTGAAAGAATACAGTTTGTGCTTGTGGAGGTAACACACCTCCACACCTGACCAAGCMAATAACGGCAAGGATGGGACA
TACTTAACTACAAGGCTACCCCTACCAAGACCTCCGAACATGGCAACCTGGCACAGGGACCCAGAATTGAACTTCAAGTMCCTCCT
GAAGCTGCTGGATCAAATAGAGGGCAAGTGGCTGCCAACCTGGCAACATTGAGGACAGGGCACGGATGCTTTCCC
ATCCAGGTAAATAATTCACTGAAATCAAGTCGCCCAATGATAATGTATTCGAGTCAGGATGGCCACCTGGGAGGGAGGGCACACCCG
TTGGTACAAACAGAGCCATCCCTATCTGGTCCGACCCCAAACTGGCTCAATTGGAGAACGGGCTTGGCGTACCCGAAACTATTGGAGACAAACCGA
CCCTGGGACACGGTGGAAAGAGTCATTGGGGCTCTCTGGTGTGATTGGTAACTCGCCAGCCCAAAAGAGGATGGCTAACGAGTGGCTATT
ACTTTGGAGGTGGAGGGCTGGCAAGTCATGGTTATTACCGAGCCTAAGAATAGGAAAGGGGGATTACAAACGGTGTGCGCTTACAACCTGGGCTGGAGTG
ATGGCAGTCCTGGTGTGTTACTGGGAGAGATACTACCGAGGGATGACAAACGGTGTGCGCTTACAACCTGGGCTGGAGTG
CTCTGATGAAACAGGATATCCGGGAGCTTTGGCATTTACCTTGCCATCTGGCAACTGGGAGGATGGCAAGGGGATGGCAACCCGGTGGCTCTGGGGCTGCTTT
GGGGMCTCCCAAGGAAACGGCTGGCTGATGCTATAATTGCTGTAATGCTGAAATGTTACAGTGGATGACATGCTGCGACCTTGG
CGGGTTGAAACAGGGGGCGTGTGCGTGGCGACAGGGCCAGGATGAGGGTATTACTTTGGGAAACCTGGCTTCACTCTGGTCCGAAC
GAGGATTAATGGCATCTGAAACCTGAAATACCGCTTAATGGGATTGGAGACTTTGAGMAAGGACACGGATACCTGGATGGTCTGCGATGG
ATGAGCTTACTGGTCAAACTGAAAGGAGCCAGTACCCAGGTGTCAATGGGAACTCCGGAAATGCCAGAAAGTTGATCAGGTGCAATTATGTT
GCAAAATGGGAGGGCTCTGTTATGCCGAGGGGTTACATCCCACAGTTGGATGACAGGATGGCTGCCCTTCACTGAAATATCTCTAGC
TTTTCCCTGGGACATCTGGAGGTTGGCAGGAGGGTTACTTGCGGGGAGTGTAAAGTGTCTGCAACGGCTTACCCGAGGCTG
AGTCCAGGATTCTCTATGCCAACGGAGGTGTAATCAAGGCGGTGGTATTGTTAAAGGCCTTCACTGCCAACAGACTCCGGCTCGATGCCGCT
TGACACAGCTAAATAACGGAGGATACACAAATTCAACTGAAATTACGGATACACCAATGGGAGGGACGGATGACGGAGCTGACTGGGTTA
GTTATCATTGIAATTGGATATGCCGCTTATTCTAG

FIGURE 16

TcoTS-D2

ATGATGGATAATTCTAAATCGTTGGCAGACGTTGATAAGCCCTCTTGTGGCGATTGACACGTATCACTGCACAAACGGGTATGGATCGGCAA
TCAGGGGGAGGAGGAGAAGAACGAAAGCTTGTGCCCTCCAGGGGGCTTGTACAAGAAAGATGAGTCGAAGGATGCAATTGGCTGCAATCTAA
AGACTGGAAAGCCCGGGTATGCCGTGTGCGCCGCTGGTCCAAATAAAACTATCGTCGCTCCGGAAAGAACTTGGCCGCAAGGAAATGG
GACTCTCAGAGGCAAAAAGCATACACACTCAAGCCAGGGAAACCGTCTCTTAAAGGAGAGCACTGCCACAAGAGAAATGCCCGGGTCACTCCT
TCGCGCATACCTTCCATGCGTTGAGCCGAATGCTGTGTTAAATTGCCATTGCGAGATGCCGAATATTGAGCTGCCCGGACTTCACATTTCATGACACGGT
TGCCAACTACAGTGTGACGGCGGTGAGACGGAAAAACTGAAAGTCATCAATTGAAAAATGCTCGTGTGGATTGCTTCACTCCGGCTGGATGCC
ACAGTGCCTGAAAGCATACAGTATTGCTCTTGGAAAGGTACAAACATCTCCAAACCGTATTGAGTCGACCTCGCTATTAAACCTTAAGTCGCT
ACATACTTATGCAAGGCTACCGTAAACCAGACGCTCGGAAGATGCCAAACCGTCCCGCAAAACATTGAGTCGACCTCGCTATTAAACCTTAAGTCGCT
TCTGGAAACCGGACTTACCGTGGACGGCATGACCGTACCCACTTTCTGCTGCTGAGCTAAACTGCTGTTAACCCGACGGGACGGGACGGATGCTT
CCCATTCCAGCTAAAAATTCACTGAAATCATGCTGCTGCTGCAATGATAATGTTACCGTACGGATGCGCCACCTGGCGAGGCGCAACAC
CCGTTGGCACACAGGCATCCGCTATCTGGTGGCACGGAAACCTGCTTCAATTGCAACCGCAACTTGGCTACCGCAAAAGTATTGAGACACAC
CGACCTTGGCACAAACCTGCAAAAGAGTCATACCGCCCTCTCTCTGAGTGGTMACTGCCAGACCCCAACCGCCCTGGCACGCTCAGGCACTGCT
ATTACTTGGAGGCTGAGGGTGTGCAAGTCATGCTTATTACCGACCCCTAAAGAAATACAAAAACGAGCTATTGCTGATGCCCTACAACTGTGGCTGGA
GTCATGCACTGCTGTTGGCTGCAACGAGCTGAGCTTACAGTATTACCGTGTGCTGCTGCTGCTGAGCTAACGAGCTAACGAGCTGAGATTATGG
AAGGAGCAGGATGCACTCTCTCTGGCAATTGCACTGCGAGGATGGCAACGGCTGGCCACCCCTGGCTGGCTGGCTGC
TTTCCGGACCTGGCMAGGAAACGGCTGGCTGATGATATAATTGCTGAAATGCGCTCGTGAATGTCAGGATGCTGAGCT
TAGCGGCTTGAACACAGGGCGGTGTTGCGCCGCTGCGTCAACAGGGCCAGGATCAGCGTATTACTTGGGACCCGTTGGCTCACTGCTGGCTGCA
ACTGTCAGTTAATGAAAGGCACCAAAATAGCAACCTGCGCTGTTGGGCTTGGAAATTCAAAAGGAGAAATCTGACCTTGTCGGCTGGCAACTACAA
CGTGGACCCCTGACGCTACGGAGGGAGAGAAAAAAAATTGTTGGCGCCGCTCACTGAGCAGGATGCACTGGCTGAGTACTTTAATGGCTGG
CACTGCTGCTGCTGATGCGTACAGTACCCCAACTCGATAAAGGCTTGTGCTGCTGCTGAGCTAACGAGCTAACGAGCTAACGAGCTAACGAGCT
GAAAGCAACTTATGGAGCATACAAACATATTGACAAACATGCTCTCTGTTAAACGGAAACTGAGTCGAAACGGACCTCAAACGAGCTGGCTCC
TCAACCCGGAGGCTATAAGAGCTGAGCTGACGGGTTAAATTACTTAAAGAGCAGGCAAGGTTGGCTCCGGAGAGGAAATAAAACTCTACTTTGGGATTCCAA
CGTCAGGGCATCTGCTGCAACGAGACCTCTGAAAGATGTTCTGCAACTGCGCTGCTGATTTGGCTTCTGCTGAGTACCGACAGGAAACTGCA

FIGURE 17

TaoTS-A1

KMPVNCYALLALVVAGQCCGPMHATAAVGTTHQALLNGSKWALKKTPKOGEVWWGEPQPGWKEVYDDWEENFMEQECPGVGDGVRAEWTYRHO
GXILVCGPKLMSFDMMNSTGMEMMTVHSYTRIPSIIVEVGGVLMCVGARYITSDYFTOTVAAYSTDQGRTWKREVLLIPNGCKVDAHYSRVDPTVVAK
GNNIYVLVGRYNVTGTYWHNQDDEAAIAADWEPFVYKGTVNVCZKDNATDVSISWERTALKSLYNFFVSGSPCTOFLCGACGGVVT8NGTIVLPVQAR
HKANRVRVSMILYSADDGRSWHFGKGEAGVGTSEAALTWDGKLLISARSIDGQQGYRMIFESSDLGATVKEMLNSIISRVIGNSPGRSGPGSSSGFITY
TVEGVPTVMLLTWPKXLEGVYTSRDKLQMMNTGNNHHVGVSEGGDNASAYKELLYTFDGVLYCLNEQMIDEVYSLMLVRLVDELKXKIKSTALVWKAQ
DELLLCLC1PCDGYDPOCDICIFTAGLAGLIVGPLTEKTXPDAYRCVMAATSCAVSTAEGVRLVCOOGHVWWVSEQQDQRYYFTNSEFTLAVTVR
FDEMFRGELPLLGTVRRXGRVNRKILKVSLSGVEWLLAYGNEYNSTAAEPLSYREH3QVVLTLHDGIVSLLSVBGGHTATVSVRVASPEELLNIHLF
VGTPVIXGAKEHVWITVSVNLVYHREPLRGVELLGLFAAIRGRIRVPGSDNGVLSGG*

FIGURE 18

TcoTS-A2

MIPVNCYALLALVVACQCCGPNAATAAVGTTIQALLNGSIKALKRKTTPKDGEVNNENPOPGWKEVYDDDEWEEMPTMEQEGPTGVNCVRGEWYRKTQ
GYILVCGPXLNEPDNNSTGTITMRTVHSYRIPSIVEVGGVLMCVGDARYITSIDYFFITDVAYSTDGGATWKREVIILPNGRVDAHYSRVDFIVVAK
GNNLTYVLVGRINVTAGTWHRNOKAAJADWEFPVYKGTIVHGTKGATDVSISWERTALKSLYMFPPVSGSPCTQFLGGAGGGVVTSNGTIVLPLVQAR
NKANHVSEMLYSADDCKSNHFCGGEAGVCTSEAALTEDGKLLIISTRSDCCOCYRMIFESSOLCATWKEMLNSISERVICNSPGRSGPGSSSCFITY
TVEGVPVMLLTHPKNLKGVYSRORLQMMTLCRTRMHEVGQVSEGDDNSAYSSLLYTFDGVLVCLHQNIDEVYSLHLVRLVDELSIKSTALWRAQ
DELLLGNCCLPGDKYDPGCDGIFTAGLAGLLVGPLTEXTWFDAYRCVNAATGAVSTAEGRVLDVGGGGHVVWFVSEQGQDQRYIFTNSEFTLAVTVR
FOEMPRGELPLLGFVNRRGKVKKILKVSLSGVEWLLAYGNEYNSTAEEPLMVNESHQVVLTLHDGIVSLHVDGCMZATVSVRVASPAELLNIHELP
VGTPVDCGAKEHANITYSMVLVYURPLRGVELLGLFANRGRIRVPGSDNSVLSGG

FIGURE 19

TcoTS-A3

MRFVVCYALLALVVAGQCCGEMHATAAVCTTBQALLWGSKHALRKKTPKGELVWWNSNPQPGKKEVYDDEWEEMYMEQEGPTCVNCVRGEWYRAMKD
CYILVCGPFLMSPDMMNSTGTTMRTVRSYRIPSIIVEVGGVLMCVGDARYITSTIDYFFTDTVAAYSTDGGKTWKTEVIIIPNGRVDAHYSRVVDPVVAK
CMMIYVLVGRYNVTGTYWHINQNEAAIAADNEPPVYRGTIVVGTGTATDVSIISWERTALKSLYNNFPVSGSPGTQPLGGACCCVVTENETIVLFWQAR
EKAANRUVVSMILYGAADDGKSWHFGECEAGVCTSEAALTEDGKLLISTRSIDCGQCYHMLFESSDLGATWKEMLNISIERSVIGMSFKRNGPCSSSCFITY
TVEGVFVHLLTPEPKSLEGSYYRDRQLQWWKTKCKRMHHIVCQVSEGDDONASAYSSLLYTPDGVLYCLHEQNIDEVYGLILVRLVDELKSIKSTALVWXAQ
DELLLCNCLFGICKYDPOCCCGIPTAGLAGLLVGFLTEKTVPGAYRCVMAATSGAVSTACGVRLDVGGGGHVVWPVSEQQDQRYYPTREFTLAVTVR
FDENMPGELPLLGFVNHKGVNKKILKVSLSGVEWLLAYGNEYNSTAAEPLSVWESHQVVLTLBDGIVSLHVDCGNTTACVSVRVVASFEELLNISHLF
VGTGVDCGAKEHANITVENVLVYERPLRGVELLGLPAHGRKRYPGSDNGYLSGG

FIGURE 20

TcoTS-B1

MENCFVPAWSKALCILLLSMCLYLAHSNCRTTRFLFLOGCHWVVGKECLAVHEEGSAARTLECXGNCSPDDEDSQRRAADONDGLQESTINCYLEP
RDKOLQVAKDMEGKRVVOSFRIPSIVEVGVLIITVSDVRYLNGNOLSFIDTVARYSADGGRTWETEVIIINARVXAEHGRVVDPPTVVVKNNNTIFVLV
CRYNESDAYXTVQCGCCGNDILMHECTVTKSLLRGCKPSVNIENDEPQNLKYLLSTVCKIDGRSLIQYIGCYGNCVVTCPNCTIVLPVQVLNTNRSVNA
MILYSTDGESESQFSKSATPVGTTESSIVWWDDKLILLNGRTNMNDLAGYRKVYESBDLATTWTEVVGTCISRVIGNSPGHNPQPCSSGSSIAITLEGHRVM
LITQPKRIKGSNMRKDRLOLWLTDGNKVWLVOQIIEGDDNGPTSSLLYTSINGTYCLYECDRAAVLSTYLAKLEDDELESIKSIVALKRQDALLSGNC
SSPOGDTTECCVGIPTAGLVGLLSGPSOEDVNHAYRCVDASVEEVVXVADGLQLSGNNSSRVLNTVSSQGDXQKYHPADVHPTLVAMLRLVVGAPKG
DFBLVQFENYEGETRKTVKLSAIKSALWMCHTDLTRGSRGSLPCDEVHQVALTLRNGVISVYANGRULSVLDTRVAGANELLNISMFTVGEPPGVG
GALIWGSAVYKDVLLYRPLHETELESLYLNGDVIKVVHNGAAGISAAARDAAELLEHVRCDDGDKPDAVPLXLAIIITGCVVVRFCGLYQMASLVLLGLM
LS

FIGURE 21

TcoTS-B2

MENCFYDVWCKALCLLLLSEKLYLAHAGCNGRTTRELELQCGGHWVNGECLAVNKEGSVHQTLCECNCGCSDEEDERIRGADDMDGLOEETINCALED
RSEQLGVAKDMECKHIVDSFRIPSIVEVDGVLLTVSDVRYLMSNOLSFIDTVARYSADGGRTWETEVLIKNARVNAEHSRVVDFPTVVVKNSTIFVLV
GRYNKSDAYWTNGGGGDWDILNNGTYTKSLRGKPSVNIIEWDEPQNLKYLLSTVGKIDGRSLIQQIGGVNCVVTPTGTIVLPVQVLNTNGSVMA
MIXISTDEGESWQTSEKSAATTFTVGTTESSIVWWDDKILLNNGRTNNOLGYTRKVYES20LOTTWKZVVCTISRVICMSPCRNQPGSSGSSIAITLEGMRVM
LITQPKNIKGSWHRORLQLWLTOGKRVWLVCQISEGDDGPySSLLYTSNGTLYCLYEQCKSAVLSIYLIKEDKLESIKSIVKLWKOQDALLSGNC
SSPOGDTTEGCVGIPTAGLVGILLSGPSOGDVWHDAYRCVDASVDIVVNADGLQLSGWNSSSRVLMPVSSQQDQKXHFANVSHFTLVANLRLVGA
DPFLVGFETYEGERRKTVKLSAIKSAFWEMCHTDLTTRGSRGSPPCDEVHOVALTLNDGVISVYANGHULSVLOTKIANELLNITNF
DALPWGSAVVROVLLYHRPLHETELESLYLNGDV1KVVNRGAAGISAARDAEILHVREDGGOKPNAVPLKLALITODGVARFRGLYQMASLHLLGLM
LS

FIGURE 22

TcoTS-C

NPSYLLPAAVVLYCFGGLVPAAQCISTTREAFMEAGQWSVNKOCLITAEGSRSKASGSYERGYASVESTTKDNWTRGGVQTSEACTLEPEVRDNST
SCDGKERULIHSFRIPSLVBDGVLIATFTDTRYLHASDSSSLIDTAMKYSAQGXTWKTBIIIIKNARLTONESRVVDPTVVVKGDNLFIFVGRNTSS
TPWWXQKNGKDWDVLLYKAKVREEBAGGVPSVSPTWDPLALKHLLTSGKIDGRSLIQQYIGGVGNNGIVTPKGTTIVPPVQLINTNKSVMXNLLY38R
DGKTWEFSKTSTPAGTTEASLVWWDGQLLLTSRTTPDVGSRKVYLTSDLGTSMNEAIGSISRVIGNSKYNNCPGGSS61AITV2GVVYMLVTPEN
AKGRUNNRMRMLWLTOGNRMKLVCQISEGDDNSAYSYLLYTNGTLLCLYERNIREIYSIYLARLEDEMEDIKSTVRLWKADELLSDCQLMKKRA
SGCTGIPITGLVULLLAGLPKRSVWPDAYNCVDASISKNNKQVSHDPP8RSTMKRRVYVWPVGCGQGQDQRYHFWNTHFPTPVATITYFDRAPQEVSIMGFE
XMEESTKTLTVSIGNGRLVITYGGLEEIPMTSLDWSVTBQUALTNGEVSLXVDGNPSIANVRLKJHEBCALLNSNLFTST2APVKTGKGSITV
VNNVILYHRMLNETELARLFNSRDLLIDEVGDVHPVSGCCGVGEWRFHVVILLAAAYVLVAY

FIGURE 23

TcoTS-D1

MGYSKSVRQTLICLLVAIDTYCTTAYGSGIRGHEEKRSRLFLPGGLWYKDEWKDMNWLQSKEWKAGYANWFWRSWCNKTIGASGRTELCKEWD
SQREKGTYLEPRESVLFRESSGTKMRMRVHSFRIPSMVKANGVLIGIADARYLSSADFTFIDTVAKYSADGGETWKTIEVIIEHARVDSFHSHRVVDEP
VAVKHNGLYVLYGRAYNTGHTYWTWQNNGNMDILMYKGTVKTSEDGXPAAMIEWTGTQNLKYLLKLVDQIEGKSLTQFLGGVGNTVVT2DGTIVTP
IQVKHNSWNQVAAMIMYSSDDGATWHLCGGATPVCTTEASAIWWDGKLVLNCRTDLGYRKVFETTDLCCTTWESLGALSRVIGNSPDRQXGSSCGAI
TLEVEGVQVNLLITQPKSTRKCDYNHDLQLQWLSDGENVWLVCQISRGDDNSPTSYELLFTGDKLYCLTBQDINGVLSIYLVHLVDELEKIATVRLWK
EQDALLSGNCSATAEDGSDCNGVPTAGLVCLLSCGPAGHANTDAYNCVHASNVMVTEDADGLQLOGGLNRGRVSNMPVHAGQXQRYYFANWRITLVAT
VRLLNGISNLEIPLMGZENFKSTRDTLIVSIVODAYWSKCREGPVPGVNVDAPECOKFIHOVAIMFQNGRVSVYADGIRHIPQOLDTSIVDASALLNISS
FFLCHPEVGSEFTSADVIVKVLLYNRPPLTBGESKILYANEGVINKPGILVKGVSLATKTPASMRVDTANKRGYTNTFPLKLTIIBSNGEAPIQLSEL
VIIVLLISALP

FIGURE 24

TcoTS-D2

NCEERKSVTAFFPEVVKNSGRSVSGGHQQTWCTPILLISAIPLFLITCCSESSTOSTWLEKRRVELFRPWGKGHPMVPGASYSSDGRGVFEGNSLLEVNDQI
VTLAGARYNFWNGCYACMMKXTIRLSFGHQCPCGAADWWQEXNWGEAVIVNEKVESURYALMCTRAAVVGDKIFFLAITSXSKDALSSPSEDESNLQ
VRLYTIGTVDKSFVCGDASVHWNGPRSLLVTFMKELKRNNSWDFVEGSGRSVVMGDTIEFFPLVALTHKQRSCVIARYRHNDENWIFTRVALDIDOCN
PTILLNKHLMIVVAHNLLKNKVYRSDVDMGLTWTDASKTRRYALTRFOQHACDVDRGDILSYRVGETDLLLFAYRMPFSSATAQHARFLLLNMEDDNKRT
HCLGPPISTGHLFTGAFGALLYTREKLYSLSQESFSSLSLIFTNLTGHLRTNRPVLDTWKCADKRVMLGLYGPSSAAGTTINFKAEPSSFDPTTGLVGF
WSZIASNATHWQDEYLGMGVLHGPLKRVTTGYTMIGCAABVWPVGCGESERKVYHLISNGLTVVMSVAVHIAZKVRIPLLGVTVRSSGMWATDVGSIW
YDNKRTWAQHCCDDEVGAVLAMEVCKTYQLVFTVKCGVARTYVOGRHYGAERGIIIVPQSQSMEVDEMVICNROKAMTKCSADALIVTVFIMLLXNYELS
PAADVRTLLTMKGRSAFETIGMSGDDDEEQHAESGGGSMILWTLAVLIPAIVLLPGAAAFTLVREKRAAGTTMPPATVHUMPYENMATDDILEVSK

FIGURE 25A

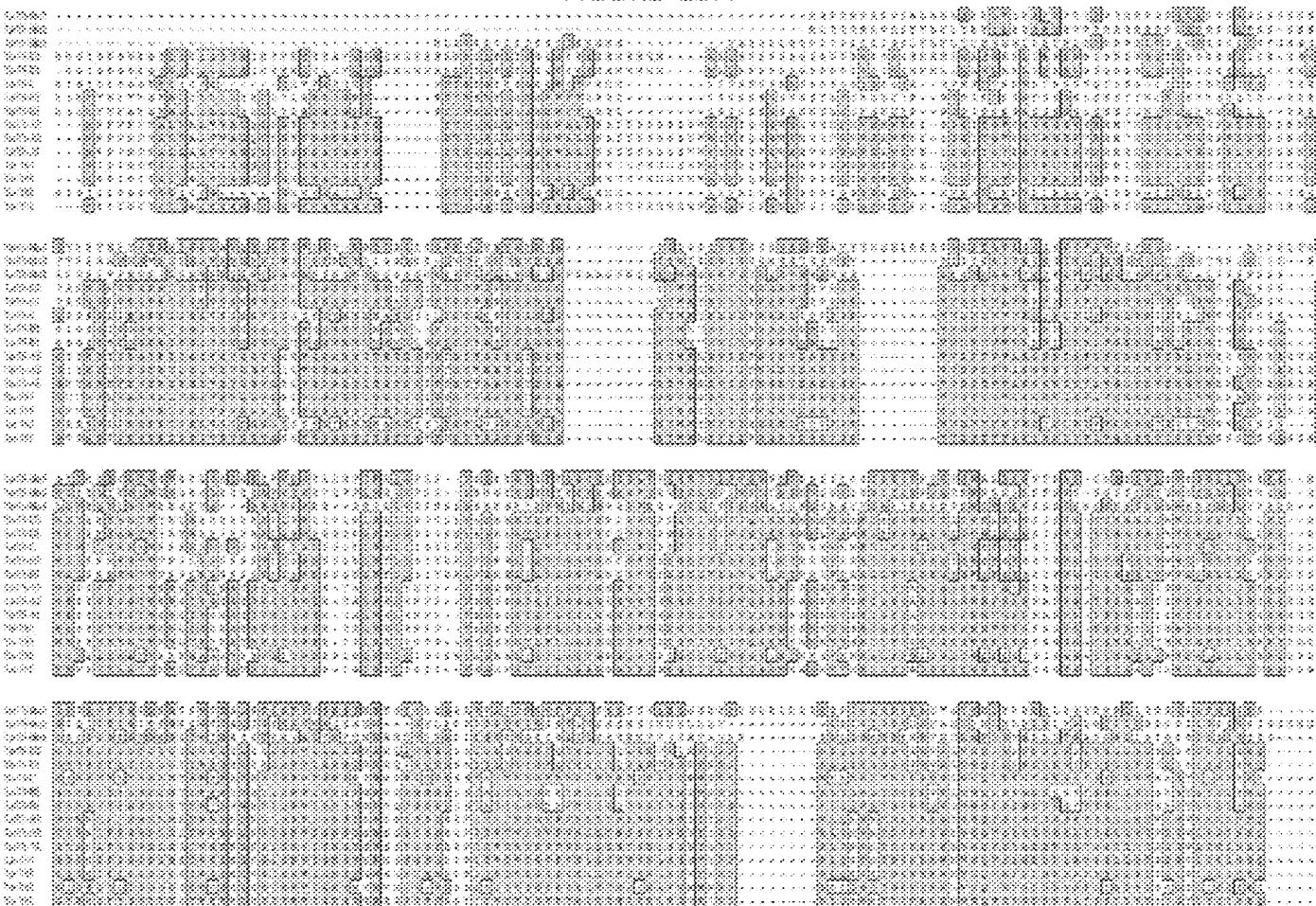


FIGURE 25B

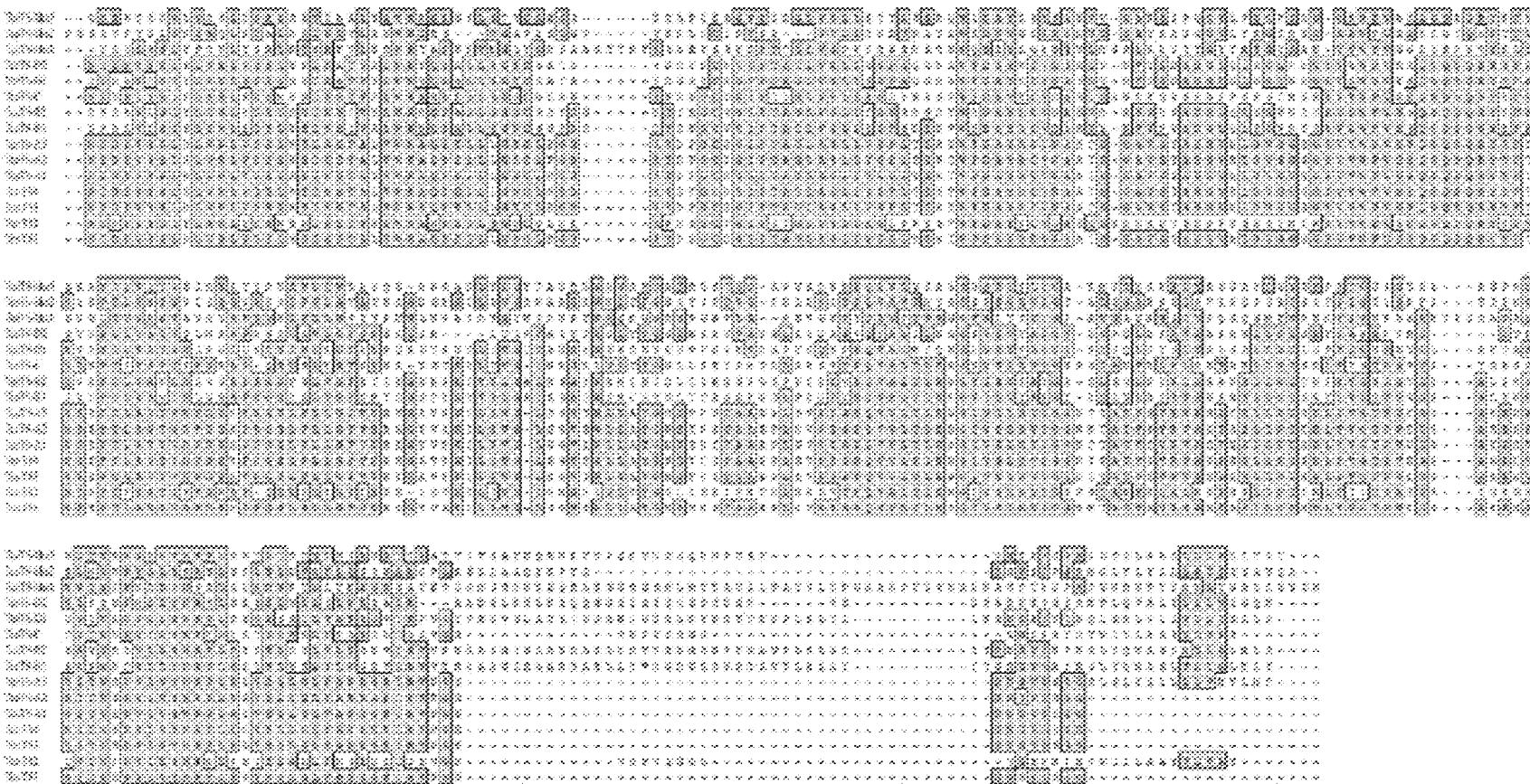


FIGURE 26

	Identity Scores (%)																				
	783-005	783-006	783-007	783-008	783-009	783-010	783-011	783-012	783-013	783-014	783-015	783-016	783-017	783-018	783-019	783-020	783-021	783-022	783-023	783-024	
783-006	99.9	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8		
783-007		99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	
783-008			99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	
783-009				99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	
783-010					99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	
783-011						99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	
783-012							99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	
783-013								99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	
783-014									99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	
783-015										99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	
783-016											99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	
783-017												99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8
783-018													99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8
783-019														99.8	99.8	99.8	99.8	99.8	99.8	99.8	99.8
783-020															99.8	99.8	99.8	99.8	99.8	99.8	99.8
783-021																99.8	99.8	99.8	99.8	99.8	99.8
783-022																	99.8	99.8	99.8	99.8	99.8
783-023																		99.8	99.8	99.8	99.8
783-024																			99.8	99.8	99.8

FIGURE 27

A

Properties	Test TS-31	Test TS-32	Test TS-33
KOGEVYVZBSPQZKXK	*	*	*
KOTKAYGOGVWQ	*	*	*
KUTWSTOPLXG	*	*	*
REINVERTESYKV	*	*	*
KUGONVAKGRR	*	*	*
KLYNVTIKVZKX	*	*	*
RISYFYIGGYOMRT	*	*	*
RONOPLLNGKV	*	*	*
KLEVSYNAOKW	*	*	*
KENPYLAZBEGDZKX	*	*	*
KLYNVTIKVZKX	*	*	*
KESTGARSGGZKX	*	*	*
KLEVSYNOGRN	*	*	*
KUTWSTOPLXG	*	*	*
RONOPPLNGKV	*	*	*
KSYLROFSSPPYDGNALGDAN	*	*	*

B

Liquified-gase	Compressed-gase	Expanded-gase
KVDVYLAZBPN		
KESTGARSGGZKX		
KUTWSTOPLXG		
REINVERTESYKV		
KUGONVAKGRR		
KLYNVTIKVZKX		
RISYFYIGGYOMRT		
RONOPPLNGKV		
KLEVSYNAOKW		
KENPYLAZBEGDZKX		
KLYNVTIKVZKX		
KESTGARSGGZKX		
KLEVSYNOGRN		
KUTWSTOPLXG		
RONOPPLNGKV		
KSYLROFSSPPYDGNALGDAN		

FIGURE 28

A

Peptides	TcoTS-A1	TcoTS-A2	TcoTS-A3
KGNNIYVLYGRY	*	*	*
RVVSMILYSADDGKS	*	*	*
KGEAGVGTSEAALTEWOGKL	*	*	

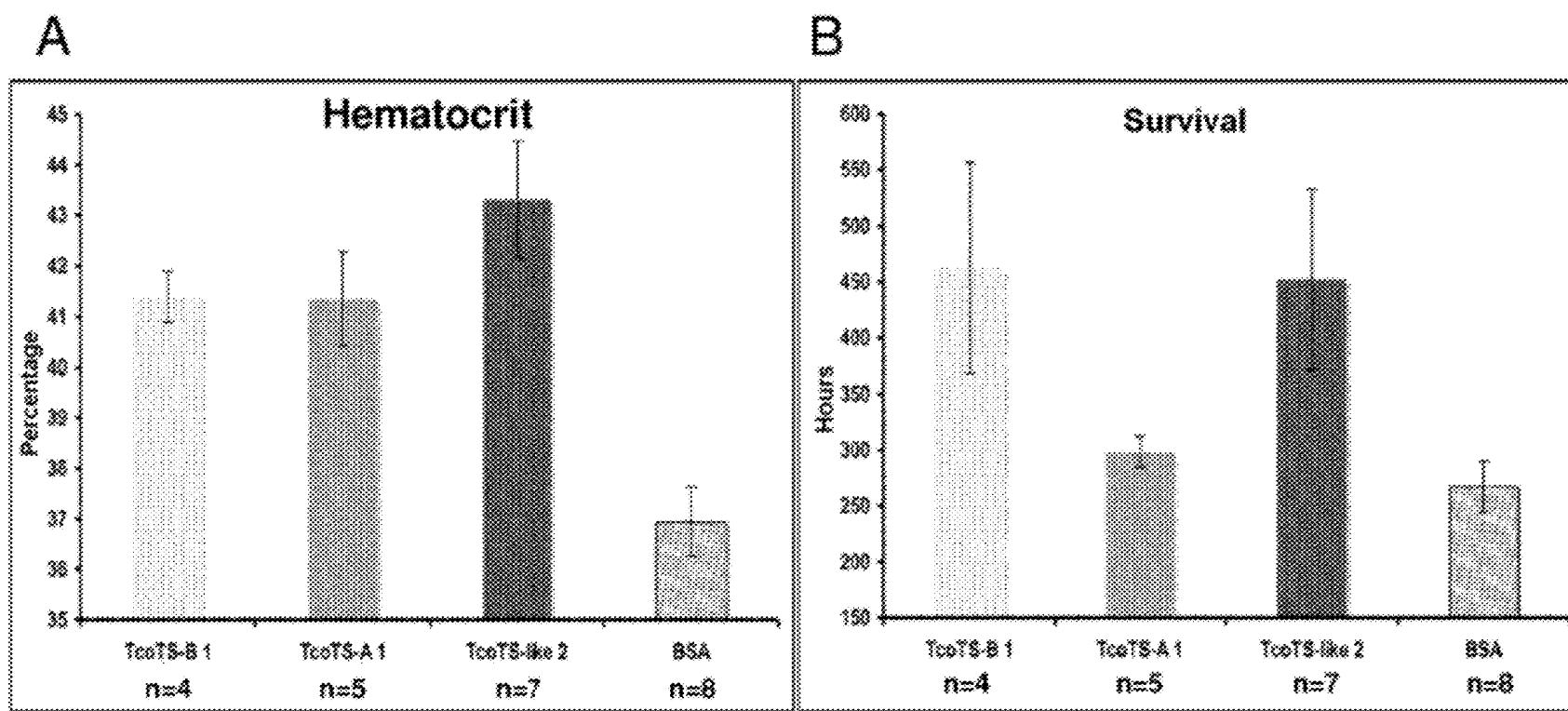
B

Peptides	TcoTS-Like2
KIESTGAVEGGGVVKN	*
REWVEAVTSYSRV	*
KLYNPFVTATVEGRR	*

C

Peptides	TcoTS-D2
KAPNSNVAVLGFGNSKG	*
KVFETTOLGTTWKE	*

FIGURE 29



ANTI-TRYPANOSOMIASIS VACCINES AND DIAGNOSTICS

[0001] The present invention relates to a novel genetic material coding for trans-sialidase-like proteins of African trypanosome parasites, and concerns the use of said genes and proteins in vaccines, therapeutics and diagnostics. The present invention also relates to the immunization of humans and/or non human animals against trypanosomosis and trypanosomiasis.

[0002] Trypanosomosis and trypanosomiasis are caused by several species of parasitic protozoa of the genus *Trypanosoma*, and African trypanosomes generally refer to trypanosomes belonging to the group Salivaria, which itself includes three principal sub-genera: *Trypanozoon*, *Duttonella* and *Nannomonas*.

[0003] Only the sub-genus *Trypanozoon* comprises, in addition to species infectious to animals, two species infectious to humans in whom they cause sleeping sickness. The other sub-genera include species that infect wild and domestic animals and are never infectious to humans, but which can have significant indirect health consequences.

[0004] The sub-genus *Trypanozoon* consists of polymorphic trypanosomes (long and short or stumpy forms), with an optional free flagellum and a small kinetoplast in the sub-terminal (posterior) position. The species of this sub-genus are *Trypanosoma (T.) brucei*, *T. evansi* and *T. equiperdum*. *T. brucei* includes three subspecies: *T. b. brucei*, *T. b. gambiense* and *T. b. rhodesiense*, which are quite similar in morphological, antigenic and biochemical terms and are distinguished by their infectious nature, their pathogenicity and their geographical distribution. *T. brucei* and its subspecies are transmitted by tsetse flies. *T. evansi* is transmitted to cattle, horses and camels by biting flies other than tsetse (Tabanidae) in Africa, South America and Southeast Asia. *T. equiperdum* has no invertebrate host (sexual transmission in horses). The latter two species extend far beyond areas with tsetse flies and are cosmopolitan. Their morphology is similar to that of *T. brucei* but they are monomorphic (long forms only).

[0005] Trypanosomes belonging to the sub-genus *Duttonella* are club shaped, with a round and broad posterior extremity and a body that narrows toward the anterior extremity. The kinetoplast is voluminous, round and in the terminal position; the undulating membrane is relatively undeveloped, narrow and terminates in a free flagellum. *T. vivax* and *T. uniforme* are species of parasites of wild and domestic ruminants. They can be transmitted mechanically or by tsetse flies, in which they colonize exclusively the proboscis and proventriculus.

[0006] Trypanosomes of the sub-genus *Nannomonas* are small (8-24 µm), and they have no free flagellum at any stage of their development. The average-size kinetoplast is in the subterminal or marginal position. The posterior extremity is round and the undulating membrane narrow. Their pathogenicity in Africa is significant for cattle, pigs and dogs. Their development in the tsetse fly takes place exclusively in the stomach and proboscis. The principal species are *T. congolense* and *T. simiae*. These trypanosomes are small with a round posterior extremity, a kinetoplast in the marginal position and a narrow undulating membrane.

[0007] Domestic ruminants in Africa are primarily infected by three species of pathogenic trypanosomes, *T. congolense*, *T. vivax* and *T. brucei*, which are responsible for a pathology

called nagana. Other animals are infected by another pathogenic trypanosome species, *T. evansi*, which is responsible for a pathology called surra. Trypanosomes are characterized by a large genetic diversity, which relates to their infectivity, virulence, pathogenicity, transmissibility and sensitivity to trypanocidal products.

[0008] *T. congolense* is the principal agent of bovine trypanosomosis in Africa, by its frequency and pathogenicity. It also adapts to various nonhuman animal species, and can thus indifferently parasitize bovids, suids, ovids, caprids, equids and canids.

[0009] *T. brucei*, and notably the subspecies *Trypanosoma brucei gambiense*, is probably the most widely known since it is responsible for the chronic form of sleeping sickness in man in Western and Central Africa. The subspecies *Trypanosoma brucei brucei* is a parasite of domestic and wild animals throughout Africa, but it is not infectious to man due to the lytic effect of apolipoprotein L, present in human serum, on the blood forms of these trypanosomes. The third subspecies is *Trypanosoma brucei rhodesiense*, which is the agent of sleeping sickness in its acute form in Africa.

[0010] Additionally, the subspecies *T. evansi* is transmitted to bovids, horses and camels and has significant economic repercussions in Africa, notably for the breeding of cattle and buffaloes.

[0011] Lastly, *T. vivax* is a parasite primarily of ungulates in tropical Africa and is transmitted by horseflies and gadflies.

[0012] Trypanosomes have a complex life cycle which includes various morphological forms. They have in general a fusiform body and a flagellum connected to the body by an undulating membrane. They reproduce asexually by binary fission. During an infection, the tsetse fly (*Glossina* sp.) injects into the dermis of the host at the puncture site the infectious metacyclics present in the mouthparts. The parasites multiply in the dermis at the inoculation point. A local reaction related to parasite multiplication in the dermis occurs, and the parasites give rise to blood forms. This stage can last from 1-3 weeks, for example, in the case of *T. congolense*. Then, the parasites invade the blood, the lymphatic system, in particular the lymph nodes, and various organs such as the liver, spleen, heart, kidneys and testicles, which then exhibit significant lesions. The tsetse becomes infected by and feeds on parasitized animals. Once infected, it remains infectious throughout its life. In the case of *T. brucei* and *T. congolense*, the trypanosome undergoes in the insect a complex cycle involving dedifferentiation in the intestine into noninfectious procyclic forms. In the salivary glands or mouthparts, trypanosomes transform into adherent epimastigote forms which actively multiply. Their differentiation leads to the infectious stage represented by metacyclic forms, which divide no further.

[0013] The *T. vivax* cycle comprises no procyclic stage. It begins with flagellum attachment in the blood forms introduced by the tsetse. They differentiate into epimastigote forms, which proliferate and then differentiate into infectious metacyclics. The total duration of the cycle in the tsetse is roughly 5-10 days for *T. vivax*, 18 days for *T. congolense* and 30 days for *T. brucei*.

[0014] The sources of infection for domestic animals are other domestic animals or wild animals that are sick or are healthy carriers. The existence of the reservoir comes from the fact that certain species are relatively unreactive to the infection, and relatively insensitive to the disease.

[0015] Potential vectors vary by trypanosome species. *T. congolense* and *T. brucei* are transmitted exclusively by biological vectors such as tsetse flies, but *T. vivax* can also be transmitted by mechanical vectors such as biting flies (gadflies or stable flies). *T. evansi* is transmitted exclusively by mechanical vectors. Transmission efficiency depends on tsetse infection rates and host-vector interactions. Generally, trypanosomes that are infectious to animals have higher infection rates than trypanosomes that infect man, which contributes to the very wide distribution of animal trypanosomiasis.

[0016] Analysis of trypanosomes by electron microscopy shows the existence of a roughly 15 nm coat covering the totality of the cell body of the parasite. This coat is present only on the surface of the blood and metacyclic forms. It is comprised essentially of a variable surface glycoprotein (VSG) with other membrane proteins in small quantities. VSGs form a very dense structure comprising a physical barrier between the plasma membrane and the host. The 3-D structure predicts that only a small part of the protein is exposed on the surface of the parasite. Thus, the principal role of the coat is to mask the invariant membrane antigens of the parasite by presenting several immunodominant motifs to the immune defenses of the host. The coat further protects blood forms against lysis by activation of the alternate complement pathway.

[0017] The fight against animal trypanosomiasis depends on the screening of animals and treatment on the basis of cost recovery. The principal chemical compounds used to treat trypanosomiasis are diminazene aceturate, homidium bromide or chloride, isometamidium chloride, quinapyramine, suramin and melarsomine. However, no new molecule has been placed on the market for at least 30 years, whereas the past few years have seen a fresh outbreak of the disease due to the appearance of trypanocide resistances and the extensive and occasionally inappropriate use of drugs leading to the selection and amplification of resistances reported notably in all regions of Africa affected by the disease.

SUMMARY OF THE INVENTION

[0018] The Applicant identified and obtained a novel genetic material coding for novel trans-sialidase-like proteins named TcoTS-like 1, 2, and 3, recognized by anti-African trypanosome antisera. The genetic material can be used to produce proteins and polypeptides intended for the development of diagnostic tests and for the preparation of vaccine or pharmaceutical compositions against infections by African trypanosomes. Similarly, the protein and any corresponding polypeptide fragment can be used for the production of specific antibodies against the parasite, for the purpose of diagnostics or passive immunization.

BRIEF DESCRIPTION OF THE FIGURES

[0019] FIG. 1: represents the nucleotide sequence coding for the trans-sialidase-like protein TcoTS-like 1;

[0020] FIG. 2: represents the nucleotide sequence coding for the trans-sialidase-like protein TcoTS-like 2;

[0021] FIG. 3: represents the nucleotide sequence coding for the trans-sialidase-like protein TcoTS-like 3;

[0022] FIG. 4: represents the peptide sequence corresponding to the trans-sialidase-like protein TcoTS-like 1;

[0023] FIG. 5: represents the peptide sequence corresponding to the trans-sialidase-like protein TcoTS-like 2;

[0024] FIG. 6: represents the peptide sequence corresponding to the trans-sialidase-like protein TcoTS-like 3;

[0025] FIG. 7: represents a sequence alignment between the trans-sialidase-like protein TcoTS-like 2 and a trans-sialidase protein of the parasite *Trypanosoma cruzi* (*T. cruzi* TS);

[0026] FIGS. 8A and 8B: represent a diagram of the five subfamilies of trans-sialidase-related proteins of the parasite *T. congolense*; the percent identities between genes of the same subfamily are indicated (FIG. 8A) with a table showing the percent identities between said proteins (FIG. 8B);

[0027] FIG. 9: represents the nucleotide sequence coding for the TcoTS-A1 protein;

[0028] FIG. 10: represents the nucleotide sequence coding for the TcoTS-A2 protein;

[0029] FIG. 11: represents the nucleotide sequence coding for the TcoTS-A3 protein;

[0030] FIG. 12: represents the nucleotide sequence coding for the TcoTS-B1 protein;

[0031] FIG. 13: represents the nucleotide sequence coding for the TcoTS-B2 protein;

[0032] FIG. 14: represents the nucleotide sequence coding for the TcoTS-C protein;

[0033] FIG. 15: represents the nucleotide sequence coding for the TcoTS-D1 protein;

[0034] FIG. 16: represents the nucleotide sequence coding for the TcoTS-D2 protein;

[0035] FIG. 17: represents the peptide sequence corresponding to the TcoTS-A1 protein;

[0036] FIG. 18: represents the peptide sequence corresponding to the TcoTS-A2 protein;

[0037] FIG. 19: represents the peptide sequence corresponding to the TcoTS-A3 protein;

[0038] FIG. 20: represents the peptide sequence corresponding to the TcoTS-B1 protein;

[0039] FIG. 21: represents the peptide sequence corresponding to the TcoTS-B2 protein;

[0040] FIG. 22: represents the peptide sequence corresponding to the TcoTS-C protein;

[0041] FIG. 23: represents the peptide sequence corresponding to the TcoTS-D1 protein;

[0042] FIG. 24: represents the peptide sequence corresponding to the TcoTS-D2 protein;

[0043] FIGS. 25A and 25B: represent a sequence alignment between 11 trans-sialidase-related proteins of the parasite *Trypanosoma congolense*;

[0044] FIG. 26: represents a table showing the percent identities between trans-sialidase-related proteins of the parasites *T. congolense* and *T. brucei*.

[0045] FIG. 27: represents a table of the various peptides identified in the immunoprecipitation experiment with anti-TcoTS-A1 serum; their relation to proteins TcoTS-A1, TcoTS-A2 or TcoTS-A3 (A), TcoTS-like 2 (B) and TcoTS-D2 (C).

[0046] FIG. 28: represents a table of the various peptides identified in the experiment involving *T. congolense* blood form membrane preparations (A), their relation to TcoTS-A1, TcoTS-A2 or TcoTS-A3 proteins is illustrated by a plus sign (+); and a table of peptides related to the TcoTS-like 2 protein identified during immunoprecipitation experiments with anti-peptide 1, anti-peptide 2 or anti-peptide 3 sera (B).

[0047] FIGS. 29A and 29B: represent measurements of hematocrit (A) and mean survival (B) in mice after immuni-

zation with TcoTS-like 2, TcoTS-A1 and TcoTS-B1 proteins or with BSA. The number of mice (n) used during the various immunizations is indicated.

DEFINITIONS

[0048] "African trypanosomes" refer to parasitic protozoa of the genus *Trypanosoma* belonging to the group Salivaria, which itself includes three principal sub-genera: *Trypanozoon*, *Duttonella* and *Nannomonas*, such as defined above. These have been described as African trypanosomes, but however are found today in Asia and South America as well as on the African continent. The most common African trypanosomes are *Trypanosoma congolense*, *Trypanosoma vivax*, *Trypanosoma evansi* and *Trypanosoma brucei*.

[0049] The terms "trypanosomosis" and "African animal trypanosomosis" (AAT) generally refer to infections of non-human animals caused by African trypanosomes, whereas the terms "trypanosomiasis" or "African trypanosomiasis" are used to refer to human infections also caused by African trypanosomes. For purposes of simplification, the terms trypanosomosis and trypanosomiasis are used indifferently herein.

DETAILED DESCRIPTION OF THE INVENTION

[0050] The present invention has as an object a DNA or RNA molecule coding for novel trans-sialidase-like proteins called TcoTS-like 1, 2, and 3, and belonging to African trypanosomes. These novel DNA or RNA molecules comprise at least one strand comprising a nucleotide sequence selected from the sequences SEQ ID NOS: 1-3, a sequence complementary, antisense or equivalent to one of the sequences SEQ ID NOS: 1-3, and notably a sequence comprising an identity of at least 70% with one of the sequences SEQ ID NOS: 1-3, or a sequence having, on a sequence of 100 contiguous nucleotides, at least 50%, preferably at least 60%, or at least 70%, or at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, or 95% homology with said sequences, or a nucleotide sequence able to hybridize with one of the sequences SEQ ID NOS: 1-3 under stringent hybridization conditions.

[0051] Stringent hybridization conditions refer to hybridization at a temperature of 65° C. overnight in a solution containing 0.1% SDS, 0.7% dried skimmed milk and 6×SSC, followed by washings at room temperature in 2×SSC—0.1% SDS and at 65° C. in 0.2×SSC—0.1% SDS.

[0052] The invention also relates to DNA or RNA fragments whose nucleotide sequence is identical, complementary, antisense or equivalent to any one of the following sequences: SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3, and notably DNA or RNA fragments, for any sequence of 30 contiguous monomers, at least 50%, preferably at least 60%, or at least 85%, 90%, 91%, 92%, 93%, 94%, or 95% homology with any one of said sequences.

[0053] Nucleotide sequence refers to at least one strand of DNA or its complementary strand, or one strand of RNA or its antisense strand or their corresponding complementary DNA. The DNA sequence as represented in one of the sequences SEQ ID NOS: 1-3 corresponds to the messenger RNA sequence, given that that the thymine (T) in DNA is replaced by uracil (U) in RNA.

[0054] According to the invention, two nucleotide sequences are said to be equivalent with respect to each other as a result of natural variability, notably spontaneous mutation of the species from which they were identified, or

induced variability, as well as homologous sequences, with homology being defined below. Variability refers to any spontaneous or induced modification of a sequence, notably by substitution and/or insertion and/or deletion of nucleotides and/or nucleotide fragments, and/or extension and/or shortening of the sequence at least one end, or unnatural variability that may result from the genetic engineering techniques used. This variability can be expressed by modifications of any starting sequence, regarded as a reference, and can be expressed by a degree of homology in relation to said reference sequence.

[0055] Homology characterizes the degree of identity of two compared nucleotide (or peptide) fragments; it is measured by percent identity, which is notably determined by direct comparison of nucleotide (or peptide) sequences in relation to reference nucleotide (or peptide) sequences.

[0056] Another object of the invention relates to proteins called TcoTS-like 1, TcoTS-like 2 and TcoTS-like 3, with apparent molecular weights of roughly 85 kDa for the TcoTS-like 1 protein, roughly 76 kDa for the TcoTS-like 2 protein and roughly 78 kDa for the TcoTS-like 3 protein, and recognized by anti-African trypanosome antisera, as well as the antigenic peptide fragments thereof or an immunological equivalent of said proteins or fragments. The amino acid sequences of said proteins are represented in the sequences SEQ ID NOS: 4-6 and further comprise protein sequences that are at least 70%, 75%, 80%, 85%, 90%, or at least 95% homologous.

[0057] The proteins newly characterized by the Applicant have at the C-terminus a conserved lectin part aimed at allowing binding to sialic acids of infected animals and at the N-terminus a catalytic part with similarity to that of trans-sialidase enzymes and thus referred to as trans-sialidase-like by the Applicant.

[0058] Immunological equivalent refers to any polypeptide or peptide able to be recognized immunologically by antibodies directed against said TcoTS-like 1, 2, and 3 proteins.

[0059] The invention further relates to any fragment of TcoTS-like 1, 2 and 3 proteins, and more particularly any antigenic peptide fragment specifically recognized by anti-African trypanosome antisera.

[0060] Said proteins and protein fragments of the invention can comprise modifications, notably chemical modifications that do not alter their immunogenicity.

[0061] The present invention thus also relates to one or more peptides whose amino acid sequence corresponds to part of the sequence of the TcoTS-like 1, TcoTS-like 2 and/or TcoTS-like 3 proteins, exhibiting alone or in mixtures reactivity with the totality of sera of nonhuman animals and/or humans infected by African trypanosomes. The peptides can be obtained by chemical synthesis, by lysis of TcoTS-like 1, TcoTS-like 2 and TcoTS-like 3 proteins, or by genetic recombination techniques.

[0062] According to a second aspect, the present invention has as an object a functional expression cassette, notably in a cell from a prokaryotic or eukaryotic organism, enabling the expression of DNA coding for the totality or a fragment of the TcoTS-like 1, TcoTS-like 2 and TcoTS-like 3 proteins as described above, in particular a DNA fragment such as defined above placed under the control of the elements required for its expression. Said protein or protein fragment thus expressed is recognized by anti-African trypanosome antisera.

[0063] Generally, any cell from a prokaryotic or eukaryotic organism can be used in the context of the present invention. Such cells are known to the person skilled in the art. As examples, mention may be made of cells from a eukaryotic organism, such as mammalian cells, notably Chinese hamster ovary (CHO) cells, insect cells or fungal cells, notably unicellular or yeast cells, notably from *Pichia*, *Saccharomyces*, *Schizosaccharomyces* and particularly selected from the group comprised of *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Schizosaccharomyces malidevorans*, *Schizosaccharomyces sloofiae* and *Schizosaccharomyces octosporus*. Similarly, among cells from prokaryotic organisms, mention may be made, without constituting a limitation in any way, of cells of a strain of *Escherichia coli* (*E. coli*) or enterobacteria cells. The cell can be wild-type or mutant. Mutations are described in the literature available to the person skilled in the art. Preferably, an *E. coli* cell is used, such as BL21 (DE3), for example.

[0064] The expression cassette of the invention is intended for the production, for example in *E. coli*, of TcoTS-like 1, TcoTS-like 2 and TcoTS-like 3 proteins, or fragments of said proteins, recognized by anti-African trypanosome antisera. Such antisera come from animals having contracted a recent or old infection by trypanosome species *T. congolense*, *T. brucei*, *T. evansi* and/or *T. vivax*, and contain immunoglobulins that specifically recognize TcoTS-like 1, TcoTS-like 2 and TcoTS-like 3 proteins. Also, TcoTS-like 1, TcoTS-like 2 and TcoTS-like 3 proteins can be recognized by other antibodies such as, for example, monoclonal or polyclonal antibodies obtained by immunization of varied species with the aforesaid natural protein, the recombinant protein, or the fragments or peptides thereof.

[0065] TcoTS-like 1, TcoTS-like 2 and TcoTS-like 3 proteins, or fragments thereof, refer to the antigen or antigenic fragment of natural African trypanosomes belonging to the species *T. congolense*, *T. brucei*, *T. evansi* and/or *T. vivax*, produced notably by the genetic recombination techniques described in the present application, or any fragment or mutant of said antigen on the condition that it is immunologically reactive with antibodies directed against the TcoTS-like 1, TcoTS-like 2 and TcoTS-like 3 proteins of said parasites.

[0066] Advantageously, said proteins have an amino acid sequence with a degree of homology of at least 70%, 75%, 80%, 85%, 90%, or at least 95% in relation to the sequences SEQ ID NOs: 4-6. In practice, one such equivalent can be obtained by deletion, substitution and/or addition of one or more amino acids of the native or recombinant protein. It is within the means of the person skilled in the art to carry out these modifications by known techniques without affecting immunological recognition.

[0067] In the context of the present invention, the TcoTS-like 1, TcoTS-like 2 and TcoTS-like 3 proteins can be modified in vitro, notably by deletion or addition of chemical groups such as phosphates, sugars or myristic acids, so as to improve its stability or the presentation of one or several epitopes.

[0068] The expression cassette of the invention enables the production of the TcoTS proteins TcoTS-like 1, TcoTS-like 2 and TcoTS-like 3, or an antigenic fragment of said proteins, having the amino acid sequences as specified above, and fragments of said proteins, which can advantageously be fused with an exogenous element able to contribute to its stability, purification, production or recognition. The choice

of one such exogenous element is within the means of the person skilled in the art. It can be notably a hapten or an exogenous peptide.

[0069] The expression cassette of the invention comprises the elements required for the expression of said DNA fragment in the cell under study. "Elements required for the expression" refer to all of the elements that enable the transcription of the DNA fragment into messenger RNA (mRNA), such as transcription promoter sequences (CMV promoter, for example) and terminator sequences, as well as elements enabling the translation of mRNA into protein.

[0070] The present invention extends to a vector comprising an expression cassette of the invention. It can also be a plasmid vector capable of autonomous replication and in particular multiplication. It can be a viral vector and notably a baculovirus-derived vector, more particularly intended for expression in insect cells, or an adenovirus-derived vector for expression in mammalian cells.

[0071] The present invention also relates to a cell from a prokaryotic or eukaryotic organism, comprising an expression cassette, either integrated in the cell genome or inserted in a vector.

[0072] A further object of the present invention is a method for preparing one or more proteins selected from TcoTS-like 1, TcoTS-like 2 and TcoTS-like 3, or antigenic fragments of said proteins, wherein: (i) a cell from a prokaryotic or eukaryotic organism, comprising the expression cassette of the invention, is cultivated under suitable conditions; and (ii) the protein expressed by said organism is recovered.

[0073] According to a third aspect, the invention relates to monoclonal or polyclonal antibodies obtained by immunological reaction of a non human animal organism with an immunogenic agent comprised of one or more natural or recombinant TcoTS-like 1, TcoTS-like 2 and TcoTS-like 3 proteins and/or the antigenic peptide fragments thereof, such as defined above. As examples, the polyclonal antibodies of the present invention can be generated by using the TcoTS-like 1, TcoTS-like 2 and TcoTS-like 3 proteins (SEQ ID NOs: 4-6), which are injected into rabbits in order to immunize them as described in Example 2. The rabbit polyclonal sera thus obtained, designated as anti-peptide antibody 1, anti-peptide antibody 2 and anti-peptide antibody 3, respectively, are also part of the present invention given that they exhibit reactivity against their inventive peptide in indirect ELISA.

[0074] According to a fourth aspect, the present invention has as an object an active immunotherapeutic composition, notably a vaccine preparation, which comprises one or more natural or recombinant TcoTS-like 1, TcoTS-like 2 and TcoTS-like 3 proteins, and/or the antigenic peptide fragments thereof, and/or a mixture of one or more TcoTS-like 1, TcoTS-like 2 and TcoTS-like 3 proteins, and/or a mixture of one or more peptide fragments such as defined above, and optionally a suitable excipient and/or adjuvant.

[0075] The vaccine or veterinary compositions of the invention are intended to treat and/or prevent an infection by African trypanosomes in humans and/or non human animals, particularly against infections by the species *T. congolense*, *T. brucei*, *T. evansi* and/or *T. vivax*.

[0076] African trypanosomosis results in syndromes of variable gravity, ranging from acute infection with mortality in 3 to 4 weeks to chronic infection lasting months or even years. The chronic progression, characterized by intermittent parasitemias, is the most frequent in cattle. The disease begins with a hyperthermia phase, and then two to three weeks after

the infecting bite the number of red blood cells and hemoglobin and hematocrit levels drop, reflecting anemia, which is the major symptom of trypanosomosis. Chronically infected animals consume less feed, become cachectic, their growth slows, and negative effects on reproduction are observed. Trypanosomosis anemia is established in two phases. During the initial phase, anemia is accompanied by parasitemia and results primarily from extra-vascular hemolysis: red blood cells are destroyed by the phagocyte system in the spleen, liver, circulating blood and bone marrow. Eventually, anemia results in bone marrow dysfunction.

[0077] Said vaccine compositions can be provided in the form of an antigenic vaccine and thus comprise a therapeutically effective quantity of one or more natural or recombinant TcoTS-like 1, TcoTS-like 2 and TcoTS-like 3 proteins, and/or the antigenic peptide fragments thereof such as described above.

[0078] The vaccine compositions can be provided in the form of DNA vaccines and can thus comprise an expression cassette, a vector, a cell from a prokaryotic or eukaryotic organism such as defined above, able to express one or more TcoTS-like 1, TcoTS-like 2 and TcoTS-like 3 proteins, and/or the antigenic peptide fragments thereof, and/or a combination thereof. The DNA vaccines can contain DNA or RNA, modified nucleotide sequences, and preferably one or more expression vectors coding for an antigenic peptide or a fragment under the control of a eukaryotic promoter sequence.

[0079] The vaccines of the present invention can be monovalent vaccines comprising a therapeutically effective quantity of one or more natural or recombinant TcoTS-like 1, TcoTS-like 2 and TcoTS-like 3 proteins, and/or the antigenic peptide fragments thereof such as described above and/or the nucleotide sequences coding for said peptide peptides or fragments.

[0080] Said monovalent vaccine prevents the infestation and thus the expression of the disease.

[0081] If said vaccine does not prevent the infestation but only the expression of the disease, it could be called an "anti-disease" vaccine. In this case, and given that differential diagnosis with other blood parasitoses is currently not systematic, the use of multivalent vaccines combining the so-called "anti-disease" vaccine with antigens of other trypanosomes and/or other therapeutic active agents and/or other vaccines commonly used in disease prevention is particularly advantageous according to the present invention.

[0082] Thus, the vaccines of the present invention can be monovalent vaccines combining one or more natural or recombinant proteins and/or peptide fragments and/or nucleotide sequence coding for said peptides and peptide fragments of one or more trypanosome species, and preferably derived from one or more similar or different trypanosome species.

[0083] Said trypanosome-derived antigenic peptides, fragments or antigenic peptide cocktails are, for example, other sialidases or trans-sialidases, tubulins, proteases, lipases and/or flagellar proteins.

[0084] As examples of trans-sialidases able to be incorporated into multivalent vaccines, mention may be made of the trans-sialidases of *T. cruzi*, *T. congolense*, *T. vivax*, *T. evansi*, *T. brucei*, *T. rhodesiense* and/or *T. gambiense*. Certain trans-sialidases of *T. congolense*, among others, are described in international application WO2004/55176 or by Tiralongo E. et al. (JBC vol. 278, No. 26, pp 23301-10, 2003). More precisely, mention may be made of *T. cruzi* trans-sialidase

chains A and B as deposited in GenBank under numbers GI:29726491, GI:29726490, GI:29726489 and GI:29726488. It is also advantageous to use inactive mutated forms of trans-sialidases. In this respect, mention may be made of the mutant *T. cruzi* trans-sialidases described in international application WO2007/107488, for example, which conserve less than 20% of their sialidase and transferase enzymatic activity.

[0085] As examples of trypanosome-derived tubulins, mention may be made of *T. brucei* alpha-tubulin (deposited in GenBank under accession number AAA30262.1), *T. brucei* beta-tubulin (deposited in GenBank under accession number AAA30261.1), *T. brucei* epsilon-tubulin (deposited in GenBank under accession number EAN77544.1), *T. brucei* TREU927 epsilon-tubulin (referenced in NCBI under numbers XP_822372.1 and XP_829157.1), *T. brucei* delta-tubulin (deposited in GenBank under accession number EAN80045.1), *T. brucei* zeta-tubulin (referenced in NCBI under number XP_001218818.1) or the *T. brucei* tubulins described in international application WO 2008/134643.

[0086] As examples of trypanosome-derived flagellar proteins, mention may be made of the *T. brucei* flagellar protein described in international application WO2002/19960 or the *T. congolense* flagellar protein described in the Applicant's French application filed on 13 Nov. 2009 under number FR09/58035. Further mention may be made of the *T. brucei* TREU927 flagellar protein or flagellar-like proteins (referenced in NCBI under numbers XP_847376.1; XP_847374.1; XP_847295.1; XP_843961.1; XP_847377.1), the *T. brucei* flagellar protein TB-44A (deposited in GenBank under accession number AAZ13310.1), the *T. brucei* flagellar protein TB-24 (deposited in GenBank under accession number AAZ13308.1) and the *T. brucei* flagellar protein deposited in GenBank under accession number AAZ13311.1.

[0087] As examples of proteases, mention may be made of trypanosome cysteine proteases such as *T. congolense* con-gopain or trypanopain-Tc, *T. rhodesiense* rhodesain and *T. cruzi* chagasin or cruzipain.

[0088] The vaccines of the present invention, whether monovalent or multivalent, can further comprise adjuvants in order to increase antigenic response. Adjuvants are well-known to the person skilled in the art. As examples of adjuvants, mention may be made of vitamin E, aluminum gels or salts such as aluminum hydroxide or aluminum phosphates, metal salts, saponins, polyacrylic acid polymers such as Carbopol®, nonionic block polymers, fatty acid amines such as pyridine and DDA, dextran-based polymers such as dextran sulfate and DEAE-dextran, liposomes, bacterial immunogens such as LPS, peptidoglycans or MDP.

[0089] The nonhuman animals that can be treated include, for example, bovids, ovids, felids, suids, camelids and/or canids.

[0090] Alternatively, the vaccines can comprise an effective therapeutic amount of a monoclonal or polyclonal antibody as described below.

[0091] The multivalent vaccines of the present invention can further contain antigens of other blood parasitoses derived, for example, from protozoa such as *Theileria parva*, *T. annulata*, *Babesia bigemina* and *B. divergens* to treat and/or prevent trypanosomes and theileriosis, anaplasmosis and/or babesiosis.

[0092] These can be further combined with other standard vaccines used for the prophylaxis and/or treatment of parasitoses in the target areas, namely against foot-and-mouth dis-

ease, clostridiosis, plague, catarrhal fever, contagious bovine pleuropneumonia (CBPP), blackleg, pasteurellosis and/or sheep pox.

[0093] The vaccines of the present invention are particularly useful for treating and/or preventing trypanosomosis-induced pathogeneses such as anemia, degradations in general health, weight loss and/or immunosuppression in humans or nonhuman animals.

[0094] The monovalent or multivalent vaccines can also be administered in combination with antiparasitic agents, anti-infective agents and/or symptomatic agents.

[0095] Antiparasitic agents include, for example, trypanocidal drugs such as diamidines (pentamidine or pentamidine mesylate, diminazene or diminazene aceturate), arsenic derivatives such as Melarsoprol®, melarsomine, eflornithine (DMFO), arsbabal, MelBdm, nitrofuran derivatives such as nifurtimox (5-nitrofuran), ornithine analogs (Eflornithine® or difluoromethylornithine), phenanthridine (isometamidium or Homidium®), a polysulfonated naphtha-urea such as Suramin®, an anti-malignancy agent such as quinapyramine, buthionine sulfoximine (BSO), azaserine, 6-diazo-5-oxo-norleucine (DON) and/or acivicin. When the vaccines are administered in combination with antiparasitic agents, the latter are preferably administered before and/or simultaneously and/or after the monovalent or multivalent vaccines described above. Other nonspecific antiparasitic agents for trypanosomes are well-known in the field, and are administered before and/or simultaneously and/or after the vaccines of the invention. Among these, mention may be made of avermectins (ivermectin, abamectin, doramectin, eprinomectin and selamectin), pyrethrins (deltamethrin, etc.) and/or anthelmintic antiparasitic agents (oxibendazole, piperazine, flubendazole).

[0096] As examples of anti-infective agents, mention may be made of antibiotics such as β -lactams, fosfomycin, glycopeptides or polypeptides with antibiotic activity, bacitracin, aminoglycosides, macrolides, lincosamides, streptogramins, tetracyclines, phenicols, fusidic acid or quinolones.

[0097] Symptomatic agents are, for example, anti-anemia agents such as iron, vitamin B12, folic acid or calcium levofolinate; or hepatoprotective agents such as flavonoid complexes (silymarin, silibinin, etc.), curcuma, *Desmodium adscendens* and/or *Chrysanthellum americanum* (carbon).

[0098] Non-steroidal anti-inflammatory drugs (NSAIDs) can include, among others, oxicams (meloxicam, piroxicam and/or tenoxicam), salicylate derivatives (methyl salicylate and acetylated lysine), 2-arylpropionic acids (profens), indole sulfonamide derivatives, selective COX-2 NSAIDs (celecoxib, etoricoxib, etc.), phenylbutazone, niflumic acid and/or fenamic acids.

[0099] According to a fifth aspect, the present invention relates to probes or primers specific for African trypanosome, and the use thereof in diagnostic tests.

[0100] The term "probe" as used in the present invention refers to DNA or RNA comprising at least one strand with a nucleotide sequence enabling hybridization with nucleic acids with at least one nucleotide sequence such as represented in the sequences SEQ ID NOs: 1-3, or a sequence complementary, antisense or equivalent to said sequence, and notably a sequence with five to 100 contiguous nucleotides that is at least 50%, preferably at least 60%, or at least 85% homologous to the sequences SEQ ID NOs: 1-3, or to a synthetic oligonucleotide enabling such hybridization, unmodified or comprising one or more modified bases such as

inosine, methyl-5-deoxycytidine, deoxyuridine, dimethylamino-5-deoxyuridine, diamino-2,6-purine, bromo-5-deoxyuridine or any other modified base. Similarly, these probes can be modified at the sugar, namely the replacement of at least one deoxyribose with a polyamide, or at the phosphate group, for example its replacement by esters notably selected from diphosphate, dialkyl and arylphosphonate esters and phosphorothioate esters.

[0101] The probes can be much shorter than the sequences identified in the sequences SEQ ID NOs: 1-3. In practice, such probes comprise at least five nucleotides, advantageously between five and 50 nucleotides, preferably roughly 20 nucleotides, having a hybridization specificity under conditions established to form a hybridization complex with the DNA or RNA having a nucleotide sequence as previously defined. The probes of the invention can be used for diagnostic purposes, as capture and/or detection probes.

[0102] The primers of the invention comprise a sequence of five to 30 monomers selected from the sequences SEQ ID NOs: 1-3, and have a hybridization specificity under predetermined conditions to initiate enzymatic polymerization, for example in an amplification technique such as the polymerase chain reaction (PCR), in an extension process such as sequencing, in a reverse transcription method or the like.

[0103] According to a sixth aspect, the present invention relates to a detection and/or monitoring reagent as well as to a method and kits for diagnosing infections by African trypanosomes, notably by *T. congolense*, *T. brucei*, *T. evansi* and/or *T. vivax*. The trypanosome detection reagents or diagnostic kits comprise as the reactive substance at least one monoclonal or polyclonal antibody as described above. Alternatively, the trypanosome detection reagents or diagnostic kit can comprise a probe and/or primer such as defined above, to detect and/or identify African trypanosomes in a biological sample, notably a capture probe and a detection probe, with one and/or the other as defined above.

[0104] The reagent above can be bound directly or indirectly to a suitable solid support. The solid support can be notably in the form of a cone, tube, well, bead or the like. The term "solid support" as used herein includes all the materials on which a reagent can be immobilized for use in diagnostic tests. Natural or synthetic materials, chemically modified or not, can be used as solid supports, notably polysaccharides such as cellulose-based materials, for example paper, cellulose derivatives such as nitrocellulose and acetate; polymers such as vinyl chloride, polyethylene, polystyrene, polyacrylate or copolymers such as vinyl chloride and propylene polymers, vinyl chloride and vinyl acetate polymers, styrene-based copolymers, natural fibers such as cotton and synthetic fibers such as nylon.

[0105] The reagent can be bound to the solid support directly or indirectly. Directly, two approaches are possible, either by adsorption of the reagent on the solid support, i.e., by noncovalent bonds (mainly hydrogen, van der Waals or ionic bonds) or by establishment of covalent bonds between the reagent and the support. Indirectly, an "anti-reagent" compound able to interact with the reagent in order to immobilize the unit on the solid support can be bound beforehand (by adsorption or covalence) to the solid support. As an example, mention may be made of an anti-TcoTS-like 1, 2, and 3 antibody, on the condition that it is immunologically reactive with a different part of the protein than that participating in the sera antibody recognition reaction; a ligand-receptor system, for example, by grafting on the TcoTS-like

1, 2, and 3 proteins a molecule such as a vitamin, and by immobilizing the corresponding receptor on the solid phase (for example the biotin-streptavidin system). Indirect approaches also include the preliminary grafting or fusion by genetic recombination of a protein, or a fragment of said protein, or a polypeptide, at one end of the TcoTS-like 1, TcoTS-like 2 and TcoTS-like 3 proteins, and immobilization of the latter on the solid support by passive adsorption or covalence of the grafted or fused protein or polypeptide.

[0106] Capture probes can be immobilized on a solid support by any suitable means, i.e., directly or indirectly, for example by covalence or passive adsorption. Detection probes are labeled by means of a label selected from radioactive isotopes, enzymes notably selected from peroxidase and alkaline phosphatase, and those able to hydrolyze a chromogenic, fluorogenic or luminescent substrate, chemical chromophores, chromogenic, fluorogenic or luminescent compounds, nucleotide basic analogs, and biotin.

[0107] The probes of the present invention used for diagnostic purposes can be implemented in any known hybridization techniques, and notably so-called "dot-blot" techniques; Southern blot; northern blot, which is a technique identical to the Southern blot technique but which uses RNA as the target; and the sandwich technique.

[0108] The method for detecting and/or monitoring an African trypanosome infection in a biological sample, such as a blood sample from a nonhuman animal capable of being infected by African trypanosomes, consists in bringing together said sample and a reagent such as defined above, under conditions enabling a possible immunological reaction, and then detecting the presence of an immune complex with said reagent.

[0109] As a nonrestrictive example, mention may be made of the one- or multi-step ELISA detection technique, which consists in reacting a first specific monoclonal or polyclonal antibody for the antigen sought, bound to a solid support, with the sample, and revealing the possible presence of an immune complex thus formed by a second antibody labeled by any suitable label known to the person skilled in the art, notably a radioactive isotope, an enzyme, for example peroxidase or alkaline phosphatase or the like, by so-called competition techniques well-known to the person skilled in the art.

[0110] Alternatively, the method for selectively detecting African trypanosomes in a biological sample and diagnosing trypanosomosis consists in taking a blood sample, exposing the DNA extracted from the sample and optionally denaturing said DNA with at least one probe such as defined above and detecting the hybridization of said probe.

[0111] Lastly, another object of the present invention relates to a kit for veterinary use for diagnosing trypanosomiasis in a biological sample, comprising a probe or a primer as described above, or an antibody such as described above, as well as a reagent for detecting an immunological reaction.

[0112] The kits of the present invention comprise at least one compartment for an optionally sterile packaging comprising an effective therapeutic quantity of a reagent such as described above, as well as instructions relating to the protocol for implementing the veterinary diagnostics of the invention.

[0113] According to another aspect, the present invention concerns sequences related to trans-sialidase-like in *T. congoense*. More precisely, 11 genes coding for sialidase-related

sequences were characterized and classified in five subfamilies according to their sequence homologies (FIGS. 8A and 8B).

[0114] The first trans-sialidase-like subfamily comprises the three genes described above and designated TcoTS-like 1, 2 and 3, which have 17-24% identity between them (FIGS. 1 to 6).

[0115] The second subfamily was named subfamily A and comprises three genes designated A1, A2 and A3 and whose nucleotide sequences are given in SEQ ID NOs: 7, 8 and 9, respectively. Genes A1, A2 and A3 have 94-97% identity between them (FIGS. 9 to 11) and code for the three proteins TcoTS-A1, TcoTS-A2 and TcoTS-A3, respectively, whose amino acid sequences are provided in SEQ ID NOs: 15, 16 and 17, respectively (FIGS. 17 to 19).

[0116] The third subfamily, designated B, comprises two genes designated hereafter B1 and B2, whose nucleotide sequences are given in SEQ ID NOs: 10 and 11, respectively, and which have 76% identity between them (FIGS. 12 and 13). The two genes B1 and B2 code for trans-sialidases TcoTS-B1 and TcoTS-B2, whose peptide sequences are represented in SEQ ID NOs: 18 and 19 (FIGS. 20 and 21).

[0117] The fourth subfamily, designated C, comprises only one gene, designated C, whose nucleotide sequence is represented in SEQ ID NO: 12 (FIG. 14), and which codes for the TcoTS-C protein whose peptide sequence is provided in SEQ ID NO: 20 (FIG. 22).

[0118] Lastly, the fifth subfamily, which was designated subfamily D, comprises two genes named D1 and D2, whose nucleotide sequences are provided in SEQ ID NOs: 13 and 14 (FIGS. 15 and 16). These two genes D1 and D2 indeed have 96% identity between them. They code for the proteins TcoTS-D1 and TcoTS-D2, whose amino acid sequences are provided in SEQ ID NOs: 21 and 22 (FIGS. 23 and 24).

[0119] The percent identities between the proteins coded by these 11 genes of the invention as described above are presented in FIGS. 8A and 8B. An alignment of the sequences is given in FIGS. 25A and 25B. Trans-sialidase-like 1 to 3 are highly divergent in relation to other genes.

[0120] According to this aspect, the present invention thus has as an object novel nucleotide sequences, coding for novel trans-sialidase-like proteins, called TcoTS-A1, TcoTS-A2, TcoTS-A3, TcoTS-B1, TcoTS-B2, TcoTS-C, TcoTS-D1 and TcoTS-D2 belonging to African trypanosomes. These novel DNA or RNA molecules comprise at least one strand comprising a nucleotide sequence selected from the sequences SEQ ID NOs: 7-14, a sequence complementary, antisense or equivalent to one of the sequences SEQ ID NOs: 7-14, and notably a sequence comprising an identity of at least 70% with one of the sequences SEQ ID NOs: 7-14, or a sequence having, on a sequence of 100 contiguous nucleotides, at least 50%, preferably at least 60%, or at least 70%, or at least 80% homology with said sequences, or a nucleotide sequence able to hybridize with one of the sequences SEQ ID NOs: 7-14 under stringent hybridization conditions, such as defined above.

[0121] The invention also relates to DNA or RNA fragments whose nucleotide sequence is identical, complementary, antisense or equivalent to any of the sequences SEQ ID NOs: 7-14, and notably DNA or RNA fragments, for any sequence of 30 contiguous monomers, at least 50%, preferably at least 60%, or at least 85% homologous to any one of said sequences.

[0122] Also, according to this aspect, the invention relates to proteins called TcoTS-A1, TcoTS-A2, TcoTS-A3, TcoTS-B1, TcoTS-B2, TcoTS-C, TcoTS-D1 and TcoTS-D2, as well as the peptide sequences of said proteins as represented in the sequences SEQ ID NOs: 15-22, respectively, and all amino acid sequences having a homology of at least 70%, 75%, 80%, 85%, 90%, or at least 95% with the peptide sequences SEQ ID NOs: 15-22. The invention also has as an object all antigenic peptide fragments of the proteins TcoTS-A1, TcoTS-A2, TcoTS-A3, TcoTS-B1, TcoTS-B2, TcoTS-C, TcoTS-D1 and TcoTS-D2 specifically recognized by anti-African trypanosome antisera, as well as all immunological functional equivalents of said proteins likely to be recognized immunologically by antibodies directed against the proteins TcoTS-A1, TcoTS-A2, TcoTS-A3, TcoTS-B1, TcoTS-B2, TcoTS-C, TcoTS-D1 and TcoTS-D2 of the present invention. Said proteins and antigenic peptide fragments of the invention can comprise modifications, notably chemical modifications that do not deteriorate their immunogenicity.

[0123] As an example, an antigenic peptide fragment of the present invention can be the peptide PKNIKG-SWHRDRLQLWLTD (SEQ ID NO: 24) belonging to the TcoTS-B1 protein or peptides at least 70%, 75%, 80%, 85%, 90%, or at least 95% homologous to said fragment.

[0124] The present invention further relates to the combination or a mixture of one or more proteins selected from TcoTS-like 1, TcoTS-like 2, TcoTS-like 3, TcoTS-A1, TcoTS-A2, TcoTS-A3, TcoTS-B1, TcoTS-B2, TcoTS-C, TcoTS-D1 and TcoTS-D2, and/or one or more antigenic peptide fragments of said proteins, and/or one or more immunological functional equivalents of said proteins. Also, it has as an object a method for preparing one or more proteins selected from TcoTS-like 1, TcoTS-like 2, TcoTS-like 3, TcoTS-A1, TcoTS-A2, TcoTS-A3, TcoTS-B1, TcoTS-B2, TcoTS-C, TcoTS-D1 and TcoTS-D2, or a mixture of said proteins, and/or one or more antigenic peptide fragments of said proteins, and/or one or more immunological functional equivalents of said proteins. These techniques for producing proteins, fragments, functional equivalents and combinations are carried out by chemical synthesis, protein lysis or genetic recombination. They are well-known to the person skilled in the art, and have been in addition described above.

[0125] According to this aspect, the invention relates to monoclonal or polyclonal antibodies obtained by immunological reaction of a nonhuman animal organism with an immunogenic agent comprised of one or more natural or recombinant TcoTS-A1, TcoTS-A2, TcoTS-A3, TcoTS-B1, TcoTS-B2, TcoTS-C, TcoTS-D1 and TcoTS-D2 proteins and the peptide fragments thereof such as described above. It also has as an object a vaccine composition comprising a mixture of one or more proteins selected from TcoTS-like 1, TcoTS-like 2, TcoTS-like 3, TcoTS-A1, TcoTS-A2, TcoTS-A3, TcoTS-B1, TcoTS-B2, TcoTS-C, TcoTS-D1 and TcoTS-D2, and/or one or more antigenic peptide fragments of said proteins, and/or one or more immunological functional equivalents of said proteins and/or a combination of said proteins, fragments or functional equivalents.

[0126] Up to the present, none of these 11 proteins has been identified in the blood forms of *T. congolense*. Indeed, Tiralongo et al. ((2003) *J. Biol. Chem.* 278(26):23301-10) as well as the international publication WO2004/055176 describe the cloning in the procyclic forms present in the insect vector of two *T. congolense* trans-sialidases, TS1 and TS2. Said proteins were only described as being expressed in the procyclic

forms present in the insect vector. Also, a study of sialidase-related genes was carried out in *T. brucei* (Montagna et al. (2006) *J. Biol. Chem.* 281(45): 33949-58). Montagna et al. describe the identification of several protein sequences of the *T. brucei* TbTS gene family (AF310231.1). It notably describes a truncated version of the TbTS gene, namely TbTSsh, the genes B and C coding for *T. brucei* trans-sialidases TbSA B and TbSA C, and finally the genes D1, D2, and E coding for *T. brucei* trans-sialidases. The percent identities between the sequences identified in *T. congolense* and *T. brucei* are presented in FIG. 26. Montagna et al. disclose that these trans-sialidases are expressed in vivo in the procyclic forms or insect forms, and likely play an important role in the transfer of sialic acid on the parasite membrane, thus ensuring the protection of the parasites and their survival when they are transported by insect vectors. However, Montagna et al. do not describe the possibility of detecting these trans-sialidases in sufficient quantity in the blood forms of parasites, i.e., in the infected animals, and thus using them as vaccines or diagnostics.

[0127] Also, up to the present no sialidase activity has been described for these 11 proteins in blood forms. On the contrary, the literature describes the absence of sialidase activity in *T. congolense* blood forms (Engstler et al. (1995) *Acta Trop.* 59: 117-29).

[0128] Whereas none of these 11 proteins has ever been identified in *T. congolense* blood forms and no sialidase activity has been described in these forms, the Applicant has demonstrated in a surprising manner sialidase activity in *T. congolense* blood forms, and has shown by immunoprecipitation followed by mass spectrometry analysis the expression of TcoTS-A1, TcoTS-A2, TcoTS-A3 and TcoTS-like 2 proteins in *T. congolense* blood forms (Example 3 and FIG. 27). The expression of these same proteins as well as the TcoTS-D2 protein was also shown by mass spectrometry analysis of *T. congolense* blood form membrane preparations (Example 4 and FIG. 28). The applicant further demonstrated during vaccination protection experiments on murine models (Example 5, FIGS. 29A and 29B) that the antigenic proteins TcoTS-A1, TcoTS-B1 and TS-like 2 produced a greater protective effect in terms of mean survival of the animals as well as in relation to hematocrit. This protection was even total (no development of parasitemia and normal hematocrit) in certain cases: three mice out of 12 in the case of TcoTS-like 2 and one out of nine in the case of TcoTS-B1.

[0129] Consequently, the present invention has as an object vaccine or veterinary compositions intended to treat and/or prevent an African trypanosome infection in a nonhuman animal, particularly against infections by the species *T. congolense*, *T. brucei*, *T. evansi* and/or *T. vivax*. Said veterinary vaccine compositions can be provided in the form of an antigenic vaccine and thus comprise a therapeutically effective quantity of one or more proteins selected from TcoTS-like 1, TcoTS-like 2, TcoTS-like 3, TcoTS-A1, TcoTS-A2, TcoTS-A3, TcoTS-B1, TcoTS-B2, TcoTS-C, TcoTS-D1 and TcoTS-D2, and/or one or more antigenic peptide fragments of said proteins, and/or one or more immunological functional equivalents of said proteins and/or a combination of said proteins, fragments or functional equivalents. Preferably, said vaccine or veterinary compositions comprise at least one protein selected from TcoTS-A1, TcoTS-B1 and TcoTS-like 2. Even more preferentially, said vaccine or veterinary compositions comprise at least the TcoTS-like 2 protein, and/or an antigenic peptide fragment, and/or an immunological

functional equivalent of TcoTS-like 2. Alternatively, the vaccine compositions can comprise an effective therapeutic quantity of a monoclonal or polyclonal antibody directed against one or more proteins selected from TcoTS-like 1, TcoTS-like 2, TcoTS-like 3, TcoTS-A1, TcoTS-A2, TcoTS-A3, TcoTS-B1, TcoTS-B2, TcoTS-C, TcoTS-D1 and TcoTS-D2. They are particularly useful for treating and/or preventing trypanosomosis-induced pathogeneses, notably such as anemia, degradations in general health, weight loss and/or immunosuppression in nonhuman animals.

[0130] Further according to this aspect, the present invention relates to a reagent for detecting and/or monitoring as well as a method and kits for diagnosing African trypanosome infections, notably by *T. congolense*, *T. brucei*, *T. evansi* and/or *T. vivax*. The trypanosome detection reagents or diagnostic kits comprise as the reactive substance at least one monoclonal or polyclonal antibody directed against one or more TcoTS-A1, TcoTS-A2, TcoTS-A3, TcoTS-B1, TcoTS-B2, TcoTS-C, TcoTS-D1 and TcoTS-D2 proteins. Preferably, the trypanosome detection reagents or diagnostic kits comprise as the reactive substance at least one monoclonal or polyclonal antibody directed against one or more proteins selected from TcoTS-A1, TcoTS-A2, TcoTS-A3 and TcoTS-like 2.

[0131] The method for detecting and/or monitoring an African trypanosome infection in a biological sample, such as a blood sample from a nonhuman animal able to be infected by African trypanosomes, consists in bringing together said sample and a reagent such as defined above, under conditions enabling a possible immunological reaction, and then detecting the presence of an immune complex with said reagent.

[0132] As a nonrestrictive example, mention may be made of the one- or multi-step ELISA detection technique, which consists in reacting a first specific monoclonal or polyclonal antibody for the antigen sought, bound to a solid support, with the sample, and revealing the possible presence of an immune complex thus formed by a second antibody labeled by any suitable label known to the person skilled in the art, notably a radioactive isotope, an enzyme, for example peroxidase or alkaline phosphatase or the like, by so-called competition techniques well-known to the person skilled in the art.

[0133] Finally, according to this aspect, the present invention has as an object a kit for veterinary use for diagnosing trypanosomosis in a biological sample, comprising an antibody such as described above as well as a reagent for detecting an immunological reaction. The kits of the present invention comprise at least one compartment for an optionally sterile packaging comprising an effective therapeutic quantity of a reagent such as described above, as well as instructions relating to the protocol for implementing the veterinary diagnostics of the invention.

EXAMPLES

Example 1

Production of Polyclonal Antibodies Directed Against the TcoTS-A1 Protein

[0134] The TcoTS-A1 protein was produced in the yeast *Pichia pastoris*. To that end, the X33 strain was transformed by the PICZ vector (Invitrogen) containing the sequence coding for the TcoTS-A1 protein lacking its first 29 amino acids. The protein secreted in the culture supernatant after 4 days of expression induction in methanol was purified by successive ion-exchange chromatographies. First, the culture superna-

tant was dialyzed against 20 mM Na acetate buffer (pH 4.5) for 16 hours, centrifuged for 30 minutes at 10,000 g, and then subjected to chromatography on one 1 ml HiTrap SP HP column (GE Healthcare). Elution was carried out according to a linear gradient of 0-1 M NaCl. Fractions containing sialidase activity (fluorometry test with the substrate 2'-(4-methylumbelliferyl)- α -D-N-acetylneurameric acid, as described in the publication by Montagna et al. (2006) *J. Biol. Chem.* 281(45): 33949-58), were combined and dialyzed for 16 hours against 20 mM Tris-HCl buffer (pH 8). After centrifugation for 30 minutes at 10,000 g, the supernatant was subjected to a second chromatography on one 1 ml HiTrap Q HP column (GE Healthcare). Elution was carried out according to a linear gradient of 0-1 M NaCl. The fractions containing sialidase activity were combined and treated with the endoglycosidase Endo Hf (Biolabs) according to the manufacturer's recommendations. The deglycosylated sample was again subjected to chromatography on one 1 ml HiTrap Q HP column (GE Healthcare) as described above. Protein integrity was verified by SDS-PAGE and staining with Coomassie blue.

[0135] This purified recombinant protein was then used to immunize BALB/c mice or rabbits. 20 μ g of recombinant protein was injected into mice on a schedule of one injection each 15 days for a total of four injections or 100 μ g of recombinant protein was injected into rabbits on a schedule of one injection each 15 days for a total of four injections. For the first injection, the recombinant protein was mixed in emulsion form with Freund's complete adjuvant and then for the following injections with Freund's incomplete adjuvant. Serum from the immunized animals was collected at the end of the experiment (anti-TcoTS-A1 serum) and its reactivity against the recombinant protein was verified by indirect ELISA.

Example 2

Production of Polyclonal Antibodies Directed Against Peptides from Sialidase-Related Sequences

[0136] The following peptides: C-RTSIDYHLIDT-VAKYSADDG (SEQ ID NO: 23), C-PKNIKG-SWHRDRLQLWLTD (SEQ ID NO: 24) and C-PVSAQQQDHRYEAANAEHT (SEQ ID NO: 25), named peptides 1, 2 and 3, respectively, were coupled via the N-terminus cysteine with a carrier protein (KLH) activated by a maleimide functional group and used to immunize rabbits on a schedule of one 100 μ g injection every 20 days for a total of five injections. For the first injection, the recombinant protein was mixed in emulsion form with Freund's complete adjuvant and then for the following injections with Freund's incomplete adjuvant. The polyclonal sera obtained, designated anti-peptide 1 antibody, anti-peptide 2 antibody and anti-peptide 3 antibody, respectively, were collected at the end of the experiment and verified for their reactivity against their respective peptide by indirect ELISA.

Example 3

Demonstration of TcoTS-A1, TcoTS-A2, TcoTS-A3 and TcoTS-Like 2 Protein Expression in *T. congolense* Blood Forms

[0137] 3 ml of rabbit serum or 1 ml of mouse serum was dialyzed against 1 l of 20 mM phosphate buffer (pH 7) for 16 hours. The dialyzed serum was centrifuged for 20 minutes at

5,000 g and then passed through one protein G sepharose Fast Flow column (GE healthcare) prepared beforehand as indicated by the manufacturer. After washing the column with 20 mM phosphate buffer (pH 7), the IgG bound to the column were eluted with 0.1 M glycine HCl buffer (pH 2.6). The IgG thus purified were dialyzed for 16 hours against 1 l of 0.1 M NaHCO₃ (pH 8.3)/0.5 M NaCl buffer. The IgG were then incubated for 2 hours at room temperature with CNBr-activated sepharose (Sigma) prepared beforehand according to the manufacturer's recommendations. After centrifugation for 1 minute at 1,000 g, the resin was washed with the previous buffer and then saturated by adding 0.1 M Tris-HCl (pH 8) for 2 hours at room temperature. After centrifugation for 1 minute at 1,000 g, the resin was washed successively with Tris-HCl (pH 8)/0.5 M NaCl buffer and then 0.1 M Na acetate (pH 4)/0.5 M NaCl buffer. The resin thus prepared for use in an immunoprecipitation experiment was equilibrated with OLB (100 mM KCl, 17% glycerol, 1 mM MgCl₂, 2.25 mM CaCl₂, 0.5% NP40, 10 mM Tris-HCl, pH 8). 10⁹ cells of the IL3000 strain were lysed in OLB for 1 hour at 4° C. and then centrifuged for 10 minutes at 20,000 g. The supernatant was incubated with the resin prepared beforehand for 16 hours at 4° C. The resin was then centrifuged for 1 minute at 1,000 g and then rinsed with OLB. The antigens bound to the IgG were eluted with 2% boiling SDS. The eluate was dialyzed against water and then freeze-dried. The lyophilizate was then taken up in Laemmli buffer (50 mM Tris-HCl (pH 6.8), 10% glycerol, 1% SDS, 2.5% γ-mercaptoethanol, 0.01% bromophenol blue) and then subjected to SDS PAGE. The gel was then stained with silver nitrate and the bands thus revealed were cut out and analyzed using tandem mass spectrometry (MS/MS).

[0138] This protocol was carried out with anti-TcoTS-A1, anti-peptide 1, anti-peptide 2 and anti-peptide 3 polyclonal sera on the procyclic forms and the blood forms of the IL3000 strain of *T. congolense*. The results for the blood forms are presented in FIG. 27. Immunoprecipitation with the anti-TcoTS-A1 serum identified TcoTS-A1, TcoTS-A2 and TcoTS-A3 proteins in the *T. congolense* procyclic forms and blood forms. Immunoprecipitations with the anti-peptide 1, anti-peptide 2 and anti-peptide 3 sera identified TcoTS-like 2 protein only in *T. congolense* blood forms. These results demonstrated for the first time the expression of TcoTS-A1, TcoTS-A2, TcoTS-A3 and TcoTS-like 2 proteins in the blood forms of the parasite.

Example 4

Demonstration of TcoTS-A1, TcoTS-A2, TcoTS-A3, TcoTS-Like 2 and TcoTS-D2 Protein Expression in *T. congolense* Blood Form Membrane Preparations

[0139] 10⁹ cells of the IL3000 strain were lysed in 1 ml of hypotonic buffer (5 mM Na₂HPO₄, 0.3 mM KH₂PO₄) for 30 minutes at 4° C. and then centrifuged for 10 minutes at 20,000 g. The pellet was subjected to the same treatment three times in a row. The last pellet is taken up at 4° C. in 100 μl of this same hypotonic lysis buffer to which is then added 0.5 ml of the following buffer: 2 mM EDTA, 15.4 mM NaOH, 0.2 mM dithiothreitol. After 10 minutes of incubation, the mixture is centrifuged for 10 minutes at 20,000 g. The supernatant is recovered (soluble fraction) and the pellet (insoluble fraction) is taken up in 50 μl of water to which is then added 50 μl of 2% SDS. 50 μl of each of these two fractions are mixed with 15 μl of 4× Laemmli buffer (200 mM Tris-HCl pH 6.8, 40% glyc-

erol, 4% SDS, 10% γ-mercaptoethanol, 0.04% bromophenol blue) heated at 100° C. for 10 minutes and then subjected to SDS-PAGE. The gel was then stained with silver nitrate and the bands thus revealed were cut out and analyzed using tandem mass spectrometry (MS/MS).

Example 5

Vaccination Tests on a Murine Model

Example 5.1

Vaccination Tests with TcoTS-like 1

[0140] Two groups of BALB/c mice were injected intraperitoneally with either 20 μg of BSA (negative control group) or recombinant TcoTS-like 1 protein (immunized mice group) on a schedule of one injection each 15 days for a total of four injections. Then, the mice were infected with 10⁴ parasites of *T. congolense* strain IL3000. Hematocrit and parasitemia were measured every 2 days for both groups of mice.

Example 5.2

Vaccination Tests with TcoTS-Like 2

[0141] Fourteen BALB/c type mice were injected intraperitoneally with 20 μg of BSA (7 negative control mice) or recombinant TcoTS-like 2 protein (7 mice) on a schedule of one injection each 15 days for a total of four injections. Then, the mice were infected with 10⁴ parasites of *T. congolense* strain IL3000. Hematocrit and parasitemia were measured every 2 days. Mean hematocrit over the entire duration of the parasitemia was calculated: it is 43.3±1.2% for the mice immunized with TcoTS-like 2 and 37.0±0.7% for the control mice immunized with BSA (FIG. 28).

[0142] Mean survival of the mice was also determined: it is 453±81 hours for the mice immunized with TcoTS-like 2 and 267±23 hours for the control mice immunized with BSA.

Example 5.3

Vaccination Tests with TcoTS-like 3

[0143] Two groups of BALB/c mice were injected intraperitoneally with either 20 μg of BSA (negative control group) or recombinant TcoTS-like 3 protein (immunized mice group) on a schedule of one injection each 15 days for a total of four injections. Then, the mice were infected with 10⁴ parasites of *T. congolense* strain IL3000. Hematocrit and parasitemia were measured every 2 days for both groups of mice.

Example 5.4

Vaccination Tests with TcoTS-A1

[0144] Thirteen BALB/c mice were injected intraperitoneally with either 20 μg of BSA (8 negative control mice) or recombinant protein TcoTS-A1 (5 mice) on a schedule of one injection each 15 days for a total of four injections. Then, the mice were infected with 10⁴ parasites of *T. congolense* strain IL3000. Hematocrit and parasitemia were measured every 2 days. Mean hematocrit over the entire duration of the parasitemia was calculated: it is 41.4±0.9% for the mice immunized with TcoTS-A1 and 37.0±0.7% for the control mice immunized with BSA (FIG. 28).

[0145] Mean survival of the mice was also determined: it is 299 ± 14 hours for the mice immunized with TcoTS-A1 and 267 ± 23 hours for the control mice immunized with BSA.

Example 5.5

Vaccination Tests with TcoTS-B1

[0146] Twelve BALB/c mice were injected intraperitoneally with either 20 µg of BSA (8 negative control mice) or recombinant protein TcoTS-B1 (4 mice) on a schedule of one injection each 15 days for a total of four injections. Next, the mice were infected with 10^4 parasites of *T. congolense* strain IL3000. Hematocrit and parasitemia were measured every 2 days.

[0147] Mean hematocrit over the entire duration of the parasitemia was calculated: it is $41.4 \pm 0.5\%$ for the mice immunized with TcoTS-B1 and $37.0 \pm 0.7\%$ for the control mice immunized with BSA (FIG. 28).

[0148] Mean survival of the mice was also determined: it is 463 ± 94 hours for the mice immunized with TcoTS-B1 and 267 ± 23 hours for the control mice immunized with BSA.

Example 5.6

Vaccination Tests with One or More Proteins Selected from TcoTS-A2, TcoTS-A3, TcoTS-B2, TcoTS-C, TcoTS-D1 and TcoTS-D2

[0149] Two groups of BALB/c mice were injected intraperitoneally with either 20 µg of BSA (negative control group) or one or more recombinant proteins selected from the proteins TcoTS-A2, TcoTS-A3, TcoTS-B2, TcoTS-C, TcoTS-D1 and TcoTS-D2 (immunized mice group) on a schedule of one injection each 15 days for a total of four injections. Next, the mice were infected with 10^4 parasites of *T. congolense* strain IL3000. Hematocrit and parasitemia are measured every 2 days for both groups of mice.

Example 6

Vaccination Tests on Cattle

[0150] Two groups of cattle were injected subcutaneously with one or more antigens such as TcoTS-like 1, TcoTS-like 2, TcoTS-like 3, TcoTS-A1, TcoTS-A2, TcoTS-A3, TcoTS-B1, TcoTS-B2, TcoTS-C, TcoTS-D1 and TcoTS-D2, mixed with two types of adjuvants, 1 mg/ml Quil A (saponin) and AdjuPhos (colloidal aluminum phosphate) volume to volume according to a final volume of 1 ml or just with the adjuvant mixture (control). One injection was given each three weeks for a total of three injections of 100 µg, 50 µg and 25 µg of antigen, respectively. The animals were infected by *T. congolense* strain IL3000 three weeks after the last injection in a ratio of 1,000 parasites per animal intradermally. Blood samples were taken daily until all the animals were recognized as infected, parasitemia being determined by buffy-coat analysis. Thereafter, weekly blood samples were taken to

monitor parasitemia and anemia, and the animals were weighed monthly. The kinetics of the response to immunization and to infection were monitored by ELISA on the various immunizing antigens.

[0151] The antigens used during this immunization experiment were TcoTS-like 1, 2 or 3 or TcoTS-A1 or TcoTS-B1, alone or in one of all possible combinations.

Example 7

Example of Diagnostic Tests on Infected Animal Blood

[0152] This test is carried out by detecting circulating antigens such as TcoTS-A1, TcoTS-A2, TcoTS-A3 and TcoTS-like 2 by the sandwich ELISA method. The so-called capture antibody is adsorbed in the wells of a 96-well plate by incubation overnight at 4°C . of 1-10 µg/ml of capture antibody diluted in 100 µl of 50 mM NaHCO₃ buffer (pH 9.6). The plate is then emptied and washed three times with 200 µl per well of PBS-Tween solution (3.2 mM Na₂HPO₄, 0.5 mM KH₂PO₄, 1.3 mM KCl, 135 mM NaCl (pH 7.4), 0.05% Tween 20). Next, 100 µl of blocking solution (0.2% gelatin in PBS-Tween) is added to each well and incubated for 30 minutes at room temperature. The plates are emptied and then 100 µl of animal sera to be tested is deposited in the wells and incubated for 2 hours at 37°C . The plate is then emptied and then washed three times with 200 µl per well of PBS-Tween solution. 100 µl of a solution containing the second antibody coupled to biotin (PBS-Tween containing 1-10 µg/ml of biotinylated antibody) is added to each well and incubated for 1 hour at 37°C . The plate is then emptied and then washed four times with 200 µl per well of PBS-Tween solution. 100 µl of PBS-Tween containing streptavidin coupled to peroxidase (Sigma) is added according to the manufacturer's recommendations. The plate is then emptied and then washed four times with 200 µl per well of PBS-Tween solution. Finally, the reaction is visualized by adding peroxidase substrate according to the manufacturer's recommendations (example of a developer substrate that can be used: ABTS (Sigma)). The result is read using a plate reader or fluorometer according to the manufacturer's recommendations.

[0153] The capture antibody used can be either an immunopurified polyclonal serum against one *T. congolense* sialidase protein or a mixture of *T. congolense* sialidase proteins such as TcoTS-like 1, TcoTS-like 2, TcoTS-like 3, TcoTS-A1, TcoTS-A2, TcoTS-A3, TcoTS-B1, TcoTS-B2, TcoTS-C, TcoTS-D1 and TcoTS-D2, or a monoclonal antibody recognizing an epitope present on one or more of these *T. congolense* sialidase proteins. The second antibody is a monoclonal antibody different than the capture antibody which recognizes a different epitope of one or more *T. congolense* sialidase proteins TcoTS-like 1, TcoTS-like 2, TcoTS-like 3, TcoTS-A1, TcoTS-A2, TcoTS-A3, TcoTS-B1, TcoTS-B2, TcoTS-C, TcoTS-D1 and TcoTS-D2.

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Leu	Thr	Trp	Thr	Asp	Ala	Ser	Lys	Thr	Arg	Arg	Tyr	Ala	Leu	Thr	Asn
		325						330						335	
Phe	Gln	His	His	Ala	Asp	Asp	Val	Asp	Arg	Gly	Asp	Ile	Leu	Ser	Val
	340				345									350	
Arg	Val	Gly	Glu	Thr	Asp	Leu	Leu	Leu	Phe	Ala	Tyr	Arg	Met	Phe	Phe
	355				360									365	
Ser	Ser	Ala	Thr	Ala	Gly	Asn	Arg	Pro	Leu	Leu	Leu	Trp	Met	Thr	Asp
	370				375									380	
Asn	Lys	Arg	Thr	His	Cys	Leu	Gly	Pro	Ile	Ser	Thr	Gly	His	Leu	Phe
	385				390									400	
Thr	Gly	Ala	Phe	Gly	Ala	Leu	Leu	Tyr	Thr	Arg	Glu	Lys	Leu	Tyr	Ser
	405					410								415	
Leu	His	Gln	Glu	Ser	Phe	Ser	Ser	Leu	Ser	Ser	Leu	Phe	Phe	Thr	Asn
	420				425									430	
Leu	Thr	Gly	Arg	Leu	Arg	Thr	Met	Arg	Pro	Val	Leu	Asp	Thr	Trp	Lys
	435				440									445	
Thr	Ala	Asp	Lys	Arg	Val	Met	Gly	Leu	Tyr	Gly	Pro	Ser	Ala	Ala	Gly
	450				455									460	
Thr	Thr	Asn	Phe	Lys	Ser	Ala	Glu	Pro	Ser	Ser	Phe	Asp	Pro	Thr	Thr
	465				470									480	
Gly	Leu	Val	Gly	Phe	Trp	Ser	Thr	Ala	Ser	Asn	Ala	Thr	His	Trp	Gln
	485					490								495	
Asp	Glu	Tyr	Leu	Gly	Met	Asp	Gly	Val	Leu	His	Gly	Pro	Leu	Lys	Arg
	500					505								510	
Val	Thr	Thr	Gly	Tyr	Thr	Met	Glu	Gly	Cys	Ala	Ala	His	Val	Val	Trp
	515					520								525	
Pro	Val	Gly	Gly	Glu	Ser	Glu	Asn	Lys	Val	Tyr	His	Ile	Ser	Asn	
	530				535									540	
Gly	Leu	Thr	Val	Val	Met	Ser	Val	Ala	Val	His	Thr	Ala	Pro	Lys	Val
	545				550									560	
Arg	Ile	Pro	Leu	Leu	Gly	Val	Thr	Val	Arg	Asn	Gly	Ser	Asn	Trp	Ala
	565					570								575	
Thr	Asp	Val	Gly	Ile	Trp	Tyr	Asp	Asn	Lys	Thr	Trp	Ala	Gln	Met	Gly
	580				585									590	
Gly	Asp	Glu	Val	Gly	Ala	Val	Leu	Ala	Met	Glu	Val	Gly	Lys	Thr	Tyr
	595					600								605	
Gln	Leu	Val	Phe	Thr	Val	Lys	Gly	Gly	Val	Ala	Arg	Thr	Tyr	Val	Asp
	610				615									620	
Gly	Arg	Arg	Val	Gly	Ala	Glu	Arg	Gly	Ile	Ile	Val	Pro	Gln	Ser	Gln
	625					630								640	
Ser	Met	Glu	Val	Asp	Glu	Met	Tyr	Ile	Gly	Asn	Arg	Asp	Lys	Ala	Met
	645					650								655	
Thr	Lys	Cys	Ser	Ala	Asp	Ala	Leu	Asn	Val	Thr	Val	Phe	Asn	Met	Leu
	660					665								670	
Leu	Tyr	Asn	Tyr	Glu	Leu	Ser	Pro	Ala	Asp	Val	Arg	Thr	Leu	Leu	Thr
	675				680									685	
Met	Lys	Gly	Arg	Ser	Ala	Phe	Glu	Thr	Ile	Gly	Met	Ser	Gly	Asp	Asp
	690					695								700	
Glu	Glu	Gln	Glu	Ala	Glu	Ser	Gly	Gly	Gly	Ser	Met	Leu	Trp	Thr	Leu

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705	710	715	720
Ala Val Leu Ile Pro Ala Ile Val Leu Leu Phe Gly Ala Ala Ala Phe			
725		730	735
Phe Leu Val Arg Arg Arg Ala Gly Thr Thr Met Pro Pro Ala Thr			
740	745		750
Val His His Asn Pro Tyr Phe Met Asn Ala Thr Asp Asp Thr Leu Glu			
755	760	765	
Val Ser Lys			
770			
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<211> LENGTH: 699			
<212> TYPE: PRT			
<213> ORGANISM: Trypanosoma congolense			
 <400> SEQUENCE: 5			
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His Tyr Ile Leu Glu Gln Ala Arg Val Gly Val Thr Pro Asn Ala Gly			
20	25	30	
His Glu Pro Asn Ser Val Asn Glu Phe Thr Leu Phe Ala Glu Gly Glu			
35	40	45	
Glu His Thr Tyr Arg Leu Ala Ala Val Asp Ser Val His Ile His Ser			
50	55	60	
Leu Val Lys Val Gly Asp Val Leu Val Ala Ile Gly Glu Arg Arg Tyr			
65	70	75	80
Arg Leu Ala Gly Glu Met Arg Leu Asn Thr Phe Ser Leu Cys Ser Val			
85	90	95	
Asp Gly Gly Thr Trp Thr Lys Asp Val Ile Ala Val Gly Met Gly			
100	105	110	
Ser Thr Ser Tyr His Ser Tyr Pro Ile Leu Tyr Glu Ala Ile Val Lys			
115	120	125	
Glu Asn Ser Ile Tyr Leu Phe Ala Gly Gly Tyr Asp Ile Asp Thr Val			
130	135	140	
Gly Thr Gly Asn Ile Asn Ile Ser Ser Arg Gly Trp Asp Pro Leu Leu			
145	150	155	160
Ile Val Gly Lys Val Glu Val Ser Arg Gly Leu Phe Ser Gln Ser Ala			
165	170	175	
Lys Val Thr Trp Gly Thr Gln Val Pro Leu Lys Gly Ser Ile Pro Asp			
180	185	190	
Gly Leu Arg Met Gly Pro Val Ser Lys Phe Tyr Arg Gly Val Lys Gly			
195	200	205	
Ala Val Val Thr Glu Val Gly Ser Leu Val Phe Leu Val Glu Leu Thr			
210	215	220	
Asn Ser His Asn Gln Asp Val Pro Val Val Ile Tyr Ser Thr Asn Asp			
225	230	235	240
Gly Glu Asn Trp Asn Leu Glu Pro Leu Asp Pro Gly Val Cys Lys Gly			
245	250	255	
Tyr Cys His Ile Phe Val Trp Asn Gly Arg Leu Met Leu Gly Asn Gln			
260	265	270	
Ser Ser Lys Gly His Gln Ile Val Tyr Glu Ser Ile Asn Phe Gly Arg			
275	280	285	

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Glu Trp Val Glu Ala Val Thr Ser Tyr Ser Arg Val Trp Ala Ile Glu
 290 295 300

 Ala Glu His Gly Lys Leu Tyr Asn Phe Val Thr Ala Thr Val Glu Gly
 305 310 315 320

 Arg Arg Val Leu Val Phe Ala Gln Arg Ser Ile Asn Asp Lys Leu Arg
 325 330 335

 Glu Val Leu Arg Ile Trp Leu Ser Asp Gly Asp His Phe Ala Glu Ile
 340 345 350

 Asp His Ile His Leu Asp Asp Asp Ile Val Gly Glu Gly Thr Leu Leu
 355 360 365

 Phe Asp Glu Asn Thr Leu Leu Tyr Phe Tyr Arg Lys Ile Gly Tyr Leu
 370 375 380

 Arg Asp Glu Phe Ser Ser Ser Val Pro Tyr Asp Ile Gly Asn Ile Ala
 385 390 395 400

 Gln Leu Asp Asp Ala Leu Ala Lys Ile Lys Ser Val Leu Arg Met Trp
 405 410 415

 Lys Ile Glu Ser Thr Gly Ala Val Glu Gly Gly Val Val Lys Asn
 420 425 430

 Leu Arg Cys Ile Asp Val Ser Pro Val Val Leu Leu Ser Asn Asp Val
 435 440 445

 Asn Ala Thr His Trp Lys Asp Val Tyr Gly Thr Ala Asn Ile Asn Val
 450 455 460

 Thr Gly Ala Thr Lys Ala Asp Gly Gly Val Leu Phe Arg Gly Thr Asn
 465 470 475 480

 Arg Gly Ala Ala Trp Tyr Val Gly Glu Arg Ser Gly Thr Gln Met Tyr
 485 490 495

 Thr Phe Val Asn Tyr Glu Phe Thr Leu Val Met Thr Val Val Ile Ser
 500 505 510

 Glu Gly Val Lys Glu Asn Ile Pro Val Leu Ala Val Ala Val Asn Glu
 515 520 525

 Gly Asp Ser Asn Lys Ile Leu Glu Val Ser Tyr Asn Ala Asp Gly Arg
 530 535 540

 Trp His Leu Thr Phe Gly Gly Lys Tyr Val Pro Thr Val Gly Phe His
 545 550 555 560

 Leu His Asn Ser Thr His Gln Val Ala Val Thr Met Tyr Gly Gly Ser
 565 570 575

 Phe Ser Val Lys Val Asp Gly Thr Ala Leu Ser Ser Ala Arg Asn Ser
 580 585 590

 Ile Lys Val Leu Lys Gln Pro Ser Arg Ile Ser Tyr Phe Tyr Ile Gly
 595 600 605

 Gly Tyr Gly Asn Pro Arg Thr Thr Pro Asn Gly Glu Leu Met Val Arg
 610 615 620

 Asn Val Ala Leu Tyr Lys Arg Glu Leu Ser Ser Leu Glu Leu Asp Val
 625 630 635 640

 Met Phe Leu Gln Ser Tyr Trp Ala Arg Cys Pro Ala Lys Ser Leu Leu
 645 650 655

 Ala Ala Gln Glu Lys Pro Thr Gly Asp Gly Val Glu Ala Pro Gly Arg
 660 665 670

 Met Gly Leu Phe Leu Tyr Leu Leu Ala Ile Ile Ser Tyr Ala Val
 675 680 685

 Gln Ala Gly Gly Gly Thr Asn Arg Val Ala

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690

695

<210> SEQ ID NO 6
<211> LENGTH: 717
<212> TYPE: PRT
<213> ORGANISM: Trypanosoma congolense

<400> SEQUENCE: 6

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Met Arg Leu Val Ser Phe Ala Phe Val Val Leu Ile Arg Val Ser His
20 25 30

Met Pro Ser Glu Gly Leu Ala Arg Val Asp Ala Gly Asp Lys Gly Asn
35 40 45

His Glu Val Gly Val Asn Arg Thr Gly Pro Tyr Ser Tyr Arg Ser Pro
50 55 60

Ser Leu Leu Ala Val Gln Gly Ser Leu Ile Thr Val Ser Glu Thr Trp
65 70 75 80

Asp Thr Thr Asp Glu Lys Tyr Val Asp Val Ile Thr Glu Tyr Ser
85 90 95

Arg Asp Tyr Gly Thr Ser Leu Val Thr Gln Val Ala Ile Arg Ser Asp
100 105 110

Lys Ala Asp Phe His Ala Val Tyr Thr His Gln Glu Asp Arg Glu Ser
115 120 125

Thr Leu His Pro Thr Ala Val Ala Ser Gly Asp Lys Val Tyr Val Leu
130 135 140

Val Phe Cys Lys Asn Ile Gly Ala Asn Asp Ser Leu Thr Gly Asp Gln
145 150 155 160

Val Ile Met Pro Tyr Val Ala Thr Gly Thr Val Leu Pro Leu Gly Ala
165 170 175

Ile Gly Glu Thr Trp Val Asp Trp Thr Ala Leu Asn Pro Ile Arg Ala
180 185 190

Leu Leu Pro Gly Phe Val Gly Gly Lys Arg Ala Ser Arg Phe Phe Gly
195 200 205

Gly Gly Gly Asn Gly Ile Ala Thr Pro Gln Gly Thr Ile Ile Ile Pro
210 215 220

Val Gln Val Val Arg Thr Asp Asp Glu Tyr Phe Ala Ser Ile Ile Tyr
225 230 235 240

Ser Thr Asn Gly Gly Ser Ser Trp Ala Leu Ala Lys Gly Val Thr Asp
245 250 255

Ala Gly Cys Arg Glu Ser Ser Val Leu Glu Trp Lys Gly Lys Leu Leu
260 265 270

Leu Val Ser Arg Ser Asn Asp Gly Phe Thr Lys Val Tyr Glu Ser Gly
275 280 285

Asp Met Gly Thr Lys Trp Thr Glu Ala Leu Gly Thr Ile Ser Arg Val
290 295 300

Phe Gly Asn Ser Pro Asn Arg Thr Gly Pro Gly Asn Gln Gly Ser Ala
305 310 315 320

Val Val Ala Asn Ile Asp Asn Val Pro Val Met Ile Phe Ser His Thr
325 330 335

Thr Val Leu His Gly Gly Asp Gly Asp Asp Ser Gly Arg Ile Arg
340 345 350

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Glu Ile His Gln Arg Ile Trp Leu Ser Asp Gly Asn Arg Ile Val Lys
 355 360 365
 Val Gly His Ile Tyr Trp Asp Asp His Leu Gln Ser Ser His Asn Asn
 370 375 380
 Leu Leu Tyr Asp Lys Gly Lys Leu Phe Cys Ala Tyr Glu Ala Gly Ala
 385 390 395 400
 Glu Lys Thr Ser Ala Val Leu Val Arg Ser Leu Asp Asp Glu Leu Ser
 405 410 415
 Lys Val Glu Ala Ala Leu Glu Ala Trp Lys Arg Gln Asp Ser Tyr Leu
 420 425 430
 Ser Thr Val Cys Ala Ser Gly Ser Asp Thr Ala Pro Cys Glu Ser Gly
 435 440 445
 Val Pro Ile Asp Gly Leu Val Gly Leu Leu Ser Thr Thr Leu Ser Glu
 450 455 460
 Arg Gln Trp Ile Asp Ala Tyr Leu Ser Val Ser Ala Glu Val Val Gly
 465 470 475 480
 Ala Arg Ser Ile Pro Gln Gly Val Leu Phe Glu Gly Pro Ile Arg Gly
 485 490 495
 Gly Arg Trp Pro Val Ala Ala Gln Gly Gln Asn Gln Arg Tyr His Phe
 500 505 510
 Val Ser Lys His Phe Thr Leu Val Ile Thr Val Ser Ile His Glu Arg
 515 520 525
 Thr Thr Asp Arg Ala Pro Leu Leu Val Leu Arg Pro Gln Glu Asp Ala
 530 535 540
 Gly Ala Asp Leu Glu Leu Ser Tyr Thr Ala Asp His Arg Trp His Val
 545 550 555 560
 Arg His Gly Asn Glu His Gly Ser Thr Ser Gly Ala Trp Val Lys Asp
 565 570 575
 Arg Glu His Gln Leu Val Leu Val Cys Glu Ala Gly Asp Ala Ser Leu
 580 585 590
 Tyr Leu Asp Gly Lys Arg Met Pro Thr Met Gly Arg Arg Leu Val Glu
 595 600 605
 Ser Gly Ala Pro Leu Gly Val Ser His Phe Ser Ile Gly Gly Tyr Gly
 610 615 620
 Leu Glu Lys Arg Ser Pro Asn Gly Lys Leu Thr Val Arg Asn Val Met
 625 630 635 640
 Leu Tyr Asn Arg Pro Leu Asn Lys Thr Glu Ile Asp Thr Val Phe His
 645 650 655
 Val Arg Asp Lys Ile Thr Ala Ala Thr Ile Val Lys Ala Phe Glu
 660 665 670
 Gln Lys Asn Arg Val Asn Val Gln Met Val Asn Ser Lys Gln Asp His
 675 680 685
 Pro Thr Ala Pro Asn Asn Glu Glu Ala Cys Gly Ala Leu Ser Thr Ser
 690 695 700
 Leu Ala Ala Leu Leu Leu Ala Leu Leu Thr Leu Thr
 705 710 715

<210> SEQ ID NO 7
 <211> LENGTH: 2205
 <212> TYPE: DNA
 <213> ORGANISM: Trypanosoma congolense

<400> SEQUENCE: 7

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tgggctctga ggaacaagac gaccccgaaa gatggcgagg tgtggggag caacccca	180
ccgggctgga aggagggtgtc cgacgatgag tggggagggt ggtttatggc gcaggaggga	240
ccaacgggtg tggatggtgt gctgtgtgag tggtaccgtc gcatgaagga tgggtatata	300
ctcggtggc gaccgaagct gaactcaccc gacatgaaca gcacccggcat gacgatgcgg	360
actgtgcaact cgtaccgtcat accctcaatt gttggggatg ggggtgtgt aatgtgtgtt	420
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gcccaactact cccgcgtcg tgcgttgcact gttgttgcga agggtaataa cattatgtt	600
ctcggtggc ggtacaatgt cacgcggggc tactggcaca atcagaacga cgaggctgcg	660
atagccgatt gggggccctt cgtgtacaag ggcacgggtga acgtgggcac gaaggacaat	720
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<210> SEQ ID NO 8
<211> LENGTH: 2205
<212> TYPE: DNA
<213> ORGANISM: Trypanosoma congolense

<400> SEQUENCE: 8

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<211> LENGTH: 2202	
<212> TYPE: DNA	
<213> ORGANISM: Trypanosoma congolense	
<400> SEQUENCE: 9	
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gtgctaacgc tccacgacgg gatcgctcc ctgcacgtt acggggtaa cacgacggcg	1980
actgtgagcg tgccgcgtggc gagccctgag gagctgctga acattcatca tcttttcgtt	2040
ggcaccccaag ttgatggagg cgccaaggag cacgccaaca tcacagttagt caatgtcctg	2100
gtgtacaatc gaccgctgctg tggcgtggag ctgcttggc tatcgcaatg caggggacgg	2160
attcggtgtgc ctgggagcga caacgggtgtc ctcagtggcg gg	2202

<210> SEQ_ID NO 10

<211> LENGTH: 2337

<212> TYPE: DNA

<213> ORGANISM: Trypanosoma congolense

<400> SEQUENCE: 10

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tgcctttact tggcgcacgc ctctggaaac ggacgaacga cacgttagttt gtttctggga	120
ggggggcatt gggcgtggg taaggagtgc cttggcgta acgaagaagg ttccggccgc	180
cggaactctgg aatgcaatgg gaactgcagc cccgatgaag actcacagag acgaagtgcg	240
gatgacaatg atggcttaca ggaggagacc attaatttgcg tactagagcc cagatcgaag	300
caacttggtg ttgctaaaga tatggaaagg aagcatgtt tggactcatt tcgtattcca	360
tcgatcggtt aagtggacgg ttttatttgc accgttatctg acgtgcgtta cctcaactct	420
aacgacccccc cttttatttgc cacgggttgc agatacgtg cagatgggtt gaggacgtgg	480
gagactgaag taataatcaa gaatgttgcg agtgcattgc agcactctcg cgtcggttgc	540
cccaactgttgc ttgtcaaaaa caacaccata tttttctcg ttggggaggta caacaagagt	600
gatgcgtact ggacgtggca gggagggtggt ggcgattggg atattctcat gcacaagggc	660
actgtgacga agtgcgttgc aggtggaaa ccattgttac acattgttgc ggatgagcca	720
caaaacttgc agtattttgc gggccatgtt ggttaatgcg atggtaggtt actgatttgc	780
tatattggcg gagttggca ctgtgtcgta acggccaaacg gtaccatcg actccctgtt	840
caagtccgttgc acacgaacag atctgtcatg gccatgttac ttactcaac agatgaagg	900
gaatcgtggc agtttagcaa ggtgttgcata ccgggtggga caacagatgc ctctattgtt	960
tggggatgc acaaaacttgc cctgtatggt agaacaaca atgatggatgc ctaccgcac	1020
gtttacgaat caagcgact tggcacaaca tggacagaag tggatggac catttcacgc	1080
gtgatcgaa actcgccggg ccgcaatcaa ccggaaatgtt cagggatgttgc tattggcata	1140
acattggaaat ggtgtgtgtt gatgttgcata actcaaccga agaatataaa aggttctgg	1200
caccgtgacc gtttgcgttgc tgggttgcac gacggtaatc ggtgtggctt ggtggggcag	1260
atttccgttgc gtgtatgttgc cggccatgtt agtccctgt ttttgcgttgc gaatggacc	1320
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gaagacgttgc tggaaagcat taagtccata gtgtatgttgc ggtgttgc ggtgttgc	1440
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actgtgtggcc tggatggatgc ttttgcgttgc ctttgcgttgc ggtgttgc ggtgttgc	1560
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agtgggttgc acacgttgc ttttgcgttgc ctttgcgttgc ggtgttgc ggtgttgc	1680
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aaggcgact tctcaactggg	gggttttag atgtacgaag gagagacgag gaaaactgta	1800
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ggatccaggg gctcttcc	ctgcgacgaa gttcatcaag tcgcactcac acttcgtaat	1920
ggtgtgatat ctgttatgc	caacggaaga cacctatcg tgctggatac gaaggttgcg	1980
ggcgccaatg agcttctaaa	tatatctaacttcttgg ggcatccggg agtggggggt	2040
gcttgccgt gggcagcgc	agtggtacga gatgtgctgc tctataaccg cccgctgcat	2100
gagactgaac tggagtca	ttacctaacs gggatgtaa taaaagtggt gaaccacggc	2160
gcagctggca tatecgctgc	tcgtgatgct gaaactgctgc atgtccgagg agatggtgga	2220
gacaagcccg atgtgttacc	attgaagcta gcaataatta ccggtgatgg tgtggtgcgg	2280
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<210> SEQ ID NO 11

<211> LENGTH: 2337

<212> TYPE: DNA

<213> ORGANISM: Trypanosoma congolense

<400> SEQUENCE: 11

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tgccttact tggcgacgc	ctctggaaac ggacgaacga cacgtgagtt gtttctggga	120
ggggccatt gggcgtggg	taaggagtgc cttgtgtta acaaggaagg ttccgtcgcc	180
cagactctgg aatgcaatgg	gaactgcagc cccgatgaag actcacggat acgaagtgcg	240
gatgacaatg atggcttaca	ggaggagacc attaatttgcg cactagagcc cagatcgag	300
caacttggtg ttgccaaaga	tatggaaagg aagcatattt gggactcatt tcgtattcca	360
tcgatcggtt aagtggacgg	tgttattt accgtatctg acgtgcgtta cctcaactct	420
aacgacccctt cttttattga	cacgggtgcc agatacgtg cggatggtgg gaggacgtgg	480
gagactgaag taatcatcaa	gaacgccaga gtgaatgcag agcactctcg tgctgttgc	540
cccaactgtt ttgtcaaaaa	caacaccata ttgttctcg ttgggaggtt caacaagagt	600
gatgcgtact ggacgtggca	gggaggtggat ggcgttggg atattctcat gcacaagggc	660
actgtgacga agtgcgttgc	aggcggggaaa ccatctgtaa acattgagtg ggatgagcca	720
caaaaacttga agtatttgc	gagcacagtt ggtaagatag atggtaggtc actgattcag	780
tatattggcg gagttggaaa	ctgcgtcgta acgcccgaacg gtaccatcg actccctgtt	840
caagtccctga acacgaacag	atctgtgttgc gccatgataa ttactcaac agatgaagg	900
gaatcgtggc agttcagcaa	gagtgttaca ccgggtgggaa caacagatgc ctctattgtt	960
tgggtggatg acaaactgct	cctgaatggt agaacaaca acgatttagg ctaccgcaag	1020
gtgtacgaat cgagcgaccc	tggcacaaca tggaaagaag tcgttggac gatttcacgc	1080
gtgatcgaa actcgccggg	ccgcaatcaa ccggaaagtt cagggagttc tatttgcata	1140
acatttggaaag ggatgcgtgt	gtgtcaata actcaaccga agaatataaa aggttcttgg	1200
caccgtgacc gtttgcagtt	gtgggttgcg acggtaatc gegtgtggcgt ggtggggcag	1260
atttccgaag gtgtatgttgc	cgaccctac agtccctgc tgcgtatcgta gaatgggacc	1320
ctctactgct tgcgttgc	ggacaaatcc gccgttttga gtatccat tataaagcta	1380
gaagacgagc tggaaagcat	taagtccata gtgtactat ggaaggatca ggacgcgtt	1440

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ctctcaggga	actgttccct	accggatgg	gattatactg	agggctgcgt	cggcattccc	1500
actgtggcc	tcgttgggc	gctctctgga	ccttcagatg	gggatgtgtg	gcacgcacgc	1560
taccgttgt	tcgatgccag	tgtcgataat	gtggtaat	ttgcagatgg	cctgcactg	1620
agtgggtgga	acagcagccg	tgtgttttg	ccgcgcagca	gtcaggggca	ggacaaaaaa	1680
taccacttg	ccaatgttca	cttcacgcgt	gttgc当地	tgcaactggt	aggggcgcca	1740
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aaactgtcg	ctataaaagag	cgccttttg	gagatgtgtc	acaccgattt	aaccacgagg	1860
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ggcgccaaatg	agtttctaaa	cataactaat	ttctttgtt	ggcatccgg	agtggggac	2040
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gagactgaac	tggagtca	ttacctcaac	ggggatgtaa	taaaaagtgg	gaaccacggc	2160
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<210> SEQ_ID NO 12
<211> LENGTH: 2217
<212> TYPE: DNA
<213> ORGANISM: Trypanosoma congolense

SEQUENCE: 12	
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gaccggcgag	gcattcatgg
aggccggtca	gtggtcggta
120	
aataaaagatt	gtctcatcac
tgcggggggt	agtgcgacgtt
ctaaggcttc	aggctttac
180	
aaaaagggt	acgcttcgt
cgaaaagtaca	acaaaagatg
tgaacactcg	aggtggtgta
240	
cagacaagcg	aggcctgcac
gttggAACCC	gaggtgcgcg
acaactctac	ttcaggtgat
300	
gggaaggaaa	gacatttgat
tcactccctt	cgaattccct
cacttggtga	gatagacggc
360	
gtgcttatcg	cgacattcga
tacacgttat	cttcacgcctt
ccgacagcag	tctcatagac
420	
acagctatga	aatacagtgc
cgatcagggg	aagacgtgga
aaactgaaat	cataataaaa
480	
aatgcttagac	taactgataa
ctttccccgc	gtcgttgatc
caacggttgt	tgttaagggt
540	
gataacttgt	ttatTTTGT
tgggaggtac	aacacctcat
ctaccccatg	ggtctggcag
600	
aaaaacggca	aagactggga
tgtactgtt	tacaaggcca
aggtaaggaa	ggaatcagcg
660	
gggtggggta	catcagttag
ctttacatgg	gacgaacccc
tacacctgaa	gcatctgctc
720	
acctctgtcg	gtaaaaataga
cggcagggtcc	ctcatacaat
acattggtgg	cgttggaaat
780	
ggtattgtaa	caccgaaagg
tactatcg	tttccagttc
aggttttaaa	caccaacaaa
840	
tccgtcatga	acatgcttct
gtattcaagt	aacgacggaa
aaacctggga	gttcagcaaa
900	
acttccacac	ccgcgggcac
aactgaggcc	tccctgttt
ggtgggatgg	acaactactt
960	
ctcacaagca	gaacaactcc
ggatgtcg	agccgcaag
tatatttaac	aagcgac
1020	
ggaacttcat	ggaatgaagc
gatcggaaagt	atctctcg
taattggtaa	ctcgcggtac
1080	
cqtaacqatc	ctqqqqqqt
aqqtqaqctc	attqcataa
ctqtqqadqq	aqtaccqqtq
1140	

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atgctcgtaa	ctcacccgga	aatgcgaag	ggtaggtgga	atcgcgaccg	aatgcgactg	1200
tggctcacgg	acggcaatcg	gatgtggctt	gttggacaga	tttctgaagg	ggacgcacaac	1260
agtgcctaca	gctaccttgt	atacactaaa	aatgggacgt	tattgtgtct	ctacgagcga	1320
aacattcgtg	agatatacag	catttatcta	gcccggtgg	aggatgagat	ggaggatata	1380
aagtcaactg	tgaggctatg	gaaagcgcatt	gacgagctcc	tgtcgggaga	ctgtcaactg	1440
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acacatcagg	tcgcgcgtgac	acttcataat	ggggagggtga	gcctgcacgt	tgatggaaac	1920
ccttcgtattg	cgaacgtaaag	actgaagctc	cacgagcctg	acaggctact	aaacatctcc	1980
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agcaggggacc	tcatcgatga	agtggagat	gtgcaccccg	tgtctggagg	tggagtcggc	2160
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<210> SEQ ID NO 13
<211> LENGTH: 2364
<212> TYPE: DNA
<213> ORGANISM: *Trypanosoma congoense*

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<400> SEQUENCE: 13

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agcttgttcc tcccaggggg gctttggtag aagaaagatg agtggaaagga tggcaatttg 180
ctgcaatcta aagagtggaa agccgggtat gcgtggtggc cgtggcggtc gtggtgcgtcc 240
aataaaaacta tcggtgcgtc cgggaaagaa ctttgcgcga aggaatggg ctctcagagg 300
aaaaaaaggat acacactcaa gccaaggggaa agcgtgtctt ttagggagag tagtggcaca 360
aagagaatgc gccgcgttca ctcccttcgc ataccctcga tggttgaggc gaacgggtgtg 420
ttaattggca ttgcagacgc gcgatatttg agctcccgcc acttcacttt catcgacacg 480
gttgccaaagt acagtgtca cggcggttag acgtggaaaa ctgaagtcat cattgaaaaat 540
gctcggtgtgg attcgtttca ctcccccgctg gtggateccca cagttgcgt gaagaataac 600
agtattttatg tgcttgttgg aaggtacaac acctccaaaca cgtattggac catgaaaaat 660
aacggcaacg attgggacat acttatgtac aagggtaccc taaccaagac ctcccgaaatg 720
ggcaaaacctg ccgcaaaacat tgagtgtagca ggcacccaga atttgaagta cctcctgaaag 780
ctggtggttgc aaatagagggg caagtcgctc acgcagtttc ttgggtgggt aggttaatact 840
gtcgtaacac cggacggcac gatcgatatt cccatccagg taaaaaattc atggaaatcaa 900
atcgcccgaaata tggataatata ttcgacttgcgatggccatccatggccatcttggggggggcc 960

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tggaaagagt cattagggc gctcttcgt gtgattggta actcgccaga ccgcaagcag	1140
aaaggtagct caggcagtgc tattactttg gaggtcgagg gtgtcaagt catgcttatt	1200
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gatggcagcg attgcaacgg cgtgcccacc gctggcctcg tcgggctgct ttccggaccc	1560
gogcaaggaa acgcgtggcc tgcataat aattgtgtaa atgcgcgcct cgtaatgtt	1620
acaagtgtatc cagatggctc gcaatggc ggggtgaaca gggggcgtgt gtcgtggccg	1680
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aagtgttaagg aaggccagt accaggtgtc aatgtggatg ctccggatag ccagaagttt	1920
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atccccacagt tggatacaag catcggttgc gctccgcctc tactgaatat ctctagctt	2040
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ttgttgtatc cggcgctatt ctatc	2364

<210> SEQ ID NO 14
<211> LENGTH: 2319
<212> TYPE: DNA
<213> ORGANISM: Trypanosoma congolense

<400> SEQUENCE: 14

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cgaagcttgc tcccccagg ggggcttgg tacaagaaatc atgagtgaaat ggtatggcaat	180
tggctgcattt ctaaaagatgc gaaagccggg tatgcgtggt ggccgtggc gtcgtggc	240
tccaataaaa ctatcggtgc gtccgggaaa gaactttgc gcaaggaatg ggactctcag	300
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acaaagagaa tgcgcgcgt tcactcttt cgcataaccc cgtatgtca ggcgtatgg	420
gtgttaatttgcattgcaga tgcgcgatattt tggagctccg cggacttcac tttcatcgac	480
acgggttgcac agtacagtgc tgcggcggtt gggacgtggaa aaactgaatgtt catcattgt	540
aatgctcgatc tggattcgtt tcactccgc gttggggatc ccacagttgc cgtgtaaat	600

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gatggcaaac ctgccgcaaa cattgagtgg acctcggtat taaaccttaa gtcgcttctg	780
gaaaccggac tatacgttgg agggcatgag gctacgcagt ttcttggtgg tgttaggtaat	840
actgtcgtaa cacggacgg cacgatcgta ttcccattcc aggtaaaaaa ttcatggaaat	900
catgtcgctg caatgataat gtattcgagt gacgatggcg ccacgtggca cttgggaggaa	960
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ctcaattgca gaacggactt ggggtaccgc aaagtattcg agacaaccga cttgggaca	1080
acgtggaaag agtcatttagg ggctcgctct cgtgtgattt gtaactcgcc agaccgcaag	1140
cagcctggaa gtcaggcag tgcttattact ttggaggtcg aggggtgtgca agtcatgctt	1200
attacgcagc ctaagaatac aaaaacgagg tatagtcgtg atcgcctaca actgtggctg	1260
agtgtatggca gtcgtgtgtg gtttagtcgga cagatatcac gaggcgtgaa caacagtccc	1320
tacagctcgc tgctgtacac tagtgcgtat aagtttttatt ggctgtatga acaaaatatt	1380
gaagagggtt acagtattta cttgtccat ctcgtgtggc aactggagaa aatcaaagcg	1440
accgtgagat tatggaaagga gcaggatgca ctccctctcg gcaattgctc tgcaactgcc	1500
gaggatggca gcgattgcaaa cggcgtgccc accgctggcc tcgtcgggct gcttccgga	1560
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gttacaagtg atgcagatgg tctgcagctt agcgggttga acagggcccg tttgtcggtt	1680
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aattcaaaag gaaaaatctt gacattgtgg gtggcaagta caacgtggac cctgacgtac	1860
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gcgtgatac ttaatgggtt cagtgtgtcc gtgtacgtt atggcgtaca ttttgcgtt	1980
ctcgataaaa ggggtgtc caaaaataaa ttgttaataa ttgtatcattt cttcgccgaa	2040
agcaactata tgggagatac aaacaacata ttcacaaaga acatgctcct gtataaccgg	2100
aaactgagtg aaagcgagct caaactgttg tccctcaacc gggaggctat aagagctgt	2160
gacgggttaa attactaaa agagcagcaaa ggtggctccg agagcgaaat aaagtctact	2220
tccgattcca acgtcagcga tcctgtgtt aacgaaacctt ctgaaaagat gtttctgcaaa	2280
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<210> SEQ ID NO 15

<211> LENGTH: 734

<212> TYPE: PRT

<213> ORGANISM: Trypanosoma congolense

<400> SEQUENCE: 15

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Gln Cys Cys Gly Pro Met His Ala Thr Ala Ala Val Gly Thr Thr His		
20	25	30

Gln Ala Leu Leu Trp Gly Ser Lys Trp Ala Leu Arg Asn Lys Thr Thr		
35	40	45

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Pro	Lys	Asp	Gly	Glu	Val	Trp	Trp	Ser	Asn	Pro	Gln	Pro	Gly	Trp	Lys
50					55			60							
Glu	Val	Tyr	Asp	Asp	Glu	Trp	Glu	Glu	Trp	Phe	Met	Glu	Gln	Glu	Gly
65					70			75			80				
Pro	Thr	Gly	Val	Asp	Gly	Val	Arg	Ala	Glu	Trp	Tyr	Arg	Arg	Met	Lys
								85	90					95	
Asp	Gly	Tyr	Ile	Leu	Val	Gly	Gly	Pro	Lys	Leu	Asn	Ser	Pro	Asp	Met
								100	105				110		
Asn	Ser	Thr	Gly	Met	Thr	Met	Arg	Thr	Val	His	Ser	Tyr	Arg	Ile	Pro
								115	120				125		
Ser	Ile	Val	Glu	Val	Gly	Gly	Val	Leu	Met	Cys	Val	Gly	Asp	Ala	Arg
								130	135			140			
Tyr	Ile	Thr	Ser	Thr	Asp	Tyr	Phe	Phe	Thr	Asp	Thr	Val	Ala	Ala	Tyr
145							150		155			160			
Ser	Thr	Asp	Gly	Gly	Arg	Thr	Trp	Lys	Arg	Glu	Val	Ile	Ile	Pro	Asn
								165	170			175			
Gly	Arg	Val	Asp	Ala	His	Tyr	Ser	Arg	Val	Val	Asp	Pro	Thr	Val	Val
								180	185			190			
Ala	Lys	Gly	Asn	Asn	Ile	Tyr	Val	Leu	Val	Gly	Arg	Tyr	Asn	Val	Thr
								195	200			205			
Arg	Gly	Tyr	Trp	His	Asn	Gln	Asn	Asp	Glu	Ala	Ala	Ile	Ile	Asp	Trp
								210	215			220			
Glu	Pro	Phe	Val	Tyr	Lys	Gly	Thr	Val	Asn	Val	Gly	Thr	Lys	Asp	Asn
225								230		235			240		
Ala	Thr	Asp	Val	Ser	Ile	Ser	Trp	Glu	Arg	Thr	Ala	Leu	Lys	Ser	Leu
								245	250			255			
Tyr	Asn	Phe	Pro	Val	Ser	Gly	Ser	Pro	Gly	Thr	Gln	Phe	Leu	Gly	Gly
								260	265			270			
Ala	Gly	Gly	Val	Val	Thr	Ser	Asn	Gly	Thr	Ile	Val	Leu	Pro	Val	
								275	280			285			
Gln	Ala	Arg	Asn	Lys	Ala	Asn	Arg	Val	Val	Ser	Met	Ile	Leu	Tyr	Ser
								290	295			300			
Ala	Asp	Asp	Gly	Lys	Ser	Trp	His	Phe	Gly	Lys	Gly	Glu	Ala	Gly	Val
305								310		315			320		
Gly	Thr	Ser	Glu	Ala	Ala	Leu	Thr	Glu	Trp	Asp	Gly	Lys	Leu	Leu	Ile
								325	330			335			
Ser	Ala	Arg	Ser	Asp	Gly	Gly	Gln	Gly	Tyr	Arg	Met	Ile	Phe	Glu	Ser
								340	345			350			
Ser	Asp	Leu	Gly	Ala	Thr	Trp	Lys	Glu	Met	Leu	Asn	Ser	Ile	Ser	Arg
								355	360			365			
Val	Ile	Gly	Asn	Ser	Pro	Gly	Arg	Ser	Gly	Pro	Gly	Ser	Ser	Ser	Gly
								370	375			380			
Phe	Ile	Thr	Val	Thr	Val	Glu	Gly	Val	Pro	Val	Met	Leu	Leu	Thr	His
385								390		395			400		
Pro	Lys	Asn	Leu	Lys	Gly	Val	Tyr	Ser	Arg	Asp	Arg	Leu	Gln	Met	Trp
								405	410			415			
Met	Thr	Asp	Gly	Asn	Arg	Met	Trp	His	Val	Gly	Gln	Val	Ser	Glu	Gly
								420	425			430			
Asp	Asp	Asn	Ser	Ala	Tyr	Ser	Ser	Leu	Leu	Tyr	Thr	Pro	Asp	Gly	Val
								435	440			445			
Leu	Tyr	Cys	Leu	His	Glu	Gln	Asn	Ile	Asp	Glu	Val	Tyr	Ser	Leu	His

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450	455	460
Leu Val Arg Leu Val Asp Glu Leu Lys Ser Ile Lys Ser Thr Ala Leu		
465	470	475
480		
Val Trp Lys Ala Gln Asp Glu Leu Leu Gly Asn Cys Leu Pro Gly		
485	490	495
Asp Lys Tyr Asp Pro Gly Cys Asp Gly Ile Pro Thr Ala Gly Leu Ala		
500	505	510
Gly Leu Leu Val Gly Pro Leu Thr Glu Lys Thr Trp Pro Asp Ala Tyr		
515	520	525
Arg Cys Val Asn Ala Ala Thr Ser Gly Ala Val Ser Thr Ala Glu Gly		
530	535	540
Val Arg Leu Asp Val Gly Gly Gly His Val Val Trp Pro Val Ser		
545	550	555
560		
Glu Gln Gly Gln Asp Gln Arg Tyr Tyr Phe Thr Asn Ser Glu Phe Thr		
565	570	575
Leu Ala Val Thr Val Arg Phe Asp Glu Met Pro Arg Gly Glu Leu Pro		
580	585	590
Leu Leu Gly Phe Val Asn Arg Lys Gly Lys Val Asn Lys Ile Leu Lys		
595	600	605
Val Ser Leu Ser Gly Val Glu Trp Leu Leu Ala Tyr Gly Asn Glu Tyr		
610	615	620
Asn Ser Thr Ala Ala Glu Pro Leu Ser Val Asn Glu Ser His Gln Val		
625	630	635
640		
Val Leu Thr Leu His Asp Gly Ile Val Ser Leu His Val Asp Gly Gly		
645	650	655
Asn Thr Thr Ala Thr Val Ser Val Arg Val Ala Ser Pro Glu Glu Leu		
660	665	670
Leu Asn Ile His His Leu Phe Val Gly Thr Pro Val Asp Gly Gly Ala		
675	680	685
Lys Glu His Val Asn Ile Thr Val Ser Asn Val Leu Val Tyr Asn Arg		
690	695	700
Pro Leu Arg Gly Val Glu Leu Leu Gly Leu Phe Ala Asn Arg Gly Arg		
705	710	715
720		
Ile Arg Val Pro Gly Ser Asp Asn Gly Val Leu Ser Gly Gly		
725	730	

<210> SEQ ID NO 16

<211> LENGTH: 734

<212> TYPE: PRT

<213> ORGANISM: Trypanosoma congolense

<400> SEQUENCE: 16

Met Trp Pro Val Asn Cys Tyr Ala Leu Leu Ala Leu Val Val Ala Gly		
1	5	10
		15
Gln Cys Cys Gly Pro Met His Ala Thr Ala Ala Val Gly Thr Thr His		
20	25	30
Gln Ala Leu Leu Trp Gly Ser Lys Trp Ala Leu Arg Asn Lys Thr Thr		
35	40	45
Pro Lys Asp Gly Glu Val Trp Trp Ser Asn Pro Gln Pro Gly Trp Lys		
50	55	60
Glu Val Tyr Asp Asp Glu Trp Glu Glu Trp Phe Met Glu Gln Glu Gly		
65	70	75
		80

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Pro Thr Gly Val Asn Gly Val Arg Gly Glu Trp Tyr Arg Arg Met Thr
 85 90 95

 Asp Gly Tyr Ile Leu Val Gly Gly Pro Lys Leu Asn Ser Pro Asp Met
 100 105 110

 Asn Ser Thr Gly Thr Thr Met Arg Thr Val His Ser Tyr Arg Ile Pro
 115 120 125

 Ser Ile Val Glu Val Gly Val Leu Met Cys Val Gly Asp Ala Arg
 130 135 140

 Tyr Ile Thr Ser Thr Asp Tyr Phe Thr Asp Thr Val Ala Ala Tyr
 145 150 155 160

 Ser Thr Asp Gly Gly Arg Thr Trp Lys Arg Glu Val Ile Ile Pro Asn
 165 170 175

 Gly Arg Val Asp Ala His Tyr Ser Arg Val Val Asp Pro Thr Val Val
 180 185 190

 Ala Lys Gly Asn Asn Ile Tyr Val Leu Val Gly Arg Tyr Asn Val Thr
 195 200 205

 Arg Gly Tyr Trp His Asn Arg Asn Asp Lys Ala Ala Ala Asp Trp
 210 215 220

 Glu Pro Phe Val Tyr Lys Gly Thr Val Asn Val Gly Thr Lys Gly Thr
 225 230 235 240

 Ala Thr Asp Val Ser Ile Ser Trp Glu Arg Thr Ala Leu Lys Ser Leu
 245 250 255

 Tyr Asn Phe Pro Val Ser Gly Ser Pro Gly Thr Gln Phe Leu Gly Gly
 260 265 270

 Ala Gly Gly Val Val Thr Ser Asn Gly Thr Ile Val Leu Pro Val
 275 280 285

 Gln Ala Arg Asn Lys Ala Asn Arg Val Val Ser Met Ile Leu Tyr Ser
 290 295 300

 Ala Asp Asp Gly Lys Ser Trp His Phe Gly Lys Gly Glu Ala Gly Val
 305 310 315 320

 Gly Thr Ser Glu Ala Ala Leu Thr Glu Trp Asp Gly Lys Leu Ile
 325 330 335

 Ser Thr Arg Ser Asp Gly Gly Gln Gly Tyr Arg Met Ile Phe Glu Ser
 340 345 350

 Ser Asp Leu Gly Ala Thr Trp Lys Glu Met Leu Asn Ser Ile Ser Arg
 355 360 365

 Val Ile Gly Asn Ser Pro Gly Arg Ser Gly Pro Gly Ser Ser Ser Gly
 370 375 380

 Phe Ile Thr Val Thr Val Glu Gly Val Pro Val Met Leu Leu Thr His
 385 390 395 400

 Pro Lys Asn Leu Lys Gly Val Tyr Ser Arg Asp Arg Leu Gln Met Trp
 405 410 415

 Met Thr Asp Gly Asn Arg Met Trp His Val Gly Gln Val Ser Glu Gly
 420 425 430

 Asp Asp Asn Ser Ala Tyr Ser Ser Leu Leu Tyr Thr Pro Asp Gly Val
 435 440 445

 Leu Tyr Cys Leu His Glu Gln Asn Ile Asp Glu Val Tyr Ser Leu His
 450 455 460

 Leu Val Arg Leu Val Asp Glu Leu Lys Ser Ile Lys Ser Thr Ala Leu
 465 470 475 480

 Val Trp Lys Ala Gln Asp Glu Leu Leu Gly Asn Cys Leu Pro Gly

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485	490	495
Asp Lys Tyr Asp Pro Gly Cys Asp Gly Ile Pro Thr Ala Gly Leu Ala		
500	505	510
Gly Leu Leu Val Gly Pro Leu Thr Glu Lys Thr Trp Pro Asp Ala Tyr		
515	520	525
Arg Cys Val Asn Ala Ala Thr Ser Gly Ala Val Ser Thr Ala Glu Gly		
530	535	540
Val Arg Leu Asp Val Gly Gly Gly His Val Val Trp Pro Val Ser		
545	550	555
Glu Gln Gly Gln Asp Gln Arg Tyr Tyr Phe Thr Asn Ser Glu Phe Thr		
565	570	575
Leu Ala Val Thr Val Arg Phe Asp Glu Met Pro Arg Gly Glu Leu Pro		
580	585	590
Leu Leu Gly Phe Val Asn Arg Lys Gly Lys Val Lys Lys Ile Leu Lys		
595	600	605
Val Ser Leu Ser Gly Val Glu Trp Leu Leu Ala Tyr Gly Asn Glu Tyr		
610	615	620
Asn Ser Thr Ala Ala Glu Pro Leu Asn Val Asn Glu Ser His Gln Val		
625	630	635
Val Leu Thr Leu His Asp Gly Ile Val Ser Leu His Val Asp Gly Gly		
645	650	655
Asn Met Thr Ala Thr Val Ser Val Arg Val Ala Ser Pro Ala Glu Leu		
660	665	670
Leu Asn Ile His His Leu Phe Val Gly Thr Pro Val Asp Gly Gly Ala		
675	680	685
Lys Glu His Ala Asn Ile Thr Val Ser Asn Val Leu Val Tyr Asn Arg		
690	695	700
Pro Leu Arg Gly Val Glu Leu Leu Gly Leu Phe Ala Asn Arg Gly Arg		
705	710	715
Ile Arg Val Pro Gly Ser Asp Asn Ser Val Leu Ser Gly Gly		
725	730	

<210> SEQ_ID NO 17

<211> LENGTH: 734

<212> TYPE: PRT

<213> ORGANISM: Trypanosoma congolense

<400> SEQUENCE: 17

Met Arg Pro Val Asn Cys Tyr Ala Leu Leu Ala Leu Val Val Ala Gly		
1	5	10
Gln Cys Cys Gly His Met His Ala Thr Ala Ala Val Gly Thr Thr His		
20	25	30
Gln Ala Leu Leu Trp Gly Ser Lys Trp Ala Leu Arg Asn Lys Thr Thr		
35	40	45
Pro Lys Asp Gly Glu Val Trp Trp Ser Asn Pro Gln Pro Gly Trp Lys		
50	55	60
Glu Val Tyr Asp Asp Glu Trp Glu Glu Trp Phe Met Glu Gln Glu Gly		
65	70	75
80		
Pro Thr Gly Val Asn Gly Val Arg Gly Glu Trp Tyr Arg Arg Met Lys		
85	90	95
Asp Gly Tyr Ile Leu Val Gly Gly Pro Lys Leu Asn Ser Pro Asp Met		
100	105	110

-continued

Asn Ser Thr Gly Thr Thr Met Arg Thr Val His Ser Tyr Arg Ile Pro
 115 120 125
 Ser Ile Val Glu Val Gly Gly Val Leu Met Cys Val Gly Asp Ala Arg
 130 135 140
 Tyr Ile Thr Ser Thr Asp Tyr Phe Phe Thr Asp Thr Val Ala Ala Tyr
 145 150 155 160
 Ser Thr Asp Gly Gly Lys Thr Trp Lys Thr Glu Val Ile Ile Pro Asn
 165 170 175
 Gly Arg Val Asp Ala His Tyr Ser Arg Val Val Asp Pro Thr Val Val
 180 185 190
 Ala Lys Gly Asn Asn Ile Tyr Val Leu Val Gly Arg Tyr Asn Val Thr
 195 200 205
 Arg Gly Tyr Trp His Asn Gln Asn Asp Glu Ala Ala Ile Ala Asp Trp
 210 215 220
 Glu Pro Phe Val Tyr Lys Gly Thr Val Asn Val Gly Thr Lys Gly Thr
 225 230 235 240
 Ala Thr Asp Val Ser Ile Ser Trp Glu Arg Thr Ala Leu Lys Ser Leu
 245 250 255
 Tyr Asn Phe Pro Val Ser Gly Ser Pro Gly Thr Gln Phe Leu Gly Gly
 260 265 270
 Ala Gly Gly Val Val Thr Ser Asn Gly Thr Ile Val Leu Pro Val
 275 280 285
 Gln Ala Arg Asn Lys Ala Asn Arg Val Val Ser Met Ile Leu Tyr Ser
 290 295 300
 Ala Asp Asp Gly Lys Ser Trp His Phe Gly Glu Gly Ala Gly Val
 305 310 315 320
 Gly Thr Ser Glu Ala Ala Leu Thr Glu Trp Asp Gly Lys Leu Leu Ile
 325 330 335
 Ser Thr Arg Ser Asp Gly Gly Gln Gly Tyr Arg Met Ile Phe Glu Ser
 340 345 350
 Ser Asp Leu Gly Ala Thr Trp Lys Glu Met Leu Asn Ser Ile Ser Arg
 355 360 365
 Val Ile Gly Asn Ser Pro Lys Arg Asn Gly Pro Gly Ser Ser Ser Gly
 370 375 380
 Phe Ile Thr Val Thr Val Glu Gly Val Pro Val Met Leu Leu Thr His
 385 390 395 400
 Pro Lys Asn Leu Lys Gly Ser Tyr Tyr Arg Asp Arg Leu Gln Met Trp
 405 410 415
 Met Thr Asp Gly Asn Arg Met Trp His Val Gly Gln Val Ser Glu Gly
 420 425 430
 Asp Asp Asn Ser Ala Tyr Ser Ser Leu Leu Tyr Thr Pro Asp Gly Val
 435 440 445
 Leu Tyr Cys Leu His Glu Gln Asn Ile Asp Glu Val Tyr Ser Leu His
 450 455 460
 Leu Val Arg Leu Val Asp Glu Leu Lys Ser Ile Lys Ser Thr Ala Leu
 465 470 475 480
 Val Trp Lys Ala Gln Asp Glu Leu Leu Gly Asn Cys Leu Pro Gly
 485 490 495
 Asp Lys Tyr Asp Pro Gly Cys Asp Gly Ile Pro Thr Ala Gly Leu Ala
 500 505 510
 Gly Leu Leu Val Gly Pro Leu Thr Glu Lys Thr Trp Pro Asp Ala Tyr

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515	520	525
Arg Cys Val Asn Ala Ala Thr Ser Gly Ala Val Ser Thr Ala Glu Gly		
530	535	540
Val Arg Leu Asp Val Gly Gly Gly His Val Val Trp Pro Val Ser		
545	550	555
560		
Glu Gln Gly Gln Asp Gln Arg Tyr Tyr Phe Thr Asn Ser Glu Phe Thr		
565	570	575
Leu Ala Val Thr Val Arg Phe Asp Glu Met Pro Arg Gly Glu Leu Pro		
580	585	590
Leu Leu Gly Phe Val Asn His Lys Gly Lys Val Asn Lys Ile Leu Lys		
595	600	605
Val Ser Leu Ser Gly Val Glu Trp Leu Ala Tyr Gly Asn Glu Tyr		
610	615	620
Asn Ser Thr Ala Ala Glu Pro Leu Ser Val Asn Glu Ser His Gln Val		
625	630	635
640		
Val Leu Thr Leu His Asp Gly Ile Val Ser Leu His Val Asp Gly Gly		
645	650	655
Asn Thr Thr Ala Thr Val Ser Val Arg Val Ala Ser Pro Glu Glu Leu		
660	665	670
Leu Asn Ile His His Leu Phe Val Gly Thr Pro Val Asp Gly Gly Ala		
675	680	685
Lys Glu His Ala Asn Ile Thr Val Ser Asn Val Leu Val Tyr Asn Arg		
690	695	700
Pro Leu Arg Gly Val Glu Leu Leu Gly Leu Phe Ala Asn Arg Gly Arg		
705	710	715
720		
Ile Arg Val Pro Gly Ser Asp Asn Gly Val Leu Ser Gly Gly		
725	730	

<210> SEQ ID NO 18

<211> LENGTH: 778

<212> TYPE: PRT

<213> ORGANISM: Trypanosoma congolense

<400> SEQUENCE: 18

1	5	10	15
Met Ser His Cys Phe Val Pro Ala Trp Ser Lys Ala Leu Cys Leu Leu			
Leu Leu Ser His Cys Leu Tyr Leu Ala His Ala Ser Gly Asn Gly Arg			
20	25	30	
Thr Thr Arg Glu Leu Phe Leu Gly Gly His Trp Val Val Gly Lys			
35	40	45	
Glu Cys Leu Ala Val Asn Glu Glu Gly Ser Ala Ala Arg Thr Leu Glu			
50	55	60	
Cys Asn Gly Asn Cys Ser Pro Asp Glu Asp Ser Gln Arg Arg Ser Ala			
65	70	75	80
Asp Asp Asn Asp Gly Leu Gln Glu Glu Thr Ile Asn Cys Val Leu Glu			
85	90	95	
Pro Arg Ser Lys Gln Leu Gly Val Ala Lys Asp Met Glu Gly Lys His			
100	105	110	
Val Val Asp Ser Phe Arg Ile Pro Ser Ile Val Glu Val Asp Gly Val			
115	120	125	
Leu Ile Thr Val Ser Asp Val Arg Tyr Leu Asn Ser Asn Asp Leu Ser			
130	135	140	

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Phe	Ile	Asp	Thr	Val	Ala	Arg	Tyr	Ser	Ala	Asp	Gly	Gly	Arg	Thr	Trp
145				150		155									160
Glu	Thr	Glu	Val	Ile	Ile	Lys	Asn	Ala	Arg	Val	Asn	Ala	Glu	His	Ser
				165			170								175
Arg	Val	Val	Asp	Pro	Thr	Val	Val	Val	Lys	Asn	Asn	Thr	Ile	Phe	Val
				180		185									190
Leu	Val	Gly	Arg	Tyr	Asn	Lys	Ser	Asp	Ala	Tyr	Trp	Thr	Trp	Gln	Gly
				195		200									205
Gly	Gly	Gly	Asp	Trp	Asp	Ile	Leu	Met	His	Lys	Gly	Thr	Val	Thr	Lys
				210		215									220
Ser	Leu	Arg	Gly	Gly	Lys	Pro	Ser	Val	Asn	Ile	Glu	Trp	Asp	Glu	Pro
				225		230									240
Gln	Asn	Leu	Lys	Tyr	Leu	Leu	Ser	Thr	Val	Gly	Lys	Ile	Asp	Gly	Arg
				245			250								255
Ser	Leu	Ile	Gln	Tyr	Ile	Gly	Gly	Val	Gly	Asn	Cys	Val	Val	Thr	Pro
				260		265									270
Asn	Gly	Thr	Ile	Val	Leu	Pro	Val	Gln	Val	Leu	Asn	Thr	Asn	Arg	Ser
				275		280									285
Val	Met	Ala	Met	Ile	Ile	Tyr	Ser	Thr	Asp	Glu	Gly	Glu	Ser	Trp	Gln
				290		295									300
Phe	Ser	Lys	Ser	Ala	Thr	Pro	Val	Gly	Thr	Thr	Glu	Ser	Ser	Ile	Val
				305		310									320
Trp	Trp	Asp	Asp	Lys	Leu	Leu	Asn	Gly	Arg	Thr	Asn	Asn	Asp	Leu	
				325			330								335
Gly	Tyr	Arg	Lys	Val	Tyr	Glu	Ser	Ser	Asp	Leu	Gly	Thr	Thr	Trp	Thr
				340			345								350
Glu	Val	Val	Gly	Thr	Ile	Ser	Arg	Val	Ile	Gly	Asn	Ser	Pro	Gly	Arg
				355		360									365
Asn	Gln	Pro	Gly	Ser	Ser	Gly	Ser	Ser	Ile	Ala	Ile	Thr	Leu	Glu	Gly
				370		375									380
Met	Arg	Val	Met	Leu	Ile	Thr	Gln	Pro	Lys	Asn	Ile	Lys	Gly	Ser	Trp
				385		390									400
His	Arg	Asp	Arg	Leu	Gln	Leu	Trp	Leu	Thr	Asp	Gly	Asn	Arg	Val	Trp
				405			410								415
Leu	Val	Gly	Gln	Ile	Ser	Glu	Gly	Asp	Asp	Asn	Gly	Pro	Tyr	Ser	Ser
				420			425								430
Leu	Leu	Tyr	Thr	Ser	Asn	Gly	Thr	Leu	Tyr	Cys	Leu	Tyr	Glu	Gln	Asp
				435			440								445
Lys	Ala	Ala	Val	Leu	Ser	Ile	Tyr	Leu	Ile	Lys	Leu	Glu	Asp	Glu	Leu
				450		455									460
Glu	Ser	Ile	Lys	Ser	Ile	Val	Lys	Leu	Trp	Lys	Asp	Gln	Asp	Ala	Leu
				465		470									480
Leu	Ser	Gly	Asn	Cys	Ser	Ser	Pro	Asp	Gly	Asp	Tyr	Thr	Glu	Gly	Cys
				485			490								495
Val	Gly	Ile	Pro	Thr	Ala	Gly	Leu	Val	Gly	Leu	Leu	Ser	Gly	Pro	Ser
				500			505								510
Asp	Glu	Asp	Val	Trp	His	Asp	Ala	Tyr	Arg	Cys	Val	Asp	Ala	Ser	Val
				515			520								525
Glu	Asn	Val	Val	Asn	Val	Ala	Asp	Gly	Leu	Gln	Leu	Ser	Gly	Trp	Asn
				530		535									540
Ser	Ser	Arg	Val	Leu	Trp	Pro	Val	Ser	Ser	Gln	Gly	Gln	Asp	Gln	Lys

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545	550	555	560
Tyr His Phe Ala Asp Val His Phe Thr Leu Val Ala Asn Leu Arg Leu			
565	570	575	
Val Gly Ala Pro Lys Gly Asp Phe Ser Leu Val Gly Phe Glu Met Tyr			
580	585	590	
Glu Gly Glu Thr Arg Lys Thr Val Lys Leu Ser Ala Ile Lys Ser Ala			
595	600	605	
Leu Trp Glu Met Cys His Thr Asp Leu Thr Thr Arg Gly Ser Arg Gly			
610	615	620	
Ser Leu Pro Cys Asp Glu Val His Gln Val Ala Leu Thr Leu Arg Asn			
625	630	635	640
Gly Val Ile Ser Val Tyr Ala Asn Gly Arg His Leu Ser Val Leu Asp			
645	650	655	
Thr Lys Val Ala Gly Ala Asn Glu Leu Leu Asn Ile Ser Asn Phe Phe			
660	665	670	
Val Gly His Pro Gly Val Gly Gly Ala Leu Pro Trp Gly Ser Ala Val			
675	680	685	
Val Arg Asp Val Leu Leu Tyr Asn Arg Pro Leu His Glu Thr Glu Leu			
690	695	700	
Glu Ser Leu Tyr Leu Asn Gly Asp Val Ile Lys Val Val Asn His Gly			
705	710	715	720
Ala Ala Gly Ile Ser Ala Ala Arg Asp Ala Glu Leu Leu His Val Arg			
725	730	735	
Gly Asp Gly Gly Asp Lys Pro Asp Ala Val Pro Leu Lys Leu Ala Ile			
740	745	750	
Ile Thr Gly Asp Gly Val Val Arg Phe Arg Gly Leu Tyr Gln Met Ala			
755	760	765	
Ser Leu Val Leu Leu Gly Leu Met Leu Ser			
770	775		

<210> SEQ ID NO 19
<211> LENGTH: 778
<212> TYPE: PRT
<213> ORGANISM: Trypanosoma congolense

<400> SEQUENCE: 19

Met Ser His Cys Phe Val Pro Val Trp Ser Lys Ala Leu Cys Leu Leu			
1	5	10	15
Leu Leu Ser His Cys Leu Tyr Leu Ala His Ala Ser Gly Asn Gly Arg			
20	25	30	
Thr Thr Arg Glu Leu Phe Leu Gly Gly His Trp Val Val Gly Lys			
35	40	45	
Glu Cys Leu Ala Val Asn Lys Glu Gly Ser Val Ala Gln Thr Leu Glu			
50	55	60	
Cys Asn Gly Asn Cys Ser Pro Asp Glu Asp Ser Arg Ile Arg Ser Ala			
65	70	75	80
Asp Asp Asn Asp Gly Leu Gln Glu Glu Thr Ile Asn Cys Ala Leu Glu			
85	90	95	
Pro Arg Ser Glu Gln Leu Gly Val Ala Lys Asp Met Glu Gly Lys His			
100	105	110	
Ile Val Asp Ser Phe Arg Ile Pro Ser Ile Val Glu Val Asp Gly Val			
115	120	125	

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Leu Ile Thr Val Ser Asp Val Arg Tyr Leu Asn Ser Asn Asp Leu Ser
 130 135 140

Phe Ile Asp Thr Val Ala Arg Tyr Ser Ala Asp Gly Gly Arg Thr Trp
 145 150 155 160

Glu Thr Glu Val Ile Ile Lys Asn Ala Arg Val Asn Ala Glu His Ser
 165 170 175

Arg Val Val Asp Pro Thr Val Val Lys Asn Asn Thr Ile Phe Val
 180 185 190

Leu Val Gly Arg Tyr Asn Lys Ser Asp Ala Tyr Trp Thr Trp Gln Gly
 195 200 205

Gly Gly Asp Trp Asp Ile Leu Met His Lys Gly Thr Val Thr Lys
 210 215 220

Ser Leu Arg Gly Gly Lys Pro Ser Val Asn Ile Glu Trp Asp Glu Pro
 225 230 235 240

Gln Asn Leu Lys Tyr Leu Leu Ser Thr Val Gly Lys Ile Asp Gly Arg
 245 250 255

Ser Leu Ile Gln Tyr Ile Gly Gly Val Gly Asn Cys Val Val Thr Pro
 260 265 270

Asn Gly Thr Ile Val Leu Pro Val Gln Val Leu Asn Thr Asn Arg Ser
 275 280 285

Val Met Ala Met Ile Ile Tyr Ser Thr Asp Glu Gly Glu Ser Trp Gln
 290 295 300

Phe Ser Lys Ser Ala Thr Pro Val Gly Thr Thr Glu Ser Ser Ile Val
 305 310 315 320

Trp Trp Asp Asp Lys Leu Leu Asn Gly Arg Thr Asn Asn Asp Leu
 325 330 335

Gly Tyr Arg Lys Val Tyr Glu Ser Ser Asp Leu Gly Thr Thr Trp Lys
 340 345 350

Glu Val Val Gly Thr Ile Ser Arg Val Ile Gly Asn Ser Pro Gly Arg
 355 360 365

Asn Gln Pro Gly Ser Ser Gly Ser Ser Ile Ala Ile Thr Leu Glu Gly
 370 375 380

Met Arg Val Met Leu Ile Thr Gln Pro Lys Asn Ile Lys Gly Ser Trp
 385 390 395 400

His Arg Asp Arg Leu Gln Leu Trp Leu Thr Asp Gly Asn Arg Val Trp
 405 410 415

Leu Val Gly Gln Ile Ser Glu Gly Asp Asp Asp Gly Pro Tyr Ser Ser
 420 425 430

Leu Leu Tyr Thr Ser Asn Gly Thr Leu Tyr Cys Leu Tyr Glu Gln Asp
 435 440 445

Lys Ser Ala Val Leu Ser Ile Tyr Leu Ile Lys Leu Glu Asp Glu Leu
 450 455 460

Glu Ser Ile Lys Ser Ile Val Lys Leu Trp Lys Asp Gln Asp Ala Leu
 465 470 475 480

Leu Ser Gly Asn Cys Ser Ser Pro Asp Gly Asp Tyr Thr Glu Gly Cys
 485 490 495

Val Gly Ile Pro Thr Ala Gly Leu Val Gly Leu Leu Ser Gly Pro Ser
 500 505 510

Asp Gly Asp Val Trp His Asp Ala Tyr Arg Cys Val Asp Ala Ser Val
 515 520 525

Asp Asn Val Val Asn Val Ala Asp Gly Leu Gln Leu Ser Gly Trp Asn

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530	535	540
Ser Ser Arg Val Leu Trp Pro Val Ser Ser Gln Gly Gln Asp Gln Lys		
545	550	555
Tyr His Phe Ala Asn Val His Phe Thr Leu Val Ala Asn Leu Arg Leu		
565	570	575
Val Gly Ala Pro Lys Gly Asp Phe Ser Leu Val Gly Phe Glu Thr Tyr		
580	585	590
Glu Gly Glu Arg Arg Lys Thr Val Lys Leu Ser Ala Ile Lys Ser Ala		
595	600	605
Phe Trp Glu Met Cys His Thr Asp Leu Thr Thr Arg Gly Ser Arg Gly		
610	615	620
Ser Pro Pro Cys Asp Glu Val His Gln Val Ala Leu Thr Leu Arg Asp		
625	630	635
Gly Val Ile Ser Val Tyr Ala Asn Gly Arg His Leu Ser Val Leu Asp		
645	650	655
Thr Lys Ile Ala Gly Ala Asn Glu Leu Leu Asn Ile Thr Asn Phe Phe		
660	665	670
Val Gly His Pro Gly Val Gly Asp Ala Leu Pro Trp Gly Ser Ala Val		
675	680	685
Val Arg Asp Val Leu Leu Tyr Asn Arg Pro Leu His Glu Thr Glu Leu		
690	695	700
Glu Ser Leu Tyr Leu Asn Gly Asp Val Ile Lys Val Val Asn His Gly		
705	710	715
720		
Ala Ala Gly Ile Ser Ala Ala Arg Asp Ala Glu Leu Leu His Val Arg		
725	730	735
Glu Asp Gly Gly Asp Lys Pro Asn Ala Val Pro Leu Lys Leu Ala Ile		
740	745	750
Ile Thr Asp Asp Gly Val Ala Arg Phe Arg Gly Leu Tyr Gln Met Ala		
755	760	765
Ser Leu Met Leu Leu Gly Leu Met Leu Ser		
770	775	

<210> SEQ_ID NO 20

<211> LENGTH: 738

<212> TYPE: PRT

<213> ORGANISM: Trypanosoma congolense

<400> SEQUENCE: 20

Met Pro Ser Tyr Leu Leu Pro Ala Ala Val Val Leu Tyr Cys Phe Gly		
1	5	10
15		
Gly Leu Val Pro Ala Ala Gln Cys Ile Ser Thr Thr Arg Glu Ala Phe		
20	25	30
Met Glu Ala Gly Gln Trp Ser Val Asn Lys Asp Cys Leu Ile Thr Ala		
35	40	45
Glu Gly Ser Arg Arg Ser Lys Ala Ser Gly Ser Tyr Glu Lys Gly Tyr		
50	55	60
Ala Ser Val Glu Ser Thr Thr Lys Asp Val Asn Thr Arg Gly Gly Val		
65	70	75
80		
Gln Thr Ser Glu Ala Cys Thr Leu Glu Pro Glu Val Arg Asp Asn Ser		
85	90	95
Thr Ser Gly Asp Gly Lys Glu Arg His Leu Ile His Ser Phe Arg Ile		
100	105	110

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Pro	Ser	Leu	Val	Glu	Ile	Asp	Gly	Val	Leu	Ile	Ala	Thr	Phe	Asp	Thr
115								120							125
Arg	Tyr	Leu	His	Ala	Ser	Asp	Ser	Ser	Leu	Ile	Asp	Thr	Ala	Met	Lys
130								135							140
Tyr	Ser	Ala	Asp	Gln	Gly	Lys	Thr	Trp	Lys	Thr	Glu	Ile	Ile	Ile	Lys
145								150							160
Asn	Ala	Arg	Leu	Thr	Asp	Asn	Phe	Ser	Arg	Val	Val	Asp	Pro	Thr	Val
								165							175
Val	Val	Lys	Gly	Asp	Asn	Leu	Phe	Ile	Phe	Val	Gly	Arg	Tyr	Asn	Thr
								180							190
Ser	Ser	Thr	Pro	Trp	Val	Trp	Gln	Lys	Asn	Gly	Lys	Asp	Trp	Asp	Val
								195							205
Leu	Leu	Tyr	Lys	Ala	Lys	Val	Arg	Lys	Glu	Ser	Ala	Gly	Gly	Val	Pro
								210							220
Ser	Val	Ser	Phe	Thr	Trp	Asp	Glu	Pro	Leu	His	Leu	Lys	His	Leu	Leu
								225							240
Thr	Ser	Val	Gly	Lys	Ile	Asp	Gly	Arg	Ser	Leu	Ile	Gln	Tyr	Ile	Gly
								245							255
Gly	Val	Gly	Asn	Gly	Ile	Val	Thr	Pro	Lys	Gly	Thr	Ile	Val	Phe	Pro
								260							270
Val	Gln	Val	Leu	Asn	Thr	Asn	Lys	Ser	Val	Met	Asn	Met	Leu	Leu	Tyr
								275							285
Ser	Ser	Asn	Asp	Gly	Lys	Thr	Trp	Glu	Phe	Ser	Lys	Thr	Ser	Thr	Pro
								290							300
Ala	Gly	Thr	Thr	Glu	Ala	Ser	Leu	Val	Trp	Trp	Asp	Gly	Gln	Leu	Leu
								305							320
Leu	Thr	Ser	Arg	Thr	Thr	Pro	Asp	Val	Gly	Ser	Arg	Lys	Val	Tyr	Leu
								325							335
Thr	Ser	Asp	Leu	Gly	Thr	Ser	Trp	Asn	Glu	Ala	Ile	Gly	Ser	Ile	Ser
								340							350
Arg	Val	Ile	Gly	Asn	Ser	Arg	Tyr	Arg	Asn	Asp	Pro	Gly	Gly	Ser	Gly
								355							365
Ser	Ser	Ile	Ala	Ile	Thr	Val	Glu	Gly	Val	Pro	Val	Met	Leu	Val	Thr
								370							380
His	Pro	Glu	Asn	Ala	Lys	Gly	Arg	Trp	Asn	Arg	Asp	Arg	Met	Arg	Leu
								385							400
Trp	Leu	Thr	Asp	Gly	Asn	Arg	Met	Trp	Leu	Val	Gly	Gln	Ile	Ser	Glu
								405							415
Gly	Asp	Asp	Asn	Ser	Ala	Tyr	Ser	Tyr	Leu	Leu	Tyr	Thr	Lys	Asn	Gly
								420							430
Thr	Leu	Leu	Cys	Leu	Tyr	Glu	Arg	Asn	Ile	Arg	Glu	Ile	Tyr	Ser	Ile
								435							445
Tyr	Leu	Ala	Arg	Leu	Glu	Asp	Glu	Met	Glu	Asp	Ile	Lys	Ser	Thr	Val
								450							460
Arg	Leu	Trp	Lys	Ala	His	Asp	Glu	Leu	Leu	Ser	Gly	Asp	Cys	Gln	Leu
								465							480
Asn	Lys	Lys	Arg	Arg	Ser	Gly	Cys	Thr	Gly	Ile	Pro	Ile	Thr	Gly	Leu
								485							495
Val	Gly	Leu	Leu	Ala	Gly	Leu	Pro	Arg	Lys	Ser	Val	Trp	Pro	Asp	Ala
								500							510
Tyr	Asn	Cys	Val	Asp	Ala	Ser	Ile	Ser	Lys	Asn	Asn	Lys	Gln	Val	Ser

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515	520	525
His Asp Pro Pro Ser Arg Ser Thr Met Lys Arg Arg Val Val Trp Pro		
530	535	540
Val Gly Asp Gln Gly Gln Asp Gln Arg Tyr His Phe Val Asn Thr His		
545	550	555
560		
Phe Thr Phe Val Ala Thr Ile Tyr Phe Asp Arg Ala Pro Gln Glu Val		
565	570	575
Ser Leu Met Gly Phe Glu Asn Asn Glu Glu Ser Thr Lys Thr Leu Thr		
580	585	590
Val Ser Ile Gly Asn Gly Arg Leu Val Leu Thr Tyr Gly Gly Leu Leu		
595	600	605
Glu Glu Ile Pro Met Thr Arg Leu Asp Trp Ser Val Thr His Gln Val		
610	615	620
Ala Leu Thr Leu His Asn Gly Glu Val Ser Leu His Val Asp Gly Asn		
625	630	635
640		
Pro Ser Ile Ala Asn Val Arg Leu Lys Leu His Glu Pro Asp Arg Leu		
645	650	655
Leu Asn Ile Ser Asn Leu Phe Thr Ser Thr Pro Ala Pro Val Lys Thr		
660	665	670
Gly Lys Gly Ser Thr Val Thr Val Asn Asn Val Ile Leu Tyr Asn Arg		
675	680	685
Met Leu Asn Glu Thr Glu Leu Ala Arg Leu Phe Asn Ser Arg Asp Leu		
690	695	700
Ile Asp Glu Val Gly Asp Val His Pro Val Ser Gly Gly Gly Val Gly		
705	710	715
720		
Glu Trp Arg Phe His Val Trp Ile Leu Leu Ala Ala Tyr Val Leu Val		
725	730	735

Ala Tyr

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<210> SEQ ID NO 21
<211> LENGTH: 787
<212> TYPE: PRT
<213> ORGANISM: Trypanosoma congolense

<400> SEQUENCE: 21

Met Gly Tyr Ser Lys Ser Val Arg Gln Thr Leu Ile Cys Leu Leu Leu
1 5 10 15

Val Ala Ile Asp Thr Tyr His Cys Thr Thr Ala Tyr Gly Ser Gly Ile
20 25 30

Arg Gly Glu Glu Glu Lys Asn Arg Ser Leu Phe Leu Pro Gly Gly Leu
35 40 45

Trp Tyr Lys Lys Asp Glu Trp Lys Asp Gly Asn Trp Leu Gln Ser Lys
50 55 60

Glu Trp Lys Ala Gly Tyr Ala Trp Trp Pro Trp Arg Ser Trp Cys Ser
65 70 75 80

Asn Lys Thr Ile Gly Ala Ser Gly Lys Glu Leu Cys Arg Lys Glu Trp
85 90 95

Asp Ser Gln Arg Glu Lys Gly Tyr Thr Leu Lys Pro Arg Glu Ser Val
100 105 110

Leu Phe Arg Glu Ser Ser Gly Thr Lys Arg Met Arg Arg Val His Ser
115 120 125

Phe Arg Ile Pro Ser Met Val Glu Ala Asn Gly Val Leu Ile Gly Ile

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130	135	140
Ala Asp Ala Arg Tyr Leu Ser Ser Ala Asp Phe Thr Phe Ile Asp Thr		
145	150	155
160		
Val Ala Lys Tyr Ser Ala Asp Gly Gly Glu Thr Trp Lys Thr Glu Val		
165	170	175
Ile Ile Glu Asn Ala Arg Val Asp Ser Phe His Ser Arg Val Val Asp		
180	185	190
Pro Thr Val Ala Val Lys Asn Asn Ser Ile Tyr Val Leu Val Gly Arg		
195	200	205
Tyr Asn Thr Ser Asn Thr Tyr Trp Thr Met Gln Asn Asn Gly Asn Asp		
210	215	220
Trp Asp Ile Leu Met Tyr Lys Gly Thr Val Thr Lys Thr Ser Glu Asp		
225	230	235
240		
Gly Lys Pro Ala Ala Asn Ile Glu Trp Thr Gly Thr Gln Asn Leu Lys		
245	250	255
Tyr Leu Leu Lys Leu Val Asp Gln Ile Glu Gly Lys Ser Leu Thr Gln		
260	265	270
Phe Leu Gly Gly Val Gly Asn Thr Val Val Thr Pro Asp Gly Thr Ile		
275	280	285
Val Phe Pro Ile Gln Val Lys Asn Ser Trp Asn Gln Val Ala Ala Met		
290	295	300
Ile Met Tyr Ser Ser Asp Asp Gly Ala Thr Trp His Leu Gly Gly Gly		
305	310	315
320		
Ala Thr Pro Val Gly Thr Thr Glu Ala Ser Ala Ile Trp Trp Asp Gly		
325	330	335
Lys Leu Val Leu Asn Cys Arg Thr Asp Leu Gly Tyr Arg Lys Val Phe		
340	345	350
Glu Thr Thr Asp Leu Gly Thr Thr Trp Lys Glu Ser Leu Gly Ala Leu		
355	360	365
Ser Arg Val Ile Gly Asn Ser Pro Asp Arg Lys Gln Lys Gly Ser Ser		
370	375	380
Gly Ser Ala Ile Thr Leu Glu Val Glu Gly Val Gln Val Met Leu Ile		
385	390	395
400		
Thr Gln Pro Lys Asn Thr Lys Gly Asp Tyr Asn Arg Asp Arg Leu Gln		
405	410	415
Leu Trp Leu Ser Asp Gly Ser Arg Val Trp Leu Val Gly Gln Ile Ser		
420	425	430
Arg Gly Asp Asp Asn Ser Pro Tyr Ser Ser Leu Leu Tyr Thr Ser Asp		
435	440	445
Asp Lys Leu Tyr Cys Leu Tyr Glu Gln Asp Ile Arg Gly Val Leu Ser		
450	455	460
Ile Tyr Leu Val His Leu Val Asp Glu Leu Glu Lys Ile Lys Ala Thr		
465	470	475
480		
Val Arg Leu Trp Lys Glu Gln Asp Ala Leu Leu Ser Gly Asn Cys Ser		
485	490	495
Ala Thr Ala Glu Asp Gly Ser Asp Cys Asn Gly Val Pro Thr Ala Gly		
500	505	510
Leu Val Gly Leu Leu Ser Gly Pro Ala Gln Gly Asn Ala Trp Pro Asp		
515	520	525
Ala Tyr Asn Cys Val Asn Ala Ser Leu Val Asn Val Thr Ser Asp Ala		
530	535	540

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Asp Gly Leu Gln Leu Gly Gly Leu Asn Arg Gly Arg Val Ser Trp Pro
545           550           555           560

Val Arg Ala Gln Gln Asp Gln Arg Tyr Tyr Phe Ala Asn Val Arg
565           570           575

Phe Thr Leu Val Ala Thr Val Arg Leu Asn Gly Ile Ser Asn Leu Glu
580           585           590

Ile Pro Leu Met Gly Phe Glu Asn Phe Gln Lys Ser Thr Arg Asp Thr
595           600           605

Leu Ile Val Ser Ile Val Asp Asp Ala Tyr Trp Ser Lys Cys Lys Glu
610           615           620

Gly Pro Val Pro Gly Val Asn Val Asp Ala Pro Glu Cys Gln Lys Phe
625           630           635           640

His Gln Val Ala Ile Met Phe Gln Asn Gly Arg Val Ser Val Tyr Ala
645           650           655

Asp Gly Ile His Ile Pro Gln Leu Asp Thr Ser Ile Val Asp Ala Ser
660           665           670

Ala Leu Leu Asn Ile Ser Ser Phe Phe Leu Gly His Pro Glu Val Gly
675           680           685

Ser Arg Phe Thr Ser Ala Asp Val Ile Val Lys Asn Val Leu Leu Tyr
690           695           700

Asn Arg Pro Leu Thr Glu Gly Glu Ser Lys Ile Leu Tyr Ala Asn Glu
705           710           715           720

Gly Val Ile Lys Pro Val Gly Ile Val Lys Gly Val Ser Leu Ala Thr
725           730           735

Lys Thr Pro Ala Ser Met Arg Val Asp Thr Ala Asn Lys Arg Gly Tyr
740           745           750

Thr Asn Phe Pro Leu Lys Leu Thr Ile Ile His Ser Asn Gly Glu Ala
755           760           765

Pro Ile Arg Gln Leu Ser Arg Leu Val Ile Ile Val Leu Leu Ile Ser
770           775           780

Ala Leu Phe
785

<210> SEQ ID NO 22
<211> LENGTH: 771
<212> TYPE: PRT
<213> ORGANISM: Trypanosoma congolense

<400> SEQUENCE: 22

Met Cys Glu Arg Lys Ser Val Phe Ala Phe Pro Glu Val Val Lys Asn
1           5           10           15

Gly Arg Ser Val Ser Gly Gly His Gln Gly Thr Trp Cys Thr Pro Leu
20          25           30

Leu Leu Ile Ser Ala Ile Phe Leu Pro Leu Thr Cys Cys Ser Glu Ser
35          40           45

Thr Asp Ser Thr Trp Leu Glu Lys Arg Arg Val Glu Leu Phe Arg Pro
50          55           60

Trp Gly Lys Gly Asn Pro Asn Val Pro Gly Ala Ser Tyr Ser Ser Asp
65          70           75           80

Gly Arg Gly Val Phe Glu Gly Asn Ser Leu Leu Glu Val Asn Asp Gln
85          90           95

Ile Val Thr Leu Ala Gly Ala Arg Tyr Asn Ser Trp Val Asp Gly Tyr

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100	105	110
Ala Gly Met Trp Met Lys Thr Ile Arg Leu Ser Glu Gly His Gln Gly		
115	120	125
Pro Gly Ala Ala Asp Trp Met Gln Glu Lys Asn Trp Lys Gly Glu Ala		
130	135	140
Val Ile Val Asn Glu Lys Val Glu Ser His Arg Tyr Ala Leu Met Gly		
145	150	155
160		
Pro Arg Ala Ala Val Val Gly Asp Lys Ile Phe Phe Leu Ser Ile Thr		
165	170	175
Ser Asn Lys Ser Lys Asp Ala Leu Ser Ser Pro Ser Asp Glu Ser Asn		
180	185	190
Leu Asp Val Arg Leu Tyr Ile Gly Thr Val Asp Lys Ser Phe Val Gly		
195	200	205
Asp Ala Ser Val His Trp Asn Gly Pro Arg Ser Leu Leu Val Thr Phe		
210	215	220
Met Lys Glu Leu Lys Lys Asn Ser Trp Lys Asp Phe Val Glu Gly Ser		
225	230	235
240		
Gly Lys Ser Val Val Met Gly Asp Thr Ile Phe Phe Pro Leu Val Ala		
245	250	255
Leu Thr His Lys Gln Ser Arg Ser Cys Val Ile Ala Arg Tyr Arg His		
260	265	270
Asn Asp Glu Asn Trp Thr Phe Thr Arg Val Ala Leu Asp Ile Asp Asp		
275	280	285
Cys Thr Asn Pro Thr Leu Leu Trp Lys Asn Glu Leu Met Ile Val		
290	295	300
Val Ala His Asn Leu Lys Asn Lys Val Tyr Arg Ser Val Asp Met Gly		
305	310	315
320		
Leu Thr Trp Thr Asp Ala Ser Lys Thr Arg Arg Tyr Ala Leu Thr Asn		
325	330	335
Phe Gln His His Ala Asp Asp Val Asp Arg Gly Asp Ile Leu Ser Val		
340	345	350
Arg Val Gly Glu Thr Asp Leu Leu Leu Phe Ala Tyr Arg Met Phe Phe		
355	360	365
Ser Ser Ala Thr Ala Gly Asn Arg Pro Leu Leu Leu Trp Met Thr Asp		
370	375	380
Asn Lys Arg Thr His Cys Leu Gly Pro Ile Ser Thr Gly His Leu Phe		
385	390	395
400		
Thr Gly Ala Phe Gly Ala Leu Leu Tyr Thr Arg Glu Lys Leu Tyr Ser		
405	410	415
Leu His Gln Glu Ser Phe Ser Ser Leu Ser Ser Leu Phe Phe Thr Asn		
420	425	430
Leu Thr Gly Arg Leu Arg Thr Met Arg Pro Val Leu Asp Thr Trp Lys		
435	440	445
Thr Ala Asp Lys Arg Val Met Gly Leu Tyr Gly Pro Ser Ala Ala Gly		
450	455	460
Thr Thr Asn Phe Lys Ser Ala Glu Pro Ser Ser Phe Asp Pro Thr Thr		
465	470	475
480		
Gly Leu Val Gly Phe Trp Ser Thr Ala Ser Asn Ala Thr His Trp Gln		
485	490	495
Asp Glu Tyr Leu Gly Met Asp Gly Val Leu His Gly Pro Leu Lys Arg		
500	505	510

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Val Thr Thr Gly Tyr Thr Met Glu Gly Cys Ala Ala His Val Val Trp
515 520 525

Pro Val Gly Gly Glu Ser Glu Asn Lys Val Tyr His Leu Ile Ser Asn
530 535 540

Gly Leu Thr Val Val Met Ser Val Ala Val His Thr Ala Pro Lys Val
545 550 555 560

Arg Ile Pro Leu Leu Gly Val Thr Val Arg Asn Gly Ser Asn Trp Ala
565 570 575

Thr Asp Val Gly Ile Trp Tyr Asp Asn Lys Thr Trp Ala Gln Met Gly
580 585 590

Gly Asp Glu Val Gly Ala Val Leu Ala Met Glu Val Gly Lys Thr Tyr
595 600 605

Gln Leu Val Phe Thr Val Lys Gly Val Ala Arg Thr Tyr Val Asp
610 615 620

Gly Arg Arg Val Gly Ala Glu Arg Gly Ile Ile Val Pro Gln Ser Gln
625 630 635 640

Ser Met Glu Val Asp Glu Met Tyr Ile Gly Asn Arg Asp Lys Ala Met
645 650 655

Thr Lys Cys Ser Ala Asp Ala Leu Asn Val Thr Val Phe Asn Met Leu
660 665 670

Leu Tyr Asn Tyr Glu Leu Ser Pro Ala Asp Val Arg Thr Leu Leu Thr
675 680 685

Met Lys Gly Arg Ser Ala Phe Glu Thr Ile Gly Met Ser Gly Asp Asp
690 695 700

Glu Glu Gln Glu Ala Glu Ser Gly Gly Ser Met Leu Trp Thr Leu
705 710 715 720

Ala Val Leu Ile Pro Ala Ile Val Leu Leu Phe Gly Ala Ala Ala Phe
725 730 735

Phe Leu Val Arg Arg Arg Ala Gly Thr Thr Met Pro Pro Ala Thr
740 745 750

Val His His Asn Pro Tyr Phe Met Asn Ala Thr Asp Asp Thr Leu Glu
755 760 765

Val Ser Lys
770

<210> SEQ ID NO 23
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Trypanosoma vivax

<400> SEQUENCE: 23

Arg Thr Ser Ile Asp Tyr His Leu Ile Asp Thr Val Ala Lys Tyr Ser
1 5 10 15

Ala Asp Asp Gly
20

<210> SEQ ID NO 24
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Trypanosoma congolense

<400> SEQUENCE: 24

Pro Lys Asn Ile Lys Gly Ser Trp His Arg Asp Arg Leu Gln Leu Trp
1 5 10 15

-continued

Leu Thr Asp

<210> SEQ ID NO 25
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Trypanosoma vivax
<400> SEQUENCE: 25

Pro Val Ser Ala Gln Gly Gln Asp His Arg Tyr Glu Ala Ala Asn Ala
1 5 10 15

Glu His Thr

<210> SEQ ID NO 26
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Trypanosoma congolense

<400> SEQUENCE: 26

Lys Val Asp Gly Thr Ala Leu Ser Ser Ala Arg Asn
1 5 10

<210> SEQ ID NO 27
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Trypanosoma congolense

<400> SEQUENCE: 27

Lys Ile Glu Ser Thr Gly Ala Val Glu Gly Gly Val Val Lys Asn
1 5 10 15

<210> SEQ ID NO 28
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Trypanosoma congolense

<400> SEQUENCE: 28

Lys Val Thr Trp Gly Thr Gln Val Pro Leu Lys Gly
1 5 10

<210> SEQ ID NO 29
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Trypanosoma congolense

<400> SEQUENCE: 29

Arg Glu Trp Val Glu Ala Val Thr Ser Tyr Ser Arg Val
1 5 10

<210> SEQ ID NO 30
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Trypanosoma congolense

<400> SEQUENCE: 30

Lys Val Gly Asp Val Leu Val Ala Ile Gly Glu Arg Arg
1 5 10

<210> SEQ ID NO 31
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Trypanosoma congolense

-continued

<400> SEQUENCE: 31

Lys Leu Tyr Asn Phe Val Thr Ala Thr Val Glu Gly Arg Arg
1 5 10

<210> SEQ ID NO 32

<211> LENGTH: 15

<212> TYPE: PRT

<213> ORGANISM: Trypanosoma congolense

<400> SEQUENCE: 32

Arg Ile Ser Tyr Phe Tyr Ile Gly Gly Tyr Gly Asn Pro Arg Thr
1 5 10 15

<210> SEQ ID NO 33

<211> LENGTH: 12

<212> TYPE: PRT

<213> ORGANISM: Trypanosoma congolense

<400> SEQUENCE: 33

Arg Gly Trp Asp Pro Leu Leu Ile Val Gly Lys Val
1 5 10

<210> SEQ ID NO 34

<211> LENGTH: 12

<212> TYPE: PRT

<213> ORGANISM: Trypanosoma congolense

<400> SEQUENCE: 34

Lys Ile Leu Glu Val Tyr Asn Ala Asp Gly Arg Trp
1 5 10

<210> SEQ ID NO 35

<211> LENGTH: 19

<212> TYPE: PRT

<213> ORGANISM: Trypanosoma congolense

<400> SEQUENCE: 35

Lys Glu Asn Ile Pro Val Leu Ala Val Ala Val Asn Glu Gly Asp Ser
1 5 10 15

Asn Lys Ile

<210> SEQ ID NO 36

<211> LENGTH: 30

<212> TYPE: PRT

<213> ORGANISM: Trypanosoma congolense

<400> SEQUENCE: 36

Lys Ile Gly Tyr Leu Arg Asp Glu Phe Ser Ser Ser Val Pro Tyr Asp
1 5 10 15

Ile Gly Asn Ile Ala Gln Leu Asp Asp Ala Leu Ala Lys Ile
20 25 30

<210> SEQ ID NO 37

<211> LENGTH: 16

<212> TYPE: PRT

<213> ORGANISM: Trypanosoma congolense

<400> SEQUENCE: 37

Lys Asp Gly Glu Val Trp Trp Ser Asn Pro Gln Pro Gly Trp Lys Glu
1 5 10 15

-continued

<210> SEQ ID NO 38
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Trypanosoma congolense

<400> SEQUENCE: 38

Lys Gly Asn Asn Ile Tyr Val Leu Val Gly Arg Tyr
1 5 10

<210> SEQ ID NO 39
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Trypanosoma congolense

<400> SEQUENCE: 39

Arg Val Val Ser Met Ile Leu Tyr Ser Ala Asp Asp Gly Lys Ser
1 5 10 15

<210> SEQ ID NO 40
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Trypanosoma congolense

<400> SEQUENCE: 40

Arg Met Ile Phe Glu Ser Ser Asp Leu Gly Ala Thr Trp Lys Glu
1 5 10 15

<210> SEQ ID NO 41
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Trypanosoma congolense

<400> SEQUENCE: 41

Arg Leu Gln Met Trp Met Thr Asp Gly Asn Arg Met
1 5 10

<210> SEQ ID NO 42
<211> LENGTH: 23
<212> TYPE: PRT
<213> ORGANISM: Trypanosoma congolense

<400> SEQUENCE: 42

Arg Leu Asp Val Gly Gly Gly His Val Val Trp Pro Val Ser Glu
1 5 10 15

Gln Gly Gln Asp Gln Arg Tyr
20

<210> SEQ ID NO 43
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Trypanosoma congolense

<400> SEQUENCE: 43

Arg Gly Val Glu Leu Leu Gly Leu Phe Ala Asn Arg Gly
1 5 10

<210> SEQ ID NO 44
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Trypanosoma congolense

-continued

<400> SEQUENCE: 44

Lys Gly Thr Ala Thr Asp Val Ser Ile Ser Trp Glu Arg Thr
1 5 10

<210> SEQ ID NO 45

<211> LENGTH: 14

<212> TYPE: PRT

<213> ORGANISM: Trypanosoma congolense

<400> SEQUENCE: 45

Arg Met Lys Asp Gly Tyr Ile Leu Val Gly Gly Pro Lys Leu
1 5 10

<210> SEQ ID NO 46

<211> LENGTH: 26

<212> TYPE: PRT

<213> ORGANISM: Trypanosoma congolense

<400> SEQUENCE: 46

Arg Val Ala Ser Pro Glu Glu Leu Leu Asn Ile His His Leu Phe Val
1 5 10 15

Gly Thr Pro Val Asp Gly Gly Ala Lys Glu
20 25

<210> SEQ ID NO 47

<211> LENGTH: 24

<212> TYPE: PRT

<213> ORGANISM: Trypanosoma congolense

<400> SEQUENCE: 47

Arg Tyr Ile Thr Ser Thr Asp Tyr Phe Phe Thr Asp Thr Val Ala Ala
1 5 10 15

Tyr Ser Thr Asp Gly Gly Arg Thr
20

<210> SEQ ID NO 48

<211> LENGTH: 10

<212> TYPE: PRT

<213> ORGANISM: Trypanosoma congolense

<400> SEQUENCE: 48

Arg Val Ile Gly Asn Ser Pro Gly Arg Ser
1 5 10

<210> SEQ ID NO 49

<211> LENGTH: 28

<212> TYPE: PRT

<213> ORGANISM: Trypanosoma congolense

<400> SEQUENCE: 49

Arg Ser Gly Pro Gly Ser Ser Ser Gly Phe Ile Thr Val Thr Val Glu
1 5 10 15

Gly Val Pro Val Met Leu Leu Thr His Pro Lys Asn
20 25

<210> SEQ ID NO 50

<211> LENGTH: 13

<212> TYPE: PRT

<213> ORGANISM: Trypanosoma congolense

<400> SEQUENCE: 50

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Arg Gly Glu Leu Pro Leu Leu Gly Phe Val Asn Arg Lys
1 5 10

<210> SEQ ID NO 51
<211> LENGTH: 28
<212> TYPE: PRT
<213> ORGANISM: Trypanosoma congolense

<400> SEQUENCE: 51

Arg Asn Gly Pro Gly Ser Ser Ser Gly Phe Ile Thr Val Thr Val Glu
1 5 10 15

Gly Val Pro Val Met Leu Leu Thr His Pro Lys Asn
20 25

<210> SEQ ID NO 52
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Trypanosoma congolense

<400> SEQUENCE: 52

Arg Gly Glu Leu Pro Leu Leu Gly Phe Val Asn His Lys Gly
1 5 10

<210> SEQ ID NO 53
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Trypanosoma congolense

<400> SEQUENCE: 53

Arg Met Thr Asp Gly Tyr Ile Leu Val Gly Gly Pro Lys Leu
1 5 10

<210> SEQ ID NO 54
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Trypanosoma congolense

<400> SEQUENCE: 54

Lys Ala Ala Ile Ala Asp Trp Glu Pro Phe Val Tyr Lys Gly
1 5 10

<210> SEQ ID NO 55
<211> LENGTH: 26
<212> TYPE: PRT
<213> ORGANISM: Trypanosoma congolense

<400> SEQUENCE: 55

Arg Val Ala Ser Pro Ala Glu Leu Leu Asn Ile His His Leu Phe Val
1 5 10 15

Gly Thr Pro Val Asp Gly Gly Ala Lys Glu
20 25

<210> SEQ ID NO 56
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Trypanosoma congolense

<400> SEQUENCE: 56

Arg Ile Arg Val Pro Gly Ser Asp Asn Ser Val Leu Ser Gly Gly
1 5 10 15

-continued

<210> SEQ ID NO 57
<211> LENGTH: 26
<212> TYPE: PRT
<213> ORGANISM: Trypanosoma congolense

<400> SEQUENCE: 57

Lys	Glu	Val	Tyr	Asp	Asp	Glu	Trp	Glu	Glu	Trp	Phe	Met	Glu	Gln	Glu
1															
							5		10					15	

Gly	Pro	Thr	Gly	Val	Asp	Gly	Val	Arg	Ala						
				20					25						

<210> SEQ ID NO 58
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Trypanosoma congolense

<400> SEQUENCE: 58

Lys	Gly	Glu	Ala	Gly	Val	Gly	Thr	Ser	Glu	Ala	Ala	Leu	Thr	Glu	Trp
1															
							5		10				15		

Asp	Gly	Lys	Leu												
			20												

<210> SEQ ID NO 59
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Trypanosoma congolense

<400> SEQUENCE: 59

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Gly															

<210> SEQ ID NO 60
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<212> TYPE: PRT
<213> ORGANISM: Trypanosoma congolense

<400> SEQUENCE: 60

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1															
							5		10						

What is claimed:

1-30. (canceled)

31. A DNA or RNA molecule comprising at least one nucleotide sequence coding for a trans-sialidase-like of an African trypanosome, selected from the sequences SEQ ID NOS: 1-3, a sequence complementary to a sequence selected from one of the sequences SEQ ID NOS: 1-3, a sequence comprising an identity of at least 70% with one of the sequences SEQ ID NOS: 1-3, a fragment of said sequences, or a nucleotide sequence able to hybridize with one of the sequences SEQ ID NOS: 1-3 under stringent hybridization conditions.

32. A protein encoded by the nucleotide sequence of claim **31.**

33. A protein comprising a sequence selected from SEQ ID NOS: 4-6, designated TcoTS-like 1, 2 and 3, respectively, or an antigenic peptide fragment of said protein.

34. An expression cassette comprising a DNA molecule of claim **31.**

35. A recombinant vector comprising an expression cassette of claim **34.**

36. A recombinant host cell comprising a nucleic acid of claim **31.**

37. A host cell of claim **36**, wherein said cell is of eukaryotic origin, such as notably mammalian cells, insect cells, fungal cells or yeast cells, or said cell is of prokaryotic origin, such as notably *E. coli* cells or enterobacteria cells.

38. A protein of claim **32** or claim **33**, or an antigenic peptide fragment thereof, wherein said protein or fragment exhibits reactivity with the sera of animals infected by an African trypanosome, preferably selected from *Trypanosoma congolense*, *Trypanosoma vivax*, *Trypanosoma evansi* and/or *Trypanosoma brucei*.

39. A vaccine comprising an effective amount of one or more proteins of claim **32** or **33**.

40. A method for preventing and/or treating trypanosomiasis or pathogeneses induced by trypanosomosis in non human

animals, or for preventing and/or treating trypanosomiasis or pathogeneses induced by trypanosomiasis in humans, comprising administering to said human or non-human subject a vaccine of claim 39.

41. A vaccine of claim 39 for protection against infections by *Trypanosoma congolense*, *Trypanosoma vivax*, *Trypanosoma evansi* and/or *Trypanosoma brucei*.

42. A method of claim 40, wherein said induced pathogeneses comprise anemia, degradations in general health, weight loss and/or immunosuppression in said animals and/or human.

43. A method of claim 40, wherein said non human animals are selected among bovids, ovids, felids, suids, camelids and/or canids.

44. A vaccine of claim 39, wherein said vaccine is a multivalent vaccine which further comprises one or more antigenic peptides and/or antigenic fragments and/or nucleotide sequences coding for said peptides derived from one or more African trypanosome species, preferably derived from flagellar proteins, sialidases, trans-sialidases, lipases, proteases and/or tubulins.

45. A vaccine of claim 39, which further comprises (i) at least one antiparasitic agent, preferably selected from a trypanocide and/or a nonspecific antiparasitic agent for trypanosomes, (ii) at least one anti-infective agent, preferably selected from β -lactams, fosfomycin, glycopeptides or polypeptides with antibiotic activity, bacitracin, aminoglycosides, macrolides, lincosamides, streptogramins, tetracyclines, phenicols, fusidic acid or quinolones, and/or (iii) at

least one symptomatic agent, preferably selected from an anti-anemia agent, a hepatoprotective agent and/or a non-steroidal anti-inflammatory drug.

46. A vaccine of claim 39, which further comprises an adjuvant.

47. A vaccine comprising the vaccine of claim 39 and a vaccine and/or or an antigen directed against theileriosis, anaplasmosis, babesiosis, foot-and-mouth disease, clostridiosis, plague, catarrhal fever, contagious bovine pleuropneumonia (CBPP), blackleg, pasteurellosis and/or sheep pox.

48. A monoclonal or polyclonal antibody which binds a protein or an antigenic peptide fragment of claim 33, and/or is obtained by immunological reaction of a non human animal organism and/or a human with at least one protein or an antigenic peptide fragment of claim 33.

49. A probe for identifying African trypanosome parasites, wherein said probe comprises a nucleotide sequence that enables hybridization with a nucleic acid of claim 31.

50. A method for detecting trypanosomosis in a biological sample, such as the blood of a non human animal and/or a human able to be infected by an African trypanosome, wherein said sample and an antibody of claim 48 are brought together under conditions enabling a possible immunological reaction, and the presence or absence of an immune complex is then detected.

51. A kit for diagnosing trypanosomosis in a biological sample, comprising an antibody of claim 48 or a probe of claim 49.

* * * * *

Trypanosoma vivax GM6 Antigen: A Candidate Antigen for Diagnosis of African Animal Trypanosomosis in Cattle

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Abstract

Background: Diagnosis of African animal trypanosomosis is vital to controlling this severe disease which hampers development across 10 million km² of Africa endemic to tsetse flies. Diagnosis at the point of treatment is currently dependent on parasite detection which is unreliable, and on clinical signs, which are common to several other prevalent bovine diseases.

Methodology/Principle Findings: the repeat sequence of the GM6 antigen of *Trypanosoma vivax* (TvGM6), a flagellar-associated protein, was analysed from several isolates of *T. vivax* and found to be almost identical despite the fact that *T. vivax* is known to have high genetic variation. The TvGM6 repeat was recombinantly expressed in *E. coli* and purified. An indirect ELISA for bovine sera based on this antigen was developed. The TvGM6 indirect ELISA had a sensitivity of 91.4% (95% CI: 91.3 to 91.6) in the period following 10 days post experimental infection with *T. vivax*, which decreased ten-fold to 9.1% (95% CI: 7.3 to 10.9) one month post treatment. With field sera from cattle infected with *T. vivax* from two locations in East and West Africa, 91.5% (95% CI: 83.2 to 99.5) sensitivity and 91.3% (95% CI: 78.9 to 93.1) specificity was obtained for the TvGM6 ELISA using the whole trypanosome lysate ELISA as a reference. For heterologous *T. congolense* field infections, the TvGM6 ELISA had a sensitivity of 85.1% (95% CI: 76.8 to 94.4).

Conclusion/Significance: this study is the first to analyse the GM6 antigen of *T. vivax* and the first to test the GM6 antigen on a large collection of sera from experimentally and naturally infected cattle. This study demonstrates that the TvGM6 is an excellent candidate antigen for the development of a point-of-treatment test for diagnosis of *T. vivax*, and to a lesser extent *T. congolense*, African animal trypanosomosis in cattle.

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Introduction

African animal trypanosomosis (AAT) is a devastating livestock disease costing approximately \$1 - 2 billion per annum in Africa [1]. AAT is caused by the tsetse-fly-transmitted

(*Glossina* sp.) protozoan parasites *Trypanosoma congolense*, *T. vivax* and to a lesser extent, *T. brucei brucei* and by the non-tsetse transmitted trypanosomes such as *T. evansi*, the most widely distributed animal pathogenic trypanosome, which is the causative agent of surra [2]. Furthermore, *T. vivax*, in addition

to being transmitted by tsetse flies, can also be transmitted by biting flies in South America and regions of Africa non-endemic to tsetse [3,4].

Disease progression is dependent on host factors as well as on parasite species and strain. The most prevalent clinical signs include anaemia, weight loss, reduced productivity, infertility and abortion [5]. However, symptoms are too varied and non-specific to be a reliable basis for diagnosis of AAT.

Unlike human African trypanosomosis, where a lateral flow device prototype has recently been developed, no point-of-treatment test exists for AAT [6,7]. The current gold standard for diagnosis of AAT is examination of blood by light microscopy for the presence of parasites. The blood can be concentrated (usually by centrifugation) to improve sensitivity [8]. However, the usefulness of parasitological diagnosis is limited in chronic infections where the parasitaemia is low and intermittent. Even during acute infection, antigenic variation results in waves of parasitaemia which could easily be missed if sampling is only performed a single time [9].

In terms of serological diagnosis, the indirect fluorescent antibody test (IFAT) was one of the first antibody detection tests to be used for diagnosis of AAT. The IFAT is both sensitive and specific, but not species-specific [10]. Furthermore, the IFAT is not quantitative, requires fluorescent-enabled microscopes, and antigen preparation is not standardised. Antibody ELISA using whole trypanosomal lysate (WTL) was subsequently developed as a serological test for AAT [11]. Although it shows high sensitivity and specificity, the WTL ELISA is not species-specific and, again, standardisation of antigen production proves difficult. For these reasons, the WTL ELISA is not readily adaptable to an immune-chromatographic test format required for point of treatment diagnosis in field situations. However, partial purification of the crude lysate allows higher specificity [12]. Still, the general problem with antibody detection tests is that they do not only detect active infections, since antibodies against trypanosomes persist after treatment or self-cure.

On the other hand, antigen detection ELISAs developed for AAT suffer from low sensitivity and low species-specificity as confirmed with experimental infections [13,14].

The GM6 antigen was originally identified in African trypanosomes by screening a *T. b. gambiense* cDNA library with infected bovine sera [15]. It has been shown that the GM6 antigen is an invariant antigen, associated with the flagellum and expressed in both procyclic and bloodstream forms (BSF) of the parasite [15]. The GM6 antigen contains a 68 amino acid repeat motif which is partially conserved in *T. brucei*, *T. congolense* and *T. vivax*. It has been noted that the GM6 antigens are part of the calpain superfamily, albeit with unusual repeat sequences and are unlikely to be active enzymes [16]. To date, the *T. vivax* GM6 and *T. congolense* GM6 antigens have not been tested in an ELISA to diagnose bovine trypanosomosis. Furthermore, previous studies of the GM6 antigen ELISA have been limited to small sets of infected sera, and almost exclusively from experimental infections.

Currently, the treatments available for AAT are not species-specific. However, since no field diagnostic test is currently available for any animal trypanosome infections, diagnosis of

T. vivax infection would be a good beginning. Primarily, the goal would be to incorporate antigens from both *T. congolense* and *T. vivax* into a pan-trypanosome field diagnostic test. Secondly, specific detection of *T. vivax* would be useful since this parasite is prevalent in both East and West Africa, in both tsetse endemic and non-endemic regions, as well as in South America. *T. vivax* is also responsible for haemorrhagic outbreaks of AAT, which would benefit from quick diagnosis [17]. Also, the natural habitat of tsetse is being reduced by climate change, encroaching human settlements and tsetse eradication programs [18]. For this reason, it is foreseeable that *T. vivax* could become more prevalent than *T. congolense* given that it does not require tsetse for transmission. Indeed, this has already been observed in the northern arid Djibo region of Burkina Faso [19].

For these reasons, in the current study, the repeat sequences of the GM6 proteins of *T. vivax* (TvGM6: TvY486_1101010) and *T. congolense* (TcoGM6: TcIL3000.11.1030) were recombinantly expressed, and purified. Sequencing of the TvGM6 genes from isolates from both East and West Africa showed high conservation despite the fact that *T. vivax* is known to be highly genetically diverse [20,21,22,23,24,25]. The purified GM6 antigens were subsequently used in an indirect ELISA that was optimised for detection of trypanosome infection in bovine sera. Sera from experimental infections using strains of *T. vivax* and *T. congolense* from both East and West Africa were tested in an indirect ELISA with the two GM6 antigens to determine the kinetics of infection. In addition, large collections of field sera were tested in order to determine the specificity and sensitivity of the TvGM6 indirect ELISA for both homologous and heterologous infections.

Materials and Methods

Ethics statement

All mice procedures were carried out in strict accordance with the French legislation (Rural Code articles L 214-1 to L 214-122 and associated penal consequences) and European Union (Directive 2010/63/EU Protection of Animals Used for Scientific Purposes) guidelines for the care of laboratory animals and were approved by the Ethical Committee of Centre National de la Recherche Scientifique, Région Aquitaine and by the University of Bordeaux 2 animal care and use committee. All efforts were made to minimize animal suffering.

For the cattle infections at ClinVet in South Africa, the study plan was submitted to the ClinVet Animal Ethics Committee (CAEC) and an approval certificate was issued authorizing the research facility to conduct the study. The study plan was designed to allow the use of the study animals in compliance with the ClinVet Policy on the ethical use of animals (CVI 08/03) using the South African National Standard "SANS 10386:2008 "The care and use of animals for scientific purposes" as a reference.

The protocol for cattle studies conducted by CIRDES (Centre International de Recherche-Développement sur l'Elevage en Zone subhumide, Bobo-Dioulasso, Burkina Faso) were reviewed and approved by the Scientific Committee of

CIRDES, and complied with the requirements of 'European Union Directive 2010/63/EU Protection of animals for scientific purposes; Requirements for establishments and for the care and accommodation of animals.

The research protocol for cattle infections at CB-UEM (Biotechnology Centre at the University Eduardo Mondlane, Maputo, Mozambique) was approved by the Scientific Board of the Veterinary Faculty of the Eduardo Mondlane University. The study was reviewed by The Mozambican Livestock National Directorate and handling of the animals and blood sampling were performed by approved staff, namely animal technicians and veterinary surgeons, according to the World Organization for Animal Health (OIE) guidelines for use of animals in research and education.

Additionally, the cattle studies conducted at CIRDES, ClinVet and CB-UEM were approved by the Scientific Committee of GALVmed (Global Alliance for Livestock Veterinary Medicine) in the frame of the Animal African Trypanosomosis Programme (*Aries* code 202040-101).

GM6 cloning, expression and purification

The *T. vivax* Y486 strain was initially isolated from a Zebu in West Africa (Nigeria) [26] and was kindly provided by the International Livestock Research Institute, Nairobi, Kenya. A fragment containing four copies of the repeat (270 bp) was amplified from the *T. vivax* GM6 gene (TvGM6: TvY486_1101010) using specific primers: Fwd: 5' GAA ATA CAG CAG CAA CAC GAT 3'; Rv: 5' GAA CTG CTC GTC CGC GTC AAG 3'. The amplicon was cloned into pGEX-4T-1 (GE Healthcare) in frame with the 5' GST-tag. A similar fragment (220 bp) of the *T. congolense* GM6 (TcoGM6: TcIL3000.11.1030) was synthesised commercially, due to cloning difficulties, (ProteoGenix, Oberhausbergen, France) and cloned into pGEX-4T-1. Recombinant vectors were used to transform *Escherichia coli* BL21 Star™ (DE3) (Invitrogen, Saint-Aubin, France) for expression. Cultures in mid-exponential growth phase were induced with 0.4 mM IPTG for 3–4 hrs. Recombinant fusion protein was present in the supernatant of cell lysate. Cells were lysed with an extraction buffer (50 mM Tris-Cl, pH 8.5, 100 mM NaCl, 1 mM EDTA) supernatants bound to Glutathione Sepharose 4B (GE Healthcare) for 1 hr at room temperature (RT) with gentle agitation. The resin was washed five times in extraction buffer (10 column volumes) and resuspended in 1 ml thrombin cleavage buffer (50 mM Tris pH 8.0, 150 mM NaCl, 2.5 mM CaCl₂). Thrombin (10 units, Sigma) was added to the resin and incubated overnight at RT with gentle agitation. Fractions containing cleaved GM6 protein were collected, and concentration estimated by Bradford protein assay [27].

Trypanosome strains and serum origins

Sera were obtained from several sources. Sera from *T. congolense* experimental infections with the strain MozO2J (isolated in Mozambique; L. Neves, 2012) and KONT2/133 (isolated in Cameroon; [28]) were obtained from novel trypanocide efficacy studies conducted at ClinVet (Bloemfontein, South Africa) by GALVmed (Global Alliance for Livestock Veterinary Medicine). For these studies, animals

were treated with either 7 mg/kg diminazene diaceturate or 1 mg/kg isometamidium chloride and novel compounds under evaluation for efficacy against *T. congolense* and *T. vivax*. *T. vivax* infections were conducted at CIRDES (Centre International de Recherche-Développement sur l'Elevage en Zone subhumide, Bobo-Dioulasso, Burkina Faso) by GALVmed, using strains isolated in West Africa (Komborodougou and Napie, isolated in Ivory Coast by S. Yao Loukou; Gando Bongaly, isolated in Togo by S. Boma) provided by Z. Bengaly. Animals were treated with 3.5 mg/kg of diminazene diaceturate. *T. vivax* experimental infection sera were also obtained from infections conducted at ILRI (Nairobi, Kenya) using the strains IL2172 and IL3769 (Ugandan origin [26]) and were provided by a co-author and E. Authié. Additional *T. vivax* experimental infections were conducted in Mozambique at CB-UEM using a local isolate (175J) and the Y486 reference strain [29]. Corresponding parasitaemia was estimated by phase contrast buffy coat [30]. *T. vivax*-infected field sera from Western Senegal, characterised by whole trypanosome lysate ELISA were provided by co-authors [31]. *T. vivax*-infected field sera from Ethiopia characterised by ITS-PCR were provided by co-authors [3]. *T. congolense*-positive field sera (buffy coat and 18s PCR) and negative sera from animals in a tsetse-free region were collected in the South of Mozambique (Biotechnology Centre, University Eduardo Mondlane, Maputo, Mozambique).

ELISA

Indirect ELISA was optimised for type and concentration of blocking agent, coating antigen concentration and secondary antibody concentration. Recombinant GM6 antigen was purified as described above. *T. congolense* (IL3000) and *T. brucei brucei* (AnTat 1) BSF parasites were obtained from *in vitro* culture [32,33]. *T. vivax* (Y486) BSF parasites were propagated in mice and purified either by centrifugation [34] or DE-52 ion-exchange chromatography [35], from which whole parasite lysate was prepared by osmotic lysis. Briefly, antigen (4 µg/ml for TvGM6, 10 µg/ml for TcoGM6 and whole trypanosome lysate) was diluted in carbonate coating buffer (50 mM carbonate buffer, pH 9.6) and plates coated with 100 µl per well and incubated overnight at 4°C. Blocking buffer (1% horse serum in PBS) was added to the wells (200 µl/well) and the plate incubated at 37°C for 1 h. Primary sera diluted in blocking buffer (1/100) were added to the wells in duplicate (100 µl/well) and incubated at 37°C for 2 h. The plates were washed with 0.05% PBS-Tween-20 using either a squeeze bottle or an automated microplate washer (ThermoFisher Scientific WellWash 4 Mk 2 MicroPlate Washer). Secondary antibody, rabbit anti-bovine horse-radish peroxidase conjugate (Sigma) diluted in blocking buffer (1/4000), was added to the wells (100 µl/well). Plates were washed as before and commercial ABTS substrate-chromogen solution (KPL) added (100 µl/well). Optical density (OD 405 nm) was measured approximately 10–15 min after addition of the substrate (FLUOStar OPTIMA fluorescence plate reader). Readings were considered acceptable when the OD values for the positive and negative control samples fell within specific ranges, with a coefficient of variance less than 10%.

Known strong positive and negative bovine serum samples (based on previous ELISAs) were added to each plate allowing calculation of the percent positivity (PP) for each sample [36]. For experimental infection sera, combined weighted estimates of sensitivity from sequential sera of nine infected animals and the weighted standard errors with 95% confidence limits were calculated according to Eisler et al., [13]. The significance of the differences observed between the TvGM6 and TvWTL ELISA was compared using the McNemar test using GraphPad Software (GraphPad Software Inc.). For field sera, cut-offs were established using a minimum of 10 PCR negative bovine sera from areas non-endemic to tsetse (peri-urban Maputo, Mozambique). Cut-offs were calculated as the mean PP added to two standard deviations. Sera were tested in duplicate, and each experiment was performed at least twice, allowing estimation of the standard error for positive and negative samples from each region.

Immunofluorescence

Anti-TvGM6 and anti-TcoGM6 sera were obtained by immunising mice at two week intervals with initially 50 µg of purified recombinant protein (in Freund's Complete adjuvant), followed by two boosters of 25 µg (Freund's Incomplete adjuvant). Parasite pellets were washed in phosphate saline with glucose (PSG), resuspended in 320 µl fixing solution (3% formaldehyde in PBS, freshly prepared) and incubated at RT for 10 min. Fixing was blocked using 1 M glycine-Cl (80 µl) and incubation at RT for 10 min. Fixed parasite suspension (20 µl) was added to slide wells. Dried slides were blocked using 0.5% BSA-PBS (50 µl/well) at RT for 10 min, followed by permeabilisation of the cells using 0.1% Triton X-100 in PBS (20 µl) for the same time. Primary antibody diluted in blocking buffer (20 µl) was added to each well and incubated in a humidified atmosphere for 1 hr. Primary antibodies used were either mouse anti-TvGM6 sera (1/2000) or mouse anti-TcoGM6 sera (1/2000), and rabbit anti-parafagellar rod (1/50). Slide wells were washed using blocking solution (3x50 µl). Secondary antibody diluted 1/100 in blocking buffer (20 µl) was added to slide wells and incubated for 60 min. Secondary antibodies used were Alexa Fluo 488 conjugated goat anti-mouse IgG and Texas Red® conjugated goat anti-mouse IgG (Invitrogen, Carlsbad, CA, USA). DAPI (20 µl/ slide well) diluted in PBS (final 0.5 µg/ml) was added to each slide well. Slides were viewed using a Zeiss Axio Imager Z1 fluorescent microscope and images captured using the MetaMorph® software (Molecular Devices, CA, USA) at a total magnification of 100x.

Results

TvGM6 is conserved within *T. vivax* isolates and is possibly flagellar-associated

TvGM6 is a homolog of the genes found in *T. brucei brucei* (Tb11.57.0008) and *T. congolense* (TcIL3000.11.1030). However, the TvGM6 repeat sequence only shares 51 and 55% identity and 72 and 64% similarity with the homologs of *T. b. brucei* and *T. congolense*, respectively (Figure 1A). Furthermore, the number of repeats of the 68 amino acid motif

differs between the different species. The TvGM6 has 11 copies of the repeat compared to 60 in *T. b. brucei* and 9 in *T. congolense*. In order to determine the level of variability in the TvGM6 gene within the *T. vivax* species, the repeat was sequenced from several *T. vivax* strains isolated in different regions. As is evident from Figure 1B there were, at most, two amino acid substitutions in a single copy of the TvGM6 repeat. *T. vivax* strains from Burkina Faso had only one amino acid substitutions in comparison to the *T. vivax* Y486 reference strain.

Immuno-localisation studies (Figure 1C) showed that anti-TvGM6 antibodies partially co-localised with anti-parafagellar rod antibodies. This indicated that the TvGM6, like the homologs in other trypanosome species, is likely to be associated with the flagellum. The co-localisation with the anti-PFR antibodies was only partial, indicating that the localisation of the TvGM6 is not entirely flagellar, and it is possible that the TvGM6 may be present in the flagellar attachment zone. TvGM6 was detected in both bloodstream form and procyclic parasites (data not shown). An identical localisation pattern was observed using anti-TcoGM6 antibodies (data not shown).

TvGM6 and TcoGM6: Detection of infection during *T. vivax* and *T. congolense* experimental infections

In total, sera from nine *T. vivax* experimental infections were tested with the TvGM6 ELISA: three with West African isolates, two with East African isolates from Uganda, two with a Mozambican isolate and two with the Y486 reference strain (Nigerian origin [29]). A representative result (only one animal per infection) of the TvGM6 indirect ELISA with a *T. vivax* experimental infection compared to the ELISA using whole trypanosome lysate of *T. vivax* (TvWTL) is shown in Figure 2A and 2B.

The TvGM6 ELISA was positive 20 to 30 days post infection (DPI), and reduced to baseline approximately 20 to 30 days post-treatment when the animal was successfully treated (Figure 2A). In Figure 2B, the TvGM6 ELISA was tested with samples from an infection using a drug-resistant strain. In this case, the parasitaemia decreased post-treatment and no parasites were detected between 35 and 90 DPI. However, a relapse was observed around 91 DPI, when parasites were again detected in the blood. The antibody response against TvGM6 decreased at 70 DPI (approximately one month after treatment) and increased again 80 DPI, 15 days prior to the detection of parasites by microscopy. These results suggest that this antigen could be used to diagnose an active infection, and, furthermore, would be a good indicator of the efficacy of treatment. The TvWTL ELISA did not decrease as quickly (or at all) as the TvGM6 ELISA after treatment.

Indirect ELISA using TcoGM6 during *T. congolense* experimental infection is shown in Figures 2C (drug-sensitive strain) and 2D (drug-resistant strain). A peak of antibody response was observed at 25 DPI, and decreased dramatically post-treatment (Figure 2C), however, this was only observed for sera from five out of a total of eleven experimental infections. In the other six experimental infections, no ELISA response to TcoGM6 was observed (data not shown). Therefore, detection of early infection using TcoGM6 ELISA,

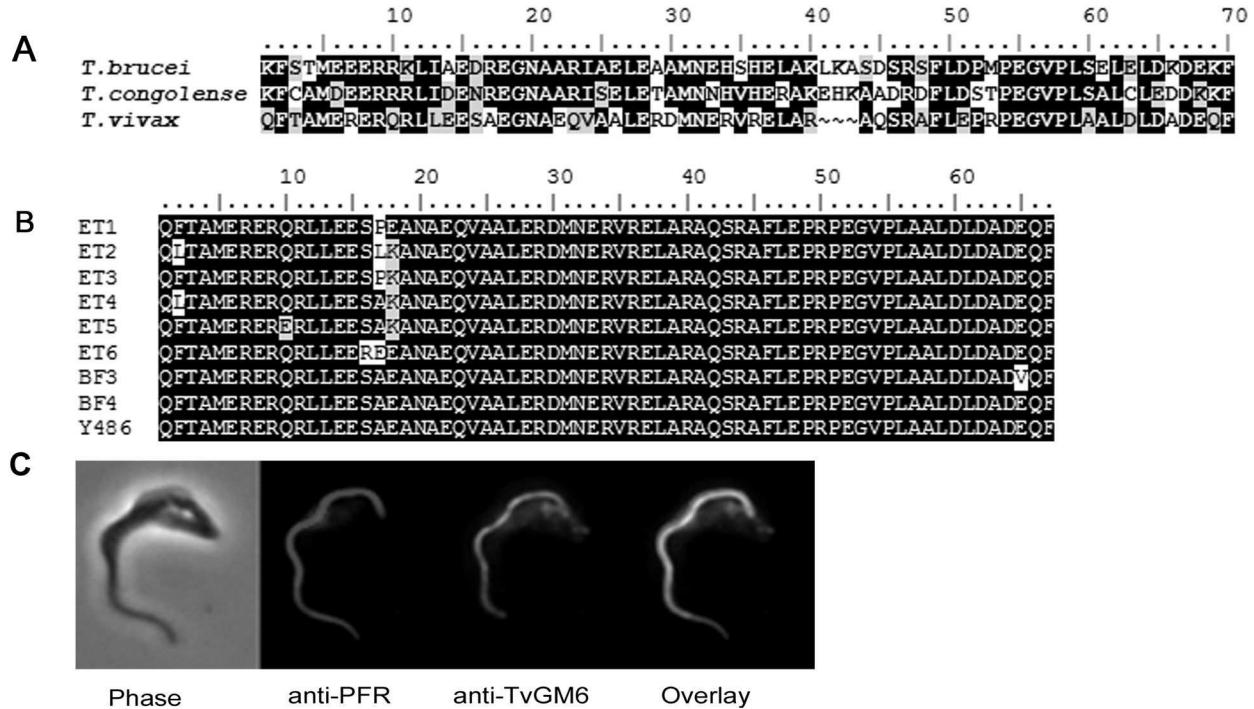


Figure 1. T.vGM6 is a homolog of the protein found in other trypanosomes, is conserved within *T. vivax* isolates and is flagellar associated. (A) Alignment of the GM6 repeat motif from *T. brucei* (427), *T. congolense* (IL3000) and *T. vivax* (Y486). (B) Sequence alignment of a single repeat of the T.vGM6 sequence from *T. vivax* isolates originating from Burkina Faso (BF) and Ethiopia (ET). The *T. vivax* Y486 reference strain is shown. Background colour indicates conservation (black), similarity (grey) and differences (white) in amino acid sequence. (C) Immunofluorescence microscopy of *T. vivax* bloodstream forms showing partial co-localisation of anti-TvGM6 antibodies (green) with anti-pfR antibodies (red).

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during the first waves of parasitaemia, was inconsistent. As can be seen in Figure 2D, the initial antibody response is barely detectable 25 DPI, whereas the response continues to increase after several waves of parasitaemia.

Comparative values for positivity of the T.vGM6 ELISA, whole trypanosome lysate (T.vWTL) ELISA, and buffy coat during experimental infection with *T. vivax* are shown in Table 1. The T.vWTL ELISA showed approximately double the sensitivity of either the T.vGM6 ELISA or the buffy coat method very early in infection. However, the two-tailed P-value calculated using the McNemar test was 0.2482 indicating that the difference between the T.vWTL and T.vGM6 ELISAs was not significant. Later than 10 DPI, the T.vGM6 ELISA and the T.vWTL ELISA were comparable at approximately 90% of sensitivity (no significant difference P-value = 0.6831) while the buffy coat method was only 24% sensitive. Finally, the T.vGM6 ELISA results were 4.5 times less likely to be positive 30 days post-treatment than the T.vWTL ELISA results. The McNemar test P-value was 0.0133 indicating that the T.vGM6 ELISA was significantly less likely to detect false positives post-treatment than the T.vWTL ELISA.

TvGM6: High specificity and sensitivity in field infections

TvGM6 indirect ELISA was used to test sera from cattle infected with *T. vivax* originating from Ethiopia, Senegal and Mozambique (Table 2). TvGM6 ELISA showed a mean (weighted) sensitivity of 91.5% (95% CI: 83.2 to 99.5) and a mean (weighted) specificity of 91.3% (95% CI: 78.9 to 93.1) in comparison to the T.vWTL ELISA. In terms of a comparison to PCR (not shown), for the Ethiopian field sera, the T.vGM6 ELISA had a sensitivity of 79% compared to PCR positive samples (268 tested), whereas the WTL ELISA had a lower sensitivity of 68%. The lower specificity for the Senegalese and Ethiopian sera ($85.4 \pm 6.9\%$) could be attributed to the fact that the negative sera were obtained from an endemic region, i.e. it cannot be excluded that animals were previously infected and treated. Since the T.vGM6 indirect ELISA requires a minimum of 20–30 days post treatment to return to baseline values (as seen from the experimental infections), it is possible that some animals which test serologically positive had been infected and treated within one month of serum collection.

In addition, the T.vGM6 ELISA was negative (below the cut off for positivity) when tested with bovine sera from animals infected with *Anaplasma marginale*, *Babesia bigemina* and *Theileria buffeli* (Marula) (results not shown).

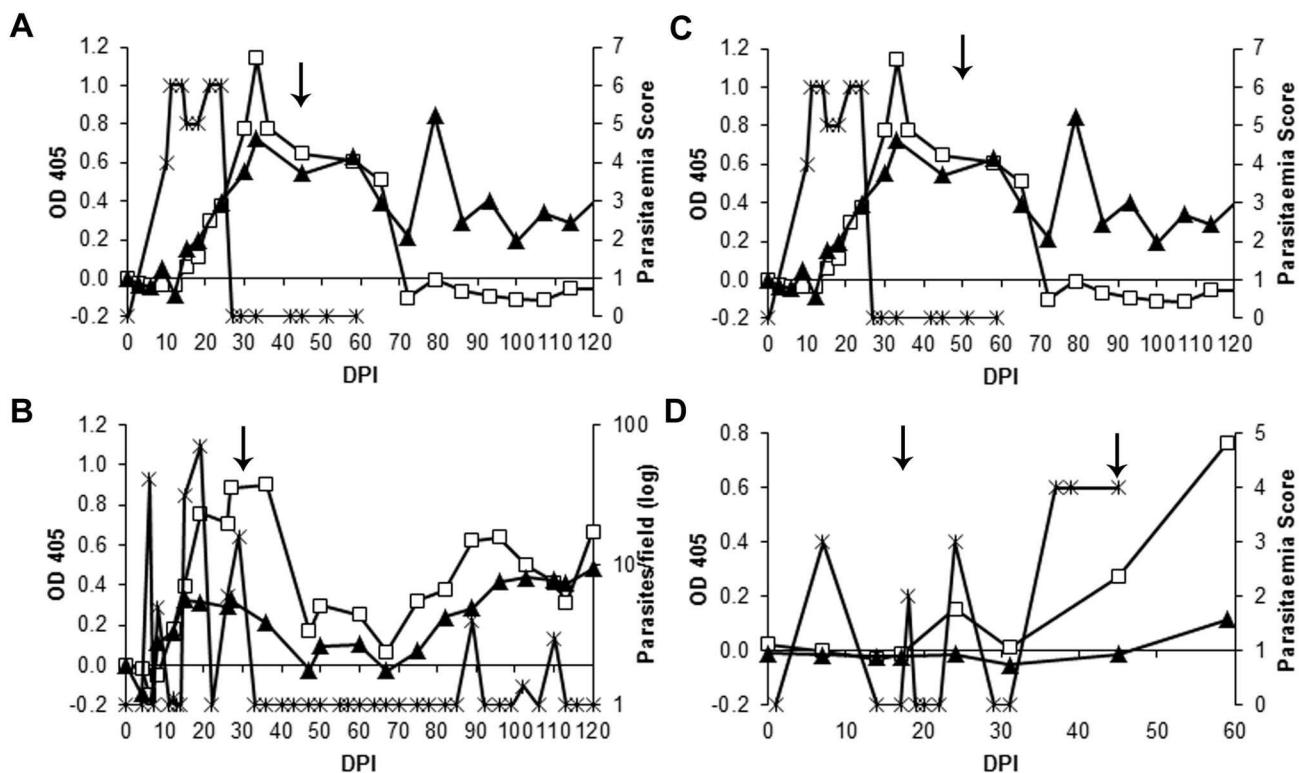


Figure 2. Representative TvGM6 and TcoGM6 ELISA analysis of longitudinal experimental infection sera with (A, B) *T. vivax* and (C,D) *T. congolense* in individual animals. TvGM6 ELISA using sera from infections with (A) *T. vivax* IL2172 (drug-sensitive) and (B) *T. vivax* Kombokorodougou (drug-resistant). TcoGM6 ELISA using sera from infections with (C) *T. congolense* O2J (drug-sensitive) and (D) *T. congolense* KONT2/133 (drug-resistant). All animals were treated with 3.5mg/kg of diminazene diacetate at the dates indicated by the arrows, (D) was treated a second time with 1 mg/kg isometamidium chloride. For figures (A), (C) and (D) parasitaemia score can be related to approximate amounts of parasites as follows: 2 (1-10/preparation), 3(1-2/field), 4 (1-10/field), 5 (10-50/>>50 field), 6 (>100/field). TvGM6 or TcoGM6 ELISA OD (£), whole trypanosome lysate ELISA OD () and parasitaemia () are indicated. Arrows indicate trypanocidal treatment, and the x-axis is the threshold for positivity.

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TvGM6 : Cross-reactions with heterologous *T. congolense* infection

The TvGM6 ELISA was also tested with sera from *T. congolense* experimental infections, and produced a pattern similar to the TcoGM6 indirect ELISA (data not shown), with a peak of antibody response 25 DPI and a rapid decrease below the threshold post-treatment. However, the TvGM6 ELISA was consistently weaker than the TcoGM6 ELISA when detecting heterologous infection. Due to this initial finding of a cross-reaction, it was decided to test the TvGM6 indirect ELISA with *T. congolense*-infected field sera to determine the sensitivity of this test for the heterologous infection and the results are shown in Table 3. The sensitivity of TvGM6 ELISA with *T. congolense* field infections gave a mean sensitivity of 85% (95% CI: 76.8 to 94.4) in comparison to the whole trypanosome lysate ELISA.

Discussion

Diagnosis of AAT is currently made on the basis of clinical signs, which are common to several other bovine pathogens, resulting in frequent misdiagnosis. Currently, no point-of-treatment diagnostic tool exists for diagnosis for either *T. congolense* or *T. vivax* infections. Furthermore, detection of either parasite would require the same intervention since there is no difference in treatment. In the current study, the immunodiagnostic potential of the *T. vivax* GM6 antigen for the detection of *T. vivax* has been explored as the first step towards a pan-trypanosome point-of-treatment diagnostic tool.

In the current study, the repeat motif of the GM6 antigens of *T. vivax* (TvGM6) and *T. congolense* (TcoGM6) were expressed and purified and their immunodiagnostic potential tested in an indirect ELISA with sera from cattle infected with either *T. vivax* or *T. congolense*. *T. vivax* is known to be quite genetically diverse and several studies have shown that West African and South American *T. vivax* strains are genetically distinct from East African isolates [21,22,23,37]. For this

Table 1. Sensitivity of TvGM6 ELISA compared to TvWTL ELISA and buffy coat in *T. vivax* experimental infections.

Point of Infection	Test	No. of cattle	No. of Samples	Sensitivity ^a	%95% CI ^b
< 10 DPI ^d	TvGM6 ELISA	9	26	24.0	17.5 to 30.4
	TvWTL ^c ELISA	9	26	34.6	29.8 to 39.4
	Buffy Coat	9	32	15.6	14.7 to 16.6
> 10 DPI ^d	TvGM6 ELISA	9	116	91.4	91.3 to 91.6
	TvWTL ELISA	9	116	90.5	90.4 to 90.7
	Buffy Coat	9	213	23.9	23.8 to 24.0
30 DPT ^e	TvGM6 ELISA	4	21	9.1	7.3 to 10.9
	TvWTL ELISA	4	21	42.9	9.9 to 75.5
	Buffy Coat	2	15	0	0

^aCombined sensitivity values were weighted for the number of samples per animal^b95% confidence interval^c*T. vivax* whole trypanosome lysate^ddays post infection^edays post treatment

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Table 2. Sensitivity and specificity of TvGM6 ELISA compared to the whole trypanosome lysate ELISA in *T. vivax* field infections.

	Region	Samples	Sensitivity (%) ± SD ^a	Mean (95% CI) ^b
Positives	Ethiopia	179	94.4 ± 5.5	91.5 (83.2 to 99.5)
	Senegal	211	89.1 ± 7.4	
Negatives	Region	Samples	Specificity (%) ± SD ^a	Mean (95% CI) ^b
	Mozambique	84	96.4 ± 9.0	91.3 (78.9 to 93.1)
	Ethiopia	36	86.1 ± 2.0	
	Senegal	41	85.4 ± 6.9	

^astandard deviation^b95% confidence interval

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Table 3. Sensitivity of TvGM6 ELISA against *T. congolense* field sera from two different regions.

Region	Total	Sensitivity (%) ± SD ^a	Mean (95% CI) ^b
Ethiopia	28	89.6 ± 4.7	85.1 (76.8 to 94.4)
Mozambique	165	81.7 ± 2.7	

^a standard deviation^b 95% confidence interval

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reason, sequencing of the TvGM6 gene from isolates originating from both East and West Africa was deemed necessary to confirm that the sequence was sufficiently conserved to allow detection of infected animals in both regions.

As shown with the *T. brucei* GM6, this study confirmed that the TvGM6 was present in both bloodstream form and procyclic parasites. Immuno-localisation of the TvGM6 indicated that, similar to the TbGM6, the *T. vivax* antigen was likely to be located in the flagellar attachment zone.

Previous preliminary diagnostic studies had been done with the GM6 antigen from different trypanosome species, including a recombinant beta-galactosidase-*T. b. gambiense* GM6 fusion protein which showed high immunodiagnostic sensitivity with sera from *T. brucei* and *T. congolense*-infected cattle [15]. The *T. b. brucei* GM6 (TbbGM6) antigen was tested in an antibody ELISA for *T. evansi* infection, but was not sufficiently sensitive [38]. However, it was useful in a competitive ELISA using *T. evansi* infected bovine or buffalo sera, but not wallaby, pig, dog or horse-infected sera [38]. Thuy et al., (2011) searched for repeat antigens of *T. congolense* for use in a diagnostic test for *T. evansi*. They identified TcoGM6 and TbbGM6 as potential antigens since both showed higher reactivity to *T. evansi* – infected water buffalo sera than other repeat antigens [39].

It is known that repeat antigens are good targets for B-cell responses [40]. This may explain why the TvGM6, which is a minor, insoluble antigen, has a high sensitivity in an indirect ELISA. In fact, antibody responses against repeat proteins of several protozoan parasites have been found, including for malaria [41], Chagas disease [42] and leishmaniasis [43].

In the current study, sera from longitudinally-followed experimental infections allowed definition of the kinetics of the antibody response to these antigens, including the length of the period post-infection before antibodies became detectable (pre-patent period) and, most importantly, the time necessary for the antibody response to decrease below the threshold post-treatment. Sera obtained from naturally infected animals in the field were tested in order to determine the level of sensitivity and specificity of the GM6 antigen indirect ELISA. To ensure that the GM6 ELISA was not strain or isolate specific, sera from distinct geographic regions were tested. This is especially significant in the case of *T. vivax*, since the TvGM6 ELISA gave similar results for experimental infections conducted with strains originating from Burkina Faso, Nigeria, Uganda and Mozambique.

Based on the experimental infections, it is clear that the antibody response against TvGM6 decreases to baseline approximately one month after treatment. This could imply that certain field sera which tested negative on PCR were positive on the GM6 indirect ELISA due to persistence of antibodies after treatment. Previous studies have shown that antibodies against the WTL can persist for 10–13 months post treatment [44,45]. However, Authié et al. (1993) indicated that although animals treated 10 months previously tested positive in a WTL ELISA, a western blot with the WTL indicated that only antibodies recognising a few specific antigens were still present. Given that the GM6 is a relatively minor, insoluble antigen, it is probable that a certain level of parasitaemia is necessary to stimulate a B-cell response. In the absence of this stimulation, when the parasitaemia drops beneath the necessary parasite load, the antibody response is short-lived.

Experimental infections showed that the antibody response to the TvGM6 was detected at the earliest, 10 days post

infection, during which period the PCR results were likely to be positive. Therefore, the TvGM6 indirect ELISA may show false negatives for animals which have been recently (less than 10 days) infected. The onset of anaemia, the most prevalent clinical sign, occurs at approximately at the same time as the emergence of detectable parasitaemia (1-3 weeks depending on infecting strain, infective dose and host genetics) [5]. Therefore, the detection of infection using the TvGM6 ELISA would be similar to the clinical pre-patent period. It was found that the TvGM6 ELISA has a similar sensitivity to the WTL ELISA later than 10 days post infection, but the antibody response to TvGM6 decreased less than one month post treatment, whereas the antibodies against the WTL tended to persist for a longer period.

The case of the TvGM6 antigen cross-reacting with *T. congolense*-infected sera is probably due to the few regions which are sufficiently conserved to provide common epitopes between the two species. However, the cross-reaction detected is lower than with the homologous antigen. Furthermore, this cross-reaction of the TvGM6 ELISA with *T. congolense*-infected sera indicates that the TvGM6 ELISA alone cannot be used for species-specific diagnosis. However, since the GM6 ELISA was consistently stronger when the homologous antigen was used, testing sera with both the TcoGM6 ELISA and TvGM6 ELISA would allow a relative response to be measured and, therefore, tentative diagnosis of the trypanosome species.

TcoGM6 gave inconsistent results when tested with sera from experimental *T. congolense* infections, and subsequently did not always detect early infection. However, the antibody response did increase following several waves of parasitaemia which may imply that the TcoGM6 would only detect secondary or re-current infections. Although the mechanism responsible is unknown, this phenomenon has already been encountered with the HSP70-based indirect ELISA and inhibition ELISA [46,47].

In summary, the TvGM6 ELISA allowed reliable detection of antibody response around 10-20 DPI with *T. vivax* infections, and decreased below the threshold less than one month following treatment, to date, the best characteristic of a trypanosome indirect ELISA. These results indicate the TvGM6 ELISA would essentially allow detection of active *T. vivax* infection, making it ideal for a point-of-treatment diagnostic tool. This would be of particular significance in South America, where a high proportion of the cattle are infected but display no symptoms [48]. Given that the TvGM6 ELISA does not detect infections one month post failed treatment prior to relapse (when the parasite is not detectable in the blood), it is likely that the test would not be positive for the apparent silent infections in South America and this would allow the discrimination of only active infections requiring treatment. However, due to the fairly rapid antibody decay post treatment, the TvGM6 ELISA would not be suitable as a test for exposure to *T. vivax*. Furthermore, the TvGM6 ELISA detected a failure of treatment at least 15 days prior to the buffy coat method. Finally, the TvGM6 indirect ELISA showed high sensitivity and specificity values in field infections for *T. vivax* infections and slightly lower values for *T. congolense* infection.

For *T. congolense*, it was noted that the TvGM6 ELISA worked better in field conditions than experimental infections,

possibly due to the fact that only secondary or recurrent infections are detected using this test. This hypothesis still needs to be confirmed. As mentioned previously, the ultimate goal would be to incorporate antigens from both *T. congolense* and *T. vivax* into a pan-trypanosome point-of-treatment diagnostic tool. To this end, the data presented in this study motivate that the TvGM6 would be a good antigen for the detection of *T. vivax* infections, but not necessarily for *T. congolense* infections. For detection of *T. evansi*, it was shown that the *T. brucei* GM6 did not give sufficiently high specificity (approximately one-third) [38], therefore, it is unlikely that the *T. vivax* or *T. congolense* GM6 antigens would perform better. A difficulty with the field sera was first to determine which method to use as a reference since the different sera collections had been characterised using different techniques (either ITS-PCR, 18s PCR, whole trypanosome lysate ELISA, or buffy coat). Given that these methods are not infallible, comparative sensitivity and specificity values of the TvGm6 ELISA should be interpreted carefully. It is possible that some field infections detected as false negatives with the TvGM6 ELISA were at a very early point in infection, since the experimental infections indicated that TvGM6 ELISA showed a very low sensitivity less than 10 days post infection. Conversely, a possible explanation for some false positives with the field sera is that some animals could have been treated less than one month prior to serum collection. Based on the TvGM6 ELISA in experimental infections, these animals would continue to test positive for one month post treatment even if treatment was successful. Although sera from *T. vivax* infections across East and West Africa were analysed during the course of this study, no analysis was done of South American *T. vivax* infections. This analysis would be useful *T. vivax* infection is estimated to be the third most economically important bovine parasitic infection in South America [4].

This study represents the first analysis of the GM6 antigen of *T. vivax* as a diagnostic candidate for AAT, and is the first to test the GM6 antigen with a wide range of both experimental and field *T. vivax*-infected sera from various locations. The data reported here demonstrate the potential of the TvGM6 antigen for the development of a point-of-treatment test for diagnosis of *T. vivax* in cattle. The TvGM6 ELISA could also be used for detection of *T. congolense*, albeit with lower sensitivity.

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Author Contributions

Conceived and designed the experiments: DP AB VC TB. Performed the experiments: DP JI. Analyzed the data: DP JI

References

1. Kristjanson PM, Swallow BM, Rowlands GJ, Kruska RL, Leeuw PND (1999) Measuring the costs of African animal trypanosomosis, the potential benefits of control and returns to research. *Agric Syst* 59: 79-98. doi:10.1016/S0308-521X(98)00086-9.
2. Habila N, Inuwa MH, Aimola IA, Udeh MU, Haruna E (2012) Pathogenic mechanisms of *Trypanosoma evansi* infections. *Res Vet Sci* 93: 13-17. doi:10.1016/j.rvsc.2011.08.011. PubMed: 21940025.
3. Fikru R, Goddeeris BM, Deleespaux V, Moti Y, Tadesse A et al. (2012) Widespread occurrence of *Trypanosoma vivax* in bovines of tsetse- as well as non-tsetse-infested regions of Ethiopia: A reason for concern? *Vet Parasitol* 190: 355-361. doi:10.1016/j.vetpar.2012.07.010. PubMed: 22858227.
4. Osorio AL, Madruga CR, Desquesnes M, Soares CO, Ribeiro LR et al. (2008) *Trypanosoma* (Duttonella) *vivax*: its biology, epidemiology, pathogenesis, and introduction in the New World - a review. *Mem Inst Oswaldo Cruz* 103: 1-13. PubMed: 18368231.
5. Taylor KA, Authie E (2004) Pathogenesis of African Trypanosomiasis. In: I MaudlinPH HolmesMA Miles. *The Trypanosomiases*. Wallingford: CAB International. pp. 331-353.
6. Sullivan L, Wall SJ, Carrington M, Ferguson MA (2013) Proteomic selection of immunodiagnostic antigens for human African trypanosomiasis and generation of a prototype lateral flow immunodiagnostic device. *PLoS Negl Trop. Drosophila Inf Serv* 7: e2087.
7. Büscher P, Gilman Q, Lejon V (2013) Rapid diagnostic test for sleeping sickness. *N Engl J Med* 368: 1069-1070. doi:10.1056/NEJMc1210373. PubMed: 23484849.
8. Woo PT (1970) The haematocrit centrifuge technique for the diagnosis of African trypanosomiasis. *Acta Trop* 27: 384-386. PubMed: 4396363.
9. Nantulya VM (1990) Trypanosomiasis in domestic animals: the problems of diagnosis. *Rev Sci Tech* 9: 357-367. PubMed: 2132685.
10. Luckins AG, Mehlitz D (1978) Evaluation of an indirect fluorescent antibody test, enzyme-linked immunosorbent assay and quantification of immunoglobulins in the diagnosis of bovine trypanosomiasis. *Trop Anim Health Prod* 10: 149-159. doi:10.1007/BF02235328. PubMed: 360539.
11. Luckins AG (1977) Detection of antibodies in trypanosome-infected cattle by means of a microplate enzyme-linked immunosorbent assay. *Trop Anim Health Prod* 9: 53-62. doi:10.1007/BF02297393. PubMed: 333677.
12. Ijagbone IF, Staak C, Reinhard R (1989) Fractionation of trypanosome antigens for species-specific sero-diagnosis. *Vet Parasitol* 32: 293-299. doi:10.1016/0304-4017(89)90040-X. PubMed: 2506689.
13. Eisler MC, Lessard P, Masake RA, Moloo SK, Peregrine AS (1998) Sensitivity and specificity of antigen-capture ELISAs for diagnosis of *Trypanosoma congolense* and *Trypanosoma vivax* infections in cattle. *Vet Parasitol* 79: 187-201. doi:10.1016/S0304-4017(98)00173-3. PubMed: 9823059.
14. Rebeski DE, Winger EM, Van Rooij EM, Schöchl R, Schuller W et al. (1999) Pitfalls in the application of enzyme-linked immunoassays for the detection of circulating trypanosomal antigens in serum samples. *Parasitol Res* 85: 550-556. doi:10.2307/3285794. PubMed: 10382604.
15. Müller N, Hemphill A, Imboden M, Duvallet G, Dwinger RH et al. (1992) Identification and characterization of two repetitive non-variable antigens from African trypanosomes which are recognized early during infection. *Parasitology* 104: 1: 111-120. doi:10.1017/S0031182000060856. PubMed: 1614728.
16. Ersfeld K, Barraclough H, Gull K (2005) Evolutionary relationships and protein domain architecture in an expanded calpain superfamily in kinetoplastid parasites. *J Mol Evol* 61: 742-757. doi:10.1007/s00239-004-0272-8. PubMed: 16315106.
17. Magona JW, Walubengo J, Odimini JT (2008) Acute haemorrhagic syndrome of bovine trypanosomosis in Uganda. *Acta Trop* 107: 186-191. doi:10.1016/j.actatropica.2008.05.019. PubMed: 18599006.
18. McDermott JJ, Kristjanson PM, Kruska RL, Reid RS, Robinson TP et al. (2002) Effects of Climate, Human Population and Socio-economic Changes on Tsetse-transmitted Trypanosomiasis to 2050. In: SJ BlackJR Seed. *The African Trypanosomes*. New York: Kluwer Publishing House Academic Publishers. pp. 25-38.
19. Pagabeleguem S, Sangaré M, Bengaly Z, Akoudjin M, Belem AM et al. (2012) Climate, cattle rearing systems and African Animal Trypanosomosis risk in Burkina Faso. *PLOS ONE* 7: e49762. doi:10.1371/journal.pone.0049762. PubMed: 23166765.
20. Fasogbon AI, Knowles G, Gardiner PR (1990) A comparison of the isoenzymes of *Trypanosoma* (Duttonella) *vivax* isolates from East and West Africa. *Int J Parasitol* 20: 389-394. doi:10.1016/0020-7519(90)90156-H. PubMed: 2358323.
21. Hamilton PB (2012) Is *Trypanosoma vivax* genetically diverse? *Trends Parasitol* 28: 173. doi:10.1016/j.pt.2012.02.003. PubMed: 22459431.
22. Cortez AP, Ventura RM, Rodrigues AC, Batista JS, Paiva F et al. (2006) The taxonomic and phylogenetic relationships of *Trypanosoma vivax* from South America and Africa. *Parasitology* 133: 159-169. doi:10.1017/S0031182006000254. PubMed: 16650339.
23. Rodrigues AC, Neves L, Garcia HA, Viola LB, Marcili A et al. (2008) Phylogenetic analysis of *Trypanosoma vivax* supports the separation of South American/West African from East African isolates and a new *T. vivax*-like genotype infecting a nyala antelope from Mozambique. *Parasitology* 135: 1317-1328. PubMed: 18752705.
24. Adams ER, Hamilton PB, Rodrigues AC, Malele II, Deleespaux V et al. (2010) New *Trypanosoma* (Duttonella) *vivax* genotypes from tsetse flies in East Africa. *Parasitology* 137: 641-650. doi:10.1017/S0031182009991508. PubMed: 19961657.
25. Morrison LJ, McLellan S, Sweeney L, Chan CN, MacLeod A et al. (2010) Role for parasite genetic diversity in differential host responses to *Trypanosoma brucei* infection. *Infect Immun* 78: 1096-1108. doi:10.1128/IAI.00943-09. PubMed: 20086091.
26. Leeflang P, Buys J, Blotkamp C (1976) Studies on *Trypanosoma vivax*: infectivity and serial maintenance of natural bovine isolates in mice. *Int J Parasitol* 6: 413-417. doi:10.1016/0020-7519(76)90027-8. PubMed: 965146.
27. Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 72: 248-254. doi:10.1016/0003-2697(76)90527-3. PubMed: 942051.
28. Mamoudou A, Zoli A, Tanenbe C, Andrikaye JP, Bourdanne et al. (2006) Evaluation sur le terrain et sur souris de la résistance des trypanosomes des bovins du plateau de l'Adamaoua au Cameroun à l'acéturate de diminazène et au chlorure d'isométabamidium. *Rev Elev Méd Vét Pays Trop* 59: 11-16.
29. Gibson W (2012) The origins of the trypanosome genome strains *Trypanosoma brucei brucei* TREU 927, *T. b. gambiense* DAL 972, *T. vivax* Y486 and *T. congolense* IL3000. *Parasit Vectors* 5: 71.
30. Murray M (1983) Livestock productivity and trypanotolerance: Network Training Manual; JCM TrailDA TurnerY Wissocq. Addis Ababa: ILCA.
31. Seck MT, Bouyer J, Sall B, Bengaly Z, Vreyen MJ (2010) The prevalence of African animal trypanosomoses and tsetse presence in Western Senegal. *Parasite* 17: 257-265. doi:10.1051/parasite/2010173257. PubMed: 21073148.
32. Coustou V, Guegan F, Plazolles N, Baltz T (2010) Complete in vitro life cycle of *Trypanosoma congolense*: development of genetic tools. *PLoS Negl Trop. Drosophila Inf Serv* 4: e618.
33. Baltz T, Baltz D, Giroud C, Crockett J (1985) Cultivation in a semi-defined medium of animal infective forms of *Trypanosoma brucei*, *T. equiperdum*, *T. evansi*, *T. rhodesiense* and *T. gambiense*. *Embo J* 4: 1273-1277.
34. D'Archivio S, Medina M, Cosson A, Chamond N, Rotureau B et al. (2011) Genetic engineering of *Trypanosoma* (Duttonella) *vivax* and in vitro differentiation under axenic conditions. *PLoS Negl Trop. Drosophila Inf Serv* 5: e1461.
35. Lanham SM, Godfrey DG (1970) Isolation of salivarian trypanosomes from man and other mammals using DEAE-cellulose. *Exp Parasitol* 28: 521-534. doi:10.1016/0014-4894(70)90120-7. PubMed: 4993889.
36. Wright PF, Nilsson E, Van Rooij EM, Lelement M, Jeggo MH (1993) Standardisation and validation of enzyme-linked immunosorbent assay techniques for the detection of antibody in infectious disease diagnosis. *Rev Sci Tech* 12: 435-450. PubMed: 8400384.
37. Auty H, Anderson NE, Picozzi K, Lembo T, Mubanga J et al. (2012) Trypanosome diversity in wildlife species from the serengeti and

- luangwa valley ecosystems. PLoS Negl Trop. Drosophila Inf Serv 6: e1828.
38. Smuts CM (2009) Development of tools to improve the detection of *Trypanosoma evansi* in Australia [PhD Thesis]. Perth: Murdoch University.
 39. Thuy NT, Goto Y, Lun ZR, Kawazu SI, Inoue N (2011) Tandem repeat protein as potential diagnostic antigen for *Trypanosoma evansi* infection. Parasitol Res 110: 733-739. PubMed: 21927872.
 40. Goto Y, Carter D, Guderian J, Inoue N, Kawazu S et al. (2010) Upregulated expression of B-cell antigen family tandem repeat proteins by *Leishmania* amastigotes. Infect Immun 78: 2138-2145. doi:10.1128/IAI.01102-09. PubMed: 20160013.
 41. Coppel RL, Cowman AF, Anders RF, Bianco AE, Saint RB et al. (1984) Immune sera recognize on erythrocytes *Plasmodium falciparum* antigen composed of repeated amino acid sequences. Nature 310: 789-792. doi:10.1038/310789a0. PubMed: 6382025.
 42. Gruber A, Zingales B (1993) *Trypanosoma cruzi*: characterization of two recombinant antigens with potential application in the diagnosis of Chagas' disease. Exp Parasitol 76: 1-12. doi:10.1006/expr.1993.1001. PubMed: 8467895.
 43. Bhatia A, Daifalla NS, Jen S, Badaro R, Reed SG et al. (1999) Cloning, characterization and serological evaluation of K9 and K26: two related hydrophilic antigens of *Leishmania chagasi*. Mol Biochem Parasitol 102: 249-261. doi:10.1016/S0166-6851(99)00098-5. PubMed: 10498181.
 44. Van den Bossche P, Chigoma D, Shumba W (2000) The decline of anti-trypanosomal antibody levels in cattle after treatment with trypanocidal drugs and in the absence of tsetse challenge. Acta Trop 77: 263-270. doi:10.1016/S0001-706X(00)00138-8. PubMed: 11114388.
 45. Authié E, Muteti DK, Williams DJ (1993) Antibody responses to invariant antigens of *Trypanosoma congolense* in cattle of differing susceptibility to trypanosomiasis. Parasite Immunol 15: 101-111. doi:10.1111/j.1365-3024.1993.tb00589.x. PubMed: 8446463.
 46. Bossard G, Boulange A, Holzmüller P, Thévenon S, Patrel D et al. (2010) Serodiagnosis of bovine trypanosomosis based on HSP70/BIP inhibition ELISA. Vet Parasitol 173: 39-47. doi:10.1016/j.vetpar.2010.06.016. PubMed: 20637547.
 47. Boulangé A, Katende J, Authié E (2002) *Trypanosoma congolense*: expression of a heat shock protein 70 and initial evaluation as a diagnostic antigen for bovine trypanosomosis. Exp Parasitol 100: 6-11. doi:10.1006/expr.2001.4667. PubMed: 11971648.
 48. Desquesnes M (2004) Livestock trypanosomes and their vectors in Latin America. Paris: OIE (World Organisation for animal health)