b|e|i RESOURCES
SUPPORTING INFECTIOUS DISEASE RESEARCH

Certificate of Analysis for MRA-1000

Plasmodium falciparum, Strain NF54 (Patient Line E)

Catalog No. MRA-1000

Product Description: The E line of NF54 stock parasites was amplified in a volunteer patient "E", who participated in a clinical trial in February 1995 at Walter Reed Army Institute of Research (WRAIR). The parent NF54 strain of *Plasmodium falciparum* (*P. falciparum*) was isolated from a patient living near an airport in the Netherlands, who had never left the country.

Lot[1]: 63834916 **Manufacturing Date: 25OCT2015**

TEST	SPECIFICATIONS	RESULTS
Identification by Giemsa Stain Microscopy[2,3]	Blood-stage parasites present	Blood-stage parasites present
Antimalarial Susceptibility Profile (*in vitro*)[2]		
Half-maximal Inhibitory Concentration (IC_{50}) by		
SYBR green I® drug sensitivity assay[4]		
Chloroquine	Report results	7.9 ± 0.4 nM
Artemisinin	Report results	4.4 ± 0.3 nM
Quinine	Report results	77.8 ± 10.8 nM
Cycloguanil	Report results	13.5 ± 0.6 nM
Pyrimethamine	Report results	65.1 ± 9.0 nM
Sulfadoxine	Report results	216400 ± 50269 nM
Genotypic Analysis[2]		
Sequencing of Merozoite Surface Protein 2 (MSP2) gene (~ 750 base pairs)	≥ 99% sequence identity to *P. falciparum*, strain NF54 (GenBank: AMYQ01000292)	100% sequence identity to *P. falciparum*, strain NF54 GenBank: AMYQ01000292) (Figure 1)
Functional Activity by PCR Amplification[2]		
MSP2 PCR amplicon analysis[5]	~ 600 to 900 base pair amplicon	~ 900 base pair amplicon
Phenotypic Analysis		
Gametocyte production[6]	Positive	Positive (Figure 2)
Level of Parasitemia		
Pre-freeze[7]	Report results	3.44%
Post-freeze[8]	> 1%	3.78%
Viability[2,9]	Growth in infected red blood cells	Growth in infected red blood cells
Sterility (21-day incubation)[2]		
Harpo's HTYE broth[10], 37°C and 26°C, aerobic	No growth	No growth
Tryptic Soy broth, 37°C and 26°C, aerobic	No growth	No growth
Sabouraud Dextrose broth, 37°C and 26°C, aerobic	No growth	No growth
DMEM with 10% FBS, 37°C, aerobic	No growth	No growth
Sheep Blood agar, 37°C, aerobic	No growth	No growth
Sheep Blood agar, 37°C, anaerobic	No growth	No growth
Thioglycollate broth, 37°C, anaerobic	No growth	No growth
Mycoplasma Contamination[2]		
DNA Detection by PCR	None detected	None detected

[1]MRA-1000 was produced by cultivation of MR-MRA-1000 lot 58607195 in fresh human erythrocytes in RPMI 1640 medium, adjusted to contain 10% (v/v) heat-inactivated human serum (pooled Type A), 25 mM HEPES, 2 mM L-glutamine, 4 g/L D-glucose, 0.005 µg/mL hypoxanthine and 2.5 µg/mL gentamicin. The culture was incubated at 37°C in sealed flasks outgassed with blood-gas atmosphere (90% N_2, 5% CO_2, 5% O_2) and monitored for parasitemia daily for 9 days. Every 1 to 3 days, uninfected, leukocyte filtered, Type O erythrocytes in complete culture medium were added dropwise to the culture to maintain 2% hematocrit.

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[2]Testing completed on vialed post-freeze material.
[3]Blood-stage malaria parasites (rings, trophozoites, schizonts +/- gametocytes) were examined by microscopic Giemsa-stained blood smears of an *in vitro* human blood culture over 5 days.
[4]A SYBR Green I® anti-malarial drug sensitivity assay in 96-well plates was used to determine IC_{50} values of an active (> 70% ring stage) parasite culture in the presence of each antimalarial drug [Hartwig, C. L., et al. "XI: I. SYBR Green I®-Based Parasite Growth Inhibition Assay for Measurement of Antimalarial Drug Susceptibility in *Plasmodium falciparum*." In Methods in Malaria Research Sixth Edition. (2013) Moll, K., et al. (Ed.), EVIMalaR, pp. 122-129. Available at: https://www.mr4.org/Publications/MethodsinMalariaResearch.aspx].
[5]Primer sequences and conditions for PCR are available upon request.
[6]Gametocyte production was measured using an Olympus microscope at 1000x magnification.
[7]Pre-freeze parasitemia was determined after 9 days post infection by microscopic counts of Giemsa-stained blood smears.
[8]Post-freeze parasitemia was determined after 5 days post infection by microscopic counts of Giemsa-stained blood smears.
[9]Viability was confirmed by examination of infected erythrocytes for parasitemia at 5 days post infection.
[10]Atlas, Ronald M. Handbook of Microbiological Media. 3rd ed. Ed. Lawrence C. Parks. Boca Raton: CRC Press, 2004, p. 798.

Figure 1: MRA-1000 MSP2 Sequence

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TATTATAAAT TTCTTTATTT TTGTTACCTT TAATATTAAA AATGAAAGTA AATATAGCAA CACATTCATA AACAATGCTT
ATAATATGAG TATAAGGAGA AGTATGGCAG AAAGTAAGCC TTCTACTGGT GCTGGTGGTA GTGCTGGTGG TAGTGCTGGT
GGTAGTGCTG GTGGTAGTGC TGGTGGTAGT GCTGGTGGTA GTGCTGGTTC TGGTGATGGT AATGGTGCAG ATGCTGAGGG
AAGTTCAAGT ACTCCCGCTA CTACCACAAC TACCAAAACT ACCACAACTA CCACAACTAC TAATGATGCA GAAGCATCTA
CCAGTACCTC TTCAGAAAAT CCAAATCATA AAAATGCCGA AACAAATCCA AAAGGTAAAG GAGAAGTTCA AGAACCAAAT
CAAGCAAATA AAGAAACTCA AAATAACTCA AATGTTCAAC AAGACTCTCA AACTAAATCA AATGTTCCAC CCACTCAAGA
TGCAGACACT AAAAGTCCTA CTGCACAACC TGAACAAGCT GAAAATTCTG CTCCAACAGC CGAACAAACT GAATCCCCCG
AATTACAATC TGCACCAGAG AATAAAGGTA CAGGACAACA TGGACATATG CATGGTTCTA GAAATAATCA TCCACAAAAT
ACTTCTGATA GTCAAAAAGA ATGTACCGAT GGTAACAAAG AAAACTGTGG AGCAGCAACA TCCCTCTTAA ATAACTCTAG
TAATATTGCT TCAATAAATA AATTTGTTGT TT
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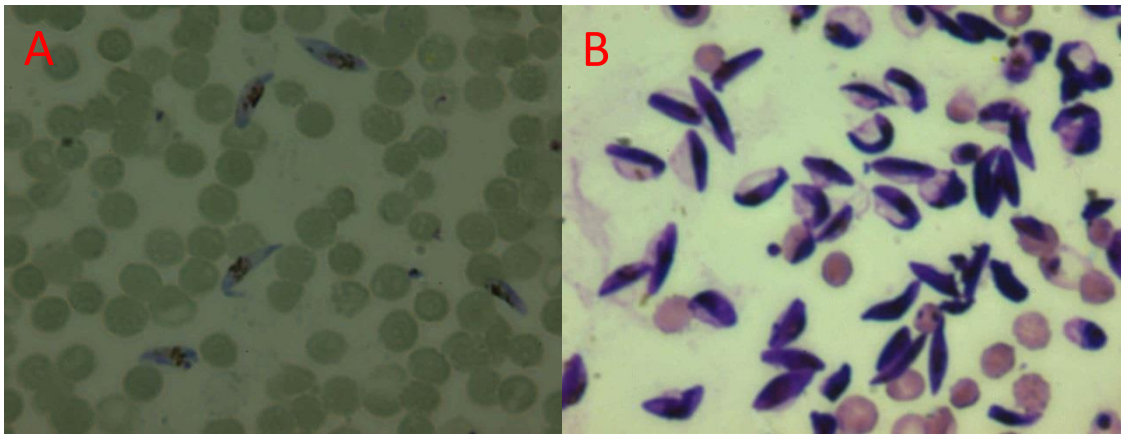
Figure 2: Gametocyte Production by MRA-1000



A. Late stage gametocytes from *in vitro* culture on day 16
B. Day 16 gametocyte culture purified using MACS magnetic column

/Heather Couch/
Heather Couch 03 JAN 2019
Program Manager or designee, ATCC Federal Solutions

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