

**Supplemental Table 1. Samples per patient.**

| Patient ID | Clinical condition | Isolate ID | Isolate LRV1 Status | Biopsy ID | Biopsy LRV1 Status |
|------------|--------------------|------------|---------------------|-----------|--------------------|
| 1          | CLTF               | 2          | Positive            | NS        | -                  |
| 2          | CLTF               | 11         | Negative            | B11       | Positive           |
| 3          | CLTF               | 18         | Positive            | NS        | -                  |
| 4          | CLTF               | 41         | Negative            | B41       | Negative           |
| 5          | CLTF               | 8          | Negative            | B8        | Positive           |
| 6          | CLTF               | 9          | Negative            | B9        | Positive           |
| 7          | CLTF               | 26         | Negative            | NS        | -                  |
| 8          | CLTF               | 44         | Negative            | B44       | Positive           |
| 9          | CLTF               | 36         | Positive            | NS        | -                  |
| 10         | CLTF               | 45         | Positive            | NS        | -                  |
| 11         | CLTF               | 60         | Negative            | B60       | Negative           |
| 12         | CLTF               | 53         | Positive            | NS        | -                  |
| 13         | CLTF               | 61         | Positive            | NS        | -                  |
| 14         | CLTF               | 63         | Negative            | B63       | Negative           |
| 15         | ML                 | 576        | Positive            | M354      | Negative           |
| 16         | ML                 | 707        | Positive            | NS        | -                  |
| 17         | ML                 | 127        | Negative            | NS        | -                  |
| 18         | ML                 | 463        | Negative            | NS        | -                  |
| 19         | ML                 | 384        | Positive            | NS        | -                  |
| 20         | ML                 | 235        | Negative            | NS        | -                  |
| 21         | ML                 | NS         | -                   | M312      | Positive           |
| 22         | ML                 | NS         | -                   | M323      | Negative           |
| 23         | ML                 | NS         | -                   | M326      | Negative           |
| 24         | ML                 | NS         | -                   | M333      | Positive           |
| 25         | ML                 | NS         | -                   | M340      | Negative           |
| 26         | ML                 | NS         | -                   | M343      | Negative           |
| 27         | ML                 | NS         | -                   | M348      | Negative           |
|            |                    |            |                     | M351A     | Negative           |

NS: non available sample  
CLTF: Cutaneous Leishmaniasis with Therapeutic Failure  
ML: Mucosal Leishmaniasis

**Supplemental Table 2.** Primers used in this study and their characteristics.

| ID  | Sequence               | Genome region                | Initial position in the genome (nt) | Final position in the genome (nt) | Amplicon length (pb) | Technique     | Utility               | Sample type | Reference |
|-----|------------------------|------------------------------|-------------------------------------|-----------------------------------|----------------------|---------------|-----------------------|-------------|-----------|
| Fw  | CCAAAGTGTGGAGATCGAAG   | 18S ribosomal gen Leishmania | ~531                                | 551                               | ~150                 | RT-qPCR       | Parasite load         | Isolates    | 36        |
| Rev | GGCCGGTAAAGGCCGAATAG   |                              | ~701                                | ~721                              |                      |               | cDNA quality          | Biopsies    |           |
| Fw  | TBRTWGCRCACAGTGAYGAAGG | ORF2 LRV                     | ~1089                               | 1111                              | ~485                 | RT-PCR        | Viral detection       | Isolates    | 26        |
| Rev | CWACCCARWACCABGGBGCCAT |                              | ~1552                               | 1574                              |                      |               |                       |             |           |
| Fw  | ATGCCTAAGAGTTTGGATTCCG | ORF1 LRV                     | ~16                                 | 37                                | ~245                 | RT-qPCR       | Viral load            | Isolates    | 27        |
| Rev | ACAACCAGACGATTGCTGTG   |                              | ~219                                | 239                               |                      |               |                       |             |           |
| Fw  | CTGACTGGACGGGGGTAAT    | ORF1 LRV                     | ~153                                | 173                               | ~125                 | Nested RT-PCR | Viral detection       | Biopsies    | 24        |
| Rev | CAAACACTCCCTTACGC      |                              | ~259                                | 277                               |                      |               |                       |             |           |
| Fw  | GGTAATCGAGTGGGAGTCC    | ORF1 LRV                     | ~167                                | 186                               | ~90                  | RT-qPCR       | Viral load            | Isolates    | 24        |
| Rev | GCGGCAGTAACCTGG        |                              | ~240                                | 255                               |                      | Nested RT-PCR | Viral detection       | Biopsies    |           |
| Fw  | GACGGTGCCTGCCTACTTCAA  | Hsp70 gen                    | ~1009                               | 1029                              | 1,422                | Nested PCR    | Specie identification | Isolates    | 37, 38    |
| Rev | CCGCCATGCTCTGGTACATC   |                              | ~2410                               | 2410                              |                      |               |                       | Biopsies    |           |
| Fw  | ACTTCAACGACTCGCAGCGCCA | Hsp70 gen                    | ~1022                               | 1044                              | 1,378                | Nested PCR    | Specie identification | Biopsies    | 39        |
| Rev | ATCGGGTTGCATGTGCTCTCCA |                              | ~2379                               | 2401                              |                      |               |                       |             |           |

**Supplemental Table 3.** Evaluation the performance of RT-qPCR used.

| <b>Target</b>  | <b>18S</b> | <b>ORF1 LRV<br/>Ito et al</b> | <b>ORF1 LRV<br/>Ramos et al</b> |
|----------------|------------|-------------------------------|---------------------------------|
| Slope          | -3.5       | -3.3                          | -3.3                            |
| Y-Intercept    | 26.24      | 37.62                         | 37.46                           |
| R <sup>2</sup> | 0.99       | 0.93                          | 0.95                            |
| Efficiency (%) | 91.1       | 100.4                         | 100.8                           |

**Supplemental Table 4. Calculation of viral load by interpolating from the PCR amplification curve of the plasmid.**

| Instruction  | Calculation   |
|--|---|
| <p>The total plasmid length in base pairs was obtained by adding the length of the PCR™4-TOPO vector (3956 bp) and the ORF1 LRV amplified fragment (420 bp) <b>(1)</b>.</p>  | $3,956 \text{ pb} + 420 \text{ pb} = \mathbf{4,376 \text{ pb (1)}}$   |
| <p>The total plasmid weight in Daltons (g/mol) was calculated by multiplying the number of total bases by 650 Da (Average weight of a DNA base pair) <b>(2)</b>.</p>   | $4,376 \text{ pb} \times 650 \text{ Da} = 2.84 \times 10^6 \text{ g/mol (2)}$ $\mathbf{1 \text{ plasmid mol} = 2.84 \times 10^6 \text{ g}}$   |
| <p>The molecules number (plasmid copies)/g was calculated using the Avogadro number and the plasmid concentration <b>(3-4)</b>. After that, first dilution was made in 1,000 times, subsequently a serial dilution was followed 10 times per round, ranging from <math>6.6 \times 10^7</math> to 66 molecules.</p> | $1 \text{ plasmid mol} = 6.022045 \times 10^{23} \text{ molecules (3)}$ $\frac{2.84 \times 10^6 \text{ g}}{1 \text{ mol}} \left( \frac{1 \text{ mol}}{6.022045 \times 10^{23} \text{ molecules}} \right) = \frac{2.84 \times 10^6 \text{ g}}{6.022045 \times 10^{23} \text{ molecules}} =$ $\mathbf{4.71 \times 10^{-18} \text{ g/molecules}}$ $4.71 \times 10^{-18} \text{ g/molecules} = 4.57 \times 10^{-9} \text{ ng/molecules}$ $\text{Plasmid concentration} = 301.3 \text{ ng/}\mu\text{l (4)}$ $\frac{1 \text{ molecule}}{4.57 \times 10^9 \text{ ng}} \left( \frac{301.3 \text{ ng}}{1 \mu\text{l}} \right) =$ $\mathbf{6.6 \times 10^{10} \text{ molecules/}\mu\text{l}}$ |