LCMV Viral Load Quantification by GP-Based SYBR Green qPCR

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Purpose

This protocol describes a quantitative PCR (qPCR) assay for measuring lymphocytic choriomeningitis virus (LCMV) RNA in mouse serum and tissues, adapted from McCausland and Crotty, J Virol Methods 2008. The assay uses gene-specific reverse transcription with SuperScript III and SYBR Green qPCR targeting the GP segment, which provides improved specificity and sensitivity compared with NP-based assays.

⚠ LCMV biosafety and handling

LCMV is a zoonotic arenavirus capable of causing severe disease in humans, including meningitis and congenital infection. Work with live LCMV must:

- Be conducted under the appropriate institutional **biosafety level (typically BSL-2 with additional local conditions)** and approved animal and biosafety protocols.
- Use **dedicated infectious CO incubators** and clearly labelled containment for all cultures and waste.
- Include suitable **PPE** (lab coat/gown, gloves, eye protection as appropriate) and measures to avoid sharps injuries, aerosol formation, and spills.
- Exclude or take special precautions for **pregnant or immunocompromised personnel** according to institutional policies.

All waste (liquid and solid) must be inactivated according to local regulations (e.g. chemical disinfection and/or autoclaving) **before** disposal.

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Principle

- Total RNA is isolated from serum or homogenized tissue.
- cDNA is generated using SuperScript III and a gene-specific primer (GP-R).
- qPCR is performed with SYBR Green detection using GP-specific primers (GP-F and GP-R).
- Absolute viral RNA copy numbers are calculated from a plasmid standard curve containing the LCMV GP gene (e.g. pSG5-GP).

Sample Types

- Serum (typically 50 uL per sample).
- Tissue homogenates:
  - Spleen: 10 mg
  - Liver: 50 mg
  - Kidney: 50 mg
  - Brain: 50 mg
  - Lymph node: 5 mg

All values are taken from the Methods section of McCausland & Crotty. Samples were snap-frozen on dry ice at harvest and stored at -80 C until RNA extraction.

Reagents and Consumables

- LCMV-infected mouse tissues or serum.
- RNAqueous Mini Kit (Ambion) or equivalent silica spin-column RNA kit.
- SuperScript III Reverse Transcriptase (Invitrogen).
- iTaq SYBR Green Supermix with ROX (Bio-Rad) or similar SYBR Green master mix with ROX passive reference.
- Gene-specific primers (see below).
- Plasmid standard containing the LCMV GP gene (e.g. pSG5-GP).
- Nuclease-free water.
- 1.5 mL microcentrifuge tubes.
- 0.2 mL PCR tubes or 96-well qPCR plates with optical seals.

Equipment

- Tissue homogenizer (e.g. Tissuemiser or similar rotor-stator homogenizer).
- Microcentrifuge (refrigerated).
- Class II biosafety cabinet or laminar flow hood.
- qPCR instrument capable of SYBR Green detection (e.g. ABI GeneAmp 5700 or equivalent).
- Standard laboratory pipettes and filtered tips.
- -80 C freezer.

Primers

All primer sequences are taken from the "LCMV viral load quantitative PCR (QPCR)" Methods section.

Recommended **GP primers (optimized set)**:

- GP-F (S 877–901):
  `CATTCACCTGGACTTTGTCAGACTC`
- GP-R (S 970–991):
  `GCAACTGCTGTGTTCCCGAAAC`

Alternative NP-based primer sets (historical, not recommended for new assays):

- NP2-F (S 2601–2623):
  `CAGAAATGTTGATGCTGGACTGC`
- NP2-R (S 2697–2720):
  `CAGACCTTGGCTTGCTTTACACAG`

Use GP-F and GP-R for all new experiments unless you have a specific reason to reproduce NP-based data.

Primer Stocks

- Prepare 100 uM stocks of GP-F and GP-R in nuclease-free water.
- Prepare 10 uM working stocks for routine use.

Sample Collection

1. **Serum**
   - Collect whole blood via retro-orbital bleed under appropriate anesthesia (e.g. isoflurane).
   - Use heparinized capillary tubes.
   - Transfer to 1.5 mL tubes and centrifuge 20 min at 12,000 g, 4 C.
   - Aliquot serum (minimum 50 uL) and store at -80 C.
2. **Tissues**
   - Harvest tissues with sterile instruments.
   - Wipe instruments with 70 percent ethanol between mice and always process uninfected controls first.
   - For qPCR, immediately snap-freeze small tissue pieces on dry ice.
   - Store at -80 C.

RNA Isolation

Based on RNAqueous Mini Kit usage:

1. Thaw serum or tissue on ice.
2. For serum:
    - Use 50 uL serum directly in the kit lysis buffer according to manufacturer instructions.
3. For tissues:
    - Weigh 5–50 mg tissue into appropriate tubes.
    - Add lysis buffer from the RNAqueous kit.
    - Homogenize thoroughly with a rotor-stator homogenizer.
4. **Critical decontamination step for homogenizer** between samples:
    - Wash once with PBS.
    - Wash once with 10 percent bleach.
    - Wash once again with PBS.
    - Do not rely on ethanol + PBS alone; this was reported as insufficient to prevent cross-contamination.
5. Proceed with the RNAqueous spin-column protocol.
6. Elute RNA in **20 uL** nuclease-free water.
7. Store RNA at -80 C until use.

cDNA Synthesis (Reverse Transcription)

Using SuperScript III and gene-specific priming:

1. For each sample, set up a **20 uL** RT reaction containing:
    - 10 uL RNA (from the 20 uL eluate).
    - 1 gene-specific primer (usually GP-R at 0.5–1 uM final).
    - dNTPs (as supplied or 0.5 mM each).
    - Reaction buffer and DTT per SuperScript III instructions.
    - SuperScript III Reverse Transcriptase (as per manufacturer recommendation, often 200 U per reaction).
2. Incubate:
    - 55 C for 60 min (50 C can also be used but 55 C is preferred in the paper for specificity).
    - Then inactivate at 70 C for 15 min or according to kit instructions.
3. Include:
    - At least two tissue-matched RNA samples from uninfected mice (negative controls).
    - No-template control (water instead of RNA).
4. Store cDNA at -20 C if not used immediately.

qPCR Reaction Setup

Standard reaction (25 uL total volume):

- 5 uL cDNA template.
- 12.5 uL iTaq SYBR Green Supermix with ROX (2x).
- 0.5 uL GP-F (10 uM; final 0.2 uM).
- 0.5 uL GP-R (10 uM; final 0.2 uM).
- 6.5 uL nuclease-free water.

Notes: - 50 uL reactions can also be used; scale reagents proportionally. - Prepare a master mix for all wells to minimise pipetting error. - Run all samples in **duplicate**.

qPCR Cycling Conditions

On ABI GeneAmp 5700 or similar:

- Initial denaturation: 95 C, 3 min (or per master mix recommendations).
- 40 cycles of:
  - 95 C, 15 s
  - 60 C, 30 s (combined annealing/extension; fluorescence read step)

After amplification, perform a melt-curve analysis to verify a single specific product.

Plasmid Standard Curve

1. Use a plasmid containing the LCMV GP gene (e.g. pSG5-GP).
2. Linearize the plasmid with an appropriate restriction enzyme.
3. Quantify DNA by gel electrophoresis against known DNA standards.
4. Convert mass to copy number using molecular weight.
5. Prepare 10-fold serial dilutions covering the desired range (e.g. 10^7 to 10^0 genome copies per reaction).
6. Aliquot each dilution and store at -80 C (single-use aliquots).
7. In every run:
   - Thaw a fresh aliquot series.
   - Run each standard in duplicate.
8. Generate a Ct vs log10(copy number) standard curve; R^2 should typically be > 0.98.

Data Analysis

1. For each plate, fit a linear regression of Ct versus log10(copy number) using the plasmid standards.
2. For each sample, convert Ct to copy number using the standard curve.
3. Correct for:
   - Fraction of RNA used (10 uL of 20 uL).
   - Volume or mass of starting material.

4. Report viral load as:
    - LCMV RNA copies per mL serum, or
    - LCMV RNA copies per mg tissue.
5. Limit of detection:
    - The assay reproducibly detects approximately 1–5 LCMV genomes per qPCR reaction.
    - In practice, set a Ct cutoff at 35–36 cycles; values above this should be considered below detection limit or flagged as unreliable.
6. Use negative controls (uninfected tissue and no-template wells) to define background; any sample with signal overlapping control levels should be treated as negative.

Controls and QC

Include in every run:

- No-template controls (water instead of cDNA).
- At least two uninfected control tissue samples per tissue type.
- A positive control cDNA sample from a known LCMV-positive tissue.
- Full plasmid standard curve.

Check:

- Single peak in melt curve for all positive wells.
- No amplification in no-template controls within the Ct window used for positivity.

Notes and Tips

- Work in a laminar flow hood where possible; use UV decontamination between runs.
- Handle plasmid standards last and avoid placing them adjacent to unknown samples on the plate.
- For very high viral loads, a 1:200 dilution of cDNA may be required to bring Ct values into the dynamic range of the standard curve.

Safety considerations

- Confirm that all work with LCMV and infected animals/samples is covered by approved **biosafety and animal use protocols**.
- Perform all live-virus manipulations (thawing virus stocks, infection steps, handling of unfixed plates) in a **certified Class II biosafety cabinet**.
- Use **dedicated infectious incubators** and clearly label plates, flasks, and waste containers with the virus strain and date.
- Wear appropriate **PPE** at all times (lab coat/gown, gloves, eye/face protection as required).
- Decontaminate all liquid waste (e.g. with freshly prepared bleach) before disposal. Solid waste (tips, plates, tubes) must be autoclaved or otherwise inactivated according to institutional guidelines.
- OPD and its solutions are handled as **chemical hazardous waste**; follow chemical safety rules in addition to biosafety rules.

- Pregnant or immunocompromised personnel should not work with LCMV unless explicitly allowed under institutional policy with additional safeguards.

Version History

Version	Date	Description
v1.0	2025-12-01	Initial adaptation of McCausland & Crotty GP qPCR assay.

Citation

McCausland MM, Crotty S. Quantitative PCR (QPCR) technique for detecting lymphocytic choriomeningitis virus (LCMV) in vivo. J Virol Methods. 2008;147(1):167-176. doi:10.1016/j.jviromet.2007.08.025.