LCMV Viral Load Quantification by GP-Based SYBR Green qPCR

Dillon Corvino

2025-12-01

Protocol ID: MUS-LCMV-QPCR
Version: v1.1
Author: Dillon Corvino

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.

Table of contents

Purpose 1

Principle 3

Sample Types 3

Reagents and Consumables 3

Equipment 3

Primers 4

Primer Stocks 4

Sample Collection 4

RNA Isolation 5

cDNA Synthesis (Reverse Transcription) 5

qPCR Reaction Setup 6

qPCR Cycling Conditions 6

Plasmid Standard Curve 6

Data Analysis 6

Controls and QC 7

Notes and Tips 7

Buffers Required 7

Safety considerations 8

Version History 8

Citation 8

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.

Buffers Required

Full composition, preparation, storage, and safety information for each buffer lives in its own linked buffer document — only a concise per-buffer summary is reproduced here, generated automatically from that document.

None

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.
v1.1	2026-09-18	Dillon Corvino - Replaced `# Buffers used` with an auto-generated `# Buffers Required` section (one heading + one small table per buffer, sourced from each buffer's own `summary:` data), moved to the correct position after Procedure, and fixed leaked R package-startup warnings.

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.