

Assays for studying Listeria-Containing Vacuoles version 2

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Abstract

Listeria monocytogenes is a bacterial pathogen that enters and proliferates in the cytosol of mammalian cells. Following entry, bacteria disrupt the invasion vacuole and reach the cytoplasm, where they replicate and use the actin cytoskeleton to propel themselves from cell to cell [1, 2]. To achieve this cytosolic lifestyle, *Listeria* deploys virulence effectors that hijack diverse cellular processes [3, 4]. Human epithelial cells grown *in vitro* can be used to study the *L. monocytogenes* infectious process. However, rapid multiplication and dissemination of bacteria may induce cell death and detachment, thereby forming lytic plaques. Thus, *in vitro* infections with *L. monocytogenes* have been restricted to short time courses (usually from a few minutes to one day). In order to study *L. monocytogenes* long-term infections, we have set up a protocol, with several modifications to the gentamicin protection assay previously used for short-term infections with this pathogen [5]. In a subset of human cells, such as hepatocytes or trophoblast cells, this protocol enables to observe and study the entrapment of *L. monocytogenes* within vacuoles, termed “Listeria-Containing Vacuoles” (LisCVs), after the intercellular dissemination phase of *Listeria*.

Here, this protocol describes the assays used to study LisCVs in a JEG3 trophoblast cell monolayer.

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Protocol

Warnings

- Containment level: all manipulations with *L. monocytogenes* must be conducted within a laminar flow class II Biological Safety Cabinet (BSC).
- Personal Protective Equipment (PPE): laboratory coat; gloves; safety glasses. Ensure the PPE is maintained in working order and properly used.
- Disinfection: disinfect all work surfaces and materials both prior to and immediately following all work practices and procedures.
- All operations involving Paraformaldehyde must be carried out in a certified chemical fume hood.