

Application in microbiology/ infectiology:

Isolation of bacterial phagosomes from infected cells

The analysis of isolated phagosomes from cells infected with invasive germs represents a central cell biological method for the analysis of defense mechanisms of cells against infections with invasive bacteria. The HOKImag magnetic chamber system offers an ideal technology for this purpose.

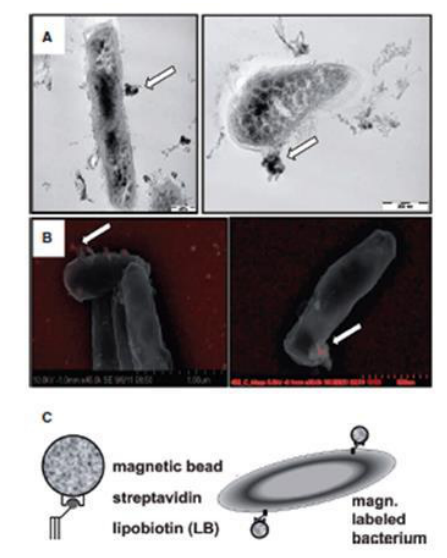
For immunomagnetic isolation of bacterial phagosomes, the germs are coated with lipobiotin and subsequently labeled with streptavidin magnetic microbeads. After infection of host cells, the magnetized germs in the formed intracellular phagosomes can be isolated from the cell homogenates at different time points using HOKImag and biochemically analyzed for proteins involved in the specific host-germ interaction.

Advantages of the "free flow" method with HOKImag:

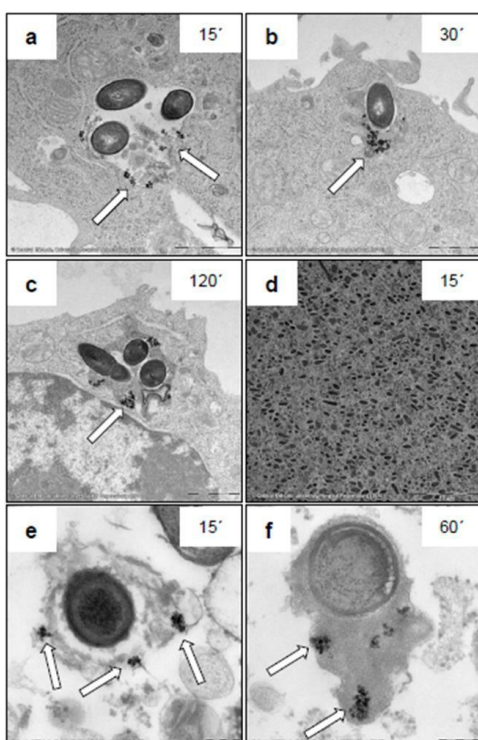
Due to the strong magnetic field, smallest superparamagnetic beads can be used in low concentrations to label the bacteria. Thus, the characteristics of the germ-host cell interaction, the uptake of the germs and the intracellular migration of the magnetized bacterial phagosomes in the cells are not hindered. The isolated phagosomes are morphologically and functionally intact.

Example of the application of the HOKImag magnetic chamber system for the isolation and characterization of phagosomes:

Source: Steinhäuser et al., (2013). Lipid-labeling facilitates a novel magnetic isolation procedure to characterize of pathogen-containing phagosomes. *Traffic*, 14, 321-336



1. Magnetic labeling of bacteria using lipobiotin and streptavidin microbeads.

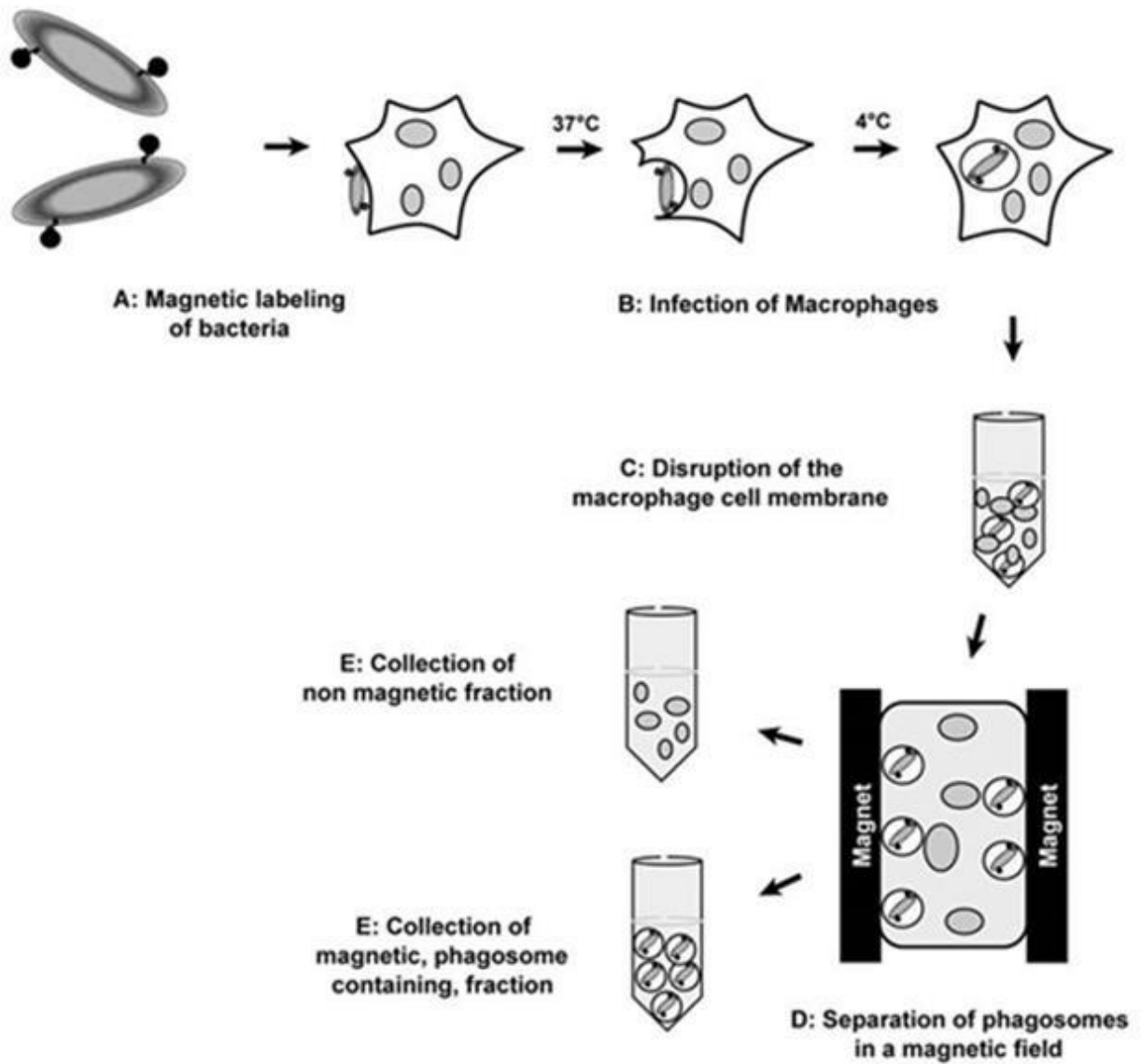


- II. Magnetic isolation of *Listeria monocytogenes*-containing phagosomes from macrophages.

a-c: Infection of macrophages

d-f: Isolated phagosomes

III. Isolation scheme for the separation of bacteria phagosomes from infected cells using the HOKImag magnetic chamber



Some of the results in which the HOKImag magnetic chamber system has found substantial use for the isolation and characterization of phagosomes:

- Steinhäuser, C., Dallenga, T., Tchikov, V., Schaible, U., Schütze, S., Reiling, N. (2014). Immunomagnetic isolation of mycobacteria-containing phagosomes and apoptotic blebs from primary macrophages. *Curr. Protocols Immunol.* 105:14.36.1-14.36.26
- Steinhäuser, C., Heigl, U., Tchikov, V., Schwarz, J., Gutschmann, T., Seeger, K., Fritsch, J., Schroeder, J., Wiesmüller, K.-H., Rosenkrands, I., Pott, J., Krause, E., Ehlers, S., Schneider-Brachert, W., Schütze, S., Reiling, N. (2013). Lipid-labeling facilitates a novel magnetic isolation procedure to characterize of pathogen-containing phagosomes. *Traffic*, 14, 321-336
- Chang, Y.-Y., Stevenin, V., Duchateau, M., Gianetto, Q.G., Hourdel, V., Rodrigues, C.D., Matondo, M., Reiling, N., Enninga, J. (2020). Shigella hijacks the exocyst to duster macropinosomes for efficient vacuolar escape. *PLoS Pathog.* 2020 Aug; 16(8): e1008822. Published online 2020 Aug 31. doi: 10.1371/journal.ppat.1008822
- Stevenin, V., Chang, V.-Y., Le Toquin, V., Duchateau, M., Gianetto, Q.G., Luk, C.H., Salles, A., Sohst, V., Matondo, M., Reiling, N., Enninga, J. (2020) Dynamic Growth and Shrinkage of the Salmonella-Containing Vacuole Determines the Intracellular Pathogen Niche. *Cell Rep.* 2019 Dec 17; 29(12): 3958-3973.e7. Published online 2019 Dec 17. doi: 10.1016/j.celrep.2019.11.049
- Reiling, N., Homolka, S., Kohl, T.-A., Steinhäuser, C., Kolbe, K., Schütze, S., Brandenburg, L. (2017). Shaping the niche in macrophages: Genetic diversity of the M. tuberculosis complex and its consequences of the infected host. *Int. J. Med. Microbiol.*, 2017 Sep 14. pii: S1438-4221(17)30294-1. doi: 10.1016/ j.ijmm. 2017.09.009. [Epub ahead of print]
- Depke, M., Michalik, S., Rabe, A., Surmann, K., Brinkmann, L., Jehmlich, N., Bernhardt, J., Hecker, M., Wollseheid, B., Sun, Z., Moritz, R.-L., Völker, U., Schmidt, F. (2015) A peptide resource for the analysis of Staphylococcus aureus in host pathogen interaction studies. *Proteomics* 2015, 15 (21): 3648-3661