



## Versatile Oral Vaccine Delivery System (ORT-VAC)

Cobra Biologics, with locations in Matfors and Södertälje, Sweden and Keele, UK, provides a full range of services from gene cloning to cGMP manufacturing for pre-clinical through to Phase III clinical trials and commercial supply. In support of customer programs, the company has developed a range of expression technologies. ORT-VAC is an oral attenuated bacterial delivery technology that significantly advances vaccine production, stability, administration and efficacy.

### Features and Benefits

- Increased vaccine efficacy: greatly improved immunogenicity compared to injection
- Potency: multi-copy plasmids produce more antigen than competing technologies
- Oral delivery: ease of distribution and administration using bile-adsorbing resin (BAR) capsules
- Reduced manufacturing time and cost: through minimal downstream purification
- Mucosal immunity: mucosal surfaces are important routes of entry for many pathogens
- Versatility: protein or DNA vaccine delivery against a range of pathogens
- Stability: stable plasmid maintenance without selectable marker gene expression
- Safety: attenuated *Salmonella* vectors with proven safe use in humans

### Potential Indications

- Infectious diseases (proof-of-principal results have been obtained using vaccines against bubonic plague, anthrax, tuberculosis and influenza)
- Cancer vaccines and therapies

### Technical Design

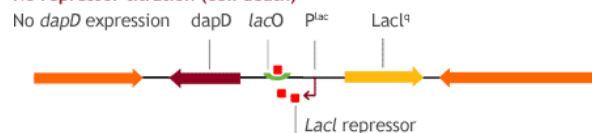
ORT-VAC utilises the ORT<sup>®</sup> (Operator-Repressor Titration) system to enable marker gene-free plasmid selection and maintenance.

In an ORT-VAC strain, the promoter of the gene *dapD* (which produces an amino acid that is an essential component of the cell wall) is replaced with an inducible operator/promoter system.

With no plasmid present, the repressor binds to the operator and shuts down expression, leading to cell death.

When the cell is transformed with a multi-copy plasmid that possesses the operator (*lacO*), the repressor is titrated by the operator sequence on the plasmid, enabling *dapD* expression and cell growth.

#### No repressor titration (cell death)



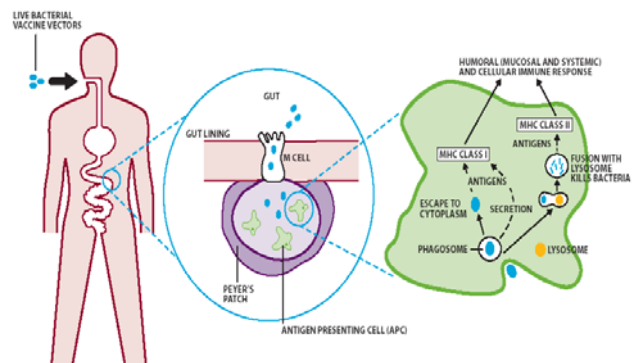
#### Repressor titration (essential gene expression)

*dapD* expression by ORT



### Mechanism of action

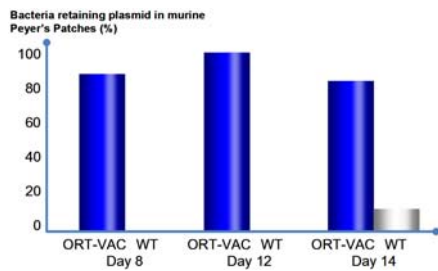
ORT-VAC uses attenuated *Salmonella* (modified to make them safe) which are transformed with the vaccine plasmid and formulated for ingestion. They pass through the stomach and invade the lining of the small intestine, entering lymphatic nodules called Peyer's patches, where they are phagocytosed by antigen-presenting cells. Antigens can be released by surface presentation, secretion or cell lysis. ORT-VAC delivery induces strong antibody and cell-mediated responses via the mucosal immune system.





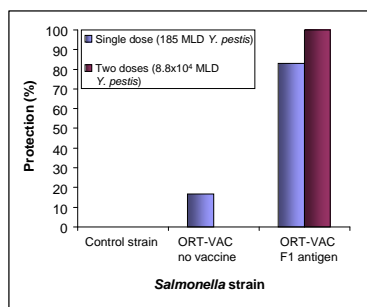
### Superior plasmid stability

Plasmid instability *in vivo* is a major problem when using live bacterial vectors. This is due to the metabolic burden from the plasmid, primarily the continuous selectable marker gene expression. As ORT-VAC is free from unnecessary marker genes, the burden is significantly reduced. The figure shows the proportion of plasmid-containing cells from orally delivered unmodified (grey) and ORT-VAC (blue) *Salmonella* extracted from murine Peyer's patches following oral administration.

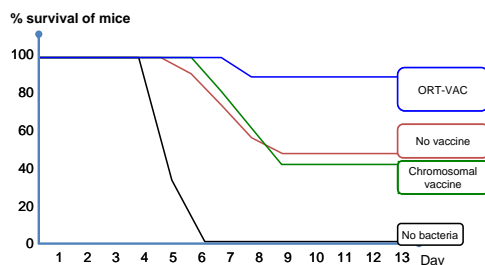


### Effective protein vaccine delivery

The F1 antigen from *Yersinia pestis* (bubonic plague) was expressed from a plasmid in ORT-VAC *Salmonella* and evaluated in a murine model. This provided good protection against a challenge with lethal *Y. pestis*. (Garmory et al. 2005, IAI 73: 2005-2007).

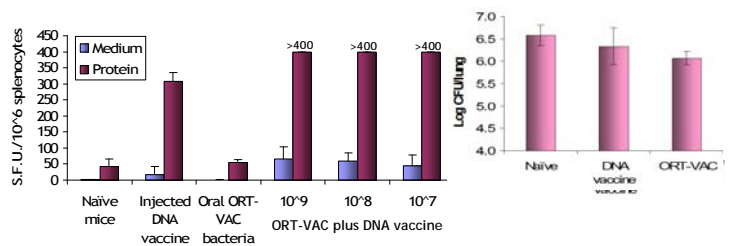


The alternative to multi-copy plasmids is chromosomal integration of a single-copy antigen gene, but this limits antigen production. The protective antigen of *Bacillus anthracis* was inserted in a plasmid in ORT-VAC and into the chromosome of unmodified *Salmonella*; ORT-VAC gave greatly enhanced protection against an anthrax challenge (Leckenby et al. 2009, *Microb. Pathog.* 46: 201-206).



### High copy-number DNA vaccine delivery

The TB antigen MPT64 expressed from a high copy-number DNA vaccine plasmid stimulated higher cellular immune responses than the injected DNA vaccine (by IFN- $\gamma$  ELISPOT, left). This reduced the *M. tuberculosis* count in murine lungs (right) following an aerosol challenge (Huang et al. 2010, *Vaccine* 28:7523-7528).



Capsules containing lyophilised bacteria can be protected against the stomach acid by an enteric coating, but are vulnerable to bile when first released into the small intestine. Encapsulation using BAR allows bacterial hydration whilst protecting from bile salts (Edwards and Slater 2009, *Vaccine* 27: 3897-3903).

